

TASK REPORT

Supplementary Testing of 16 Chemicals in the Recombinant Aromatase Assay

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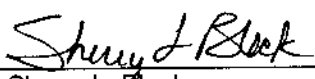
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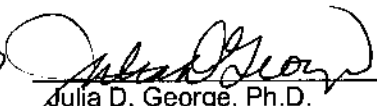
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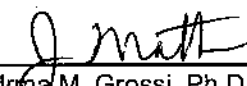
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Quality Assurance Statement

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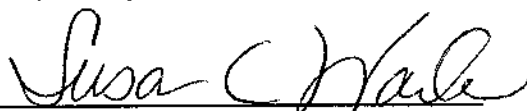
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This study was audited by the Sciences and Engineering – Health Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the Study Director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
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1.0 EXECUTIVE SUMMARY

The response of the aromatase activity in recombinant microsomes to the presence of 16 chemicals was determined in this task order using assay conditions developed during previous assay validation tasks. Each chemical was assayed in triplicate tubes for each of eight concentrations in three replicates of the aromatase assay. The known aromatase inhibitor, 4-hydroxyandrostenedione (4-OH ASDN, formestane, at 8 concentrations), was included as a positive control along with other appropriate controls in each replicate.

All technicians were qualified for participation through successful completion of a positive control (4-OH ASDN) assay.

Full activity and background controls were run with each assay. The mean ($\pm$ SD) full aromatase activity for this batch of recombinant microsomes was 0.313 ± 0.085 nmol/mg/min. In only 1 (of 50 total conducted) assay was the full activity <0.1 nmol/mg/min, and this assay was excluded from analysis. The mean ($\pm$ SD) uncorrected background activity was 0.002 ± 0.001 nmol/mg/min, which corresponded to $0.7 \pm 0.3\%$ of control.

Proposed performance criteria for the four model fit parameters (Top, Bottom, LogIC50, and HillSlope for the positive control assay) were met in 39 of 48 assays. Most of the failures involved only one of the four parameters, and the values were usually just outside the criteria ranges. Data and curve fits for the assays which had performance criteria failures were examined and did not indicate any serious flaw in the functioning of the aromatase assay. It may be advisable to either widen some of the performance criteria ranges slightly, especially those for Top and LogIC50, or to allow acceptance of data sets where only one of the four parameters fails the performance criteria (within certain bounds) in these positive control assays.

Percent of control data from each replicate for each reference chemical were fitted to both a 4- and 2-parameter logistic equation to calculate estimates for Top, Bottom, Log IC50, and HillSlope. Top and Bottom were fixed at 100 and 0%, respectively, for the 2-parameter equation. The data indicate that requiring $\text{Top} = 100 \pm 10\%$ is an acceptable performance criterion for the assay of test chemicals.

The two model curve fits were compared to determine if they were statistically different. In about half of the cases where Prism was able to fit both models, there were significant

differences in the model fits. There was no discernible pattern in the data that would allow prediction of the best model for a particular data set. When the data nearly span the range 0-100% of control, either model can produce good parameter estimates. The increased flexibility of the 4-parameter model usually leads to better curve fits when the inhibited range is limited to less than 0-100%. However, care must be taken in interpreting Prism results from the 4-parameter equation in cases where the 95% confidence intervals of Top and Bottom do not encompass 100 and 0%, respectively. Prism estimates 'LogEC50' as the concentration corresponding to the median of Top and Bottom, and this is not equivalent to the concentration corresponding to 50% inhibition (LogIC50) when Top and Bottom are not encompassed by 100 and 0%, respectively. Equation 3, a modification to the 4-parameter model described in the Results section, can be used to accurately estimate LogIC50 in those cases.

In order to develop a second performance criterion for the test chemical assays, within-replicate coefficients of variation (CV) for the percent of control values at each concentration were examined. Often the CVs were less than 10%, but in some cases they ranged to 15% or higher. High (>15%) CVs are most common at low percent of control activity because of the higher measurement variance in the liquid scintillation spectrometry (LSS) data at these near background levels. The mean CVs across all concentrations within a replicate were <15% in 47 of the 48 assays conducted and were ≤10% in 41 of the 48 assays. Of the seven with CV>10%, only two assays involved chemicals that inhibited aromatase. The 95% confidence interval bands on the curve fits for those two replicates were narrow, indicating a good fit for the model. This suggests that data sets should not be rejected automatically even when CVs range up to about 15%, but rather that higher CVs should prompt further review of the data, including review of percent of control values for outliers that might indicate experimental error and review of the 95% confidence intervals for the fitted curve and parameter estimates.

Only 6 of the 16 test chemicals inhibited aromatase activity >50% and thus were all classified as inhibitors in the present work. These six compounds were: bisphenol A, flavone, triadimefon, imazalil, apigenin, and nitrofen. None of the test chemicals had a maximal inhibition in the range 75-50% of control, so none of them was classified as a chemical for which the data were "equivocal". Ten of the test chemicals were classified as noninhibitors since they did not inhibit aromatase activity more than 25% at any tested concentration (up to 1 mM). The

absence of assay data, with the highest degree of inhibition near the classification boundaries of 75% or 50%, makes evaluation of any data interpretation criterion difficult. In order to generate data that might be useful for data interpretation at the classification boundaries, the data sets from the chemicals classified as inhibitors were limited so that the maximal inhibition was ca. 40-60 % of control. For some replicates, this resulted in there not being enough data to fit a curve. When the curve fit was successful, the 95% confidence interval bands were reviewed in order to assess their utility in data evaluation to 'classify' the chemicals. The 95% confidence interval bounds were narrow, and in all cases where the lowest mean percent of control activity was <50%, both confidence interval bounds also fell below 50%, which would allow the classification of inhibitor to be assigned. Also, in each case when the lowest mean percent of control activity was between 75% and 50% of control, both confidence interval bounds fell completely within that range and the chemical could be classified as an equivocal inhibitor.

Assessment of any aromatase assay data set for test chemicals should begin with a review of the tube-to-tube variance in percent of control. Variances >10-15% should be reviewed for any contribution from experimental error and outliers excluded as appropriate. Concentrations that yield percent of control values <10% can be expected to have higher CVs between tubes. Except in cases where the test chemical was not soluble (which should always be excluded from analysis), data from these concentrations should be allowed to remain in the data set. The data sets should be examined to determine the mean maximum inhibition. Chemicals for which the mean maximum inhibition is >80% of control will likely lead to model fit failures or meaningless curve fits and may be classified as noninhibitors without further analysis. When the mean maximum inhibition is <80% of control, the data should be subjected to curve fitting so that the proper classification can be made. The data show that the 4-parameter model will yield good parameter estimates when the inhibited range spans 100-0% of control; in cases where the inhibited range is less than 0-100%, an alternative model (Equation 3) is preferred. The fitted curve, 95% confidence bounds for the curve, and estimated parameters Top and LogIC50 should be examined. Overall review of the data from the test chemicals that were classified as inhibitors indicates that it is reasonable to keep $Top = 100 \pm 10\%$ as a performance criterion. Bottom and Hillslope are not useful in evaluating assay performance because, especially in the case of partial inhibition curves where a bottom plateau is not defined by the data, there can be high variances

in these two parameters, even when other parameters (Top, LogIC50) have narrow variances. An important indicator of a good curve fit is whether the 95% confidence interval bounds conform to the shape of the fitted curve along its entire length. If the 95% confidence interval bounds diverge from the fitted curve, further examination of the data is warranted. In that case, it might be necessary to choose a different model (such as Equation 3 instead of the 4-parameter equation) or to reject the data set. As discussed above, the 95% confidence interval bounds of the most inhibited point can be used as a data interpretation criterion to distinguish between the three classifications or to determine that the data is equivocal.

2.0 INTRODUCTION

2.1 Background

The Food Quality Protection Act of 1996 was enacted by Congress to authorize the U.S. Environmental Protection Agency (EPA) to implement a screening program on pesticides and other chemicals found in food or water sources for endocrine effects in humans. Thus, the EPA is implementing an Endocrine Disrupter Screening Program (EDSP). In this program, comprehensive toxicological and ecotoxicological screens and tests are being developed for identifying and characterizing the endocrine effects of various environmental contaminants, industrial chemicals, and pesticides. The program's aim is to develop a two-tiered approach, e.g., a combination of *in vitro* and *in vivo* mammalian and ecotoxicological screens (Tier 1) and a set of *in vivo* tests (Tier 2) for identifying and characterizing endocrine effects of pesticides, industrial chemicals, and environmental contaminants. Validation of the individual screens and tests is required, and the Endocrine Disrupter Method Validation Advisory Committee (EDMVAC) will provide advice and counsel on the validation assays.

Estrogens are sex steroid hormones that are necessary for female reproduction and affect the development of secondary sex characteristics of females. Estrogens are biosynthesized from cholesterol by a series of enzymatic steps, with the last step involving the conversion of androgens into estrogens by the enzyme aromatase. Estrogen biosynthesis occurs primarily in the ovary in mature, premenopausal women. During pregnancy, the placenta is the main source of estrogen biosynthesis and pathways for production change. Small amounts of these hormones

are also synthesized by the testes in the male and by the adrenal cortex, the hypothalamus, and the anterior pituitary in both sexes. The major source of estrogens in both postmenopausal women and men occurs in extraglandular sites, particularly in adipose tissue. One potential endocrine target for environmental chemicals is the enzyme aromatase, which catalyzes the biosynthesis of estrogens. An aromatase assay is proposed as one of the Tier 1 Screening Battery Alternate Methods. A detailed literature review (EPA, 2005) on aromatase was performed and encompassed: (1) searching the literature databases, (2) contacting individuals to obtain information on unpublished research, and (3) evaluating the literature and personal communications.

Aromatase is a cytochrome P450 enzyme complex responsible for estrogen biosynthesis and converts androgens, such as testosterone and androstenedione, into the estrogens estradiol and estrone. Aromatase is present in the ovary, placenta, uterus, testis, brain, and extraglandular adipose tissues. Two proteins, cytochrome P450arom and NADPH-cytochrome P450 reductase, are necessary for enzymatic activity, and the enzyme complex is localized in the smooth endoplasmic reticulum. The aromatase gene, designated CYP19, encodes the cytochrome P450arom and consists of 10 exons, with the exact size of the gene exceeding 70 kilobases. Aromatase is also found in breast tissue, and the importance of intratumoral aromatase and local estrogen production is the subject of current research efforts. Aromatase inhibitors have been developed as efficacious therapeutic agents for estrogen-dependent breast cancer to reduce the stimulatory effects of estrogens in the progression of breast cancer. Investigations on the development of aromatase inhibitors began in the 1970s and have expanded greatly in the past three decades.

An *in vitro* aromatase assay could easily be utilized as an alternative screening method in the Tier 1 Screening Battery to assess the potential effects of various environmental toxicants on aromatase activity. Both *in vitro* subcellular (microsomal) assays and cell-based assays are available for measuring aromatase activity. The *in vitro* subcellular assay using human placental microsomes is commonly used to evaluate the ability of pharmaceuticals and environmental chemicals to inhibit aromatase activity. In addition, human JEG-3 and JAR choriocarcinoma cell culture lines, originally isolated from cytotrophoblasts of malignant placental tissues, have been used as *in vitro* systems for measuring the effects of compounds on aromatase activity. These

cell lines are also utilized for investigations on the effects of agents in placental toxicology. The H295R human adrenocortical carcinoma cell line has been used by Sanderson and colleagues to examine the effect of the 2-chloro-S-triazine herbicides (Sanderson et al., 2000) and diindolylmethane and analogs (Sanderson et al., 2001).

Numerous flavonoids and related phytoestrogen derivatives have been extensively evaluated for their ability to inhibit aromatase activity for two primary reasons: (1) these natural plant products can serve as possible leads for the development of new nonsteroidal aromatase inhibitors; and (2) humans and other animals are exposed to these agents through the diet. In general, the flavonoids and related analogs demonstrate aromatase inhibition with IC₅₀ values in the micromolar range; however, these compounds lack both the potency and specificity of aromatase inhibitors developed for breast cancer therapy (Adlercreutz et al., 1993; Chen et al., 1997; Ibrahim and Abul-Hajj, 1990; Jeong et al., 1999, Kao et al., 1998; Le Bail et al., 1998, 2000a, 2000b, 2001). Several pesticides have also demonstrated inhibition of aromatase activity in the human placental microsomal assay system (Vinggaard, 2000), with IC₅₀ values for aromatase inhibition as low as 0.04 μ M.

Interlaboratory validation studies of the human recombinant and placental aromatase assay were conducted on 10 chemicals in four laboratories. Those studies established the equivalence of the two assays and the ability of the laboratories to successfully conduct the assay using the standardized protocols previously developed. The recombinant aromatase microsomal assay was recommended as the *in vitro* aromatase screening assay to be included in the Tier 1 Screening Battery. This assay will detect environmental contaminants that possess the ability to inhibit aromatase activity. Upon review of the validation results, EPA determined that some additional testing was necessary to more completely elucidate the ability of the assay to assess the aromatase inhibition potential of a fuller range of chemicals. Each of the 16 chemicals to be used in this task order will be tested in one laboratory with three independent replicates per chemical.

2.2 Objectives

This work assignment provided for the testing of an additional 16 chemicals to support the validation of the recombinant aromatase assay. In addition to broadening the range of classes

and potencies of the chemicals tested, this work assignment tested the performance criteria that EPA developed using the data from the interlaboratory testing, using the recombinant microsomal assay and the impact of using a four-parameter (no constraints) versus a two-parameter model in which the Top and Bottom values are set to 100% and 0%, respectively.

The performance criteria for the positive control assay are presented below. For the reference chemicals, only a performance criterion for Top ($100 \pm 10\%$) was defined. A second performance criteria was developed as part of this task.

Positive Control Assay Performance Criteria

Parameter	Criteria
BOTTOM	0 ± 5
TOP	100 ± 10
LOGIC50	-7.0 to - 7.3
HILLSLOPE	-0.8 to -1.2

3.0 MATERIALS AND METHODS

3.1 Study Protocol and QAPP

Procedures used on this study are described in the Study Protocol (Appendix A) and QAPP (Appendix B).

3.2 Substrate

The substrate, nonradiolabeled androstenedione (ASDN) and radiolabeled androstenedione ($[1\beta\text{-}^3\text{H}]$ -androstenedione, $[^3\text{H}]$ ASDN) were provided by the Chemical Repository (CR). Lot and source details are presented in Table 1 and Appendix C.

Table 1. Substrate Data

Chemical	CAS Number	Molecular Formula (Molecular Weight [g/mol])	Supplier	Lot Number	Stated Purity
ASDN	63-05-8	C ₁₉ H ₂₆ O ₂ (286.4)	Sigma	115K12921	99%
[³ H]ASDN	NA	C ₁₉ H ₂₆ O ₂ (286)	Perkin Elmer	3557306	>97%

3.2.1 Radiochemical Purity

The radiochemical purity of the [³H]ASDN was determined using high performance liquid chromatography (HPLC) and liquid scintillation counting. The HPLC system consisted of a Waters 2690 Separations Module, a Waters 2487 Dual λ Absorbance Detector, and a β -RAM Model 3 flow-through radioactivity detector (IN/US, Inc., Tampa, FL) with a 250 μ L glass scintillant cell. Data were collected using Empower2 Client/Server Chromatography Data System Software.

The HPLC method used a Zorbax SB-C₁₈ column (4.6 x 250 mm) with a mobile phase of 55:15:30 (v:v:v) distilled, deionized water: tetrahydrofuran: methanol and a flow rate of 1 mL/min. The eluant was monitored by UV absorbance at 240 nm and by a flow-through radiochemical detector. Eluant fractions were collected manually into vials containing ca. 10 mL Ultima Gold and assayed for radiochemical content by liquid scintillation spectrometry (LSS). A reference standard of nonradiolabeled ASDN was analyzed by the same method and coelution of the nonradiolabeled and radiolabeled ASDN was confirmed.

3.2.2 Preparation of Substrate Solution

The substrate solution was prepared fresh each day of assay by combining solutions of [³H]ASDN and non-radiolabeled ASDN in order to prepare a solution containing 2 μ M ASDN with ca. 1 μ Ci/mL. A 1:100 dilution of the radiolabeled [³H]ASDN stock in buffer was prepared fresh each day of assay. A solution (1 mg/mL) of ASDN in ethanol was prepared (fresh each day) and then diluted in buffer to a final concentration of 1 μ g/mL. The substrate solution was prepared by combining 4.5 mL of the 1 μ g/mL solution of ASDN, 800 μ L of the [³H]ASDN

dilution and 2.7 mL buffer to make 8 mL of substrate solution. The addition of 100 μ L of the substrate solution to each 2 mL assay volume yielded a final [^{3} H]ASDN concentration of 100 nM with ca. 0.1 μ Ci/tube.

3.3 Reference Chemicals

Reference chemical stock solutions were prepared and analyzed by the CR and shipped to RTI's Materials Handling Facility (MHF), along with MSDSs and information related to the content (including solvent), molarity, and storage requirements for each formulation. The CR supplied the MHF with solvents of the same lot used in the preparation of the stock solutions which were then used in preparation of dilutions of the stock solutions for use in the assay. Reference chemicals were formulated at a concentration of 0.1 M in DMSO, except for CR11603 which was supplied as a 0.1 M solution in EtOH. Suppliers and lot numbers for reference chemical vehicles are presented in Table 6. The CR coded the reference chemical stock solutions prior to distributing them to the MHF, and documents identifying the chemicals by name were separated from the samples prior to distribution of the solutions to the assaying laboratory, so that the technicians were blind to the identity of the chemical under testing. The chemical codes, chemical names, CAS numbers, molecular formulas, and reason for selections for testing for the reference chemicals are presented in Table 2. All reference chemical formulations were stored refrigerated (ca 4°C) except for CR11610, CR11611, and CR11612, which were stored frozen (ca. -20°C). Reference chemical custody sheets are presented in Appendix C.

Table 2. Reference Chemicals

Chemical Code	Chemical	CAS No.	Molecular Formula	Reason for Selection
CR11601	Vinclozolin	50471-44-8	286.12	Used in other validation studies and has been reported to be negative in both H295R and KGN cell-based assays.
CR11602	Bisphenol A	80-05-7	228.29	A challenging chemical used extensively in ED testing. Structurally different from the pesticides tested and reported to be an inhibitor in the KGN assay.
CR11603	Tributyltin	688-73-3	291.06	Structurally different from all other chemicals tested and reported in the literature to be an aromatase inhibitor.
CR11604	Diethylhexyl phthalate	117-81-7	390.56	Used in other validation studies. Reported to be an inhibitor in the KGN assay.
CR11605	Methoxychlor	72-43-5	345.66	Used in other validation studies. Known to be metabolized to a strongly estrogenic agent and to have antiandrogenic properties. Reported to be negative in the KGN assay.
CR11606	Aldicarb	116-06-3	190.3	Aldicarb is a carbamate and therefore structurally different from other pesticides tested in the validation program. It was reported to be an inhibitor in the KGN assay.
CR11607	Flavone	525-82-6	222.24	Flavone is the parent of a group of natural aromatase inhibitors. It is reported to be a weak inhibitor in the KGN assay and a moderate (10 μ M) inhibitor in the PM assay.
CR11608	Triadimefon	43121-43-3	293.75	A weak to moderate azole fungicide inhibitor for which several literature values were found.
CR11609	Imazalil	35554-44-0	297.20	An azole fungicide for which several literature values were found.
CR11610	Apigenin	520-36-5	270.24	A flavone with known aromatase activity.
CR11611	Ronidazole	7681-76-7	200.15	Reported to be a very weak azole fungicide inhibitor in the HT assay.
CR11612	Genistein	446-72-0	270.24	An isoflavone reported to be negative. Genestein has been used extensively in ER binding and other ED validation studies.
CR11613	p,p'-DDE	72-55-9	318.03	An organochlorine insecticide known to be estrogenic. It has been reported to be an inhibitor in the KGN assay.
CR11614	Alachlor	15972-60-8	269.8	A chlorinated herbicide reported to be negative in the KGN study.
CR11615	Nitrofen	1836-75-5	284.10	A diphenyl ether reported to be negative in the KGN assay.
CR11616	Trifluralin	1582-09-8	335.32	A dinitroaniline reported to be negative in the KGN assay.

3.3.1 Reference Chemical Dilution Preparation

The total volume of reference chemical formulation used in each assay was no more than 1% of the total assay volume (i.e., 20 μ L in a 2 mL assay) in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution were prepared in the same solvent as the stock solution on the day of use, such that the target concentration of reference chemical was achieved by the addition of 20 μ L of the dilution to a 2-mL assay volume. The dilution scheme described in Table 3 was used in the preparation of dilutions for the first replicate of each reference chemical. The results of each Replicate 1 assay were reviewed and adjustments were made as necessary (as per study protocol) to the concentrations tested in Replicates 2 and 3 of the assay in order to obtain data that better described the concentration response curve. The concentrations of each reference chemical tested in all replicates are included in Table 14 and Appendix G.

Table 3. Dilution Scheme for the First Replicate of all Reference Chemicals

Dilution ID	Volume Vehicle (μ L)	Volume Sample (μ L)	Sample Used	Solution Concentration (M)	Final Concentration in Assay (M)
1	0	1000	Stock	1.00E-01	1.00E-03
2	900	100	Diln. 1	1.00E-02	1.00E-04
3	900	100	Diln. 2	1.00E-03	1.00E-05
4	900	100	Diln. 3	1.00E-04	1.00E-06
5	900	100	Diln. 4	1.00E-05	1.00E-07
6	900	100	Diln. 5	1.00E-06	1.00E-08
7	900	100	Diln. 6	1.00E-07	1.00E-09
8	900	100	Diln. 7	1.00E-08	1.00E-10

3.4 Positive Controls

The known aromatase inhibitor, 4-hydroxyandrostenedione (4-OH ASDN, formestane, Sigma, Lot #081K2133), was used as a positive control. Stock solution (Table 4) was supplied by Battelle's CR (Appendix C) and was stored refrigerated and protected from light. Dilutions were made fresh each day in EtOH, Lot 06C08PA as described in Table 5.

Table 4. 4-OH ASDN (Positive Control) Information

Chemical	Mfr Purity	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Stock Formulation Concentration (M)	Vehicle
4-OH ASDN	99.6%	566-48-3	C ₁₉ H ₂₆ O ₃	302.4	0.01	100% ethanol

Table 5. Dilution Scheme for 4-OH ASDN

Dilution ID	Volume EtOH (μL)	Volume Sample (μL)	Sample Used	Solution concentration (M)	Final Concentration in assay (M)
1	0	1000	Stock	1.00E-03	1.00E-05
2	900	100	Diln. 1	1.00E-04	1.00E-06
3	300	300	Diln. 2	5.00E-05	5.00E-07
4	900	100	Diln. 2	1.00E-05	1.00E-07
5	300	300	Diln. 4	5.00E-06	5.00E-08
6	900	100	Diln. 4	1.00E-06	1.00E-08
7	900	100	Diln. 6	1.00E-07	1.00E-09
8	900	100	Diln. 7	1.00E-08	1.00E-10

3.5 Microsomes

Recombinant microsomes (Human CYP19 + P450 Reductase SUPERSOMES™, Lot # 67045, 6.5 mg protein/mL, purchased from Gentest, Woburn, MA) were used on this task. The product data sheet for the microsomes is presented in Appendix C. The microsomes were stored at approximately -70 to -80°C.

Twenty vials of the stock microsomes were thawed rapidly in a 37 ± 1°C water bath and immediately transferred to an ice bath. The microsomes were pooled, rehomogenized using a Potter Elvehjem homogenizer, and then were divided into 160-μL aliquots, refrozen in liquid nitrogen, and stored at approximately -70 to -80°C.

On the day of use, microsomes were thawed rapidly in a 37 ± 1°C water bath and then immediately transferred to an ice bath. The microsomes were rehomogenized by vortexing about 5 seconds prior to use. The microsomes were diluted in buffer (1:20 and then 1:40 for a

total 1:800 dilution) to an approximate protein concentration of 0.008 mg/mL, and the dilutions were stored on ice for no more than 1 h before being used in the assay.

3.6 Other Assay Components

In addition to substrate, reference or control chemicals or vehicle, and microsomes, the aromatase assay contained NADPH, propylene glycol, and phosphate buffer. Supplier and lot numbers for other aromatase assay components are presented in Table 6.

Table 6. Supplier and Lot Numbers for Aromatase Assay Components

Chemical	Supplier	Lot Number
NADPH	Sigma	096K7029
Propylene glycol	J.T.Baker	B37606
Sodium phosphate dibasic	J.T.Baker	E01465
Sodium phosphate monobasic	J.T.Baker	A12H20
EtOH (vehicle for 4-OH ASDN and Reference Chemical CR11603)	Battelle CR	06C08PA
DMSO (vehicle for all Reference Chemicals except CR11603)	Battelle CR	00743BD

3.6.1 NADPH

A solution of NADPH (β -nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt, Sigma, cat # 1630, 833.4 g/mol, 6 mM) in pH 7.4 sodium phosphate buffer was prepared fresh each day of assay and kept on ice until used.

3.6.2 Assay Buffer

The assay buffer was 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (JT Baker, catalog # 4011-01, 137.99 g/mol) and sodium phosphate dibasic (JT Baker, catalog # 4062-01, 141.96 g/mol) were used in the preparation of the buffer. Solutions of each reagent at 0.1 M were prepared in distilled, deionized water and then the solutions were combined to a final pH of 7.4. The assay buffer was stored for up to one month in the refrigerator (2-8°C).

3.7 Protein Determination

Protein content of the microsomal preparation was determined by using a DC Protein Assay kit purchased from Bio-Rad (Hercules, CA). The protein concentration was determined in 8 independent assays using the standard protein assay and a 1:20 dilution of the stock microsomes. The micro protocol was used in 5 independent assays to measure the protein content of a 1:800 dilution of the microsome stock. The protein curve standards were prepared with bovine serum albumin (BSA). Quality control (QC) samples for the standard protocol were from a set of standards supplied by Pierce (Rockford, IL). The QCs for the micro protocol were prepared by diluting the standards from that same set. Unknown and curve standards were run in triplicate and QC standards were run in duplicate.

For the standard protocol, a 6-point standard curve (0.13 to 1.5 mg/mL) was prepared. To a 25- μ L aliquot of unknown, QC or curve standard, 125 μ L of BioRad DC Protein Kit Reagent A was added and mixed. Next, 0.8 mL of BioRad DC Protein Kit Reagent B was added to each sample and the samples were vortex mixed.

For the micro protocol, a 6-point standard curve (5 to 250 μ g protein/mL) was prepared. To a 200- μ L aliquot of unknown, QC or curve standard, 100 μ L of BioRad DC Protein Kit Reagent A was added and mixed. Next, 1 mL of BioRad DC Protein Kit Reagent B was added to each sample and the samples were vortex mixed.

Both protocols continued as follows. The samples were allowed to sit at room temperature for at least 15 min to allow for color development. The absorbances are stable for about 1 h. Each sample (unknown, QCs, and standards) was transferred to disposable polystyrene cuvettes and the absorbance (@ 750 nm) was measured using a spectrophotometer. The protein concentration of the microsomal sample was determined by interpolation of the absorbance value using the curve developed from the protein standards.

3.8 Aromatase Assay Method

The assays were performed in 13x100 mm test tubes maintained at $37 \pm 1^\circ\text{C}$ in a shaking water bath. Propylene glycol (100 μ L), [^3H]ASDN, NADPH (as applicable) and buffer (0.1 M sodium phosphate buffer, pH 7.4), and reference chemical (or control or vehicle) were combined in the test tubes (total volume 1 mL). The volume of the reference or control chemical solutions

or vehicle used was 20 μ L (1% of total assay volume). The final concentrations for the assay components are presented in Table 7. The tubes and the microsomal suspension were placed in the $37 \pm 1^\circ\text{C}$ water bath for 5 min prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume was 2 mL, and the tubes were incubated for 15 min. The incubations were stopped by the addition of methylene chloride (2 mL); the tubes were vortex-mixed for ca. 5 s and placed on ice while the rest of the incubations were stopped. The tubes were then vortex-mixed an additional 10-15 s. The tubes were then centrifuged using an AllegraX-15R centrifuge with an SX4750a rotor for 10 min at a setting of 1000 rpm (ca. 230 x g) to aid in the separation of the organic and aqueous phases. In some instances, a Beckman GS-6R centrifuge with GH-3.8 rotor was used for centrifugation in lieu of AllegraX-15R. The methylene chloride layer (bottom) was removed and discarded; the aqueous layers were extracted again with methylene chloride (2 mL). This extraction procedure was performed one additional time, each time discarding the methylene chloride layer. The aqueous layers were transferred to vials and duplicate aliquots (0.5 mL) were transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) was added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot was determined by using liquid scintillation spectrometry (LSS). Radiolabel found in the aqueous fractions represented tritiated water formed. The aromatization of one mole of [^3H]ASDN resulted in the production of one mole of estrone (non-radiolabeled) and one mole of tritium associated with water.

Table 7. Optimized Aromatase Assay Conditions

Assay factor (units)	Recombinant Assay
Microsomal Protein (mg/mL) ^a	0.004
NADPH (mM) ^a	0.3
[^3H]ASDN (nM) ^a	100
Incubation Time (min)	15

^a Final concentrations

3.9 Positive Control Assays

Each technician who was participating in the testing of reference chemicals conducted a single replicate of the positive control experiment as outlined in Table 8. Data from this replicate was assessed against the performance criteria that were included in the protocol.

Table 8. Positive Control Experiment Outline

Sample Type	Repetitions (Tubes)	Description	4-OH ASDN Concentration (M)
Full Activity Control	4	Complete assay ^a with inhibitor vehicle control, no inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc 1	3	Complete assay with 4-OH ASDN added	1×10^{-5}
Positive Control Conc 2	3	Same	1×10^{-6}
Positive Control Conc 3	3	Same	5×10^{-7}
Positive Control Conc 4	3	Same	1×10^{-7}
Positive Control Conc 5	3	Same	5×10^{-8}
Positive Control Conc 6	3	Same	1×10^{-8}
Positive Control Conc 7	3	Same	1×10^{-9}
Positive Control Conc 8	3	Same	1×10^{-10}

^a The complete assay contains buffer, propylene glycol, microsomal protein, [³H] ASDN, and NADPH

3.10 Determination of the Reponse of Aromatase Activity to Reference Chemicals

Each of the 16 reference chemicals listed in Table 2 were tested in three independent replicates of the aromatase assay using a single technician per chemical as outlined in Table 9. Three to four reference chemicals were tested on each day of assay and shared a set of positive control (4-OH ASDN) dilutions. As described in Table 9, a full eight-point positive control assay, with duplicate determinations per concentration, was conducted concurrently with each reference chemical assay set. The following sets of reference chemicals were run concurrently and shared control dilution sets:

- vinclozolin, diethylhexyl phthalate, p,p'-DDE
- bisphenol A, tributyltin, flavone

- methoxychlor, aldicarb, alachlor
- triadimefon, ronidazole, nitrofen, trifluralin
- imazalil, apigenin, genistein

Each reference chemical was tested at eight concentrations and there were three (triplicate) repetitions for each concentration of a given replicate. A single replicate study of an example reference chemical is described in Table 9.

Table 9. Reference Chemical Study Design

Sample Type	Repetitions (Test Tubes)	Description	Test Chemical Concentration (M, Final)
Full Enzyme Activity Control	4	Complete assay ^a with reference chemical vehicle control, no inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc 1	2	Complete assay with 4-OH ASDN added	1×10^{-5}
Positive Control Conc 2	2	Same	1×10^{-6}
Positive Control Conc 3	2	Same	5×10^{-7}
Positive Control Conc 4	2	Same	1×10^{-7}
Positive Control Conc 5	2	Same	5×10^{-8}
Positive Control Conc 6	2	Same	1×10^{-8}
Positive Control Conc 7	2	Same	1×10^{-9}
Positive Control Conc 8	2	Same	1×10^{-10}
Reference Chem. Conc 1	3	Complete assay with Reference Chemical added	1×10^{-3}
Reference Chem. Conc 2	3	Same	1×10^{-4}
Reference Chem. Conc 3	3	Same	1×10^{-5}
Reference Chem. Conc 4	3	Same	1×10^{-6}
Reference Chem. Conc 5	3	Same	1×10^{-7}
Reference Chem. Conc 6	3	Same	1×10^{-8}
Reference Chem. Conc 7	3	Same	1×10^{-9}
Reference Chem. Conc 8	3	Same	1×10^{-10}

^a The Complete Assay contained buffer, propylene glycol, microsomal protein, [³H]ASDN, and NADPH

Two types of control samples were included with each replicate. The background activity tubes served as the negative controls. These included:

- full enzyme (aromatase) activity controls which contained substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes
- background activity controls which contained all components that were in the full aromatase activity controls, except NADPH

Four test tubes of each type of control were included with each replicate and were treated the same as the other samples. The controls sets were split so that two tubes (of each control type) were run at the beginning and two at the end of each replicate set.

3.11 Data Analysis

3.11.1 Aromatase Activity and Percent of Control Calculations

Data were entered into an Excel spreadsheet for calculation of aromatase activity and percent of control. The master spreadsheet used was titled Aromatase_Master_Version1.5.xls.

For each repeat tube (full enzyme activity and background activity controls and each positive control and reference chemical concentration), the Excel spreadsheet included total observed (uncorrected) disintegrations per minute (DPMs) per tube and total aromatase activity per tube. The DPM values were corrected for the background DPMs, as measured by the mean DPM of the background activity control tubes. The aromatase activity was calculated as the corrected DPM, normalized by the specific activity of the [³H]ASDN, the mg of protein of the aromatase, and the incubation time. The average (corrected) DPMs and aromatase activity across the four background activity control repeat tubes were necessarily equal to 0 within each replicate, and the mean activity of the full aromatase activity controls represented 100% activity.

For each tube, the remaining percent of control activity was determined by dividing the background-corrected activity for that tube by the mean activity of the full aromatase activity controls and multiplying by 100. Nominally one might expect for an inhibitor the percent of control activity values to vary between approximately 0% near the high inhibition concentrations and approximately 100% near the low inhibition concentrations. However, due to experimental

variation, individual observed percent of control values sometimes extended below 0% or above 100%.

3.11.2 Model Fitting

Percent of control data (for both the reference chemical and positive control chemical [4-OH ASDN]) obtained during the reference chemical testing were imported into GraphPad Prism version 4.03 to fit a four-parameter model and a two parameter model for each chemical. The four-parameter model fit to the data to obtain the estimated dose-response curve was:

$$Y = Bottom + \left(\frac{Top - Bottom}{1 + 10^{(\mu - X)\beta}} \right)$$

where Y is the percent of control activity remaining, X is the $\log_{10}$ of the concentration of the chemical, Top and $Bottom$ are the plateaus of the response curve, μ is the $\log_{10}IC_{50}$ and β is the slope of the response curve at $\log_{10}IC_{50}$. The two-parameter model uses the same equation with $Bottom$ fixed at 0 and Top fixed at 100. In the current study, the four-parameter model is referred to as Equation 1 and the two parameter model is referred to as Equation 2.

3.11.3 Statistical Analyses

3.11.3.1 Analysis of Fitted Curves for Differences

The sums of squares, root mean square error, and degrees of freedom results from each model fit for each chemical were used to perform a partial F-test to test for significant differences simultaneously among the four model parameters. The partial F-test was calculated as follows:

$$F = \frac{(MSS_{FM} - MSS_{RM}) / (MDF_{FM} - MDF_{RM})}{RSS_{FM} / RDF_{FM}}$$

where MSS_{FM} denotes the model sum of squares for the full model containing all four parameters, MDF_{FM} is the model degrees of freedom for the full model, RSS_{FM} and RDF_{FM} denote the residual sum of squares and degrees of freedom, respectively, for the full model and MSS_{RM} and MDF_{RM} denote the model sum of squares and degrees of freedom for the reduced model containing two parameters.

The partial F-test to compare the two models was done with GraphPad Prism Version 4.03 using that software's "extra sum-of-squares F test".

3.11.3.2 Comparison of Positive Control Values Within and Across Technicians

In order to assess the variation across and within technicians (for the reference chemical assays), a mixed effects model was fit to the positive control values with the 4-OH ASDN concentrations as a fixed effect and the technicians along with the replicates within technicians, as random effects.

3.11.3.3 Evaluation of the Suitability of the Positive Control Performance Criteria

To assess the suitability of the quality assurance measures, the dose-response model results from each run of the positive control experiments were checked to see if all of the parameter estimates met the performance criteria. The 95% confidence intervals for each parameter estimate were examined for how they compared with the performance criteria ranges to assess whether the performance criteria ranges need to be modified.

3.12 Statistical Software

Concentration response curves were fitted to the data using the nonlinear regression analysis features in the PRISM software package, Version 4.03. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons were carried out using PRISM and the SAS statistical analysis system, Version 9.1.

4.0 RESULTS

4.1 Radiochemical Purity

The HPLC radiochromatogram of the [^3H]ASDN, lot number 3557306, is presented in Figure 1. The measured radiochemical purity of the [^3H]ASDN was 98%. This surpasses the minimum radiochemical purity standard of 95%.

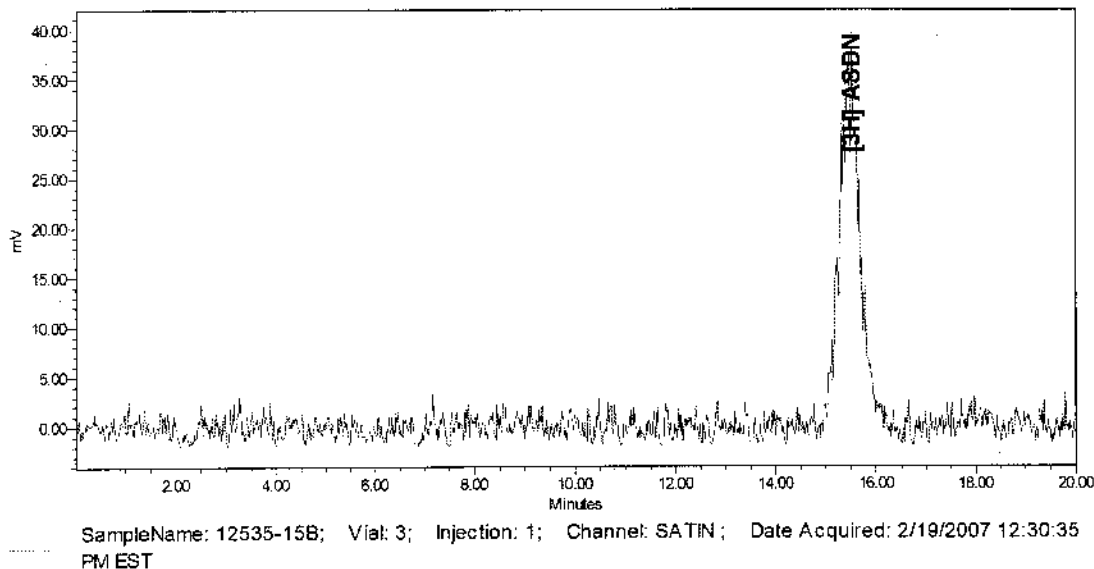


Figure 1. HPLC Radiochromatogram of [^3H]ASDN

4.2 Stock Formulation Analysis

The formulation stability and formulation analysis results for all 16 reference chemicals and the positive control chemical from the Battelle CR are included in their chemistry reports. Formulation analysis data are summarized in Table 10.

Table 10. Formulation Preparation and Use

Reference Chemical Code	Chemical ID	Manufacturer's Purity (%)	Reference Chemical Vehicle	Stock Solution Prep Date	Date of Last Use
CR11601	Vinclozolin	99.5	DMSO	2/19/07	2/28/07
CR11602	Bisphenol A	99.9	DMSO	2/26/07	3/13/07
CR11603	Tributyltin	97.9	EtOH	2/26/07	3/7/07
CR11604	Diethylhexyl phthalate	99.6	DMSO	2/19/07	2/28/07
CR11605	Methoxychlor	98	DMSO	3/5/07	3/14/07
CR11606	Aldicarb	99	DMSO	3/5/07	3/14/07
CR11607	Flavone	99.7	DMSO	2/26/07	3/7/07
CR11608	Triadimefon	99.8	DMSO	3/12/07	3/21/07
CR11609	Imazalil	99	DMSO	3/19/07	3/28/07
CR11610	Apigenin	95	DMSO	3/19/07	3/28/07
CR11611	Ronidazole	100.6	DMSO	3/12/07	3/21/07
CR11612	Genistein	98.8	DMSO	3/19/07	3/28/07
CR11613	p,p'-DDE	98.6	DMSO	2/19/07	2/28/07
CR11614	Alachlor	99.2	DMSO	3/5/07	3/14/07
CR11615	Nitrofen	98	DMSO	3/12/07	3/21/07
CR11616	Trifluralin	99.5	DMSO	3/12/07	3/21/07

4.3 Protein Analysis

Protein content of the recombinant microsomes was measured as described in Section 3.7 using both the standard and micro assay protocols. Each day of assay, the protein content of at least three independently prepared dilutions of the stock microsomes was measured. There was high variability in the QC results obtained with the microassay protocol, while the standard assay was more reliable; therefore, only data from the standard assay were used and are included in this report. Protein assay spreadsheets are presented in Appendix D.

The standard protein assay included standards ranging from 0.13 to 1.5 mg protein/mL. QC standards at concentrations of 0.25, 0.5, and 1 mg/mL were included in each assay set. A total of eight independent protein assays were conducted, and three failed the requirement that at least two-thirds of the QCs samples be within 15% of the nominal value and were thus excluded from inclusion in the determination of microsome stock protein content. The QC results for all standard protocol assays are presented in Table 11.

Table 11. Protein QC Table

Assay Date/ Notebook Ref	Nominal Concentration (mg/mL)					
	1		0.5		0.25	
	Measured (mg/mL)	% RE	Measured (mg/mL)	% RE	Measured (mg/mL)	% RE
2/20/07 12535-30	1.03	2.9	0.59	18.79	0.36	45.5
	1.05	5.4	0.50	0.38	0.29	16.9
2/22/07 12535-46	1.11	10.5	0.57	13.8	0.17	-33.9
	0.92	-7.7	0.58	16.0	0.28	10.4
2/27/07 12535-49	0.64	-35.5	0.42	-16.2	0.25	-1.0
	0.59	-40.9	0.30	-40.7	0.34	34.3
2/28/07 12535-52	1.11	11.2	0.52	5.0	0.25	1.3
	0.98	-2.4	0.60	19.1	0.21	-16.1
3/1/07 12535-55	1.07	7.3	0.52	4.4	0.26	3.6
	0.98	-2.5	0.53	6.4	0.26	5.6
3/6/07 12535-58	1.06	5.8	0.45	-10.2	0.24	-3.8
	0.81	-18.8	0.56	12.2	0.22	-10.2
3/9/07 12535-64	0.97	-2.8	0.58	15.5	0.29	16.5
	0.96	-3.9	0.62	23.5	0.18	-27.0
3/13/07 12535-67	1.11	11.2	0.57	14.6	0.35	41.7
	0.99	-0.6	0.57	14.6	0.18	-26.6

Bold designates assay passes QC requirements

All measured protein concentrations for the microsome stock are presented in Table 12. The overall average measured protein concentration for the recombinant microsomes was 8.0 ± 0.5 mg/mL (6.8 % CV). This was about 23% higher than the 6.5 mg/mL concentration reported by the supplier of the microsomes. As specified in the protocol, since the measured value was outside the range $100 \pm 10\%$ of the nominal value, the measured value was used in all calculations of aromatase activity.

Table 12. Measured Protein Concentrations

Assayed Microsome Dilution ID	Measured Protein Concentration (mg/mL)
12535-46C	7.25
12535-47G	8.73
12535-48C	8.14
12535-52C	7.78
12535-53G	8.65
12535-54C	8.36
12535-55C	7.41
12535-56G	7.48
12535-57C	8.41
12535-58C	6.88
12535-59G	7.98
12535-60C	8.48
12535-67C	8.19
12535-68G	8.33
12535-69C	8.01
Average	8.00
SD	0.5
CV%	6.8
SEM	0.1

4.4 Positive Control Assay (Task 5) Results

Each of four chemists conducted the positive control assay as described in Section 3.9. The curve fit results are presented in Table 13 and graphically in Figure 2. The four parameter response curve for the positive control (4-OH ASDN) was examined to determine if the criteria listed in Table 13 had been met.

All parameters met the performance criteria so all technicians were qualified to proceed with the reference chemical testing.

Table 13 Positive Control Curve Fit Parameters and Criteria

Parameter	Technician				Criteria
	1	2	3	4	
BOTTOM	0.2	1.4	-0.1	1.2	0 ± 5
TOP	99	92	90	108	100 ± 10
LOGIC50	-7.2	-7.1	-7.2	-7.2	-7.0 to -7.3
HILLSLOPE	-1.0	-1.1	-1.0	-1.1	-0.8 to -1.2

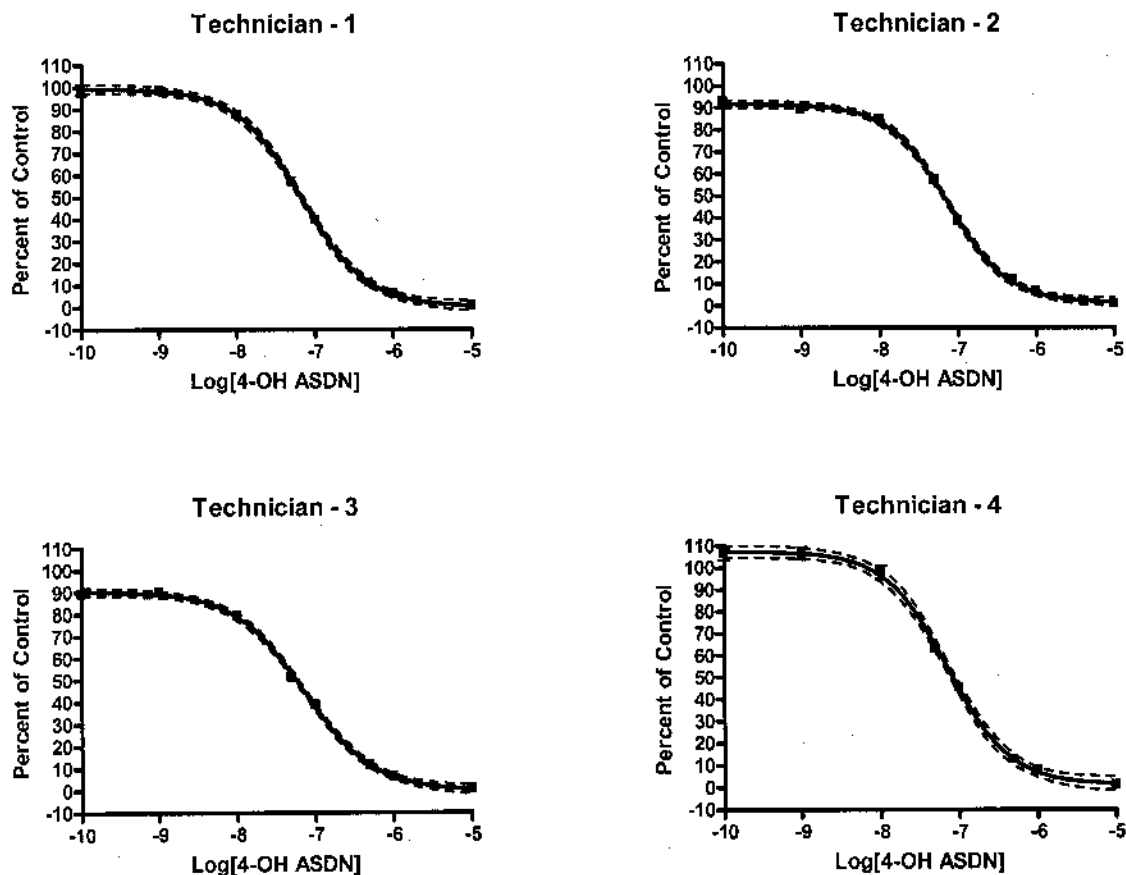


Figure 2. Positive Control Assay Concentration Response Curves

4.5 Determination of the Response of Aromatase Activity to Reference Chemicals

4.5.1 Tested Reference Chemical Concentration Ranges

Three independent replicate assays were conducted for each reference chemical and all assays for a given reference chemical were conducted by the same technician. In cases where evidence of insolubility of the reference chemical was noted in the assay tubes of Replicate 1 or where different concentrations would better describe the concentration response curve, changes were made to the reference chemical concentrations tested in succeeding replicates. Information

regarding assay dates, substrate specific activity, and tested reference chemical concentration ranges is presented in Table 14.

Table 14. Reference Chemical Assay Dates and Concentration Ranges

Chemical Code	Test Chemical ID	Technician	Replicate	Assay Date	Substrate Soln SA $\mu\text{Ci}/\mu\text{g}$ ASDN	Reference Chemical Concentration Range (M)
CR11601	Vinclozolin	1	1	2/22/07	1.461	1.00E-03 to 1.00E-10
			2	2/27/07	1.220	1.00E-04 to 1.00E-10
			3	2/28/07	1.521	1.00E-04 to 1.00E-10
CR11602	Bisphenol A	4	1*	3/1/07	1.599	1.00E-03 to 1.00E-10
			4	3/9/07	1.503	1.00E-03 to 1.00E-06
			5	3/13/07	1.603	1.00E-03 to 1.00E-06
CR11603	Tributyltin	2	1	3/1/07	1.570	1.00E-03 to 1.00E-10
			2	3/6/07	1.480	1.00E-03 to 1.00E-10
			3	3/7/07	1.478	1.00E-03 to 1.00E-10
CR11604	Diethylhexyl phthalate	2	1	2/22/07	1.454	1.00E-03 to 1.00E-10
			2	2/27/07	1.509	1.00E-04 to 1.00E-10
			3	2/28/07	1.599	1.00E-04 to 1.00E-10
CR11605	Methoxychlor	1	1	3/9/07	1.528	1.00E-03 to 1.00E-10
			2	3/13/07	1.585	1.00E-05 to 1.00E-10
			3	3/14/07	1.472	1.00E-05 to 1.00E-10
CR11606	Aldicarb	2	1	3/9/07	1.514	1.00E-03 to 1.00E-10
			2	3/13/07	1.578	1.00E-03 to 1.00E-10
			3	3/14/07	1.572	1.00E-03 to 1.00E-10
CR11607	Flavone	3	1	3/1/07	1.579	1.00E-03 to 1.00E-10
			2	3/6/07	1.580	2.50E-04 to 1.00E-07
			3	3/7/07	1.503	2.50E-04 to 1.00E-07
CR11608	Triadimefon	1	1	3/16/07	1.529	1.00E-03 to 1.00E-10
			2	3/20/07	1.526	1.00E-04 to 1.00E-08
			3	3/21/07	1.513	1.00E-04 to 1.00E-08
CR11609	Imazalil	1	1	3/22/07	1.476	1.00E-03 to 1.00E-10
			2	3/27/07	1.472	1.00E-05 to 1.00E-10
			3	3/28/07	1.515	1.00E-05 to 1.00E-10
CR11610	Apigenin	2	1	3/22/07	1.551	1.00E-03 to 1.00E-10
			2	3/27/07	1.563	5.00E-05 to 1.00E-08
			3	3/28/07	1.561	5.00E-05 to 1.00E-08
CR11611	Ronidazole	4	1	3/16/07	1.612	1.00E-03 to 1.00E-10
			2	3/20/07	1.492	1.00E-05 to 1.00E-10
			3	3/21/07	1.536	1.00E-05 to 1.00E-10
CR11612	Genistein	3	1	3/22/07	1.494	1.00E-03 to 1.00E-10
			2	3/27/07	1.542	5.00E-04 to 1.00E-09
			3	3/28/07	1.535	5.00E-04 to 1.00E-09

Table 14 (continued)

Chemical Code	Test Chemical ID	Technician	Replicate	Assay Date	Substrate Soln SA $\mu\text{Ci}/\mu\text{g}$ ASDN	Reference Chemical Concentration Range (M)
CR11613	p,p'-DDE	3	1	2/22/07	1.482	1.00E-03 to 1.00E-10
			2	2/27/07	1.500	1.00E-05 to 1.00E-10
			3	2/28/07	1.547	1.00E-05 to 1.00E-10
CR11614	Alachlor	3	1	3/9/07	1.513	1.00E-03 to 1.00E-10
			2	3/13/07	1.736	1.00E-04 to 1.00E-10
			3	3/14/07	1.703	1.00E-04 to 1.00E-10
CR11615	Nitrofen	2	1	3/16/07	1.554	1.00E-03 to 1.00E-10
			2	3/20/07	1.511	5.00E-05 to 1.00E-08
			3	3/21/07	1.510	5.00E-05 to 1.00E-08
CR11616	Trifluralin	3	1	3/16/07	1.675	1.00E-03 to 1.00E-10
			2	3/20/07	1.604	1.00E-04 to 1.00E-10
			3	3/21/07	1.566	1.00E-04 to 1.00E-10

*Replicates 2 and 3 of Bisphenol A did not meet performance criteria and were excluded from analysis

SA = specific activity

4.5.2 Full Activity and Background Activity Control Results

Each replicate set for each reference chemical included two types of controls, each run in quadruplicate. The control types were full aromatase activity and background activity controls. The overall mean activities for full and background activity controls for each assay and the percent of full activity represented by the uncorrected background activity are presented in Table 15. These data were examined to determine if they met applicable performance criteria and those that did not are noted in Table 15. The performance criterion for full activity control value require that the minimum mean activity be 0.100 nmol/mg protein/min. The performance criterion for background activity controls required that they be $\leq 1\%$ of the full activity control.

There were five failures of the background control criterion, four of which occurred in assays of a single chemical conducted by the same technician. Only two of the background control criterion failures occurred in assays that met all other performance criteria. The failures seem to be related to a lower full activity in those assays rather than to an elevated background. No technical issues were identified to explain these lower full activities. Prior to percent of control calculation, all activities are corrected by subtracting the mean background activity, and these low background activities have little effect on the magnitude of the corrected activities.

Therefore, the data from CR11602, Replicates 4 and 5 and CR11611 Replicate 1, were not removed from the data set for analysis. Data from Replicates 2 and 3 for Chemical CR11602 were excluded from analysis; Replicate 2 because of reference chemical Top values >110% and Replicate 3 because of full aromatase activity < 0.100 nmol/mg/min.

Table 15. Full Activity and Background Activity Control Results for Reference Chemicals

Chemical Code	Chemical Name	Replicate	Full Activity	Background Activity (uncorrected)	Background Control Activity as Percentage of Full Activity
CR11601	Vinclozolin	1	0.419	0.003	0.6
		2	0.417	0.003	0.7
		3	0.358	0.002	0.4
CR11602	Bisphenol A	1	0.293	0.003	1.0
		2	0.145	0.003	1.8
		3	0.056	0.001	1.4
		4	0.171	0.002	1.2
		5	0.173	0.003	1.7
CR11603	Tributyltin	1	0.279	0.002	0.5
		2	0.321	0.002	0.5
		3	0.265	0.002	0.6
CR11604	Diethylhexyl phthalate	1	0.297	0.002	0.6
		2	0.304	0.002	0.5
		3	0.297	0.002	0.6
CR11605	Methoxychlor	1	0.276	0.002	0.6
		2	0.195	0.002	0.9
		3	0.222	0.002	0.8
CR11606	Aldicarb	1	0.279	0.001	0.5
		2	0.204	0.002	0.8
		3	0.216	0.001	0.6
CR11607	Flavone	1	0.343	0.002	0.5
		2	0.355	0.002	0.5
		3	0.333	0.002	0.6
CR11608	Triadimefon	1	0.431	0.002	0.4
		2	0.400	0.001	0.3
		3	0.393	0.002	0.5
CR11609	Imazalil	1	0.405	0.001	0.4
		2	0.382	0.002	0.5
		3	0.397	0.002	0.6

Table 15 (continued)

Chemical Code	Chemical Name	Replicate	Full Activity	Background Activity (uncorrected)	Background Control Activity as Percentage of Full Activity
CR11610	Apigenin	1	0.334	0.002	0.5
		2	0.361	0.002	0.4
		3	0.218	0.002	0.7
CR11611	Ronidazole	1	0.345	0.005	1.4
		2	0.409	0.002	0.4
		3	0.367	0.002	0.4
CR11612	Genistein	1	0.444	0.002	0.4
		2	0.399	0.002	0.4
		3	0.358	0.002	0.5
CR11613	p,p'-DDE	1	0.289	0.002	0.6
		2	0.360	0.002	0.5
		3	0.357	0.002	0.5
CR11614	Alachlor	1	0.254	0.002	0.6
		2	0.244	0.002	0.6
		3	0.250	0.002	0.7
CR11615	Nitrofen	1	0.262	0.002	0.7
		2	0.341	0.003	0.8
		3	0.351	0.002	0.4
CR11616	Trifluralin	1	0.260	0.002	0.6
		2	0.373	0.002	0.5
		3	0.427	0.003	0.6
	Mean (SD)		0.313 (0.085)	0.002 (0.001)	0.7 (0.3)

^aUnits are nmol/mg protein/min

Bold = Failure to meet performance criteria

4.5.3 Positive Control Results

The positive control data were entered into spreadsheets for calculation of aromatase activity (Appendix E). The aromatase activity measured at each concentration of the positive control (4-OH ASDN) assay was divided by the average full enzyme activity control activity for each chemical and replicate to calculate percent of control values. The percent of control values ranged approximately from 0 to 100 % over the 4-OH ASDN concentration range of 1×10^{-5} M to 1×10^{-10} M. The percent of control values was fitted to the models described in Section 3.11.2

using Prism 4.03, and the results are presented in Appendix F. The fitted curves resulting from the four parameter model are shown in Figure 3. These plots also include the 95% confidence intervals for each curve which are generally very narrow, indicating a good curve fit and low variance across all concentrations.

A mixed effects model using the 4-OH ASDN concentrations as fixed effects and the technicians, along with the replicates within technicians, as random effects was fit to the percent of control values for the positive controls. Table 16 shows the variance components estimates and significance tests for the terms in the model.

The results in Table 16 show that there was not significant variation between technicians, meaning that results did not differ significantly overall from technician to technician. After removing the effects of the concentration levels for 4-OH ASDN from the model (as a fixed effect), there was still significant variation across replicates for the technicians, meaning that the positive control results vary from replicate to replicate for each technician. Figure 4 shows the percent of control means with error bars corresponding to one standard deviation above and below the means for each technician.

Table 16. Results of Random Effects ANOVA in Mixed Effects Model

Term	Parameter Estimate	Test Statistic	Degrees of Freedom	P-value
Technician	0.1239	1.20	92	0.3158
Replicate (Technician)	12.0506	5.55	665	<.0001
Residual	21.1849	-	-	-

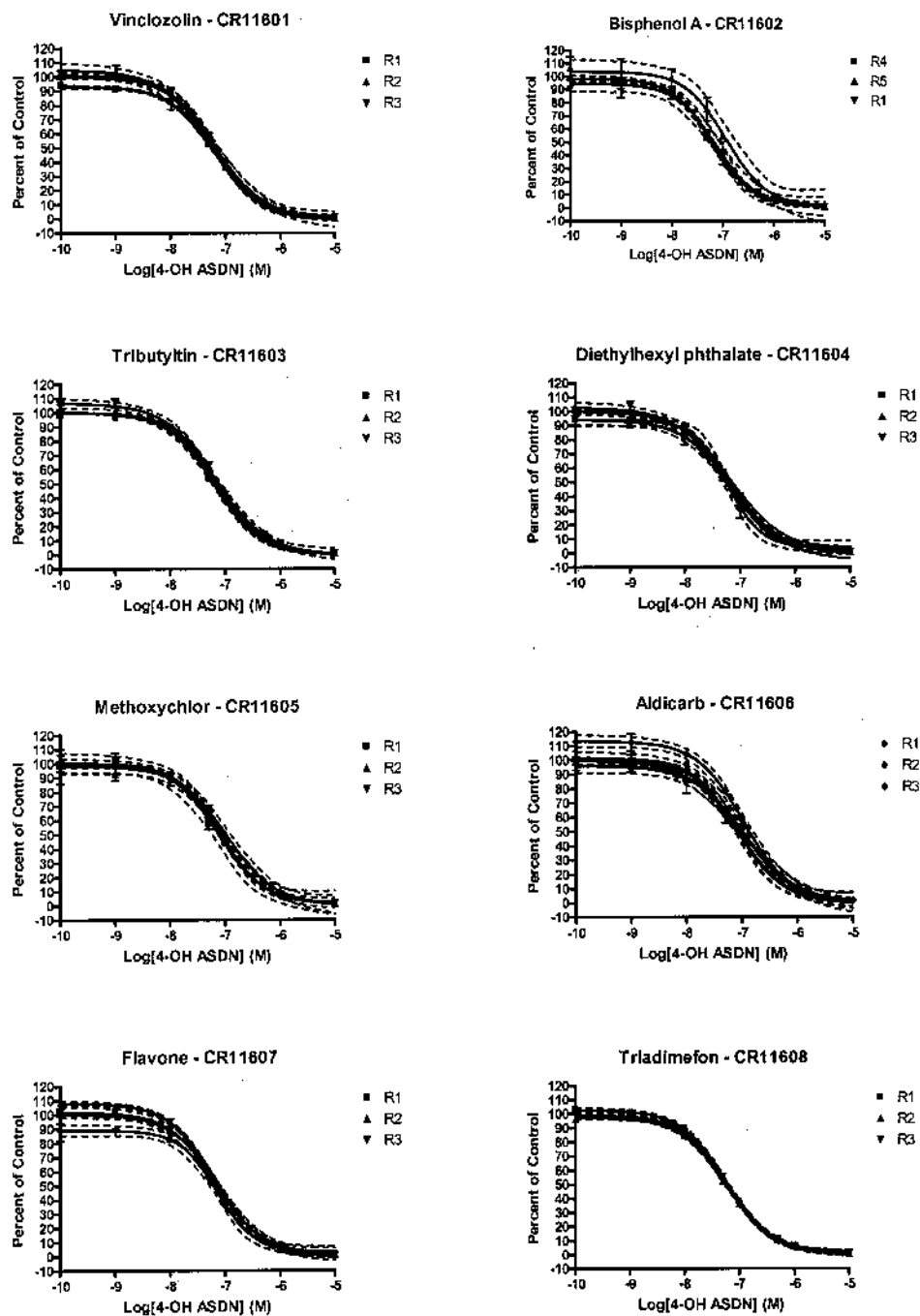


Figure 3. Concentration Response Curves for 4-OH ASDN

Chemical codes indicate the reference chemical assay conducted in conjunction with the 4-OH ASDN assay.

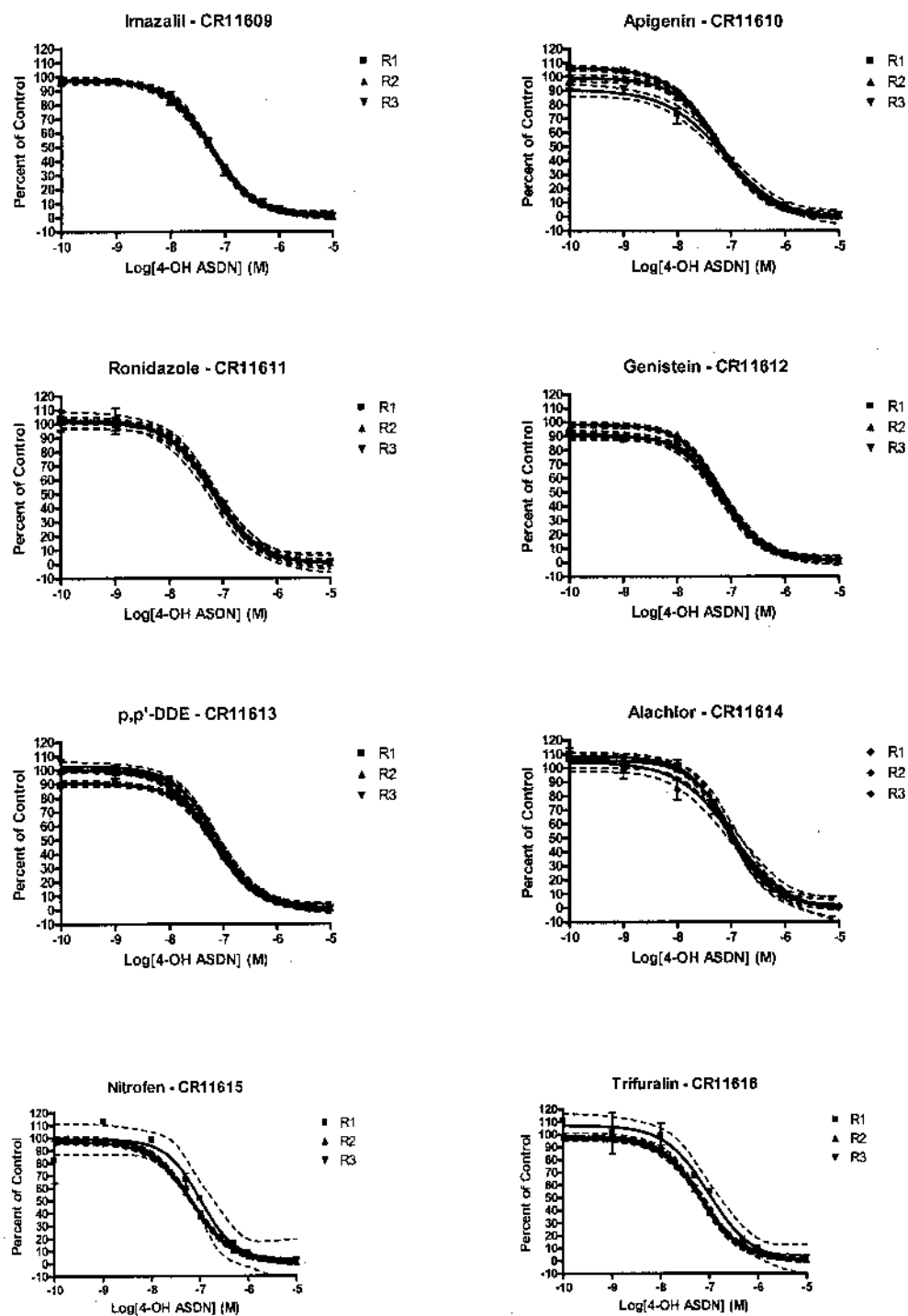
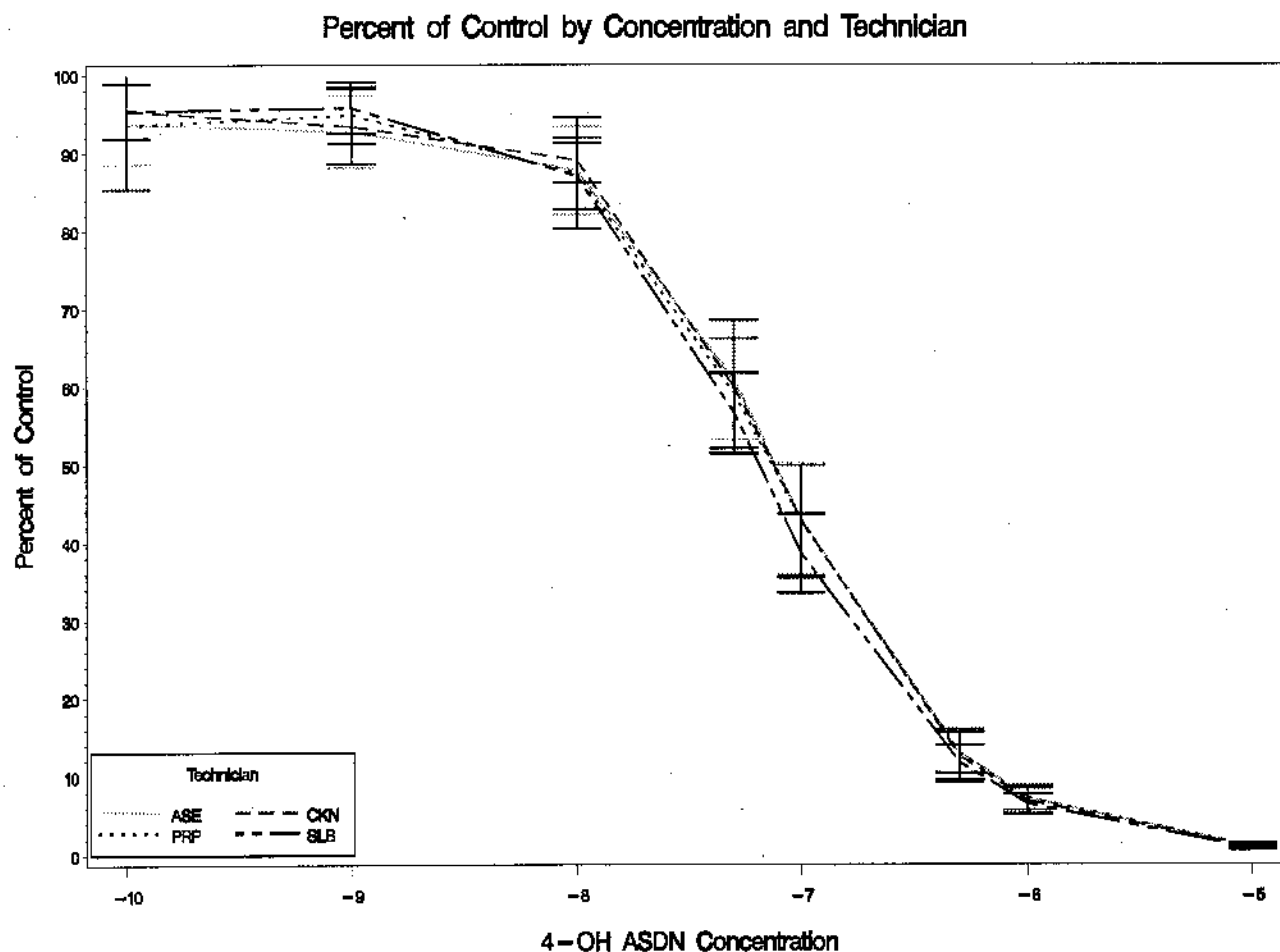


Figure 3 (continued) Concentration Response Curves for 4-OH ASDN

Chemical codes indicate the reference chemical assay conducted in conjunction with the 4-OH ASDN assay.



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Figure 4. Mean Concentration Response Curves for 4-OH ASDN by Technician

The parameters (Top, Bottom, LogIC50, and Hillslope) for each fitted curve (Table 17) were examined to determine if they met the performance criteria described in Table 13. There were 9 positive control assays (of 48) with at least one criterion failure, and these were distributed among the 4 participating technicians. Four of the assays with failures were conducted on the same day and shared positive control chemical dilutions which points to a possible problem with the dilutions as the cause of the failures. These assays included those

conducted in conjunction with the second replicates of CR11605, CR11606, CR11614 and the fifth replicate of CR11602, and each had Log[IC50] values between -6.93 and -6.97. Excluding those replicates, only 5 other replicates had any failed criteria, and those failures were just outside the acceptable range, and in only one case were there multiple failures within a replicate.

The utility of the performance criteria ranges can be assessed by comparing them with the 95% confidence intervals bands for the parameters. The 95% confidence interval ranges for the curve parameters are presented in Table 18. The parameters with the highest failure (either the lower or upper bound out of range) rates are Bottom and HillSlope with 20 and 32 (of 48), respectively. Bottom derives primarily from LSS data which are near background levels and where variance is near 15%, so it is not unexpected that many of the 95% confidence interval bounds for Bottom fall outside of the $0 \pm 5\%$ performance criterion range. HillSlope is highly dependent on the defined Bottom value, so when there are a large number of failures in Bottom, a similar finding is expected in HillSlope. There are fewer 95% confidence interval bound values for Log[IC50] and Top (14 in each case) that fall outside the criteria ranges, and many of these values are barely outside the ranges. The data show that in most cases, even the bounds of the 95% confidence intervals fall within the performance criteria ranges, indicating a high degree of certainty that the correct estimate falls within that range and that the performance criteria ranges are appropriate.

Table 17. Positive Control Assay Model Fit Parameters*

	Vinclozolin CR11601			Flavone CR11607		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
BOTTOM	0.4	-1.1	0.2	0.7	0.5	3.0
TOP	93.1	105	101	102	108	89
LOGIC50	-7.20	-7.23	-7.20	-7.17	-7.19	-7.15
HILLSLOPE	-1.1	-0.9	-1.1	-1.0	-1.0	-1.3
	Bisphenol A CR11602			Triadimefon CR11608		
	Rep 1	Rep 4	Rep 5	Rep 1	Rep 2	Rep 3
BOTTOM	0.7	1.7	0.7	0.9	0.8	0.6
TOP	94.5	98.3	104	103	97.7	99
LOGIC50	-7.13	-7.21	-6.93	-7.24	-7.24	-7.22
HILLSLOPE	-1.1	-1.1	-1.1	-1.1	-1.0	-1.1
	Tributyltin CR11603			Imazalil CR11609		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
BOTTOM	1.7	0.1	-0.3	0.9	0.7	2.0
TOP	98.3	103	107	98.1	98	97.2
LOGIC50	-7.21	-7.18	-7.17	-7.22	-7.27	-7.25
HILLSLOPE	-1.1	-1.0	-0.9	-1.1	-1.0	-1.2
	Diethylhexyl phthalate CR11604			Apigenin CR11610		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
BOTTOM	3.8	-0.4	-0.9	-0.4	0.5	-2.1
TOP	94.1	94.1	103	106	99.1	90.8
LOGIC50	-7.23	-7.14	-7.22	-7.24	-7.20	-7.13
HILLSLOPE	-1.4	-1.0	-0.9	-1.0	-1.0	-0.8
	Methoxychlor CR11605			Ronidazole CR11611		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
BOTTOM	1.4	-0.3	1.1	-0.1	0.1	0.5
TOP	100	98.5	100	103	103	101
LOGIC50	-7.14	-6.97	-7.09	-7.21	-7.18	-7.15
HILLSLOPE	-1.1	-1.0	-1.1	-1.0	-1.0	-1.0
	Aldicarb CR11606			Genistein CR11612		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
BOTTOM	2.0	0.5	-1.4	1.2	1.8	1.5
TOP	101	113	96.2	90	98.1	91.1
LOGIC50	-7.10	-6.97	-6.94	-7.11	-7.18	-7.20
HILLSLOPE	-1.1	-1.0	-0.9	-1.2	-1.2	-1.1

Table 17 (continued)

	p,p'-DDE CR11613			Nitrofen CR11615		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
BOTTOM	1.3	0.7	0.7	2.4	0.2	0.9
TOP	90.6	101	103	98.8	99.5	96.8
LOGIC50	-7.14	-7.20	-7.13	-7.00	-7.19	-7.17
HILLSLOPE	-1.1	-1.0	-1.1	-1.2	-1.1	-1.0
	Alachlor CR11614			Trifluralin CR11616		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
BOTTOM	0.1	-3.2	1.7	-0.3	1.3	0.9
TOP	106	104	108	107	98.6	96.8
LOGIC50	-6.96	-6.96	-7.03	-7.02	-7.19	-7.17
HILLSLOPE	-1.1	-0.8	-1.1	-1.0	-1.1	-1.0

*Reference chemical names and codes indicate the RC replicate that was run concurrent with each positive control assay

Values that don't meet the performance criteria are presented in **bold, italicized** type.

Table 18. Positive Control Assay 95% Confidence Intervals for Model Parameters

Chemical Code	Chemical	Technician	Replicate	Bottom		TOP		LOGIC50		HILLSLOPE	
				Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
CR11601	Vinclozolin	1	1	-0.8	1.7	92.0	94.2	-7.22	-7.17	-1.1	-1.0
			2	-7.3	5.1	99.8	110	-7.34	-7.12	-1.1	-0.7
			3	-2.1	2.5	99.0	103	-7.24	-7.16	-1.2	-0.9
CR11602	Bisphenol A	4	1	-7.2	8.5	88.2	101	-7.27	-6.99	-1.5	-0.7
			4	-1.1	4.3	95.9	101	-7.26	-7.16	-1.3	-1.0
			5	-13.1	14.6	94.5	113	-7.16	-6.71	-1.6	-0.6
CR11603	Tributyltin	2	1	-1.1	4.3	95.9	101	-7.26	-7.16	-1.3	-1.0
			2	-2.8	3.1	100	105	-7.23	-7.13	-1.2	-0.9
			3	-4.6	4.0	103	110	-7.25	-7.10	-1.1	-0.8
CR11604	Diethylhexyl phthalate	2	1	-0.9	8.5	89.6	98.6	-7.31	-7.15	-1.9	-1.0
			2	-5.4	4.6	90.3	97.9	-7.23	-7.04	-1.2	-0.8
			3	-5.8	4.0	98.7	107	-7.30	-7.13	-1.1	-0.7
CR11605	Methoxychlor	1	1	-7.8	10.5	92.9	107	-7.29	-6.98	-1.5	-0.6
			2	-7.6	7.1	93.7	103	-7.10	-6.85	-1.2	-0.7
			3	-2.6	4.8	97.3	103	-7.15	-7.03	-1.3	-0.9
CR11606	Aldicarb	2	1	-3.5	7.6	97.2	106	-7.19	-7.01	-1.4	-0.8
			2	-5.8	6.8	109	118	-7.07	-6.88	-1.3	-0.8
			3	-9.5	6.7	91.0	101	-7.08	-6.80	-1.2	-0.7
CR11607	Flavone	3	1	-4.2	5.6	97.7	106	-7.25	-7.08	-1.3	-0.8
			2	-1.4	2.4	106	110	-7.22	-7.16	-1.1	-1.0
			3	-1.4	7.4	85.2	92.8	-7.24	-7.07	-1.6	-0.9
CR11608	Triadimefon	1	1	-1.1	2.9	101.0	104	-7.27	-7.20	-1.2	-1.0
			2	-0.6	2.2	96.5	98.9	-7.27	-7.21	-1.1	-1.0
			3	-1.4	2.6	97.2	101	-7.26	-7.19	-1.2	-1.0
CR11609	Imazail	1	1	-0.4	2.2	97.0	99.3	-7.25	-7.20	-1.1	-1.0
			2	-2.0	3.4	95.6	100	-7.32	-7.21	-1.2	-0.9
			3	-0.3	4.3	95.0	99.3	-7.29	-7.20	-1.3	-1.0

Table 18 (continued)

Chemical Code	Chemical	Technician	Replicate	Bottom		TOP		LOGIC50		HILLSLOPE	
				Lower	Upper	Lower	Upper	Lower	Upper	Lower	Upper
CR11610	Apigenin	2	1	-2.6	1.8	105	108	-7.28	-7.20	-1.0	-0.9
			2	-2.5	3.6	96.6	102	-7.26	-7.15	-1.2	-0.9
			3	-8.5	4.3	86.1	95.4	-7.26	-7.00	-1.0	-0.6
CR11611	Ronidazole	4	1	-7.5	7.3	96.5	109	-7.34	-7.08	-1.3	-0.7
			2	-2.8	3.1	100	105	-7.23	-7.13	-1.2	-0.9
			3	-4.8	5.7	97.0	105	-7.24	-7.06	-1.3	-0.8
CR11612	Genistein	3	1	-2.2	4.5	87.4	92.7	-7.17	-7.05	-1.2	-1.0
			2	-0.4	4.0	96.2	100	-7.21	-7.14	-1.3	-1.0
			3	-1.9	4.9	88.2	94.0	-7.27	-7.14	-1.3	-0.9
CR11613	p,p'-DDE	3	1	-1.3	3.8	88.5	92.6	-7.18	-7.09	-1.3	-1.0
			2	-2.1	3.5	98.3	103	-7.25	-7.15	-1.2	-0.9
			3	-3.2	4.6	99.7	106	-7.20	-7.07	-1.3	-0.9
CR11614	Alachlor	3	1	-8.1	8.4	100	111	-7.09	-6.84	-1.4	-0.8
			2	-14.3	8.0	97.4	111	-7.14	-6.77	-1.1	-0.6
			3	-2.5	5.8	105	111	-7.10	-6.97	-1.3	-1.0
CR11615	Nitrofen	2	1	-15.1	19.9	86.3	111	-7.29	-6.71	-2.2	-0.3
			2	-2.1	2.6	97.6	101	-7.24	-7.15	-1.2	-0.9
			3	-1.5	3.4	94.8	98.7	-7.22	-7.13	-1.1	-0.9
CR11616	Trifluralin	3	1	-14.1	13.5	97.6	117	-7.24	-6.80	-1.4	-0.5
			2	-1.8	4.4	95.9	101	-7.24	-7.13	-1.3	-0.9
			3	-1.5	3.4	94.8	98.7	-7.22	-7.13	-1.1	-0.9

Values in **bold, italicized** type are outside the performance criteria ranges

4.5.4 Reference Chemical Assay Results

Percent of Control Values. The assay data were entered into spreadsheets for calculation of aromatase activity (Appendix E). The aromatase activity found in each assay tube was normalized to percent of control by dividing by the average full enzyme activity for the replicate. The percent of control values for each reference chemical replicate and tube, along with the mean, SD, SEM, and CV of the percent of control across tubes within a replicate, are presented in Appendix H. Generally, there was little tube-to-tube variance (CV generally less than 10%) within a replicate. For some high reference chemical concentrations where the percent of control values were near zero, the CV values were higher, in part due to increased variance in the measurement of DPM at these near background levels.

The mean percent of control values across tubes for each replicate and the overall mean, SD, SEM, and CV across replicates are presented in Table 19. Replicate-to-replicate reproducibility was good, with most CV values less than 10%. Again, some higher CVs were observed when percent of control was near zero.

Table 19. Mean Percent of Control Per Replicate and Percent of Control Across Replicates

Reference Chemical	Log[RC] (M)	Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	CV
Vinclozolin (CR11601)	-3.00	83.89	NA	NA	83.89	NC	NC	NC
	-4.00	88.44	99.20	99.08	95.57	6.18	3.57	6.46
	-5.00	93.78	104.51	106.61	101.63	6.88	3.97	6.77
	-6.00	95.92	100.84	105.30	100.68	4.69	2.71	4.66
	-6.30	NA	100.22	107.86	104.04	5.40	3.82	5.19
	-7.00	95.40	97.64	101.50	98.18	3.08	1.78	3.14
	-8.00	97.02	99.81	103.94	100.26	3.48	2.01	3.47
	-9.00	96.08	99.52	104.03	99.88	3.99	2.30	4.00
Bisphenol A* (CR11602)	-10.00	95.28	101.47	101.44	99.40	3.56	2.06	3.59
	-3.00	3.23	4.67	4.59	4.16	0.81	0.46	19.35
	-3.30	NA	19.37	20.85	20.11	1.05	0.74	5.20
	-3.60	NA	42.30	43.50	42.90	0.85	0.60	1.98
	-4.00	59.13	65.37	73.12	65.87	7.01	4.04	10.63
	-4.30	NA	89.07	90.70	89.88	1.15	0.81	1.28
	-4.60	NA	82.10	97.24	89.67	10.70	7.57	11.94
	-5.00	87.57	97.80	104.18	96.51	8.38	4.84	8.68
	-6.00	90.03	87.37	113.43	96.94	14.34	8.28	14.79
	-7.00	92.17	NA	NA	92.17	NC	NC	NC
	-8.00	92.30	NA	NA	92.30	NC	NC	NC
	-9.00	93.27	NA	NA	93.27	NC	NC	NC
	-10.00	92.93	NA	NA	92.93	NC	NC	NC

Table 19 (continued)

Reference Chemical	Log[RC] (M)	Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	CV
Tributyltin (CR11603)	-3.00	78.03	80.95	82.10	80.36	2.10	1.21	2.61
	-4.00	83.25	81.70	83.89	82.95	1.12	0.65	1.36
	-5.00	92.95	94.85	90.34	92.71	2.26	1.31	2.44
	-6.00	98.86	100.75	99.87	99.83	0.95	0.55	0.95
	-7.00	95.49	94.69	90.21	93.46	2.84	1.64	3.04
	-8.00	93.89	94.13	98.74	95.59	2.73	1.58	2.86
	-9.00	92.30	97.76	95.32	95.13	2.73	1.58	2.87
	-10.00	93.20	99.24	92.97	95.14	3.55	2.05	3.74
Diethylhexyl phthalate (CR11604)	-3.00	107.10	NA	NA	107.10	NC	NC	NC
	-4.00	109.21	100.91	110.52	106.88	5.21	3.01	4.88
	-5.00	109.20	106.35	110.48	108.68	2.11	1.22	1.94
	-6.00	97.84	97.03	102.43	99.10	2.91	1.68	2.94
	-6.30	NA	101.01	103.23	102.12	1.57	1.11	1.54
	-7.00	98.11	95.53	105.25	99.63	5.03	2.91	5.05
	-8.00	96.65	93.55	99.16	96.45	2.81	1.62	2.91
	-9.00	100.23	93.37	101.94	98.51	4.54	2.62	4.61
Methoxychlor (CR11605)	-3.00	81.16	NA	NA	81.16	NC	NC	NC
	-4.00	93.68	NA	NA	93.68	NC	NC	NC
	-5.00	96.26	109.33	94.01	99.87	8.27	4.78	8.29
	-6.00	97.67	109.09	105.14	103.97	5.80	3.35	5.58
	-7.00	91.29	116.65	103.79	103.91	12.68	7.32	12.20
	-7.30	NA	106.99	94.39	100.69	8.91	6.30	8.85
	-8.00	100.30	112.76	99.42	104.16	7.46	4.31	7.16
	-8.30	NA	110.62	103.34	106.98	5.15	3.64	4.81
	-9.00	105.41	117.60	102.25	108.42	8.10	4.68	7.47
	-10.00	102.39	116.17	102.66	107.07	7.88	4.55	7.36
Aldicarb (CR11606)	-3.00	86.29	106.30	84.92	92.50	11.97	6.91	12.94
	-4.00	94.06	116.65	106.06	105.59	11.30	6.52	10.70
	-5.00	93.84	113.30	100.16	102.43	9.93	5.73	9.69
	-6.00	83.66	113.38	101.46	99.50	14.96	8.64	15.03
	-7.00	93.01	121.81	95.22	103.35	16.03	9.25	15.51
	-8.00	92.79	115.66	95.49	101.31	12.50	7.22	12.34
	-9.00	97.68	114.52	94.93	102.38	10.60	6.12	10.36
	-10.00	82.46	117.85	98.42	99.58	17.72	10.23	17.80

Table 19 (continued)

Reference Chemical	Log[RC] (M)	Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	CV
Flavone (CR11607)	-3.00	1.88	NA	NA	1.88	NC	NC	NC
	-3.60	NA	11.25	10.90	11.07	0.25	0.18	2.24
	-4.00	24.39	25.24	26.23	25.28	0.92	0.53	3.65
	-4.30	NA	41.60	40.98	41.29	0.44	0.31	1.06
	-4.60	NA	57.88	57.87	57.87	0.01	0.00	0.01
	-5.00	76.89	77.41	76.08	76.79	0.67	0.39	0.87
	-5.30	NA	87.27	82.43	84.85	3.42	2.42	4.03
	-6.00	97.72	98.20	94.18	96.70	2.20	1.27	2.27
	-7.00	97.74	101.41	96.22	98.46	2.67	1.54	2.71
	-8.00	98.84	NA	NA	98.84	NC	NC	NC
	-9.00	98.14	NA	NA	98.14	NC	NC	NC
	-10.00	100.88	NA	NA	100.88	NC	NC	NC
Triadimefon (CR11608)	-3.00	0.16	NA	NA	0.16	NC	NC	NC
	-4.00	2.09	1.83	2.14	2.02	0.17	0.10	8.22
	-4.30	NA	3.76	4.19	3.97	0.30	0.21	7.59
	-5.00	16.36	14.85	16.75	15.99	1.01	0.58	6.29
	-5.30	NA	24.98	27.67	26.33	1.90	1.34	7.21
	-5.60	NA	39.30	42.36	40.83	2.17	1.53	5.30
	-6.00	64.74	59.06	63.82	62.54	3.05	1.76	4.87
	-7.00	94.33	90.65	95.73	93.57	2.63	1.52	2.81
	-8.00	102.78	96.66	100.73	100.06	3.12	1.80	3.11
	-9.00	101.06	NA	NA	101.06	NC	NC	NC
	-10.00	99.18	NA	NA	99.18	NC	NC	NC
Imazalil (CR11609)	-3.00	0.15	NA	NA	0.15	NC	NC	NC
	-4.00	0.07	NA	NA	0.07	NC	NC	NC
	-5.00	0.14	0.26	-0.01	0.13	0.14	0.08	103.43
	-6.00	1.12	1.78	1.14	1.35	0.38	0.22	28.08
	-7.00	8.95	14.93	11.54	11.81	3.00	1.73	25.38
	-7.60	NA	40.25	30.28	35.26	7.06	4.99	20.01
	-8.00	46.29	61.73	52.00	53.34	7.81	4.51	14.63
	-8.60	NA	85.85	77.56	81.71	5.86	4.14	7.17
	-9.00	90.75	93.30	84.29	89.45	4.64	2.68	5.19
	-10.00	98.62	98.84	90.45	95.97	4.78	2.76	4.98

Table 19 (continued)

Reference Chemical	Log[RC] (M)	Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	CV
Apigenin (CR11610)	-3.00	7.19	NA	NA	7.19	NC	NC	NC
	-4.00	16.67	NA	NA	16.67	NC	NC	NC
	-4.30	NA	18.68	12.69	15.68	4.24	3.00	27.03
	-4.60	NA	31.86	21.77	26.81	7.14	5.05	26.61
	-5.00	51.09	52.45	38.82	47.45	7.51	4.33	15.82
	-5.30	NA	69.55	54.91	62.23	10.35	7.32	16.63
	-5.60	NA	80.97	66.81	73.89	10.01	7.08	13.54
	-6.00	93.11	92.30	82.97	89.46	5.63	3.25	6.30
	-7.00	102.32	97.57	94.99	98.29	3.72	2.15	3.78
	-8.00	102.46	100.74	92.09	98.43	5.56	3.21	5.65
	-9.00	99.99	NA	NA	99.99	NC	NC	NC
	-10.00	100.61	NA	NA	100.61	NC	NC	NC
Ronidazole (CR11611)	-3.00	108.93	NA	NA	108.93	NC	NC	NC
	-4.00	113.17	NA	NA	113.17	NC	NC	NC
	-5.00	114.44	102.21	98.25	104.97	8.44	4.87	8.04
	-6.00	111.53	101.56	100.12	104.40	6.21	3.59	5.95
	-7.00	107.77	101.48	100.92	103.39	3.80	2.20	3.68
	-7.30	NA	103.13	81.64	92.38	15.19	10.74	16.45
	-8.00	111.97	97.11	97.49	102.19	8.47	4.89	8.29
	-8.30	NA	96.40	91.60	94.00	3.40	2.40	3.62
	-9.00	107.88	102.09	98.56	102.84	4.71	2.72	4.58
	-10.00	111.33	99.33	102.56	104.41	6.21	3.58	5.95
Genistein (CR11612)	-3.00	59.42	NA	NA	59.42	NC	NC	NC
	-3.30	NA	72.47	74.09	73.28	1.14	0.81	1.56
	-3.60	NA	82.90	84.20	83.55	0.92	0.65	1.10
	-4.00	86.97	91.82	85.38	88.05	3.35	1.94	3.81
	-5.00	94.04	100.53	82.87	92.48	8.93	5.16	9.66
	-6.00	96.72	97.84	84.77	93.11	7.24	4.18	7.78
	-7.00	97.48	98.67	89.26	95.13	5.13	2.96	5.39
	-8.00	98.98	96.51	88.25	94.58	5.62	3.24	5.94
	-9.00	97.28	100.07	96.51	97.95	1.87	1.08	1.91
	-10.00	97.33	NA	NA	97.33	NC	NC	NC

Table 19 (continued)

Reference Chemical	Log[RC] (M)	Percent of Control			Overall			
		Rep 1	Rep 2	Rep 3	Mean	±SD	SEM	CV
p,p'-DDE (CR11613)	-3.00	64.90	NA	NA	64.90	NC	NC	NC
	-4.00	81.78	NA	NA	81.78	NC	NC	NC
	-5.00	81.75	89.09	91.73	87.52	5.17	2.99	5.91
	-6.00	90.86	97.63	100.42	96.30	4.91	2.84	5.10
	-7.00	98.82	104.48	106.73	103.34	4.08	2.35	3.95
	-7.30	NA	108.66	106.09	107.37	1.82	1.29	1.70
	-8.00	99.59	111.49	96.76	102.61	7.82	4.51	7.62
	-8.30	NA	108.74	101.65	105.19	5.01	3.54	4.76
	-9.00	99.29	106.26	104.50	103.35	3.63	2.09	3.51
	-10.00	97.96	107.02	103.77	102.92	4.59	2.65	4.46
Alachlor (CR11614)	-3.00	53.91	NA	NA	53.91	NC	NC	NC
	-4.00	103.02	100.50	96.24	99.92	3.43	1.98	3.43
	-5.00	104.90	92.89	102.30	100.03	6.32	3.65	6.31
	-6.00	115.25	95.07	114.45	108.26	11.42	6.60	10.55
	-6.30	NA	96.01	101.57	98.79	3.93	2.78	3.98
	-7.00	119.26	99.18	109.18	109.21	10.04	5.79	9.19
	-8.00	123.86	102.22	105.42	110.50	11.68	6.74	10.57
	-9.00	99.32	109.93	101.77	103.67	5.55	3.21	5.36
	-10.00	114.21	96.99	82.38	97.86	15.93	9.20	16.28
Nitrofen (CR11615)	-3.00	37.48	NA	NA	37.48	NC	NC	NC
	-4.00	50.59	NA	NA	50.59	NC	NC	NC
	-4.30	NA	41.24	42.20	41.72	0.67	0.48	1.62
	-4.60	NA	44.79	43.47	44.13	0.93	0.66	2.11
	-5.00	69.77	57.19	56.90	61.29	7.35	4.24	11.99
	-5.30	NA	71.83	69.67	70.75	1.53	1.08	2.16
	-5.60	NA	85.72	82.89	84.30	2.00	1.41	2.37
	-6.00	107.14	97.04	89.78	97.99	8.72	5.03	8.90
	-7.00	108.11	100.33	96.81	101.75	5.78	3.34	5.68
	-8.00	101.55	100.91	97.92	100.13	1.94	1.12	1.94
	-9.00	107.24	NA	NA	107.24	NC	NC	NC
	-10.00	109.06	NA	NA	109.06	NC	NC	NC
Trifluralin (CR11616)	-3.00	102.30	NA	NA	102.30	NC	NC	NC
	-4.00	104.84	100.72	100.88	102.15	2.34	1.35	2.29
	-5.00	103.62	98.94	106.02	102.86	3.60	2.08	3.50
	-6.00	103.54	95.69	104.85	101.36	4.96	2.86	4.89
	-6.30	NA	99.54	100.87	100.20	0.94	0.66	0.94
	-7.00	105.56	99.39	102.38	102.45	3.08	1.78	3.01
	-8.00	92.98	99.19	105.97	99.38	6.50	3.75	6.54
	-9.00	106.74	96.38	102.38	101.83	5.20	3.00	5.10
	-10.00	89.54	103.85	102.92	98.77	8.01	4.62	8.11

Italicized concentrations were insoluble in the reaction mixture and data obtained are excluded from modeling.

NA Not applicable – this concentration was not assayed in this replicate

NC Not calculated – only one data point – no SD, SEM or CV calculated.

*Values under Rep 2 and Rep 3 are from Rep 4 and Rep 5, respectively, for Bisphenol A

Model Fitting. The percent of control data were fit using nonlinear regression methods in Prism 4.03 to the models described in Section 3.11.2. Prism outputs are presented in Appendix H. The Top, Bottom, LogIC50, and Slope were calculated for the full (four-parameter) model while only Log IC50 and Slope were calculated for the two-parameter model (since Top and Bottom were fixed in that reduced model). The parameters from both models are presented in Table 20. The calculated four-parameter model Top value was compared against the performance criterion that requires $\text{Top} = 100 \pm 10\%$, and all replicates (where Top was calculable) passed this requirement except genistein (CR11612), Replicate 3, for which the value for Top was 189.7. There was an extremely wide 95% confidence interval for that (and other) parameter(s) in that replicate, indicating a poor curve fit for this noninhibitor.

The model fits were compared in Prism to determine if there were significant differences between them, and the results of that analysis are presented in Table 21. As expected, the more complex models fit the data better in all cases where there were significant differences in the model fits. When the model fits are not significantly different, the preferred model is the simpler two-parameter model by default. The curve fits are presented graphically in Figure 5. The plotted curves are those of the preferred model for each replicate.

Table 20. Reference Chemical Model Fit Parameters

Chemical Name	Chemical Code	Replicate	Top		Bottom		LogIC50		Slope	
			Eq 1	Eq 2	Eq 1	Eq 2	Eq 1	Eq 2	Eq 1	Eq 2
Vinclozolin	CR11601	R1	95.95	100	88.04	0	-4.752	4.929	-1.686	-0.1089
		R2								
		R3								
Bisphenol A	CR11602	R1	92.37	100	-22.92	0	-3.575	-3.912	-0.9262	-1.104
		R4	91.92	100	-6.355	0	-3.622	-3.759	-1.418	-1.28
		R5	112.1	100	-23.86	0	-3.604	-3.689	-0.9766	-1.579
Tributyltin	CR11603	R1	94.69	100	77.73	0	-4.234	-4.1E+07	-1.387	2.46E-08
		R2	97.31	100	81.05	0	-4.659	-0.4193	-2.23	-0.2286
		R3	95.42	100	82.99	0	-4.981	1.67	-8.315	-0.1418
Diethylhexyl phthalate	CR11604	R1	109.2	100	97.73	0	-5.591	-13.51	4.929	0.3997
		R2								
		R3								
Methoxychlor	CR11605	R1	103.9	100	95.07	0	-7.981	0.5212	-8.651	-0.2356
		R2								
		R3		100		0		-4.835		-7.236
Aldicarb	CR11606	R1		100		0		-549.6		0.0018
		R2								
		R3								
Flavone	CR11607	R1	98.94	100	14.85	0	-4.665	-4.484	-1.342	-1.015
		R2	101.8	100	-3.973	0	-4.438	-4.461	-0.9307	-1.014
		R3	96.75	100	-6.247	0	-4.37	-4.482	-0.9122	-0.9194
Triadimefon	CR11608	R1	101.0	100	-0.09823	0	-5.74	-5.728	-0.9571	-0.9801
		R2	97.55	100	-0.2917	0	-5.791	-5.829	-0.9238	-0.8861
		R3	101.8	100	0.002194	0	-5.757	-5.734	-0.9391	-0.9691

Table 20 (continued)

Chemical Name	Chemical Code	Replicate	Top		Bottom		LogIC50		Slope	
			Eq 1	Eq 2	Eq 1	Eq 2	Eq 1	Eq 2	Eq 1	Eq 2
<i>Imazalil</i>	CR11609	R1	100.4	100	0.2596	0	-8.064	-8.056	-0.994	-0.9947
		R2	99.51	100	-0.03275	0	-7.776	-7.783	-0.9656	-0.9556
		R3	91.06	100	0.2206	0	-7.882	-8.005	-1.017	-0.8179
<i>Apigenin</i>	CR11610	R1	101.3	100	50.95	0	-5.783	-4.984	-3.274	-1.148
		R2	99.88	100	0.375	0	-4.948	-4.946	-0.9888	-0.9821
		R3	94.31	100	2.478	0	-5.188	-5.229	-0.9968	-0.864
Ronidazole	CR11611	R1								
		R2								
		R3								
Genistein	CR11612	R1	97.81	100	75.41	0	-3.958	-0.7823	-0.6602	-0.2648
		R2	98.72	100	81.99	0	-3.956	-2.906	-3.491	-0.9823
		R3	189.7	100	84.09	0	-11.52	6.274	-0.3524	-0.06876
p,p'-DDE	CR11613	R1	98.94	100	81.59	0	-5.972	-3.537	-2.104	-0.4385
		R2	108.4	100	87.51	0	-6.049	-4.215	-1.014	-1.158
		R3	103.0	100	91.73	0	-5.933	-4.847	-7.743	-6.85
Alachlor	CR11614	R1								
		R2								
		R3								
<i>Nitrofen</i>	CR11615	R1								
		R2	100.9	100	39.26	0	-5.265	-4.776	-1.496	-0.8813
		R3	97.44	100	31.59	0	-5.171	-4.788	-1.161	-0.7552
Trifluralin	CR11616	R1								
		R2								
		R3								

For replicates where the models did not converge, the cells are left blank.
Bold, italicized type indicates the chemical was classified as an inhibitor.

Table 21. Model Curve Fit Comparison Results

	Vinclozolin CR11601			Flavone CR11607		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Converged?	Y	DNC	DNC	Y	Y	Y
P value	0.015			0.7977	0.1050	0.0564
Preferred model	Equation 1			Equation 2	Equation 2	Equation 2
F	5.433			0.2291	2.528	3.331
	Bisphenol A CR11602			Triadimefon CR11608		
	Rep 1	Rep 1	Rep 1	Rep 1	Rep 2	Rep 3
Converged?	Y	Y	Y	Y	Y	Y
P value	P<0.0001	0.1182	0.0003	0.4240	P<0.0001	0.0033
Preferred model	Equation 1	Equation 2	Equation 1	Equation 2	Equation 1	Equation 1
F	70.71	2.381	12.28	0.9029	22.47	7.684
	Tributyltin CR11603			Imazalil CR11609		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Converged?	Y	Y	Y	Y	Y	Y
P value	P<0.0001	0.0376	0.1737	0.2510	0.6059	0.0698
Preferred model	Equation 1	Equation 1	Equation 2	Equation 2	Equation 2	Equation 2
F	23.32	3.883	1.913	1.518	0.5138	3.050
	Diethylhexyl phthalate CR11604			Apigenin CR11610		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Converged?	Y	DNC	DNC	Y	Y	Y
P value	0.0004			0.0642	0.9886	0.0001
Preferred model	Equation 1			Equation 2	Equation 2	Equation 1
F	12.55			3.363	0.01148	14.51
	Methoxychlor CR11605			Ronidazole CR11611		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Converged?	Y	DNC	Y (1 model)	DNC	DNC	DNC
P value	0.0879		No Comp			
Preferred model	Equation 2		Equation 2			
F	2.908					
	Aldicarb CR11606			Genistein CR11612		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Converged?	Y (1 model)	DNC	DNC	Y	Y	Y
P value	No Comp			0.2199	0.1320	0.0172
Preferred model	Equation 2			Equation 2	Equation 2	Equation 1
F				1.658	2.287	5.212

Table 21 (continued)

	p,p'-DDE CR11613			Nitrofen CR11615		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Converged?	Y	Y	Y	DNC	Y	Y
P value	0.2151	<i>P<0.0001</i>	0.1206		<i>0.0004</i>	<i>0.0002</i>
Preferred model	Equation 2	Equation 1	Equation 2		Equation 1	Equation 1
F	1.718	37.08	2.369		12.95	15.17
	Alachlor CR11614			Trifluralin CR11616		
	Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
Converged?	DNC	DNC	DNC	DNC	DNC	DNC
P value						
Preferred model						
F						

Y = yes, the models converged

DNC = neither model converged

F = the test statistic

No Comp = no comparison because only one model converged

Equation 1 = the four-parameter equation

Equation 2 = the two-parameter equation

Statistical significance level is set at $P < 0.05$, significant results indicated with ***bold, italicize***

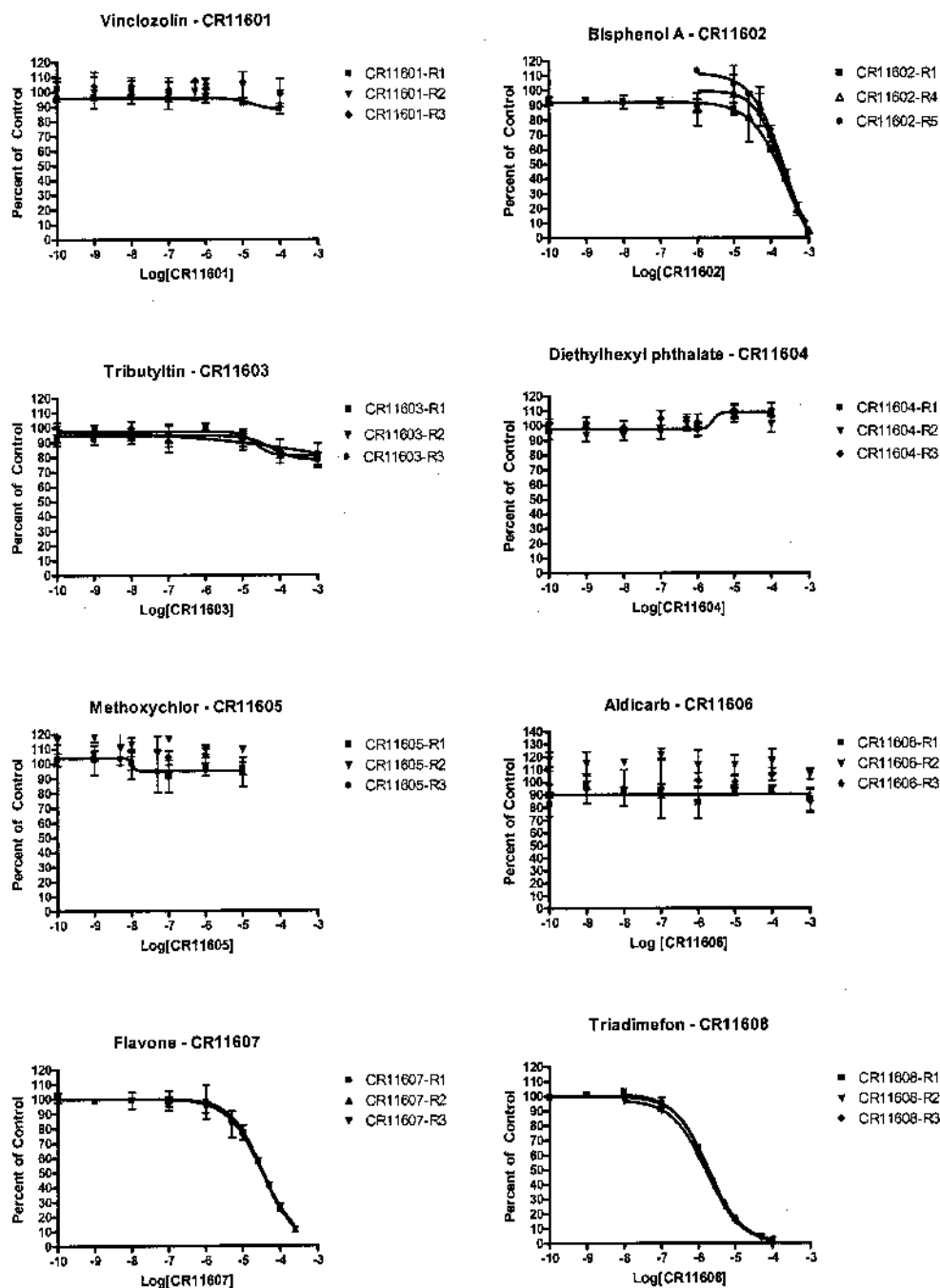


Figure 5. Reference Chemical Concentration Response Curves

Data are mean of 3 tubes per replicate with error bars denoting SEM

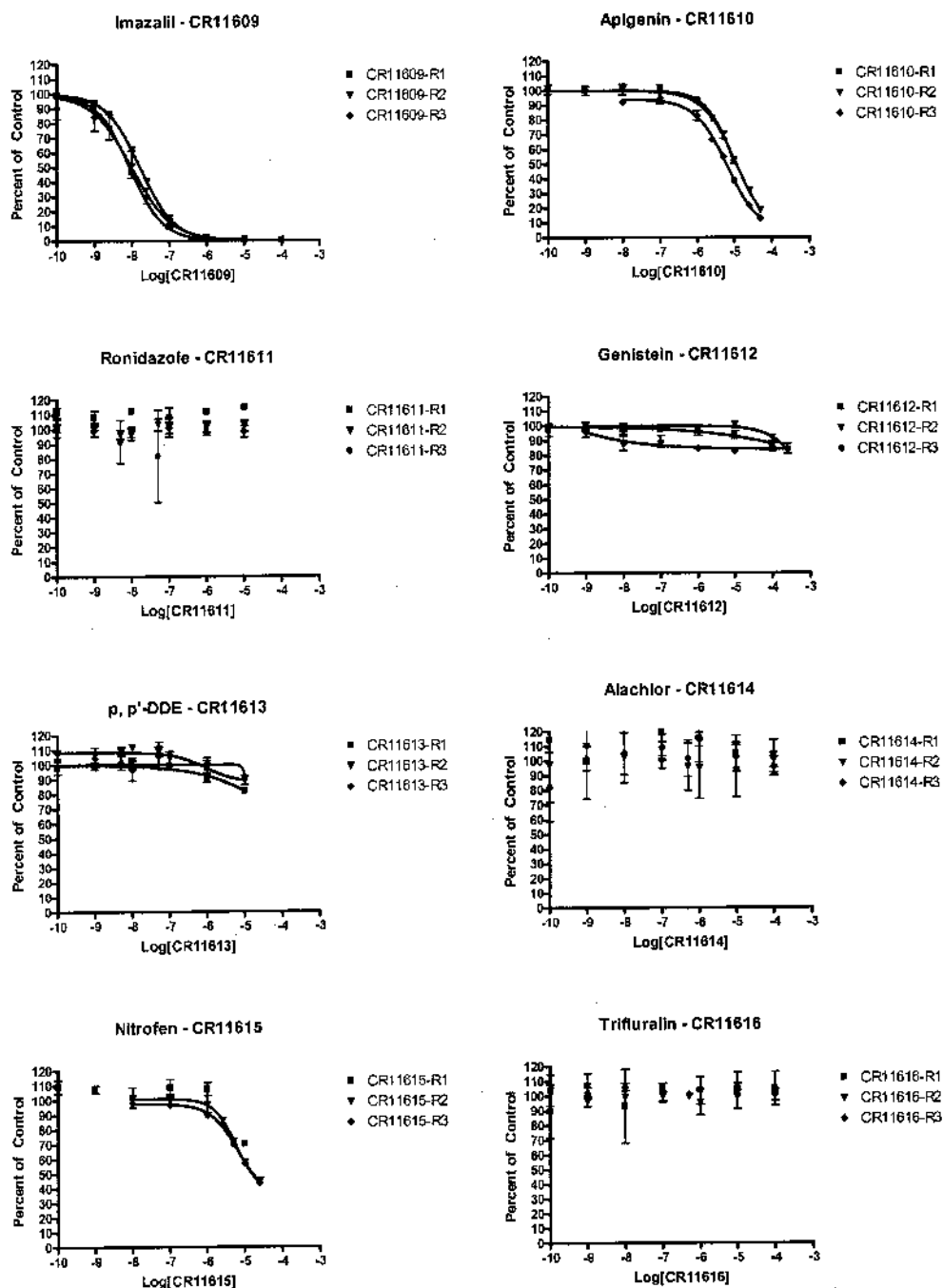


Figure 5. (continued) Reference Chemical Concentration Response Curves

Data are mean of 3 tubes per replicate with error bars denoting SEM

4.5.5 Classification of Chemicals

The classification of the reference chemicals as noninhibitors, inhibitors, or as chemicals for which the results are equivocal is presented in Table 22. Only 6 of the 16 chemicals tested had Bottom values below 75%, and for all of these Bottom was below 50%, which would lead to the classification of all six compounds as inhibitors of aromatase. These six compounds were: bisphenol A, flavone, triadimefon, imazalil, apigenin and nitrofen. The percent of control values essentially spanned the entire range 0-100% for five of the six inhibitors. Only nitrofen (CR11615) had a Bottom value (39%) that was not near 0%. The classifications are assigned based on the criteria in Table 23 below.

Table 22. Classification of Reference Chemicals

Chemical Name	Chemical Code	Highest Tested Soluble Concentration (M)	Classification
Vinclozolin	CR11601	1.00E-04	Noninhibitor
Bisphenol A	CR11602	1.00E-03	Inhibitor
Tributyltin	CR11603	1.00E-03	Noninhibitor
Diethylhexyl phthalate	CR11604	1.00E-04	Noninhibitor
Methoxychlor	CR11605	1.00E-05	Noninhibitor
Aldicarb	CR11606	1.00E-03	Noninhibitor
Flavone	CR11607	2.50E-04	Inhibitor
Triadimefon	CR11608	1.00E-04	Inhibitor
Imazalil	CR11609	1.00E-05	Inhibitor
Apigenin	CR11610	5.00E-05	Inhibitor
Ronidazole	CR11611	1.00E-05	Noninhibitor
Genistein	CR11612	5.00E-04	Noninhibitor
p,p'-DDE	CR11613	1.00E-05	Noninhibitor
Alachlor	CR11614	1.00E-04	Noninhibitor
Nitrofen	CR11615	5.00E-05	Inhibitor
Trifluralin	CR11616	1.00E-04	Noninhibitor

Table 23. Chemical Classification Criteria

Class	Criteria
Noninhibitor	The most inhibited level lies above 75% of full activity control
Equivocal	The most inhibited level lies between 75% and 50% of full activity control
Inhibitor	The most inhibited level lies below 50% of full activity control

4.5.6 Aromatase Inhibitors – Curve Fits

Data from the three replicates of each of the chemicals classified as an inhibitor were combined and fitted to the models. The resulting concentration response curves, with 95% confidence intervals, are presented in Figure 6. It is clear from the graphs that there is very little replicate-to-replicate variation in the data and the curve fits. The exception occurs at the bottom of the nitrofen (CR11615) curve where the 95% confidence interval broadens. The curve parameters (Top, Bottom, LogIC50, and Hillslope) calculated, using the two models, are presented in Table 24. There were no significant differences in the fitted curve parameters obtained from the two models for the chemicals flavone, triadimefon, imazalil, and apigenin. There were significant differences between the two models for bisphenol A and nitrofen. In the case of nitrofen, the values for LogIC50 and Hillslope, obtained with Equation 1, were markedly different from those obtained with the reduced model, and the standard errors for those parameters from Equation 1 were about 3-fold larger than those obtained with Equation 2.

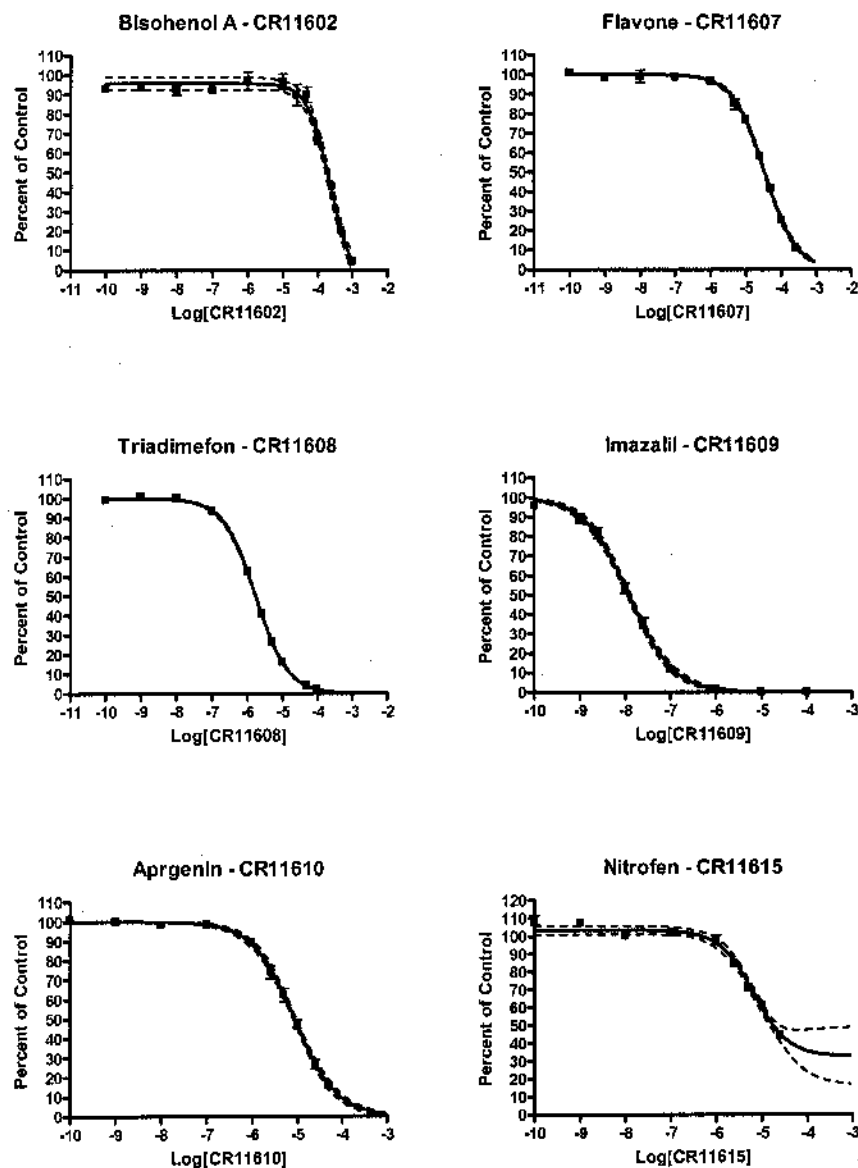


Figure 6. Reference Chemical Curve Fits Across Replicates

Symbols represent mean percent of control with error bars showing SEM

Dashed lines indicate 95% confidence intervals

Solid lines indicated the fitted curve from the preferred model.

Table 24. Comparison of Best Fit Estimates and their associated Standard Errors (SE) for the Model Parameters Across Models for Aromatase Inhibitors

Chemical	Chem Code	Model	P value	Bottom		Top		LogIC50		HillSlope	
				Best Fit	SE	Best fit	SE	Best Fit	SE	Best fit	SE
Bisphenol A	CR11602	Equation 1	0.0091	-8.1	8.4	95.7	1.66	-3.636	0.075	-1.3	0.2
		Equation 2		0.0		100.0		-3.749	0.028	-1.4	0.1
Flavone	CR11607	Equation 1	0.2467	-4.1	4.4	99.2	0.81	-4.417	0.049	-0.9	0.1
		Equation 2		0.0		100.0		-4.473	0.013	-1.0	0.0
Triadimefon	CR11608	Equation 1	0.6846	-0.1	0.7	100.5	0.54	-5.772	0.013	-0.9	0.0
		Equation 2		0.0		100.0		-5.767	0.009	-0.9	0.0
Imazalil	CR11609	Equation 1	0.2283	0.0	1.2	96.9	1.82	-7.882	0.034	-1.0	0.1
		Equation 2		0.0		100.0		-7.924	0.023	-0.9	0.0
Apigenin	CR11610	Equation 1	0.9262	-1.1	7.6	99.6	1.24	-5.047	0.086	-0.9	0.1
		Equation 2		0.0		100.0		-5.064	0.020	-0.9	0.0
Nitrofen	CR11615	Equation 1	0.0009	32.7	8.2	103.3	1.22	-5.189	0.107	-1.1	0.2
		Equation 2		0.0		100.0		-4.766	0.034	-0.9	0.1

The value returned as $\log EC_{50}$ by Prism using the 4-parameter equation is equivalent to $(\text{Top} + \text{Bottom})/2$. For data sets where the 95% confidence intervals of Top and Bottom encompass 100 and 0%, respectively, the parameter 'LogEC50' estimated by Prism is equivalent to LogIC50 which, by definition, is the concentration that yields 50% inhibition. Caution should be exercised in the reporting of IC_{50} estimates generated from data sets that do not span the 0-100% range for percent of control. Plots of the two- and four-parameter curve fits and their associated 95% confidence bands for nitrofen are presented in Figure 7. Both models fit this data set reasonably well, but the 95% confidence bands to broaden as the four-parameter model approaches lower percent of control values due to the poorly defined Bottom for that model. The Prism estimates for LogEC50 are -4.776 and -5.189 for the two- and four-parameter models, respectively. As depicted graphically in Figure 7, the estimate for LogEC50 returned by the 4-parameter model corresponds to the concentration that yields 68% of control activity $[(\text{Top} + \text{Bottom})/2 = (103.3 + 32.7)/2 = 68]$. When queried for the value of X where Y=50 (defined as LogIC50), Prism returns the estimate of -4.762 for the 4-parameter equation. Equation 3 (below) can be used in Prism to directly yield direct LogIC50 estimates, regardless of whether the range of the inhibition spans 0-100%. The LogIC50 value from Equation 3 (-4.762) matches that obtained as a point estimate for X at Y=50 from the 4-parameter equation. The advantage of using Equation 3 is the availability of confidence intervals from Prism for the true LogIC50.

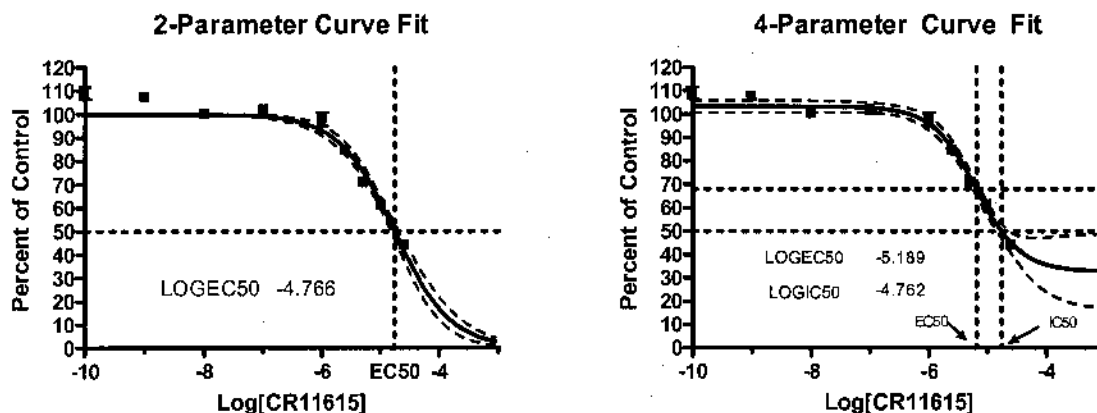


Figure 7. Comparison of the 2- and 4-Parameter Model Fits for Nitrofen

Table 25. Values for LogIC50 from Various Models

Model	LogIC50 (from curve fit)	SE of LogIC50	Concentration corresponding to Y=50 (LogIC50)
Equation 1	-5.189	0.1072	-4.762
Equation 2	-4.766	0.0337	-4.766
Equation 3	-4.762	0.0464	-4.762

$$Y = Bottom + \left(\frac{Top - Bottom}{1 + 10^{(\mu - X)\beta + \log((Top - Bottom)/(50 - Bottom) - 1)}} \right) \quad \text{Equation 3}$$

where Y is the percent of control activity remaining, X is the $\log_{10}$ of the concentration of the chemical, Top and $Bottom$ are the plateaus of the response curve, μ is the $\log_{10}IC_{50}$ and β is the slope of the response curve at $\log_{10}IC_{50}$.

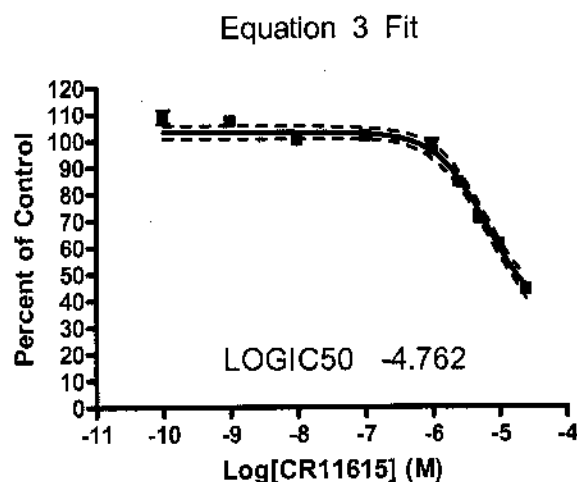


Figure 8. Concentration Response Curve for Nitrofen from Equation 3

4.5.7 Development of Second Performance Criterion for Reference Chemicals

Data Quality Criterion: In order to assess whether an individual run of the assay is acceptable, coefficients of variation (CVs) for the percent of control values at each concentration and the mean CV across all concentrations were examined. The CVs obtained for each reference chemical replicate in the current study are presented in Table 26. It is clear that within replicate CVs at each concentration are often less than 10%, but that sometimes they range to 15% or even higher. In cases where the CVs are higher than 15%, the data should be examined for outliers that might indicate experimental error. In addition, it is important to note that when the DPM data approach background values (as when percent of control is less than approximately 5%) the inherent uncertainty (measurement error) in the data rises to nearly 15% and, therefore, CVs calculated for data at very low percent of control values are primarily comprised of measurement error. There are enough CVs in the 10-15% range to warrant setting the standard for CV at each concentration to be no tighter than $\leq 15\%$. The effect of the higher individual concentration CVs on the curve fit can be assessed by examining the 95% confidence bands of the fitted curves. Only two replicates in the data set (CR11602- Rep 4 and CR11609 – Rep 3) had both individual CVs $>15\%$ and produced fitted curves in Prism. For both of these, the 95% confidence bands are narrow across the entire curve (Figure 9), indicating that the fitted curves were well defined even when there were some instances of high individual CVs in the data set. Three other replicates

(CR11611- Rep 3, CR11614-Rep 2 and CR11616- Rep 1) had individual CVs above 15%, but these chemicals were all noninhibitors.

Another approach would be to look at the mean CV across concentrations within a replicate. As can be seen in the data presented in Table 26, those mean CVs were $\leq 15\%$ for 47 of the 48 assays and were $\leq 10\%$ in 41 of the 48 assays. Of the seven replicates that had CV means greater than 10%, only two (CR11602, Rep 4 and CR11609, Rep 3) were from chemicals for which Prism could fit the model; the others were from chemicals that were clearly noninhibitors. As discussed above, the 95% confidence bands on these curve fits are narrow, indicating that well-defined curves were obtained from the data.

Table 26. Coefficients of Variance (%CV) for each Reference Chemical Replicate and Concentration

Chemical	Log[RC]*	Rep 1	Rep 2	Rep 3	Chemical	Log[RC]*	Rep 1	Rep 2	Rep 3
CR11601 Vinclozalin	-4	4.1	9.7	1.5	CR11609 Imizalil	-7	0.2	2.4	24.8
	-5	2.7	8.6	1.3		-7.6		1.9	17.2
	-6	3.1	8.2	3.2		-8	0.1	1.1	17.8
	-6.3	NA	6.1	1.2		-8.6		1.3	11.0
	-7	0.7	9.3	8.0		-9	0.3	2.3	11.3
	-8	1.4	7.7	5.7		-10	1.0	1.1	8.7
	-9	1.3	10.8	9.0					
	-10	1.0	8.1	5.4					
	Mean	2.0	8.6	4.4		Mean	0.4	1.7	15.1
	SD	1.3	1.4	3.1		SD	0.4	0.6	6.0
CR11602† Bisphenol A	-3.3	NA	23.3	2.5	CR11610 Apigenin	-4.3	NA	7.7	1.8
	-3.6	NA	9.3	2.1		-4.6	NA	2.7	5.7
	-4	1.3	4.7	4.5		-5	0.8	0.5	4.7
	-4.3	NA	14.9	7.7		-5.3	NA	3.4	2.3
	-4.6	NA	20.3	1.6		-5.6	NA	2.9	0.8
	-5	4.8	13.2	12.2		-6	1.4	1.6	4.1
	-6	4.6	12.7	1.3		-7	1.0	0.1	3.8
	-7	3.7	NA	NA		-8	1.6	3.5	1.2
	-8	4.9	NA	NA		-9	3.0	NA	NA
	-9	1.3	NA	NA		-10	3.2	NA	NA
	-10	3.0	NA	NA					
	Mean	3.4	14.0	4.6		Mean	2.0	2.8	3.1
	SD	1.6	6.3	4.0		SD	1.0	2.3	1.8

Table 26 (continued)

Chemical	Log[RC]	Rep 1	Rep 2	Rep 3	Chemical	Log[RC]	Rep 1	Rep 2	Rep 3
CR11603 Tributyltin	-3	5.8	10.0	8.8	CR11611 Ronidazole	-5	1.7	3.7	3.9
	-4	2.7	2.9	9.6		-6	1.1	3.8	4.1
	-5	6.6	2.9	6.0		-7	6.2	6.5	3.8
	-6	1.8	2.5	3.0		-7.3	NA	3.9	38.3
	-7	5.9	8.2	7.5		-8	1.7	4.5	2.8
	-8	2.6	5.4	5.7		-8.3	NA	3.2	16.0
	-9	4.5	0.8	6.8		-9	4.3	2.5	3.5
	-10	6.1	4.0	3.0		-10	3.2	4.4	3.9
	Mean SD	4.5 1.9	4.6 3.1	6.3 2.4		Mean SD	3.0 2.0	4.1 1.2	9.5 12.4
CR11604 Diethyl- phthalate	-4	1.3	5.6	4.3	CR11612 Genistein	-3.3	NA	5.8	1.1
	-5	4.1	3.8	3.9		-3.6	NA	2.3	4.1
	-6	4.8	4.5	5.1		-4	2.8	2.3	3.1
	-6.3		4.5	4.3		-5	2.5	2.0	1.5
	-7	7.1	2.4	4.6		-6	3.5	2.8	1.4
	-8	6.7	3.4	0.7		-7	1.8	2.0	4.6
	-9	1.6	4.4	3.6		-8	2.5	2.5	5.4
	-10	5.5	2.4	2.3		-9	5.0	2.7	1.6
						-10	4.3	NA	NA
	Mean SD	4.4 2.3	3.9 1.1	3.6 1.4		Mean SD	3.2 1.1	2.8 1.2	2.9 1.7
CR11605 Methoxychlor	-5	4.4	1.3	10.6	CR11613 p,p'-DDE	-5	1.2	3.6	0.3
	-6	6.4	2.8	4.7		-6	3.5	5.0	4.0
	-7	11.9	1.6	4.7		-7	1.1	4.2	1.6
	-7.3	NA	10.9	14.9		-7.3	NA	3.1	8.6
	-8	4.7	4.2	10.0		-8	3.0	1.7	8.0
	-8.3	NA	10.4	0.7		-8.3	NA	2.9	5.0
	-9	2.9	2.7	9.6		-9	2.5	5.1	3.6
	-10	1.1	2.7	4.2		-10	4.5	2.9	4.6
	Mean SD	5.2 3.7	4.6 3.9	7.4 4.6		Mean SD	2.6 1.3	3.5 1.1	4.5 2.8
CR11606 Adicarb	-3	10.6	3.9	10.5	CR11614 Alachlor	-4	10.7	5.0	6.7
	-4	3.0	8.3	4.7		-5	11.6	19.0	9.8
	-5	4.0	6.8	4.8		-6	14.0	21.8	4.1
	-6	14.7	10.4	5.8		-6.3	NA	16.7	12.2
	-7	5.9	3.9	24.9		-7	5.3	4.2	7.9
	-8	3.5	2.1	14.8		-8	8.0	16.7	14.2
	-9	3.2	8.3	12.4		-9	25.6	1.4	8.2
	-10	11.3	4.9	10.5		-10	13.3	25.9	28.6
	Mean SD	7.0 4.5	6.1 2.8	11.1 6.7		Mean SD	12.6 6.4	13.8 9.1	11.5 7.6

Table 26 (continued)

Chemical	Log[RC]	Rep 1	Rep 2	Rep 3	Chemical	Log[RC]	Rep 1	Rep 2	Rep 3
CR11607 Flavone	-3.6	NA	2.2	1.7	CR11615 Nitrofen	-4.3	NA	4.3	4.0
	-4	4.9	2.2	2.9		-4.6	NA	0.9	0.8
	-4.3	NA	3.4	0.9		-5	2.8	3.0	2.0
	-4.6	NA	2.8	1.7		-5.3	NA	3.2	1.7
	-5	6.6	1.3	1.9		-5.6	NA	2.3	1.5
	-5.3	NA	2.3	10.9		-6	4.1	5.9	0.7
	-6	11.7	1.3	4.1		-7	5.5	3.3	1.7
	-7	3.3	3.8	4.5		-8	6.7	2.0	0.4
	-8	5.6	NA	NA		-9	2.4	NA	NA
	-9	2.2	NA	NA		-10	4.2	NA	NA
	-10	3.2	NA	NA					
	Mean	5.3	2.4	3.6		Mean	4.3	3.1	1.6
	SD	3.2	0.9	3.2		SD	1.6	1.5	1.1
CR11608 Triadimefon	-5	2.7	4.2	1.4	CR11616 Trifluralin	-4	10.9	4.0	1.1
	-5.3	NA	0.4	0.6		-5	11.9	1.8	2.3
	-5.6	NA	3.9	0.4		-6	9.0	9.1	0.8
	-6	1.6	1.8	0.6		-6.3	NA	1.5	1.3
	-7	4.9	0.3	2.4		-7	2.1	3.5	5.8
	-8	2.0	0.5	1.4		-8	26.7	2.2	2.3
	-9	1.6	NA	NA		-9	7.8	3.7	3.3
	-10	1.9	NA	NA		-10	20.2	10.2	1.6
	Mean	2.5	1.8	1.1		Mean	12.7	4.5	2.3
	SD	1.3	1.8	0.8		SD	8.2	3.3	1.6

* Concentrations showing insolubility were excluded.

†CR11602 replicates reported are 1, 4 and 5.

NA Not applicable – this concentration was not assayed in this replicate

Bold, italicized type denotes CVs > 15%

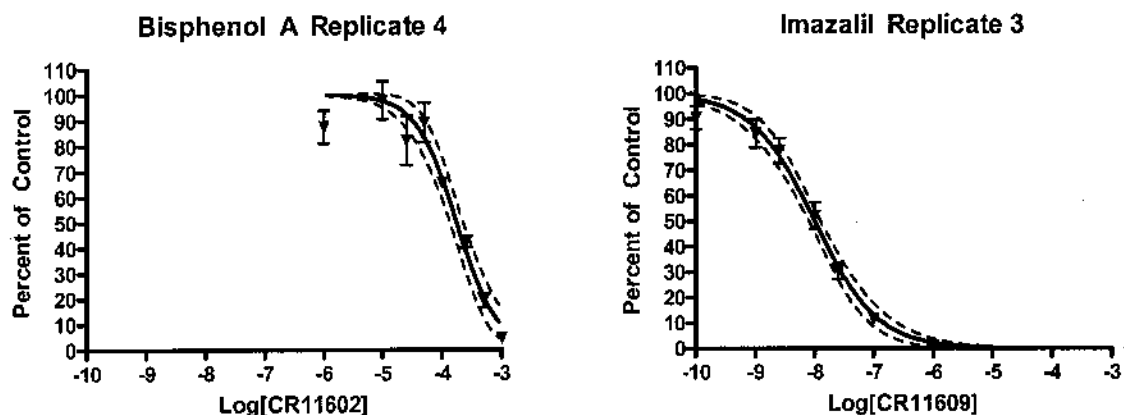


Figure 9. Concentration Response Curves for Replicates with CV%>15%

Data Interpretation Criterion. Data interpretation criteria would be used to decide upon the proper classification of a test chemical as either an inhibitor, a noninhibitor, or as a chemical for which the data are equivocal. The initial classification criteria are outlined in the Table 23.

The results of the experiment can be interpreted based on the upper and lower 95% confidence bounds on the estimated response curve. If both confidence bands for the estimated response curve extend below the 50% line on the graph, then the chemical can be classified as an inhibitor with only a small chance of a classification error. If both confidence bands for the estimated response curve stay above the 75% line on the graph, then the chemical can be classified as a noninhibitor with only a small chance of a classification error. More analyses would need to be performed to determine the classification rules if at least one of the confidence bands remains between the 50% and 75% lines for the highest concentrations of the chemical.

In the present data set, there are six compounds that are clearly inhibitors and 10 that are clearly noninhibitors. In no case was the most inhibited level between 75% and 50% of control which would lead to the classification of equivocal.

Five of the six inhibitors exhibited maximal inhibition of less than 20% of control; the other inhibitor (CRI1615) had a maximal inhibition of about 32% of control. These are all well within the 'inhibitor' classification range. The 95% confidence bands on the curve fits (Figure 5 and Appendix H) were examined to determine if both confidence bands extended below 50% of control at the highest concentration. In all cases, the confidence bands were well below 50% of control at the highest concentration.

Generally, there was no concentration-dependent response for the noninhibitors, and Prism was often unable to fit the model to the data. Under these circumstances, there can be no examination of the 95% confidence bands, and no further analysis is necessary to classify these compounds as noninhibitors. In some cases, Prism returned a fitted curve, but the 95% confidence intervals were very large and so the curve fits were meaningless.

In order to mimic data one might obtain for equivocal inhibitors and to generate a test set for evaluation of the 95% confidence interval bounds as an interpretation criterion, the data from the six inhibitors was limited to those concentrations where activity was > 40-60% of control and

Prism was used to fit the four parameter model. The resulting plots are presented in Figure 10 and include the 95% confidence intervals. The data sets for which there are wide variances in the curve fit parameters are obvious by their wide confidence intervals, and all of these would necessarily be classified as equivocal. There were not enough data in some of the limited data sets to fit a curve. The other data sets show fairly narrow confidence intervals, and in all cases where the lowest mean percent of control activity was <50%, both confidence interval bounds also fell below 50% allowing the classification of inhibitor to be assigned. Also, in each case when the lowest mean percent of control activity was between 75% and 50% of control, both confidence interval bounds fell completely within that range and the chemical would be classified as an equivocal inhibitor.

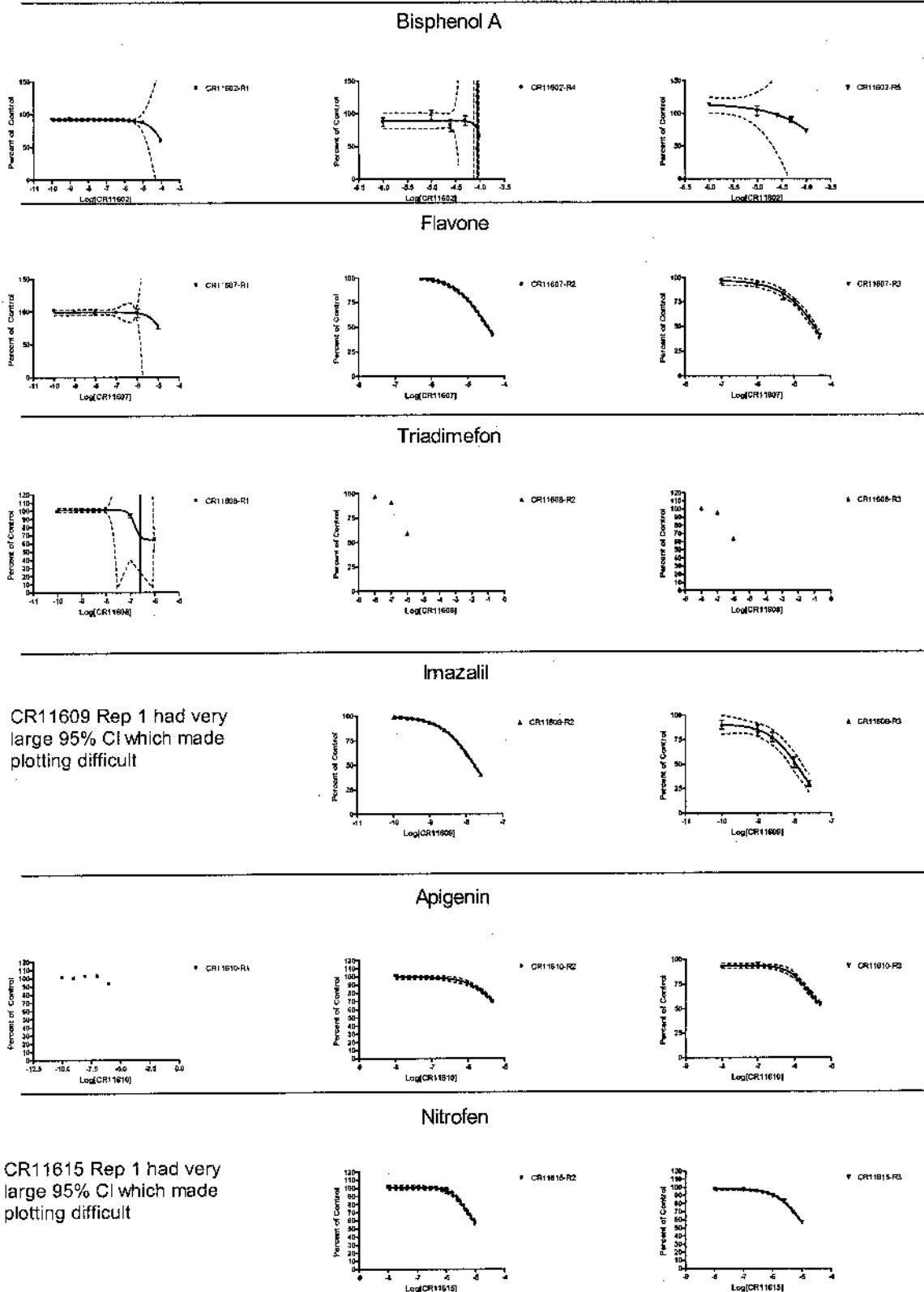


Figure 10. Concentration Response Curves from Data Sets with Percent of Control Limited to >40-60%

5.0 DISCUSSION

The response of the aromatase activity in recombinant microsomes to the presence of 16 chemicals was determined in this task order. The assay conditions used were the same as those used in the interlaboratory validation for the assay. Each chemical was assayed in triplicate tubes for each of 8 concentrations in three replicates of the aromatase assay. Controls for the assay included both full enzyme activity (no test inhibitor present) and background activity (no NADPH present) controls. In addition, each assay included duplicate tubes for each of 8 concentrations of the known aromatase inhibitor, 4-OH ASDN, which served as a positive control.

Each technician (4 total) who participated in the task order was required to obtain satisfactory results in the assay of the positive control chemical, 4-OH ASDN, before proceeding to the reference chemical testing. All technicians, each of whom had varying degrees of prior experience with the assay, successfully completed the positive control assay on the first try.

Full activity and background controls were run with each assay. The mean ($\pm$ SD) full aromatase activity for this batch of recombinant microsomes was 0.313 ± 0.085 nmol/mg/min. In only 1 (of 50 total conducted) assay was the full activity <0.1 nmol/mg/min, and this assay was excluded from analysis. The mean ($\pm$ SD) uncorrected background activity was 0.002 ± 0.001 nmol/mg/min, which corresponded to $0.7 \pm 0.3\%$ of control. There were five assays where the uncorrected background activity was more than 1% of control; the highest was at 1.8% of control. In each of these cases, there seemed to be a lower than normal full aromatase activity rather than an elevated background activity. Since background activity was in a normal range and all data is corrected for background before calculating percent of control, assays with these slightly elevated uncorrected backgrounds as percent of control values were not excluded from the data sets.

Results from the positive control assays that were run in conjunction with the reference chemical replicates indicated no significant difference in percent of control values over all concentrations between technicians. There were some statistically significant differences in percent of control values from replicate to replicate within a technician. Proposed performance criteria for the four model fit parameters (Top, Bottom, LogIC50, and HillSlope) for the positive control assay were met in 39 of 48 assays, and there was some indication that failures in 4 of the

assays were due to a solution preparation problem. Most of the failures involved only one of the four parameters, and the values were usually just outside the criteria ranges. In fact, the 95% confidence interval bands for each parameter always intersected the performance criteria ranges. Data and curve fits for the assays which had performance criteria failures were examined and did not indicate any serious flaw in the functioning of the aromatase assay. It may be advisable to allow acceptance of data sets where only one of the four parameters fails the performance criteria, especially in cases where the 95% confidence interval band for the parameter in question includes values within the performance criteria ranges.

Reference chemicals were formulated by the Chemical Repository and were coded (blind) prior to distribution. All replicates of a given reference chemical were conducted by the same technician.

Percent of control data from each replicate for each reference chemical were fitted to both a 4- and 2-parameter logistic equation to calculate estimates for Top, Bottom, Log IC50, and HillSlope. Top and Bottom were fixed at 100 and 0%, respectively, for the 2-parameter equation.

All but 1 of the 48 assays of reference chemicals met the performance criterion that required that $\text{Top} = 100 \pm 10\%$. The one failure was associated with a chemical that was not an aromatase inhibitor for which the model fit was extremely poor with huge 95% confidence intervals for all parameters. In fact, the data set for that replicate contained no percent of control values higher than 100%. The data indicate that requiring $\text{Top} = 100 \pm 10\%$ is an acceptable performance criterion for the assay of test chemicals.

The two model curve fits were compared in Prism to determine if they were statistically different. In about half of the cases where Prism was able to fit both models, there were significant differences in the model fits. There was no discernible pattern in the data that would allow prediction of the best model for a particular data set. When the data nearly span the range 0-100% of control, either model can produce good parameter estimates. The increased flexibility of the 4-parameter model usually leads to better curve fits when the inhibited range does not span the full range of 0-100%. However, care must be taken in interpreting Prism results from the 4-parameter equation in cases where the 95% confidence intervals of Top and Bottom do not encompass 100 and 0%, respectively. Prism estimates 'LogEC50' as the concentration

corresponding to the median of Top and Bottom, and this is not equivalent to the concentration corresponding to 50% inhibition (LogIC50), when Top and Bottom are not encompassed by 100 and 0%, respectively. Equation 3, a modification to the 4-parameter model described in the Results section, can be used to accurately estimate LogIC50 in those cases.

In order to develop a second performance criterion for the test chemical assays, within replicate coefficients of variation (CV) for the percent of control values at each concentration were examined. Often the CVs were less than 10%, but in some cases they ranged to 15% or higher. High (>15%) CVs are most common at low percent of control activity because of the higher measurement variance in the LSS data at these near background levels. The mean CVs across all concentrations within a replicate were <15% in 47 of the 48 assays conducted and were ≤10% in 41 of the 48 assays. Of the seven with CV>10%, only two assays involved chemicals that inhibited aromatase. The 95% confidence interval bands on the curve fits for those two replicates were narrow, indicating a good fit for the model. This suggests that data sets should not be rejected automatically even when CVs range up to about 15%, but rather that higher CVs should prompt further review of the data, including review of percent of control values for outliers that might indicate experimental error and review of the 95% confidence intervals for the fitted curve and parameter estimates.

Only 6 of the 16 test chemicals inhibited aromatase activity >50% and these were all classified as inhibitors. These six compounds were: bisphenol A, flavone, triadimefon, imazalil, apigenin, and nitrofen. None of the test chemicals had a maximal inhibition in the range 75-50% of control, so none of them was classified as a chemical for which the data were equivocal. Ten of the test chemicals were classified as noninhibitors since they did not inhibit aromatase activity more than 25% at any tested concentration. The absence of assay data with the highest degree of inhibition near the classification boundaries of 75% or 50% makes evaluation of any data interpretation criterion difficult. In order to generate data that might be useful for evaluating data interpretation at the classification boundaries, the data sets from the chemicals classified as inhibitors were limited so that the maximal inhibition was ca. 40-60 % of control. For some replicates, this resulted in there not being enough data to fit a curve. When the curve fit was successful, the 95% confidence interval bands were reviewed in order to assess their utility in data evaluation to 'classify' the chemicals. The 95% confidence interval bounds were narrow and in all cases where the lowest mean percent of control activity was <50%, both confidence

interval bounds also fell below 50% which would allow the classification of inhibitor to be assigned. Also, in each case when the lowest mean percent of control activity was between 75% and 50% of control, both confidence interval bounds fell completely within that range and the chemical could be classified as an equivocal inhibitor.

Assessment of any aromatase assay data set for test chemicals should begin with a review of the tube-to-tube variance in percent of control. Variances greater than 10-15% should be reviewed for any contribution from experimental error and outliers excluded as appropriate. Concentrations that yield percent of control values <10% can be expected to have higher CVs between tubes due to limitations of LSS data near background levels. Except in cases where the test chemical was not soluble (which should always be excluded from analysis), data from these concentrations should be allowed to remain in the data set. The data sets should be examined to determine the mean maximum inhibition. Chemicals for which the mean maximum inhibition is >80 percent of control will likely lead to model fit failures or meaningless curve fits and may be classified as non-inhibitors without further analysis. The data show that the 4-parameter model will yield good parameter estimates when the inhibited range spans approximately 100-0% of control; in cases where the full range of inhibition is less than 0-100%, an alternative model (Equation 3) is preferred. The fitted curve, 95% confidence bounds for the curve and estimated parameters Top and LogIC50 should be examined. Overall review of the data from the test chemicals that were classified as inhibitors indicates that it is reasonable to keep $Top = 100 \pm 10\%$ as a performance criterion. Bottom and Hillslope are not as useful in evaluating assay performance because, especially in the case of partial inhibition curves where a bottom plateau is not defined by the data, there can be high variances in these two parameters, even when other parameters (Top, LogIC50) have narrow variances. An important indicator of a good curve fit is whether the 95% confidence interval bounds conform to the shape of the fitted curve along its entire length. If the 95% confidence interval bounds diverge from the fitted curve, further examination of the data is warranted. In that case, it might be necessary to choose a different model (such as Equation 3 instead of the 4-parameter equation) or to reject the data set. As discussed above, the 95% confidence interval bounds of the most inhibited point can be used as a data interpretation criteria to distinguish between the three classifications or to determine that the data is equivocal.

6.0 REFERENCES

- Adlercreutz, H., Bannwart, C., Wahala, K., Makela, T., Brunow, G., Hase, T., Arosemena, P. J., Kellis, J. T., Jr., and Vickery, L. E. (1993) Inhibition of human aromatase by mammalian lignans and isoflavonoid phytoestrogens. *J.Steroid Biochem.Mol.Biol.*, *44*: 147-153.
- Chen, S., Kao, Y. C., and Laughton, C. A. (1997) Binding characteristics of aromatase inhibitors and phytoestrogens to human aromatase. *J.Steroid Biochem.Mol.Biol.*, *61*: 107-115.
- EPA (2005) http://www.epa.gov/oscpmont/oscpendo/pubs/edmv/s/aromatase_drp_final_3_30_05.pdf, accessed 6/25/2007.
- Ibrahim, A. R. and Abul-Hajj, Y. J. (1990) Aromatase inhibition by flavonoids. *J.Steroid Biochem.Mol.Biol.*, *37*: 257-260.
- Jeong, H. J., Shin, Y. G., Kim, I. H., and Pezzuto, J. M. (1999) Inhibition of aromatase activity by flavonoids. *Arch.Pharm.Res.*, *22*: 309-312.
- Kao, Y. C., Zhou, C., Sherman, M., Laughton, C. A., and Chen, S. (1998) Molecular basis of the inhibition of human aromatase (estrogen synthetase) by flavone and isoflavone phytoestrogens: A site-directed mutagenesis study. *Environ.Health Perspect.*, *106*: 85-92.
- Le Bail, J. C., Pouget, C., Fagnere, C., Basly, J. P., Chulia, A. J., and Habrioux, G. (2001) Chalcones are potent inhibitors of aromatase and 17beta-hydroxysteroid dehydrogenase activities. *Life Sci.*, *68*: 751-761.
- Le Bail, J. C., Aubourg, L., and Habrioux, G. (2000a) Effects of pinostrobin on estrogen metabolism and estrogen receptor transactivation. *Cancer Lett.*, *156*: 37-44.
- Le Bail, J. C., Champavier, Y., Chulia, A. J., and Habrioux, G. (2000b) Effects of phytoestrogens on aromatase, 3beta and 17beta-hydroxysteroid dehydrogenase activities and human breast cancer cells. *Life Sci.*, *66*: 1281-1291.
- Le Bail, J. C., Laroche, T., Marre-Fournier, F., and Habrioux, G. (1998) Aromatase and 17beta-hydroxysteroid dehydrogenase inhibition by flavonoids. *Cancer Lett.*, *133*: 101-106.
- Sanderson, J. T., Slobbe, L., Lansbergen, G. W., Safe, S., and van den, B. M. (2001) 2,3,7,8-Tetrachlorodibenzo-p-dioxin and Diindolylmethanes Differentially Induce Cytochrome

P450 1A1, 1B1, and 19 in H295R Human Adrenocortical Carcinoma Cells. *Toxicol.Sci.*, *61*: 40-48.

Sanderson, J. T., Seinen, W., Giesy, J. P., and van den, B. M. (2000) 2-Chloro-s-triazine herbicides induce aromatase (CYP19) activity in H295R human adrenocortical carcinoma cells: a novel mechanism for estrogenicity? *Toxicol.Sci.*, *54*: 121-127.

Vinggaard, A. M., Hnida, C., Breinholt, V., and Larsen, J. C. (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro. *Toxicol.In Vitro*, *14*: 227-234.

APPENDIX A

Study Protocol

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EPA Contract No.: EP-W-06-026
 Task Number: 9
 RTI Study Code: An07-996
 RTI Project Number: 0210114.009

**TITLE: Task Order 9: Supplementary Testing of 16
 Chemicals in the Recombinant Aromatase Assay**

SPONSOR: U. S. EPA
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TESTING FACILITY: RTI International
 DMPK
 3040 Cornwallis Rd.
 Research Triangle Park, NC 27709

PROPOSED EXPERIMENTAL START DATE: February 14, 2007
PROPOSED EXPERIMENTAL END DATE: April 10, 2007

AMENDMENTS:

Number	Date	Section(s)	Page(s)
1			
2			
3			

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1.0 OBJECTIVES

This work assignment will provide for the testing of an additional sixteen chemicals to support the validation of the recombinant aromatase assay. In addition to broadening the range of classes and potencies of the chemicals tested, this work assignment will test the performance criteria that EPA has developed based on the data from the interlaboratory testing using the recombinant microsomal assay and the impact of using a four-parameter (no constraints) versus a two-parameter model in which the top and bottom values are set to 100% and 0%, respectively.

1.1 Justification for Test System

The test system for this study is recombinant microsomes. This test system was selected because it provides a commercially available source of the aromatase enzyme.

1.2 Test Method

This *in vitro* test method involves combining microsomes, substrate, appropriate co-factors and reference chemicals in a common reaction vessel. The effect of the reference chemicals on microsomal enzyme activity is evaluated by measuring the amount of the product of the enzyme-catalyzed substrate oxidation that is formed.

2.0 MATERIALS RECEIPT AND/OR PREPARATION

A sufficient supply of chemical reagents, radiolabeled and non-radiolabeled androstenedione, and recombinant microsomal preparations will be obtained prior to initiation of the first set of experiments to ensure that sufficient quantities are available to conduct the studies. Appendix A contains a complete list of materials, chemicals and equipment needed for the conduct of the assay.

2.1 Substrate

2.1.1 Substrate Name/Supplier

The substrate for the aromatase assay is androstenedione (4-androstene-3,17-dione, ASDN, CAS# 63-05-8). Nonradiolabeled and radiolabeled ASDN will be used. The nonradiolabeled ASDN should be $\geq 98\%$ pure. The radiolabeled ASDN ($[1\beta\text{-}^3\text{H}(\text{N})]$ -androst-4-ene-3,17-dione, $[^3\text{H}]$ ASDN) should be $\geq 95\%$ radiochemically pure and is usually supplied at a specific activity of 20-30 Ci/mmol. The $[^3\text{H}]$ ASDN must have a specific activity of no less than 500 $\mu\text{Ci/mmol}$. The nonradiolabeled ASDN and the $[^3\text{H}]$ ASDN will be provided by the Chemical Repository (CR). The CR will forward all applicable information regarding supplier, lot numbers, and reported/measured purity for the substrate to RTI and this information will be included in study report.

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2.1.2 Radiochemical Purity The radiochemical purity of the [³H]ASDN will be determined using high performance liquid chromatography (HPLC) and liquid scintillation counting. The HPLC system consists of a Waters 2695 Separations Module, a Waters 2487 Dual λ Absorbance Detector and a β-RAM Model 3 flow-through radioactivity detector (IN/US, Inc., Tampa, FL) with a 250 μL glass scintillant cell. Data will be collected using Waters Empower 2 Client/Server Chromatography Data System Software. The HPLC method uses a Zorbax SB-C₁₈ column (4.6 x 250 mm) with a mobile phase of 55:15:30 (v:v:v) distilled, deionized water: tetrahydrofuran: methanol and a flow rate of 1 mL/min. The eluant will be monitored by UV absorbance at 240 nm and by a flow-through radiochemical detector. Eluant fractions will be collected manually into vials containing ca. 10 mL Ultima Gold and assayed for radiochemical content by liquid scintillation spectrometry (LSS). A reference standard of nonradiolabeled ASDN will be analyzed by the same method and coelution of the nonradiolabeled and radiolabeled ASDN will be confirmed. 2.1.3 Preparation of Substrate Solution for use in Aromatase Assay The specific activity of the [³H]ASDN as received from the vendor is usually 20-30 Ci/mmol which is too high for use directly in the assay. Thus, the specific activity must be lowered to about 500 μCi/mmol by the addition of nonradiolabeled ASDN. The target final concentration of ASDN in the assay is 100 nM and the amount of tritium to be added to each incubation is about 0.1 μCi. Therefore, prepare a substrate solution containing a mixture of nonradiolabeled and radiolabeled ASDN with a concentration of 2 μM and with a radiochemical content of about 1 μCi/mL. The following illustrates the preparation of a substrate solution using a stock of [³H]ASDN with a specific activity of 25.3 Ci/mmol and a concentration of 1 mCi/mL. Prepare a 1:100 dilution of the radiolabeled stock in buffer. Prepare a 1 mg/mL solution of ASDN in ethanol and then prepare dilutions in buffer to a final concentration of 1 μg/mL. Combine 4.5 mL of the 1 μg/mL solution of ASDN, 800 μL of the [³H]ASDN dilution and 2.7 mL buffer to make 8 mL of substrate solution (enough for 80 tubes). Record the weight of each component added to the substrate solution. After mixing the solution well, weigh aliquots (ca 20 μL) and combine with scintillation cocktail for radiochemical content analysis. The addition of 100 μL of the substrate solution to each 2 mL assay volume yields a final [³H]ASDN concentration of 100 nM with 0.1 μCi/tube. 2.2 Reference Chemicals The reference chemicals; their properties and rationale for selection are listed in Table 1. Each chemical will initially be tested over the concentration range 10⁻³ to 10⁻¹⁰ M (final concentration) but the range may be adjusted as described in Section 6. All reference chemical should be $\geq$97% pure.		

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Table 1. Reference Chemicals

Chemical	CAS No.	Reason for Selection
Vinclozolin	50471-44-8	Used in other validation studies and has been reported to be negative in both H295R and KGN cell-based assays.
Bisphenol A	80-05-7	A challenging chemical used extensively in ED testing. Structurally different from the pesticides tested and reported to be an inhibitor in the KGN assay.
Tributyltin	688-73-3	Structurally different from all other chemicals tested and reported in the literature to be an aromatase inhibitor.
Diethylhexyl phthalate	117-81-7	Used in other validation studies. Reported to be an inhibitor in the KGN assay.
Methoxychlor	72-43-5	Used in other validation studies. Known to be metabolized to a strongly estrogenic agent and to have antiandrogenic properties. Reported to be negative in the KGN assay.
Aldicarb	116-06-3	Aldicarb is a carbamate and therefore structurally different from other pesticides tested in the validation program. It was reported to be an inhibitor in the KGN assay.
Flavone	525-82-6	Flavone is the parent of a group of natural aromatase inhibitors. It is reported to be a weak inhibitor in the KGN assay and a moderate (10 μ M) inhibitor in the PM assay.
Triadimefon	43121-43-3	A weak to moderate azole fungicide inhibitor for which several literature values were found.
Imazalil	35554-44-0	An azole fungicide for which several literature values were found.
Apigenin	520-36-5	A flavone with known aromatase activity.
Ronidazole	7681-76-7	Reported to be a very weak azole fungicide inhibitor in the HT assay.
Genistein	446-72-0	An isoflavone reported to be negative. Genestein has been used extensively in ER binding and other ED validation studies.
p,p'-DDE	72-55-9	An organochlorine insecticide known to be estrogenic. It has been reported to be an inhibitor in the KGN assay.
Alachlor	15972-60-8	A chlorinated herbicide reported to be negative in the KGN study.
Nitrofen	1836-75-5	A diphenyl ether reported to be negative in the KGN assay.
Trifluralin	1582-09-8	A dinitroaniline reported to be negative in the KGN assay.

2.2.1 Reference Chemical Formulation and Analysis

Reference chemical stock solutions will be prepared and analyzed by the CR and shipped to RTI's Materials Handling Facility (MHF), along with MSDSs and information related to the content (including solvent) and molarity of each formulation. The CR will also supply the MHF with solvents of the same lot used in the preparation of the stock solutions which can then be used in preparation of dilutions of the stock solutions for use in the assay. Reference chemicals will be formulated at a preferred concentration of 0.1 M. The preferred solvent is dimethylsulfoxide (DMSO), but absolute ethanol or buffer may be used if necessary for adequate solubility. If a 0.1 M solution cannot be prepared, a lower concentration (0.05 or 0.01 M) will be tried. The molarity of each supplied formulation will be clearly stated on the label. The CR will code the reference chemical stock solutions prior to distributing them to the assaying laboratory, so that the technicians will be blinded to the identity of the chemical under testing. Information on storage conditions for reference chemical stock solutions will be provided by the CR.

The total volume of reference chemical formulation used in each assay should be no more than 1% of the total assay volume (i.e., 20 μ L in a 2 mL assay) in order to minimize the

potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use such that the target concentration of reference chemical can be achieved by the addition of 20 μ L of the dilution to a 2 mL assay volume.

2.3 Positive Control

The known aromatase inhibitor, 4-hydroxyandrostendione (4-OH ASDN), is used as the positive control. Table 2 contains identity and property information for 4-OH ASDN.

Table 2. Positive Control Information

Control Substance	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
4-OH ASDN	566-48-3	C ₁₉ H ₂₆ O ₃	302.4	Known aromatase inhibitor

2.3.1 Positive Control Formulation and Analysis

Positive control stock solutions will be prepared and analyzed by the CR and shipped to RTI's Materials Handling Facility (MHF). 4-OH ASDN will be formulated in absolute ethanol at a concentration of 0.01M. The total volume of control substance formulation used in each assay should be no more than 1% of the total assay volume (i.e., 20 μ L in a 2 mL assay) in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use. Dilutions will be prepared such that the target concentration of control substance (Table 4) can be achieved by the addition of 20 μ L of the dilution to a 2 mL assay volume. Information on storage conditions for control substance stock solutions will be provided by the CR.

2.4 Microsomes

Recombinant microsomes will be purchased from Gentest (Woburn, MA). The product name is Human CYP19 + P450 Reductase SUPERSOMES™, catalog number 456260. The microsomes must be stored at approximately -70 to -80 °C. The approximate protein content of the microsomes will be provided by the supplier. The product data sheet for the batch of microsomes used will be maintained in the study records.

Caution: Microsomes can be denatured by detergents. Therefore, it is important to ensure that all glassware, etc. that is used in the preparation or usage of microsomes is free of detergent residue. New disposable test tubes, bottles, vials, pipets and pipet tips may be used directly in the assay. Durable labware that may have been exposed to detergents should be rinsed with water and/or buffer prior to use in the assay.

If the recombinant microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order to minimize and standardize the number of freeze/thaw cycles each preparation undergoes. Microsomes will

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be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be pooled and rehomogenized using a Potter-Elvehjem homogenizer (about 5–10 passes). The pooled sample will be aliquoted into portions appropriate for use in a single experiment (ca. 160 µL, dependant on the protein concentration of the preparation) and the samples will be flash frozen and stored at -70 to -80 °C for future use. Each tube will provide enough protein for a single experiment and any excess thawed microsomal preparation will be discarded. On the day of use, microsomes will be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be rehomogenized using a Potter-Elvehjem homogenizer (about 5-10 passes) or by vortexing about 5 seconds prior to use. The microsomes will be diluted in buffer (serial dilutions may be necessary) to an approximate protein concentration of 0.008 mg/mL. The addition of 1 mL of that microsome dilution will result in a final approximate protein concentration of 0.004 mg/mL in the assay tubes. All microsome samples must be kept on ice until they are placed in the water bath just prior to their addition to the aromatase assay. Microsomes are not to be left on ice for longer than approximately 1 h before proceeding with the assay. Appropriate documentation of time from thaw to use must be maintained. Diluted microsomes must be used only on the day of preparation. Under no conditions should diluted microsomes be refrozen for later use in the assay. 2.5 Other Assay Components 2.5.1 Buffer The assay buffer will be 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (JT Baker, cat # 4011-01, 137.99 g/mol) and sodium phosphate dibasic (JT Baker, cat # 4062-01, 141.96 g/mol) will be used in the preparation of the buffer. Solutions of each reagent at 0.1 M will be prepared in distilled, deionized water and then the solutions will be combined to a final pH of 7.4. The assay buffer may be stored for up to one month in the refrigerator (2–8 °C). 2.5.2 Propylene Glycol Propylene glycol (JT Baker, cat # 9402-01, 76.1 g/mol) will be added to the assay directly as described below. 2.5.3 NADPH NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt, Sigma, cat # 1630, 833.4 g/mol) is the required co-factor for CYP19. The final concentration in the assay is 0.3 mM. Typically, a 6 mM stock solution will be prepared in assay buffer and then 100 µL of the stock will be added to the 2 mL assay volume. NADPH must be prepared fresh each day and will be kept on ice.		

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3.0 PROTEIN ASSAY

The protein concentration of the microsome preparation will be measured in 5 independent assays for each lot of microsomes. Appropriate QCs will be included with each assay. A standard curve (of at least 5-points) will be prepared of a suitable range to bracket the concentration of protein in the microsome dilution. The protein curve standards will be prepared with bovine serum albumin (BSA). Protein will be determined by using a DC Protein Assay kit purchased from Bio-Rad (Hercules, CA). One of two protocols will be employed and the selection between the two can be made based on the expected concentration of the microsomal dilution to be measured and the nominal protein concentration of the stock microsomes.

Sample preparation for the micro protocol, which is appropriate for use over the range 5 to 250 µg protein/mL, is conducted as follows:

- To a 200 µL aliquot of unknown, QC or curve standard, 100 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 800 µL of BioRad DC Protein Kit Reagent B will be added to each sample and the samples will be vortex mixed.

Sample preparation for the standard protocol, which is appropriate for use over the range 0.2 to 1.5 mg/mL, is conducted as follows:

- To a 25 µL aliquot of unknown, QC or curve standard, 125 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 1 mL of BioRad DC Protein Kit Reagent B will be added to each sample and the samples will be vortex mixed.

Both protocols continue as follows:

The samples will be allowed to sit at room temperature for at least 15 min to allow for color development. The absorbances are stable for about 1 h. Each sample (unknown, QCs and standards) will be transferred to disposable polystyrene cuvettes and the absorbance (@ 750 nm) will be measured using a spectrophotometer. The protein concentration of the microsomal sample will be determined by interpolation of the absorbance value using the curve developed from the protein standards.

The protein assay data can be accepted if the determined value for two-thirds of the QC samples is within 15% of the nominal value. The average measured protein concentration, SD and CV across the five assays will be determined. The CV for the set should be ≤10%. If the data do not meet this criterion, the set will be examined for outliers, that may be excluded and/or additional assays may be run to generate more data. The average measured protein value will be compared with the stated protein value from the product data sheet. If the measured value is within 100 ± 10% of the nominal value, the nominal value will be used in all calculations of aromatase activity. If the measured value is outside this range, then the measured value will be used in all calculations of aromatase activity.

4.0 AROMATASE ASSAY METHOD

The assays will be performed in 13 x 100 mm test tubes maintained at 37 ± 1 °C in a

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shaking water bath. Each test tube will be uniquely identified by applying a label or writing directly on the test tube. Propylene glycol (100 μ L), [^{3} H]ASDN, NADPH, and buffer (0.1 M sodium phosphate buffer, pH 7.4) will be combined in the test tubes (total volume 1 mL). The final concentrations for the assay components are presented in Table 3. The tubes and the microsomal suspension will be placed at 37 ± 1 °C in the water bath for 5 min prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume will be 2 mL, and the tubes will be incubated for 15 min. The incubations will be stopped by the addition of methylene chloride (2 mL); the tubes will be vortex-mixed for ca. 5 s and placed on ice while the rest of the incubations are stopped. The tubes will then be vortex-mixed an additional 10-15 s. The tubes will then be centrifuged briefly at low speed to effect separation of the organic and aqueous layers. Use of an AllegraX-15R centrifuge with an SX4750a rotor for 10 min at a setting of 1000 rpm (approx. 200 x g) has achieved sufficient separation but other low speed centrifugation protocols can also be used. The methylene chloride layer (bottom) will be removed and discarded; the aqueous layers will be extracted again with methylene chloride (2 mL). The methylene chloride may be removed by the use of a glass pipet; care should be taken to not remove the aqueous sample until the final step. This extraction procedure will be performed one additional time, each time discarding the methylene chloride layer. The aqueous layers will be transferred to vials and duplicate aliquots (0.5 mL) will be transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) will be added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot will be determined as described below.

Table 3. Optimized Aromatase Assay Conditions

Assay factor (units)	Recombinant
Microsomal Protein (mg/mL) ^a	0.004
NADPH (mM) ^a	0.3
[^{3} H]ASDN (nM) ^a	100
Incubation Time (min)	15

^a Final concentrations

Analysis of the samples will be performed using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represents ^{3} H₂O formed.

5.0 POSITIVE CONTROL ASSAYS (TASK 5)

Each technician who will be participating in the testing of reference chemicals must first conduct a single replicate of the positive control experiment as outlined in Table 4. Data from this replicate will be assessed against the following criteria and will be submitted to EPA:

1. The minimum level of mean aromatase activity (in the full activity control samples) shall be 0.100 nmol/mg protein/min.
2. The mean background control activity shall be $\leq 1\%$ of the full activity control.
3. The concentration response curve generated for 4-OH ASDN shall meet the following conditions:
 - a) The Top must fall within the range $100 \pm 10\%$ of the full activity level.

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- b) The Bottom must fall within the range $0 \pm 5\%$ of the full activity level.
- c) The Log IC_{50} must be between -7.00 and -7.30.
- d) The Slope must be between -0.8 and -1.2.

If the data from any technician do not meet the performance criteria, RTI will notify EPA and the assay will be repeated by the technician in question at least twice. Data from these replicate runs will be assessed with respect to the performance criteria and will be reported to EPA. Only technicians who produce data that meet the positive control performance criteria will participate in the reference chemical assays.

The concentrations of 4-OH ASDN chosen for the assay are intended to generate a complete inhibition curve (ranging from 100 – 0 % of control activity). In the event the concentration range is inappropriate for generation of a complete inhibition curve, RTI will notify the EPA TOPO after the first run of the assay and alternate concentrations will be discussed and incorporated into the study design as necessary.

The positive control assay and the first replicate of one reference chemical per technician may be conducted concurrently. If this study design is chosen, both 4-OH ASDN and the reference chemical would be run in three tubes per concentration for those runs. If the positive control portion of the assay meets the performance criteria, the entire assay could be accepted and would satisfy requirements for both the positive control assay (Task 5) and the first replicate of the reference chemical.

Table 4. Positive Control Experiment Outline

Sample Type	Repetitions (tubes)	Description	4-OH ASDN Concentration (M)
Full Activity Control	4	Complete assay ^a with inhibitor vehicle control, no inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc 1	3	Complete assay with 4-OH ASDN added	1×10^{-5}
Positive Control Conc 2	3	Same	1×10^{-6}
Positive Control Conc 3	3	Same	5×10^{-7}
Positive Control Conc 4	3	Same	1×10^{-7}
Positive Control Conc 5	3	Same	5×10^{-8}
Positive Control Conc 6	3	Same	1×10^{-8}
Positive Control Conc 7	3	Same	1×10^{-9}
Positive Control Conc 8	3	Same	1×10^{-10}

^a The complete assay contains buffer, propylene glycol, microsomal protein, [³H] ASDN, and NADPH

6.0 DETERMINATION OF THE RESPONSE OF AROMATASE ACTIVITY TO REFERENCE CHEMICALS (TASK 6)

Each of the 16 reference chemicals listed in Table 1 will be tested in three independent runs of the aromatase assay using a single technician per chemical as outlined in Table 5. The reference chemicals will be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all sixteen reference chemicals. As described in the Table 5, a full eight-point positive control assay, with duplicate determinations per concentration will be done concurrently with each reference chemical assay set.

Full activity and background activity controls will be included with each replicate. The background activity tubes will serve as the negative control. The make-up of these controls is described below:

- full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes)
- background activity controls (all components that are in the full aromatase activity controls, except NADPH)

Four test tubes of each type of control are included with each replicate and are treated the same as the other samples. The controls sets will be split so that two tubes (of each control type) are run at the beginning and two at the end of each replicate set.

The assay will be conducted as described in Section 4.0 with the following modification. Reference or positive control chemical solution (or vehicle) will be added to the mixture of propylene glycol, substrate, NADPH and buffer in a volume not to exceed 20 μ L prior to preincubation of that mixture. The volume of buffer used will be adjusted so the total incubation volume remains at 2 mL.

Some chemicals may not be soluble in the assay mixture over the entire range of target testing concentrations. When chemicals precipitate upon addition to the reaction mixture, it is impossible to know the true concentration of chemical in solution that is available to interact with the enzyme. Therefore, it is important to observe the reaction mixtures as test chemicals are added and to note any precipitation and/or cloudiness. If cloudiness and/or precipitation is observed at any concentration used in the first replicate of a particular chemical, the target test concentrations for the remaining replicates shall be adjusted as follows:

- Set the highest concentration to the highest concentration that appeared to be soluble.
- Add new mid-log concentration(s) near the estimated IC_{50} based on the first replicate results, so that there are still eight concentrations in the set.
- The lowest concentration to be tested is 10^{-10} M.

Based on the results of the first replicate, it may be desirable to alter the concentrations tested in the remaining replicates to better define the concentration response curve over the critical region where maximal slope is observed. When selecting concentrations for the remaining replicates, use the following guidance:

- Make any adjustments that are necessary as a result of poor solubility first, as described above.
- Keep at least 2 concentrations each that define the top and bottom plateaus. Any excess points from concentrations lower than the two needed to define the top plateau or from concentrations higher than the two needed to define the bottom plateau, may be removed from the concentration set.
- Add new mid-log concentration(s) near the estimated IC_{50} based on the first replicate results, so that there are still eight concentrations in the set.
- The lowest concentration to be tested is 10^{-10} M.

Table 5. Reference Chemical Experiment Outline

Sample Type	Repetitions (tubes)	Description	Control or Reference Chemical Concentration (M)
Full Activity Control	4	Complete assay ^a with inhibitor vehicle control, no inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc 1	2	Complete assay with 4-OH ASDN added	1×10^{-5}
Positive Control Conc 2	2	Same	1×10^{-6}
Positive Control Conc 3	2	Same	5×10^{-7}
Positive Control Conc 4	2	Same	1×10^{-7}
Positive Control Conc 5	2	Same	5×10^{-8}
Positive Control Conc 6	2	Same	1×10^{-8}
Positive Control Conc 7	2	Same	1×10^{-9}
Positive Control Conc 8	2	Same	1×10^{-10}
Reference Chem. Conc 1	3	Complete assay with reference chemical added	1×10^{-3}
Reference Chem. Conc 2	3	Same	1×10^{-4}
Reference Chem. Conc 3	3	Same	1×10^{-5}
Reference Chem. Conc 4	3	Same	1×10^{-6}
Reference Chem. Conc 5	3	Same	1×10^{-7}
Reference Chem. Conc 6	3	Same	1×10^{-8}
Reference Chem. Conc 7	3	Same	1×10^{-9}
Reference Chem. Conc 8	3	Same	1×10^{-10}

^a The complete assay contains buffer, propylene glycol, microsomal protein, [^3H] ASDN, and NADPH

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7.0 DATA ANALYSIS

7.1 Aromatase Activity and Percent of Control Calculations

Relevant data will be entered into a spreadsheet for calculation of aromatase activity and percent of control. The version of the spreadsheet used will be included in the reports. The data will be examined to determine whether the following performance criteria have been met:

- The minimum level of mean aromatase activity (in the full activity control samples) shall be 0.100 nmol/mg protein/min.
- The mean background control activity shall be $\leq 1\%$ of the full activity control.

If any one of these criteria has not been met, no further analysis for that data set will be performed. In that case, the assay would be repeated.

7.2 Model Fitting

Percent of control activity remaining at each reference (or control) chemical concentration will be imported into GraphPad Prism version 4.03 to fit a four-parameter model and a two parameter model for each chemical. The data obtained in Section 5 will only be fit to the four parameter model and will be excluded from the model fit comparison. The four-parameter model to be fit to the data to obtain the estimated dose-response curve is:

$$Y = Bottom + \left(\frac{Top - Bottom}{1 + 10^{(\mu - X)\beta}} \right)$$

where Y is the percent of control activity remaining, X is the $\log_{10}$ of the concentration of the chemical, Top and $Bottom$ are the plateaus of the response curve, μ is the $\log_{10}IC_{50}$ and β is the slope of the response curve at $\log_{10}IC_{50}$. The two-parameter model uses the same equation with $Bottom$ fixed at 0 and Top fixed at 100.

The (four parameter) response curve for the positive control (4-OH ASDN) will be examined to determine if the following criteria have been met:

- The Top must fall within the range $100 \pm 10\%$ of the full activity level.
- The $Bottom$ must fall within the range $0 \pm 5\%$ of the full activity level.
- The $\log IC_{50}$ must be between -7.00 and -7.30.
- The Slope must be between -0.8 and -1.2.

If the positive control response curve fails any of these criteria the failure will be reported promptly to the EPA TOPO. The task order leader will confer with the EPA TOPO to determine an appropriate course of action in this case.

The (four-parameter) response curve from the reference chemical will be examined to determine if the following criterion has been met:

- The Top of the test chemical curve must fall within the range $100 \pm 10\%$ of the full activity level.

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If this criterion is not met, the study director, in consultation with the TOPO, will make appropriate changes to the concentrations of reference chemical included in the assay.

7.3 Data Interpretation

Data from the assay will be used to classify chemicals according to their ability to inhibit aromatase. EPA has established the following classifications and criteria for their assignment:

Class	Criteria
Non-inhibitor	The most inhibited level lies above 75% of full activity control
Equivocal	The most inhibited level lies between 75% and 50% of full activity control
Inhibitor	The most inhibited level lies below 50% of full activity control

Classifications will be made with consideration given to the performance criterion to be developed in Section 7.5 of this protocol.

7.4 Statistical Analyses

7.4.1 Analysis of Fitted Curves for Differences

The sums of squares, root mean square error and degrees of freedom results from each model fit for each chemical will be used to perform a partial F-test to test for significant differences simultaneously among the four model parameters. The partial F-test is calculated as follows:

$$F = \frac{(MSS_{FM} - MSS_{RM}) / (MDF_{FM} - MDF_{RM})}{RSS_{FM} / RDF_{FM}}$$

where MSS_{FM} denotes the model sum of squares for the full model containing all four parameters, MDF_{FM} is the model degrees of freedom for the full model, RSS_{FM} and RDF_{FM} denote the residual sum of squares and degrees of freedom, respectively, for the full model and MSS_{RM} and MDF_{RM} denote the model sum of squares and degrees of freedom for the reduced model containing two parameters.

The partial F-test to compare the two models will be done with GraphPad Prism Version 4 using that software's "extra sum-of-squares F test". The results will be used to determine whether there are significant differences in the parameter estimates generated by the two models.

7.4.2 Comparison of Positive Control Values Within and Across Technicians

In order to assess the variation across and within technicians (for the assays in Section 6), a mixed effects model will be fit to the positive control values with the 4-OH ASDN concentrations as a fixed effect and the technicians as random effects. Tests performed on the

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variance components will determine if there is more variation in the positive control results between technicians than within technicians.

7.4.3 Evaluation of the Suitability of the Positive Control Performance Criteria

To assess the suitability of the quality assurance measures, the dose-response model results from each run of the positive control experiments will be checked to see if all of the parameter estimates meet the performance criteria. If most or all of the dose-response model parameter estimates obtained from different technicians meet all of the performance criteria, the performance criteria intervals can be determined to be sufficiently large enough for labs to be able to meet the performance criteria. If too many of the dose-response model parameter estimates do not meet the performance criteria, the performance criteria will need to be re-evaluated to provide obtainable standards. Another method of assessing the suitability of the performance criteria as quality assurance measures is to estimate the dose-response model parameters from all of the runs performed by each technician and examine the standard errors of each of the model parameters. If the 95% confidence intervals for each parameter estimate for a given technician are within the performance criteria, the technician should be able to produce positive control experiment results that meet the performance criteria in at least 95% of the experiments that the technician runs. The percentage associated with the confidence intervals for each technician and each parameter that fit inside the performance criteria intervals will determine what percentage of the experiments that the technician would run that would meet the performance criteria. If the percentage of experimental data results for a technician that meet the performance criteria is too low, the performance criteria intervals may be too narrow for labs to attain and would need to be re-evaluated. Likewise, if the 95% confidence intervals for each technician and each parameter are much smaller than the performance criteria intervals, the performance criteria intervals may be too wide and may need to be shortened to ensure only good quality experimental data will pass the performance criteria evaluations.

7.5 Development of a Second Performance Criterion for Reference Chemicals

Thus far, the only specified performance criterion for reference chemicals is that the top of the curve shall lie in the range 90% to 110% for the four parameter model. Task 8 involves the development of one or more additional criteria for reference chemical data. An additional performance criterion for the assay should evaluate the quality of the data. Data of acceptable quality (i.e., meeting all performance criteria) can then be used to classify reference chemicals as to their aromatase inhibitory characteristics. Test data sets will be used to classify chemicals as aromatase inhibitors, non-inhibitors or as chemicals for which the data are equivocal; this function falls under the category of data interpretation procedures. A separate performance criterion should be developed to apply to the data interpretation procedures. It is appropriate that both types of criteria be added as performance criteria for evaluation of test chemical data.

The new performance criterion should assess the quality of the data by examining the variation

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across all data points. The classification of a chemical as an inhibitor or non-inhibitor should depend on all of the data points, since response model estimates are fit to the experimental data collected at each tested concentration level, and larger than acceptable standard deviations at any concentration level will affect the precision of the response model.

7.5.1 Proposed Data Quality Criterion

One possible approach to assessing the quality of the experimental data is to examine the coefficients of variation (CV) of the percent of control values at each chemical concentration level. The CV is calculated as the standard deviation divided by the average of the percent of control values.

The results of the entire set of positive control and reference chemical assays conducted in Task 6 (96 assays in total) will be evaluated to determine a reasonable CV to use as a data quality criterion. Data from the positive control assays will be useful in determining a reasonable CV over time using the same chemical, while the data from the reference chemicals will be a good model for the range of CVs one can expect when testing a variety of chemicals in the assay and so these subsets of data will also be evaluated separately to determine a reasonable CV to use in each case.

7.5.2 Data Interpretation Criterion

One possible approach to interpreting the results of the experiments is to graph both the upper and lower confidence bounds on the estimated response curve. These bounds, referred to in the software manuals as the "confidence bands" will be added to the graph of the estimated response curve using GraphPad Prism version 4.03. If the 95% confidence bounds on the response curve are used, it means that if the same experiment was repeated a large number of times, 95% of the estimated response curves calculated from each of the experiments would lie within the 95% confidence bounds. If both confidence bands for the estimated response curve extend below the 50% line on the graph, then the chemical can be classified as an inhibitor with only a small chance of a classification error. If both confidence bands for the estimated response curve stay above the 75% line on the graph, then the chemical can be classified as a non-inhibitor with only a small chance of a classification error. More analyses would need to be performed to determine the classification rules if at least one of the confidence bands remains between the 50% and 75% lines for the highest concentrations of the chemical.

Additional approaches to assessing the quality of the experimental data and to interpreting the data results may be examined in conjunction with the U.S. EPA. The various approaches would be weighed against the priorities the U.S. EPA determines for the statistical tests, such as the importance of having criteria that are easily implemented by technical staff using the GraphPad Prism software.

7.6 Statistical Software

Concentration response curves will be fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 4.03 or higher. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons will be carried out using PRISM, the SAS statistical analysis system, Version 8 or higher, or other general purpose statistical packages (e.g. SPSS), as convenient.

8.0 RETENTION OF RECORDS

All records that remain the responsibility of the testing laboratory will be retained in the archives for the life of the contract.

9.0 QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

Quality control (QC) and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) that was prepared for this study. The study will be conducted in compliance with the Federal Register, 40 CFR Part 160. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practices Standards.

10.0 REPORTS

A final report that includes (at minimum) a full description of the work performed in the laboratory and during data analysis, summaries of the data in tabular (spreadsheet) and graphical format, results of the statistical analyses and conclusions drawn from the data will be prepared. Reference chemicals will be identified by name in the report. In addition, a final aromatase assay protocol which includes any modified performance criteria or data interpretation procedures will be prepared and appended to the final report. The draft report will be audited by RTI's QA unit for compliance with the QAPP and study protocol and to ensure the accuracy of the data contained therein. The draft report will be submitted to EPA for review. This draft report will be revised as appropriate and then the final, signed version will be submitted (in both hard copy and electronic formats) to the EPA TOPO.

11.0 STUDY RECORDS TO BE MAINTAINED

- All records that document the conduct of the laboratory experiments and results obtained, as well as the equipment and chemicals used
- Protocol and any Amendments
- List of any Protocol Deviations
- List of Standard Operating Procedures

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• QAPP and any Amendments • List of any QAPP Deviations		

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Appendix A Supply List for the Aromatase Assay Materials 13 x 100 mm test tubes Test tube rack Pasteur pipets 1-Dram vials with caps – silylated Assorted laboratory glassware – beakers, graduated cylinders, volumetric flasks, bottles, vials Pipet tips Ice bucket Ice Assorted glass pipets Scintillation vials Disposable microcuvettes Pipet bulbs Equipment Shaking water bath capable of maintaining 37°C Vortexer Lab timer Low speed centrifuge capable of holding test tubes Micropipettors – various volumes ranging from 1 µL to 5 mL Liquid scintillation spectrometer Uv-Vis spectrophotometer HPLC (pumps, injector, reversed phase column (suggested: Zorbax SB-C₁₈ column (4.6 x 250 mm)), uv/vis and radiochemical detectors, data collection/analysis system) Analytical balance pH meter Chemicals 4-androstene-3,17-dione (ASDN) ([1β-³H(N)]-androst-4-ene-3,17-dione ([³H]ASDN) Sodium phosphate monobasic (JT Baker, cat # 4011-01, 137.99 g/mol) Sodium phosphate dibasic (JT Baker, cat # 4062-01, 141.96 g/mol) Propylene glycol (JT Baker, cat # 9402-01, 76.1 g/mol) NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt, Sigma, cat # 1630, 833.4 g/mol) 4-hydroxyandrostendione (4-OH ASDN) formulation Reference chemical formulations DC Protein Assay kit - Bio-Rad (Hercules, CA) Bovine serum albumin Human CYP19 + P450 Reductase SUPERSOMES™, BD Biosciences, catalog number 456260 Tetrahydrofuran (HPLC grade) Methanol (HPLC grade) Ultima Gold or other scintillation cocktail Absolute ethanol Methylene chloride		

Memo to the Record

Date: 6/15/2007

To: The Record for Study RTI-996-AN, Study Code An07-996

From: Sherry Black *SBS 6/15/07*

RE: Protocol Deviation – Reference Chemical Purity

The study protocol required that reference chemicals be $\geq 97\%$ pure. Reference chemical, apigenin (CR11610) had a stated purity of 95%. This small difference in purity is unlikely to have had a significant effect on the results of the study.

APPENDIX B

Quality Assurance Project Plan (QAPP)

Quality Assurance Project Plan (QAPP)
For Task Order 9
Supplementary Testing of 16 Chemicals in the Recombinant Aromatase Assay

EPA CONTRACT NUMBER EP-W-06-026

February 2007

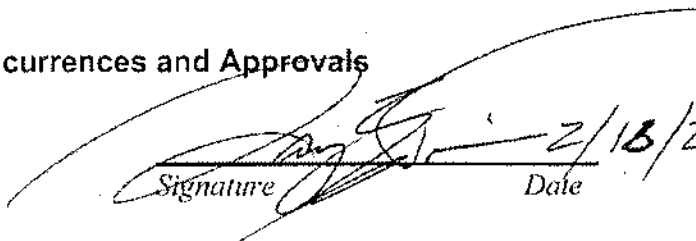
SIGNATURE PAGE

Quality Assurance Project Plan for Task 9 Supplementary Testing of 16 Chemicals in the Recombinant Aromatase Assay

EPA CONTRACT NUMBER EP-W-06-026

Concurrences and Approvals

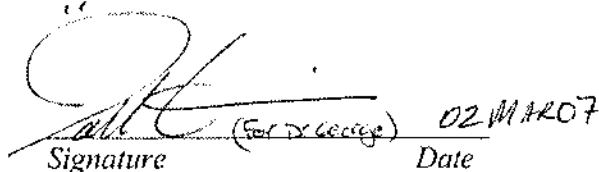
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Washington, D.C.


Signature Date 2/18/2007

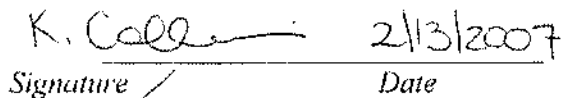
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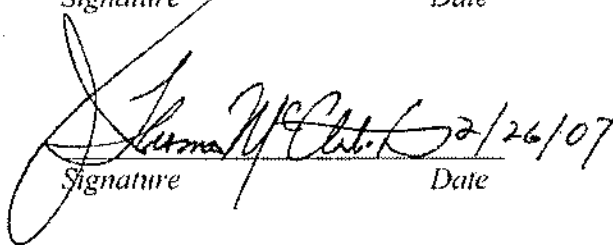
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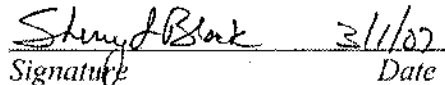

Signature Date 3/1/07

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Study Protocol for Task Order 9

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1.0 PROJECT ORGANIZATION

Table 1 lists RTI International and EPA personnel assigned to this project and their roles.

Table 1. Task Order 9 Staff

Staff	Affiliation	Role for Task Order 3
Gary Timm, M.S., M.A.	EPA	Task Order Project Officer (TOPO)
Shirlee Tan, Ph.D.	EPA	Alternate TOPO
J. Thomas McClintock, Ph.D.	EPA	QA manager
Julia George, PhD	RTI	Program Manager
Bonnie Hamby, B.S.	RTI	Task Order Manager
Sherry Black, B.S.	RTI	Task Order Leader/Study Director
Purvi Patel, B.S.	RTI	Chemist
Amy Etheridge, B.S.	RTI	Chemist
James Mathews, Ph.D.	RTI	Consultant
Jon Larson	RTI	MHF Technician
Don Hubbard, B.S.	RTI	MHF Technician
Margaret Byron, M.S.	RTI	Statistician
Gayle Bieler, M.S.	RTI	Statistician
Kimberly Collier, M.A.	RTI	Quality Assurance Specialist
Leslie MacDonald, B.S.	RTI	Quality Assurance Specialist
Cynthia Barnette, B.S.	RTI	Financial Specialist
Cathee Winkie	RTI	Administrative Coordinator

1.1 RTI International Personnel

Further descriptions of these roles and the resumes of the RTI team were included in the proposal submitted for this task order. All staff have the training and experience necessary to perform their roles.

Dr. Julia George and Ms. Bonnie Hamby will oversee general task order management. They will be responsible for tracking the expenditure of hours and funds, monitoring task progress, and communicating with the EPA TOPO on all contract issues.

Ms. Sherry Black will be the Task Order Leader, and will be responsible for technical management of the task order. She will oversee and aid in the conduct of the laboratory study. She will oversee and direct the efforts of Ms. Purvi Patel and Ms. Amy Etheridge on the laboratory work. Ms. Black will be responsible for data analysis, quality control and reporting of the laboratory work. She will consult with Dr. Mathews and Mr. Timm on technical issues and secure approval of EPA (through Mr. Timm) as required by the Task Order Statement of Work to proceed from one experimental set to the next. Ms. Black will be responsible for responding to data audits and correcting deficiencies.

Ms. Purvi Patel and Ms. Amy Etheridge will carry out experimental work in accordance with the QAPP, study protocol and instructions of Ms. Black. They will aid in the data analysis, quality control and report preparation.

Jon Larson and Don Hubbard will receive chemicals and formulations from the CR and will code the samples as necessary to blind the study of the reference chemicals.

Margaret Byron and Gayle Bieler will assist in the statistical analysis of the data.

Kimberly Collier will administer the QAPP at RTI. Her specific responsibilities include:

- Interact with the Task Order Leader to ensure that QA and QC procedures are understood by Task personnel.
- Conduct technical systems audits (TSAs) and audits of data quality (ADQs) to evaluate the implementation of the task with respect to the EDSP QMP, the task QAPP and study protocol, and applicable program and facility SOPs.
- Prepare and track reports of deficiencies and submit them to both line and program management.
- Consult with the Task Order Leader and, as necessary, the EPA EDSP QA Manager and RTI Program Manager on actions required to correct deficiencies noted during the conduct of the Task.
- Ensure that all data produced as part of the task are maintained in a secure, environmentally-protected archive.
- Ensure, during the conduct of TSAs, that all staff participating on the task are adequately trained.
- Maintain complete QA records related to the task.
- Submit a QA Statement with the final report that describes the audit and review activities completed and any outstanding issues that could affect data quality or interpretation of the results discussed in the report.
- Maintain effective communication with the EPA QA Manager.

James Mathews will serve as technical consultant for the task and will advise Ms. Black on any problems encountered during task conduct.

1.2 EPA Personnel

Mr. Gary Timm (USEPA) will serve as the Task Order Project Officer (TOPO) for this task. In this role, he will communicate with the RTI Program Manager on matters of task management and scheduling. He will consult with the Task Order Leader on experimental details that have not been defined as of the submission of the Technical Proposal, such as the selection of a control inhibitor and selection of substrate and inhibitor concentrations, etc. He will review data submitted from each group of experiments and provide the necessary approval, as appropriate, for RTI to proceed with the next set of experiments.

Dr. Shirlee Tan is the alternate TOPO for this task and can fulfill all TOPO functions if Mr. Timm is unavailable.

Dr. J. Thomas McClintock is the EPA QA manager for this task. He will review and approve the QAPP before any laboratory work can begin.

2.0 PROBLEM DEFINITION/BACKGROUND

2.1 Problem Definition

This work assignment will provide for the testing of an additional sixteen chemicals to support the validation of the recombinant aromatase assay. In addition to broadening the range of classes and potencies of the chemicals tested, this work assignment will test the performance criteria that EPA has developed based on the data from the interlaboratory testing using the recombinant microsomal assay and the impact of using a four-parameter (no constraints) versus a two-parameter model in which the top and bottom values are set to 100% and 0%, respectively. Current performance criteria will be assessed for suitability and new criteria will be developed as part of this task order.

2.2 Background

Interlaboratory validation studies of the recombinant and placental aromatase assay were conducted on 10 chemicals in four laboratories. These studies established the equivalence of the two assays and the ability of the laboratories to successfully conduct the assay using the standardized protocols previously developed. Upon review of these results, EPA determined that some additional testing was necessary to more completely elucidate the ability of the assay to assess the aromatase inhibition potential of a fuller range of chemicals. Each of the 16 chemicals to be used in this task order will be tested in one laboratory with three independent runs.

As part of this task order, the previously developed protocol will be modified. New features to be incorporated include the use of an 8-point curve for concurrent positive control versus a single concentration, performance standards for the controls as well as test chemicals, and eliminating the requirement for a separate negative control chemical. EPA intends that the

final protocol should include performance standards that ensure that the assay is being performed correctly and that data submitted to the Agency are of sufficient quality.

3.0 Technical Approach

First, each technician who will participate in the study will demonstrate their competence with the assay by producing a satisfactory concentration response curve with the known aromatase inhibitor, 4-hydroxyandrostenedione (4-OH ASDN). The main part of the study involves the testing of 16 reference chemicals in three independent runs as described in the study protocol. Controls (including a full concentration response curve set for the positive control) will be included with each reference chemical assay. The data will be analyzed and the suitability of the proposed performance criteria will be assessed. In addition, the data will be used to select an additional performance criterion for the testing of unknowns in the assay.

Full experimental details are described in the attached study protocol (Appendix).

4.0 QUALITY SYSTEMS

The following general quality systems are in place to ensure that the data produced are reliable, reproducible and of high quality.

- Experimental procedures will be conducted in accordance with the study protocol and applicable SOPs. Any modifications to procedures will be documented as protocol amendments (if they are planned) or as deviations. Amendments are approved by the Task Order Leader and the TOPO. Any deviations are documented and assessed by the Task Order Leader for their impact on the study and are included in the record and the reports.
- Data collection and recording will be done according to study protocol and SOP requirements.
- All instruments/equipment will be maintained and calibrated according to procedures described in applicable SOPs. Any instrument/equipment that does not meet criteria set forth in the applicable SOP will not be used on this task.
- Quality control (QC) procedures will include a 100% check of all transcribed data and the verification of all Excel spreadsheets used for calculation. These QC checks will be documented on printouts by the reviewer's initials and date.
- The data forms and data for each replicate will be reviewed for completeness and accuracy and any errors or omissions will be corrected or documented appropriately.
- RTI's QAU will provide data review and audits as described in Section 8.0 and applicable SOPs.

5.0 DATA ACCEPTANCE/REJECTION CRITERIA

In this task, the aromatase activity of recombinant microsomes is measured in the presence of varying concentrations of potential inhibitors (reference chemicals) using a defined experimental protocol. Relevant weights, volumes and conditions (i.e., temperature) of the assay components are collected and recorded using calibrated instruments in accordance with SOPs and the study protocol. The aromatase activity measurement is based on radioactivity remaining in the aqueous portion of the reaction mixture after extraction. Duplicate aliquots of the aqueous portion of the reaction mixture are assayed for radiochemical content using liquid scintillation counters that have been calibrated and performance-verified according to SOPs. Radiochemical content data for each assay tube, along with data on assay contents (solution concentrations, etc) are used to calculate aromatase activity. Aromatase activity (converted to percent of full activity remaining) and inhibitor concentration data are used to calculate inhibition parameters (IC_{50} , slope, etc). Therefore control of the variance of radiochemical content data will yield inhibition parameter estimates with low variability. Additionally, it is important that the enzyme preparation possesses sufficient aromatase activity so that an appropriate dynamic range of inhibition can be measured. Also, there should be a low level of background activity (that not dependent on NADPH), in order that true enzymatic activity can be measured.

The following key data will be examined and accepted or rejected as described below:

1. *Radiochemical content of aliquots of extracted reaction mixture (from a given assay tube).* The duplicate aliquots should be within the mean $\pm 10\%$. If this is not the case the following actions will be taken: 1) realiquot (duplicate aliquots) the reaction mixture and test for acceptable variance; 2) if variance is still too high, exclude the data from that tube from the data set.
2. *The minimum level of mean aromatase activity in the full activity control samples shall be 0.1 nmol/mg protein/min.* Data from any experiment that does not meet this criterion will not be used. A new tube of microsomes will be tested for adequate activity. If three tubes of microsomes (from the same batch) fail this criterion, in the absence of identifiable procedural mishaps, the batch of microsomes will be judged to be inadequate for the requirement of the study and a new batch of microsomes will be procured.
3. *Mean background activity must be $\leq 1\%$ of the mean full activity control for a particular experiment.* Data from any experiment that does not meet this criterion will not be used.
4. *For assays of inhibition of aromatase by test chemicals, the estimated 'Top' of the fitted curve shall be $100 \pm 10\%$ of full activity control.* If this criterion is not met and in the absence of identifiable procedural mishaps, changes in test chemical concentrations may be made as a result of discussions with the EPA TOPO. Data from experiments that fail to meet this criterion will not be used. In the case of

identifiable procedural mishaps, the experiment will be repeated with no changes to test chemical concentrations.

Other performance criteria (for both the positive control concentration response curve and test chemical data) will be evaluated and/or developed as described in the study protocol and technical proposal. Because the development and/or evaluation of these other criteria is part of the current work, appropriate rejection criteria can not be defined *a priori*. The methods used and the results of the analysis of these other performance criteria will be fully described in the final report. Any changes to the above described performance criteria that become necessary during the analysis of the data will be fully documented in the study record and/or through study protocol and/or QAPP amendments.

6.0 DOCUMENTS AND RECORDS

Laboratory and quality management procedures for this task order are described in this QAPP, the study protocol and applicable SOPs. Each of these documents (and any amendments/revisions to them) is distributed to study personnel. It is the responsibility of the Task Order Leader to ensure that all staff have access to the most recent versions of each document. The QAPP and SOPs are controlled by version number in the document header. Protocol amendments are sequentially numbered and distributed upon approval.

Study records to be maintained are detailed in the protocol. Study records will be maintained in the laboratories during the conduct of the study. At the conclusion of the study (i.e., when the final report is signed), records will be transferred to the RTI archives (in accordance with relevant SOPs) and will be retained for the life of the contract. Records may be transferred to the Sponsor if so directed.

Requirements for the final report and electronic data submissions are detailed in the study protocol.

Other reports that will not be included in the above-named reports include QA audit reports and status/progress reports. QA assessment reports are maintained as confidential files in the RTI's QAU. Status/progress reports will be submitted to the EPA TOPO on a monthly basis as stipulated in the contract.

7.0 APPLICABLE SOPS

Table 2 lists applicable SOPs for this Task. The current version of each of these documents is readily available to RTI project staff on RTI's intranet.

Table 2 Applicable SOPs

SOP	Subject
SEG-ADM-003	RTI Notebooks
SEG-ADM-004	Handwritten Records

SOP	Subject
SEG-ADM-005	Personnel Training Files
SEG-ADM-007	Study Protocols
SEG-EQP-004	Analytical Balances
SEG-EQP-010	pH Meters
SEG-EQP-011	Liquid Scintillation Counters
SEG-GLC-002	HPLC: Pumps
SEG-GLC-004	HPLC: UV/Vis detectors
SEG-GLC-006	HPLC: Radioactivity detectors
SEG-GLC-007	HPLC: Autosamplers
SEG-QAU-002	Initiating QAU Audits
SEG-QAU-003	Performing QAU Audits
SEG-QAU-004	Performing QAU Inspections
SEG-QAU-005	Response to QAU Reports
SEG-QAU-007	QA Statements
SEG-QAU-008	Master Schedule
SEG-QAU-010	Archival Storage
DPK-EQP-003	Dubnoff Metabolic Shaking Incubator
DPK-EQP-004	UV Spectrophotometer
DPK-LSS-002	Assaying Samples by LSS
DPK-SAM-001	Chemical Receipt
DPK-SAM-002	Test Article Use Log
CCS-EQP-003	Pipettors

8.0 ASSESSMENTS AND RESPONSE ACTIONS

RTI QA team members will perform assessments on task activities and operations affecting data quality and the raw data and final report. They will report any findings to the Task Order Leader and management to ensure that the requirements in relevant SOPs, study protocol and QAPP and the QMP are met.

9.0 RECONCILIATION AND USER REQUIREMENTS

The objective of this study is to test an additional sixteen chemicals in the recombinant aromatase assay. The data will be analyzed as described in the study protocol. The data will be used to assess the impact of the use of a two-parameter vs a four-parameter model for the determination of inhibition parameters. In addition, the data collected will be used to evaluate the suitability of the performance criteria established for the positive control and an additional performance criterion for test chemicals will be established. Any limitations that are discovered related to the use of established or proposed performance criteria will be discussed in the final report.

10.0 REFERENCES

Dean, R.B. and Dixon, W.J. (1951) Simplified statistics for small numbers of observations. *Analytical Chem.* **23**: 636-638.

APPENDIX

STUDY PROTOCOL FOR TASK ORDER 9

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EPA Contract No.: EP-W-06-026
Task Number: 9
RTI Study Code: An07-996
RTI Project Number 0210114.009

**TITLE: Task Order 9: Supplementary Testing of 16
Chemicals in the Recombinant Aromatase Assay**

SPONSOR: U. S. EPA
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Washington, DC 20460

Overnight/courier address:
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Washington, DC 20004

TESTING FACILITY: RTI International
DMPK
3040 Cornwallis Rd.
Research Triangle Park, NC 27709

PROPOSED EXPERIMENTAL START DATE: February 14, 2007
PROPOSED EXPERIMENTAL END DATE: April 10, 2007

AMENDMENTS:

Number	Date	Section(s)	Page(s)
1			
2			
3			

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Approved By:

Sherry J. Black 3/1/07^①
Sherry Black, B.S. Date
RTI, Task Order Leader, Study Director

Gary Timm 2/16/07
Gary Timm, M.S., M.A. Date
EPA, Task Order Project Officer (TOPO)

Kelly M. O'Rourke 16 Feb 2007
Linda J. Phillips, Ph.D. Date
EPA, Project Officer
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Reviewed By:

Kim Collier 2/13/2007
Kim Collier, M.A. Date
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J. Thomas McClintock 2/26/07
J. Thomas McClintock, Ph.D. Date
EPA, Quality Assurance Manager

① Signer this page when the hard copy original signature page was received 2/2/07

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1.0 OBJECTIVES

This work assignment will provide for the testing of an additional sixteen chemicals to support the validation of the recombinant aromatase assay. In addition to broadening the range of classes and potencies of the chemicals tested, this work assignment will test the performance criteria that EPA has developed based on the data from the interlaboratory testing using the recombinant microsomal assay and the impact of using a four-parameter (no constraints) versus a two-parameter model in which the top and bottom values are set to 100% and 0%, respectively.

1.1 Justification for Test System

The test system for this study is recombinant microsomes. This test system was selected because it provides a commercially available source of the aromatase enzyme.

1.2 Test Method

This *in vitro* test method involves combining microsomes, substrate, appropriate co-factors and reference chemicals in a common reaction vessel. The effect of the reference chemicals on microsomal enzyme activity is evaluated by measuring the amount of the product of the enzyme-catalyzed substrate oxidation that is formed.

2.0 MATERIALS RECEIPT AND/OR PREPARATION

A sufficient supply of chemical reagents, radiolabeled and non-radiolabeled androstenedione, and recombinant microsomal preparations will be obtained prior to initiation of the first set of experiments to ensure that sufficient quantities are available to conduct the studies. Appendix A contains a complete list of materials, chemicals and equipment needed for the conduct of the assay.

2.1 Substrate

2.1.1 Substrate Name/Supplier

The substrate for the aromatase assay is androstenedione (4-androstene-3,17-dione, ASDN, CAS# 63-05-8). Nonradiolabeled and radiolabeled ASDN will be used. The nonradiolabeled ASDN should be $\geq 98\%$ pure. The radiolabeled ASDN ($[1\beta\text{-}^3\text{H}(\text{N})]$ -androst-4-ene-3,17-dione, $[^3\text{H}]$ ASDN) should be $\geq 95\%$ radiochemically pure and is usually supplied at a specific activity of 20-30 Ci/mmol. The $[^3\text{H}]$ ASDN must have a specific activity of no less than 500 $\mu\text{Ci/mmol}$. The nonradiolabeled ASDN and the $[^3\text{H}]$ ASDN will be provided by the Chemical Repository (CR). The CR will forward all applicable information regarding supplier, lot numbers, and reported/measured purity for the substrate to RTI and this information will be included in study report.

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2.1.2 Radiochemical Purity The radiochemical purity of the [³H]ASDN will be determined using high performance liquid chromatography (HPLC) and liquid scintillation counting. The HPLC system consists of a Waters 2695 Separations Module, a Waters 2487 Dual λ Absorbance Detector and a β-RAM Model 3 flow-through radioactivity detector (IN/US, Inc., Tampa, FL) with a 250 μL glass scintillant cell. Data will be collected using Waters Empower 2 Client/Server Chromatography Data System Software. The HPLC method uses a Zorbax SB-C₁₈ column (4.6 x 250 mm) with a mobile phase of 55:15:30 (v:v:v) distilled, deionized water: tetrahydrofuran: methanol and a flow rate of 1 mL/min. The eluant will be monitored by UV absorbance at 240 nm and by a flow-through radiochemical detector. Eluant fractions will be collected manually into vials containing ca. 10 mL Ultima Gold and assayed for radiochemical content by liquid scintillation spectrometry (LSS). A reference standard of nonradiolabeled ASDN will be analyzed by the same method and coelution of the nonradiolabeled and radiolabeled ASDN will be confirmed. 2.1.3 Preparation of Substrate Solution for use in Aromatase Assay The specific activity of the [³H]ASDN as received from the vendor is usually 20-30 Ci/mmol which is too high for use directly in the assay. Thus, the specific activity must be lowered to about 500 μCi/mmol by the addition of nonradiolabeled ASDN. The target final concentration of ASDN in the assay is 100 nM and the amount of tritium to be added to each incubation is about 0.1 μCi. Therefore, prepare a substrate solution containing a mixture of nonradiolabeled and radiolabeled ASDN with a concentration of 2 μM and with a radiochemical content of about 1 μCi/mL. The following illustrates the preparation of a substrate solution using a stock of [³H]ASDN with a specific activity of 25.3 Ci/mmol and a concentration of 1 mCi/mL. Prepare a 1:100 dilution of the radiolabeled stock in buffer. Prepare a 1 mg/mL solution of ASDN in ethanol and then prepare dilutions in buffer to a final concentration of 1 μg/mL. Combine 4.5 mL of the 1 μg/mL solution of ASDN, 800 μL of the [³H]ASDN dilution and 2.7 mL buffer to make 8 mL of substrate solution (enough for 80 tubes). Record the weight of each component added to the substrate solution. After mixing the solution well, weigh aliquots (ca 20 μL) and combine with scintillation cocktail for radiochemical content analysis. The addition of 100 μL of the substrate solution to each 2 mL assay volume yields a final [³H]ASDN concentration of 100 nM with 0.1 μCi/tube. 2.2 Reference Chemicals The reference chemicals, their properties and rationale for selection are listed in Table 1. Each chemical will initially be tested over the concentration range 10^{-3} to 10^{-10} M (final concentration) but the range may be adjusted as described in Section 6. All reference chemical should be $\geq 97\%$ pure.		

Table 1. Reference Chemicals

Chemical	CAS No.	Reason for Selection
Vinclozolin	50471-44-8	Used in other validation studies and has been reported to be negative in both H295R and KGN cell-based assays.
Bisphenol A	80-05-7	A challenging chemical used extensively in ED testing. Structurally different from the pesticides tested and reported to be an inhibitor in the KGN assay.
Tributyltin	688-73-3	Structurally different from all other chemicals tested and reported in the literature to be an aromatase inhibitor.
Diethylhexyl phthalate	117-81-7	Used in other validation studies. Reported to be an inhibitor in the KGN assay.
Methoxychlor	72-43-5	Used in other validation studies. Known to be metabolized to a strongly estrogenic agent and to have antiandrogenic properties. Reported to be negative in the KGN assay.
Aldicarb	116-06-3	Aldicarb is a carbamate and therefore structurally different from other pesticides tested in the validation program. It was reported to be an inhibitor in the KGN assay.
Flavone	525-82-6	Flavone is the parent of a group of natural aromatase inhibitors. It is reported to be a weak inhibitor in the KGN assay and a moderate (10 μ M) inhibitor in the PM assay.
Triadimefon	43121-43-3	A weak to moderate azole fungicide inhibitor for which several literature values were found.
Imazalil	35554-44-0	An azole fungicide for which several literature values were found.
Apigenin	520-36-5	A flavone with known aromatase activity.
Ronidazole	7681-76-7	Reported to be a very weak azole fungicide inhibitor in the HT assay.
Genistein	446-72-0	An isoflavone reported to be negative. Genistein has been used extensively in ER binding and other ED validation studies.
p,p'-DDE	72-55-9	An organochlorine insecticide known to be estrogenic. It has been reported to be an inhibitor in the KGN assay.
Alachlor	15972-60-8	A chlorinated herbicide reported to be negative in the KGN study.
Nitrofen	1836-75-5	A diphenyl ether reported to be negative in the KGN assay.
Trifluralin	1582-09-8	A dinitroaniline reported to be negative in the KGN assay.

2.2.1 Reference Chemical Formulation and Analysis

Reference chemical stock solutions will be prepared and analyzed by the CR and shipped to RTI's Materials Handling Facility (MHF), along with MSDSs and information related to the content (including solvent) and molarity of each formulation. The CR will also supply the MHF with solvents of the same lot used in the preparation of the stock solutions which can then be used in preparation of dilutions of the stock solutions for use in the assay. Reference chemicals will be formulated at a preferred concentration of 0.1 M. The preferred solvent is dimethylsulfoxide (DMSO), but absolute ethanol or buffer may be used if necessary for adequate solubility. If a 0.1 M solution cannot be prepared, a lower concentration (0.05 or 0.01 M) will be tried. The molarity of each supplied formulation will be clearly stated on the label. The CR will code the reference chemical stock solutions prior to distributing them to the assaying laboratory, so that the technicians will be blinded to the identity of the chemical under testing. Information on storage conditions for reference chemical stock solutions will be provided by the CR.

The total volume of reference chemical formulation used in each assay should be no more than 1% of the total assay volume (i.e., 20 μ L in a 2 mL assay) in order to minimize the

potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use such that the target concentration of reference chemical can be achieved by the addition of 20 μ L of the dilution to a 2 mL assay volume.

2.3 Positive Control

The known aromatase inhibitor, 4-hydroxyandrostendione (4-OH ASDN), is used as the positive control. Table 2 contains identity and property information for 4-OH ASDN.

Table 2. Positive Control Information

Control Substance	CAS Number	Molecular Formula	Molecular Weight (g/mol)	Basis for Selection
4-OH ASDN	566-48-3	C ₁₉ H ₂₆ O ₃	302.4	Known aromatase inhibitor

2.3.1 Positive Control Formulation and Analysis

Positive control stock solutions will be prepared and analyzed by the CR and shipped to RTI's Materials Handling Facility (MHF). 4-OH ASDN will be formulated in absolute ethanol at a concentration of 0.01M. The total volume of control substance formulation used in each assay should be no more than 1% of the total assay volume (i.e., 20 μ L in a 2 mL assay) in order to minimize the potential of the solvent to inhibit the enzyme. Fresh dilutions of the stock solution will be prepared in the same solvent as the stock solution on the day of use. Dilutions will be prepared such that the target concentration of control substance (Table 4) can be achieved by the addition of 20 μ L of the dilution to a 2 mL assay volume. Information on storage conditions for control substance stock solutions will be provided by the CR.

2.4 Microsomes

Recombinant microsomes will be purchased from Gentest (Woburn, MA). The product name is Human CYP19 + P450 Reductase SUPERSOMES™, catalog number 456260. The microsomes must be stored at approximately -70 to -80 °C. The approximate protein content of the microsomes will be provided by the supplier. The product data sheet for the batch of microsomes used will be maintained in the study records.

Caution: Microsomes can be denatured by detergents. Therefore, it is important to ensure that all glassware, etc. that is used in the preparation or usage of microsomes is free of detergent residue. New disposable test tubes, bottles, vials, pipets and pipet tips may be used directly in the assay. Durable labware that may have been exposed to detergents should be rinsed with water and/or buffer prior to use in the assay.

If the recombinant microsomes are supplied in aliquots in excess of what is required to conduct a single experiment, they will be thawed, pooled, homogenized, divided into appropriate aliquots for conduct of a single experiment and refrozen as described below in order to minimize and standardize the number of freeze/thaw cycles each preparation undergoes. Microsomes will

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be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be pooled and rehomogenized using a Potter-Elvehjem homogenizer (about 5–10 passes). The pooled sample will be aliquoted into portions appropriate for use in a single experiment (ca. 160 µL, dependant on the protein concentration of the preparation) and the samples will be flash frozen and stored at -70 to -80 °C for future use. Each tube will provide enough protein for a single experiment and any excess thawed microsomal preparation will be discarded. On the day of use, microsomes will be thawed quickly in a 37 ± 1 °C water bath and then will be immediately transferred to an ice bath. The microsomes will be rehomogenized using a Potter-Elvehjem homogenizer (about 5-10 passes) or by vortexing about 5 seconds prior to use. The microsomes will be diluted in buffer (serial dilutions may be necessary) to an approximate protein concentration of 0.008 mg/mL. The addition of 1 mL of that microsome dilution will result in a final approximate protein concentration of 0.004 mg/mL in the assay tubes. All microsome samples must be kept on ice until they are placed in the water bath just prior to their addition to the aromatase assay. Microsomes are not to be left on ice for longer than approximately 1 h before proceeding with the assay. Appropriate documentation of time from thaw to use must be maintained. Diluted microsomes must be used only on the day of preparation. Under no conditions should diluted microsomes be refrozen for later use in the assay. 2.5 Other Assay Components 2.5.1 Buffer The assay buffer will be 0.1 M sodium phosphate buffer, pH 7.4. Sodium phosphate monobasic (JT Baker, cat # 4011-01, 137.99 g/mol) and sodium phosphate dibasic (JT Baker, cat # 4062-01, 141.96 g/mol) will be used in the preparation of the buffer. Solutions of each reagent at 0.1 M will be prepared in distilled, deionized water and then the solutions will be combined to a final pH of 7.4. The assay buffer may be stored for up to one month in the refrigerator (2–8 °C). 2.5.2 Propylene Glycol Propylene glycol (JT Baker, cat # 9402-01, 76.1 g/mol) will be added to the assay directly as described below. 2.5.3 NADPH NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt, Sigma, cat # 1630, 833.4 g/mol) is the required co-factor for CYP19. The final concentration in the assay is 0.3 mM. Typically, a 6 mM stock solution will be prepared in assay buffer and then 100 µL of the stock will be added to the 2 mL assay volume. NADPH must be prepared fresh each day and will be kept on ice.		

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3.0 PROTEIN ASSAY

The protein concentration of the microsome preparation will be measured in 5 independent assays for each lot of microsomes. Appropriate QCs will be included with each assay. A standard curve (of at least 5-points) will be prepared of a suitable range to bracket the concentration of protein in the microsome dilution. The protein curve standards will be prepared with bovine serum albumin (BSA). Protein will be determined by using a DC Protein Assay kit purchased from Bio-Rad (Hercules, CA). One of two protocols will be employed and the selection between the two can be made based on the expected concentration of the microsomal dilution to be measured and the nominal protein concentration of the stock microsomes.

Sample preparation for the micro protocol, which is appropriate for use over the range 5 to 250 µg protein/mL, is conducted as follows:

- To a 200 µL aliquot of unknown, QC or curve standard, 100 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 800 µL of BioRad DC Protein Kit Reagent B will be added to each sample and the samples will be vortex mixed.

Sample preparation for the standard protocol, which is appropriate for use over the range 0.2 to 1.5 mg/mL, is conducted as follows:

- To a 25 µL aliquot of unknown, QC or curve standard, 125 µL of BioRad DC Protein Kit Reagent A will be added and mixed. Next, 1 mL of BioRad DC Protein Kit Reagent B will be added to each sample and the samples will be vortex mixed.

Both protocols continue as follows:

The samples will be allowed to sit at room temperature for at least 15 min to allow for color development. The absorbances are stable for about 1 h. Each sample (unknown, QCs and standards) will be transferred to disposable polystyrene cuvettes and the absorbance (@ 750 nm) will be measured using a spectrophotometer. The protein concentration of the microsomal sample will be determined by interpolation of the absorbance value using the curve developed from the protein standards.

The protein assay data can be accepted if the determined value for two-thirds of the QC samples is within 15% of the nominal value. The average measured protein concentration, SD and CV across the five assays will be determined. The CV for the set should be ≤10%. If the data do not meet this criterion, the set will be examined for outliers, that may be excluded and/or additional assays may be run to generate more data. The average measured protein value will be compared with the stated protein value from the product data sheet. If the measured value is within 100 ± 10% of the nominal value, the nominal value will be used in all calculations of aromatase activity. If the measured value is outside this range, then the measured value will be used in all calculations of aromatase activity.

4.0 AROMATASE ASSAY METHOD

The assays will be performed in 13 x 100 mm test tubes maintained at 37 ± 1 °C in a

shaking water bath. Each test tube will be uniquely identified by applying a label or writing directly on the test tube. Propylene glycol (100 μ L), [^{3} H]ASDN, NADPH, and buffer (0.1 M sodium phosphate buffer, pH 7.4) will be combined in the test tubes (total volume 1 mL). The final concentrations for the assay components are presented in Table 3. The tubes and the microsomal suspension will be placed at 37 ± 1 °C in the water bath for 5 min prior to initiation of the assay by the addition of 1 mL of the diluted microsomal suspension. The total assay volume will be 2 mL, and the tubes will be incubated for 15 min. The incubations will be stopped by the addition of methylene chloride (2 mL); the tubes will be vortex-mixed for ca. 5 s and placed on ice while the rest of the incubations are stopped. The tubes will then be vortex-mixed an additional 10-15 s. The tubes will then be centrifuged briefly at low speed to effect separation of the organic and aqueous layers. Use of an AllegraX-15R centrifuge with an SX4750a rotor for 10 min at a setting of 1000 rpm (approx. 200 x g) has achieved sufficient separation but other low speed centrifugation protocols can also be used. The methylene chloride layer (bottom) will be removed and discarded; the aqueous layers will be extracted again with methylene chloride (2 mL). The methylene chloride may be removed by the use of a glass pipet; care should be taken to not remove the aqueous sample until the final step. This extraction procedure will be performed one additional time, each time discarding the methylene chloride layer. The aqueous layers will be transferred to vials and duplicate aliquots (0.5 mL) will be transferred to 20-mL liquid scintillation counting vials. Liquid scintillation cocktail (Ultima Gold, Packard, 10 mL) will be added to each counting vial and shaken to mix the solution. The radiochemical content of each aliquot will be determined as described below.

Table 3. Optimized Aromatase Assay Conditions

Assay factor (units)	Recombinant
Microsomal Protein (mg/mL) ^a	0.004
NADPH (mM) ^a	0.3
[^{3} H]ASDN (nM) ^a	100
Incubation Time (min)	15

^a Final concentrations

Analysis of the samples will be performed using liquid scintillation spectrometry (LSS). Radioactivity found in the aqueous fractions represents ^{3} H₂O formed.

5.0 POSITIVE CONTROL ASSAYS (TASK 5)

Each technician who will be participating in the testing of reference chemicals must first conduct a single replicate of the positive control experiment as outlined in Table 4. Data from this replicate will be assessed against the following criteria and will be submitted to EPA:

1. The minimum level of mean aromatase activity (in the full activity control samples) shall be 0.100 nmol/mg protein/min.
2. The mean background control activity shall be $\leq 1\%$ of the full activity control.
3. The concentration response curve generated for 4-OH ASDN shall meet the following conditions:
 - a) The Top must fall within the range $100 \pm 10\%$ of the full activity level.

- b) The Bottom must fall within the range $0 \pm 5\%$ of the full activity level.
- c) The Log IC_{50} must be between -7.00 and -7.30.
- d) The Slope must be between -0.8 and -1.2.

If the data from any technician do not meet the performance criteria, RTI will notify EPA and the assay will be repeated by the technician in question at least twice. Data from these replicate runs will be assessed with respect to the performance criteria and will be reported to EPA. Only technicians who produce data that meet the positive control performance criteria will participate in the reference chemical assays.

The concentrations of 4-OH ASDN chosen for the assay are intended to generate a complete inhibition curve (ranging from 100 – 0 % of control activity). In the event the concentration range is inappropriate for generation of a complete inhibition curve, RTI will notify the EPA TOPO after the first run of the assay and alternate concentrations will be discussed and incorporated into the study design as necessary.

The positive control assay and the first replicate of one reference chemical per technician may be conducted concurrently. If this study design is chosen, both 4-OH ASDN and the reference chemical would be run in three tubes per concentration for those runs. If the positive control portion of the assay meets the performance criteria, the entire assay could be accepted and would satisfy requirements for both the positive control assay (Task 5) and the first replicate of the reference chemical.

Table 4. Positive Control Experiment Outline

Sample Type	Repetitions (tubes)	Description	4-OH ASDN Concentration (M)
Full Activity Control	4	Complete assay ^a with inhibitor vehicle control, no inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc 1	3	Complete assay with 4-OH ASDN added	1×10^{-5}
Positive Control Conc 2	3	Same	1×10^{-6}
Positive Control Conc 3	3	Same	5×10^{-7}
Positive Control Conc 4	3	Same	1×10^{-7}
Positive Control Conc 5	3	Same	5×10^{-8}
Positive Control Conc 6	3	Same	1×10^{-8}
Positive Control Conc 7	3	Same	1×10^{-9}
Positive Control Conc 8	3	Same	1×10^{-10}

^a The complete assay contains buffer, propylene glycol, microsomal protein, [³H] ASDN, and NADPH

6.0 DETERMINATION OF THE RESPONSE OF AROMATASE ACTIVITY TO REFERENCE CHEMICALS (TASK 6)

Each of the 16 reference chemicals listed in Table 1 will be tested in three independent runs of the aromatase assay using a single technician per chemical as outlined in Table 5. The reference chemicals will be coded prior to distribution to the assaying technicians in order that the replicates are conducted blind for reference chemical identity. All three replicates for a given reference chemical must be conducted by the same technician. However, the same technician is not required to perform the three replicates for all sixteen reference chemicals. As described in the Table 5, a full eight-point positive control assay, with duplicate determinations per concentration will be done concurrently with each reference chemical assay set.

Full activity and background activity controls will be included with each replicate. The background activity tubes will serve as the negative control. The make-up of these controls is described below:

- full enzyme (aromatase) activity controls (substrate, NADPH, propylene glycol, buffer, vehicle [used for preparation of reference chemical solutions] and microsomes)
- background activity controls (all components that are in the full aromatase activity controls, except NADPH)

Four test tubes of each type of control are included with each replicate and are treated the same as the other samples. The controls sets will be split so that two tubes (of each control type) are run at the beginning and two at the end of each replicate set.

The assay will be conducted as described in Section 4.0 with the following modification. Reference or positive control chemical solution (or vehicle) will be added to the mixture of propylene glycol, substrate, NADPH and buffer in a volume not to exceed 20 μ L prior to preincubation of that mixture. The volume of buffer used will be adjusted so the total incubation volume remains at 2 mL.

Some chemicals may not be soluble in the assay mixture over the entire range of target testing concentrations. When chemicals precipitate upon addition to the reaction mixture, it is impossible to know the true concentration of chemical in solution that is available to interact with the enzyme. Therefore, it is important to observe the reaction mixtures as test chemicals are added and to note any precipitation and/or cloudiness. If cloudiness and/or precipitation is observed at any concentration used in the first replicate of a particular chemical, the target test concentrations for the remaining replicates shall be adjusted as follows:

- Set the highest concentration to the highest concentration that appeared to be soluble.
- Add new mid-log concentration(s) near the estimated IC_{50} based on the first replicate results, so that there are still eight concentrations in the set.
- The lowest concentration to be tested is 10^{-10} M.

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Based on the results of the first replicate, it may be desirable to alter the concentrations tested in the remaining replicates to better define the concentration response curve over the critical region where maximal slope is observed. When selecting concentrations for the remaining replicates, use the following guidance:

- Make any adjustments that are necessary as a result of poor solubility first, as described above.
- Keep at least 2 concentrations each that define the top and bottom plateaus. Any excess points from concentrations lower than the two needed to define the top plateau or from concentrations higher than the two needed to define the bottom plateau, may be removed from the concentration set.
- Add new mid-log concentration(s) near the estimated IC_{50} based on the first replicate results, so that there are still eight concentrations in the set.
- The lowest concentration to be tested is 10^{-10} M.

Table 5. Reference Chemical Experiment Outline

Sample Type	Repetitions (tubes)	Description	Control or Reference Chemical Concentration (M)
Full Activity Control	4	Complete assay ^a with inhibitor vehicle control, no inhibitor	N/A
Background Activity Control	4	Same as full activity control, but no NADPH	N/A
Positive Control Conc 1	2	Complete assay with 4-OH ASDN added	1×10^{-5}
Positive Control Conc 2	2	Same	1×10^{-6}
Positive Control Conc 3	2	Same	5×10^{-7}
Positive Control Conc 4	2	Same	1×10^{-7}
Positive Control Conc 5	2	Same	5×10^{-8}
Positive Control Conc 6	2	Same	1×10^{-8}
Positive Control Conc 7	2	Same	1×10^{-9}
Positive Control Conc 8	2	Same	1×10^{-10}
Reference Chem. Conc 1	3	Complete assay with reference chemical added	1×10^{-3}
Reference Chem. Conc 2	3	Same	1×10^{-4}
Reference Chem. Conc 3	3	Same	1×10^{-5}
Reference Chem. Conc 4	3	Same	1×10^{-6}
Reference Chem. Conc 5	3	Same	1×10^{-7}
Reference Chem. Conc 6	3	Same	1×10^{-8}
Reference Chem. Conc 7	3	Same	1×10^{-9}
Reference Chem. Conc 8	3	Same	1×10^{-10}

^a The complete assay contains buffer, propylene glycol, microsomal protein, [^3H] ASDN, and NADPH

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7.0 DATA ANALYSIS

7.1 Aromatase Activity and Percent of Control Calculations

Relevant data will be entered into a spreadsheet for calculation of aromatase activity and percent of control. The version of the spreadsheet used will be included in the reports. The data will be examined to determine whether the following performance criteria have been met:

- The minimum level of mean aromatase activity (in the full activity control samples) shall be 0.100 nmol/mg protein/min.
- The mean background control activity shall be $\leq 1\%$ of the full activity control.

If any one of these criteria has not been met, no further analysis for that data set will be performed. In that case, the assay would be repeated.

7.2 Model Fitting

Percent of control activity remaining at each reference (or control) chemical concentration will be imported into GraphPad Prism version 4.03 to fit a four-parameter model and a two parameter model for each chemical. The data obtained in Section 5 will only be fit to the four parameter model and will be excluded from the model fit comparison. The four-parameter model to be fit to the data to obtain the estimated dose-response curve is:

$$Y = Bottom + \left(\frac{Top - Bottom}{1 + 10^{(\mu - X)\beta}} \right)$$

where Y is the percent of control activity remaining, X is the $\log_{10}$ of the concentration of the chemical, Top and $Bottom$ are the plateaus of the response curve, μ is the $\log_{10}IC_{50}$ and β is the slope of the response curve at $\log_{10}IC_{50}$. The two-parameter model uses the same equation with $Bottom$ fixed at 0 and Top fixed at 100.

The (four parameter) response curve for the positive control (4-OH ASDN) will be examined to determine if the following criteria have been met:

- The Top must fall within the range $100 \pm 10\%$ of the full activity level.
- The $Bottom$ must fall within the range $0 \pm 5\%$ of the full activity level.
- The $\log IC_{50}$ must be between -7.00 and -7.30.
- The Slope must be between -0.8 and -1.2.

If the positive control response curve fails any of these criteria the failure will be reported promptly to the EPA TOPO. The task order leader will confer with the EPA TOPO to determine an appropriate course of action in this case.

The (four-parameter) response curve from the reference chemical will be examined to determine if the following criterion has been met:

- The Top of the test chemical curve must fall within the range $100 \pm 10\%$ of the full activity level.

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If this criterion is not met, the study director, in consultation with the TOPO, will make appropriate changes to the concentrations of reference chemical included in the assay.

7.3 Data Interpretation

Data from the assay will be used to classify chemicals according to their ability to inhibit aromatase. EPA has established the following classifications and criteria for their assignment:

Class	Criteria
Non-inhibitor	The most inhibited level lies above 75% of full activity control
Equivocal	The most inhibited level lies between 75% and 50% of full activity control
Inhibitor	The most inhibited level lies below 50% of full activity control

Classifications will be made with consideration given to the performance criterion to be developed in Section 7.5 of this protocol.

7.4 Statistical Analyses

7.4.1 Analysis of Fitted Curves for Differences

The sums of squares, root mean square error and degrees of freedom results from each model fit for each chemical will be used to perform a partial F-test to test for significant differences simultaneously among the four model parameters. The partial F-test is calculated as follows:

$$F = \frac{(MSS_{FM} - MSS_{RM}) / (MDF_{FM} - MDF_{RM})}{RSS_{FM} / RDF_{FM}}$$

where MSS_{FM} denotes the model sum of squares for the full model containing all four parameters, MDF_{FM} is the model degrees of freedom for the full model, RSS_{FM} and RDF_{FM} denote the residual sum of squares and degrees of freedom, respectively, for the full model and MSS_{RM} and MDF_{RM} denote the model sum of squares and degrees of freedom for the reduced model containing two parameters.

The partial F-test to compare the two models will be done with GraphPad Prism Version 4 using that software's "extra sum-of-squares F test". The results will be used to determine whether there are significant differences in the parameter estimates generated by the two models.

7.4.2 Comparison of Positive Control Values Within and Across Technicians

In order to assess the variation across and within technicians (for the assays in Section 6), a mixed effects model will be fit to the positive control values with the 4-OH ASDN concentrations as a fixed effect and the technicians as random effects. Tests performed on the

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variance components will determine if there is more variation in the positive control results between technicians than within technicians. 7.4.3 Evaluation of the Suitability of the Positive Control Performance Criteria To assess the suitability of the quality assurance measures, the dose-response model results from each run of the positive control experiments will be checked to see if all of the parameter estimates meet the performance criteria. If most or all of the dose-response model parameter estimates obtained from different technicians meet all of the performance criteria, the performance criteria intervals can be determined to be sufficiently large enough for labs to be able to meet the performance criteria. If too many of the dose-response model parameter estimates do not meet the performance criteria, the performance criteria will need to be re-evaluated to provide obtainable standards. Another method of assessing the suitability of the performance criteria as quality assurance measures is to estimate the dose-response model parameters from all of the runs performed by each technician and examine the standard errors of each of the model parameters. If the 95% confidence intervals for each parameter estimate for a given technician are within the performance criteria, the technician should be able to produce positive control experiment results that meet the performance criteria in at least 95% of the experiments that the technician runs. The percentage associated with the confidence intervals for each technician and each parameter that fit inside the performance criteria intervals will determine what percentage of the experiments that the technician would run that would meet the performance criteria. If the percentage of experimental data results for a technician that meet the performance criteria is too low, the performance criteria intervals may be too narrow for labs to attain and would need to be re-evaluated. Likewise, if the 95% confidence intervals for each technician and each parameter are much smaller than the performance criteria intervals, the performance criteria intervals may be too wide and may need to be shortened to ensure only good quality experimental data will pass the performance criteria evaluations. 7.5 Development of a Second Performance Criterion for Reference Chemicals Thus far, the only specified performance criterion for reference chemicals is that the top of the curve shall lie in the range 90% to 110% for the four parameter model. Task 8 involves the development of one or more additional criteria for reference chemical data. An additional performance criterion for the assay should evaluate the quality of the data. Data of acceptable quality (i.e., meeting all performance criteria) can then be used to classify reference chemicals as to their aromatase inhibitory characteristics. Test data sets will be used to classify chemicals as aromatase inhibitors, non-inhibitors or as chemicals for which the data are equivocal; this function falls under the category of data interpretation procedures. A separate performance criterion should be developed to apply to the data interpretation procedures. It is appropriate that both types of criteria be added as performance criteria for evaluation of test chemical data. The new performance criterion should assess the quality of the data by examining the variation		

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across all data points. The classification of a chemical as an inhibitor or non-inhibitor should depend on all of the data points, since response model estimates are fit to the experimental data collected at each tested concentration level, and larger than acceptable standard deviations at any concentration level will affect the precision of the response model. 7.5.1 Proposed Data Quality Criterion One possible approach to assessing the quality of the experimental data is to examine the coefficients of variation (CV) of the percent of control values at each chemical concentration level. The CV is calculated as the standard deviation divided by the average of the percent of control values. The results of the entire set of positive control and reference chemical assays conducted in Task 6 (96 assays in total) will be evaluated to determine a reasonable CV to use as a data quality criterion. Data from the positive control assays will be useful in determining a reasonable CV over time using the same chemical, while the data from the reference chemicals will be a good model for the range of CVs one can expect when testing a variety of chemicals in the assay and so these subsets of data will also be evaluated separately to determine a reasonable CV to use in each case. 7.5.2 Data Interpretation Criterion One possible approach to interpreting the results of the experiments is to graph both the upper and lower confidence bounds on the estimated response curve. These bounds, referred to in the software manuals as the "confidence bands" will be added to the graph of the estimated response curve using GraphPad Prism version 4.03. If the 95% confidence bounds on the response curve are used, it means that if the same experiment was repeated a large number of times, 95% of the estimated response curves calculated from each of the experiments would lie within the 95% confidence bounds. If both confidence bands for the estimated response curve extend below the 50% line on the graph, then the chemical can be classified as an inhibitor with only a small chance of a classification error. If both confidence bands for the estimated response curve stay above the 75% line on the graph, then the chemical can be classified as a non-inhibitor with only a small chance of a classification error. More analyses would need to be performed to determine the classification rules if at least one of the confidence bands remains between the 50% and 75% lines for the highest concentrations of the chemical. Additional approaches to assessing the quality of the experimental data and to interpreting the data results may be examined in conjunction with the U.S. EPA. The various approaches would be weighed against the priorities the U.S. EPA determines for the statistical tests, such as the importance of having criteria that are easily implemented by technical staff using the GraphPad Prism software.		

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7.6 Statistical Software

Concentration response curves will be fitted to the data using the non-linear regression analysis features in the PRISM statistical analysis package, Version 4.03 or higher. Supplemental statistical analyses and displays such as summary tables, graphical displays, analysis of variance, and multiple comparisons will be carried out using PRISM, the SAS statistical analysis system, Version 8 or higher, or other general purpose statistical packages (e.g. SPSS), as convenient.

8.0 RETENTION OF RECORDS

All records that remain the responsibility of the testing laboratory will be retained in the archives for the life of the contract.

9.0 QUALITY CONTROL/QUALITY ASSURANCE PROCEDURES

Quality control (QC) and quality assurance (QA) procedures will follow those outlined in the Quality Assurance Project Plan (QAPP) that was prepared for this study. The study will be conducted in compliance with the Federal Register, 40 CFR Part 160. Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) Good Laboratory Practices Standards.

10.0 REPORTS

A final report that includes (at minimum) a full description of the work performed in the laboratory and during data analysis, summaries of the data in tabular (spreadsheet) and graphical format, results of the statistical analyses and conclusions drawn from the data will be prepared. Reference chemicals will be identified by name in the report. In addition, a final aromatase assay protocol which includes any modified performance criteria or data interpretation procedures will be prepared and appended to the final report. The draft report will be audited by RTI's QA unit for compliance with the QAPP and study protocol and to ensure the accuracy of the data contained therein. The draft report will be submitted to EPA for review. This draft report will be revised as appropriate and then the final, signed version will be submitted (in both hard copy and electronic formats) to the EPA TOPO.

11.0 STUDY RECORDS TO BE MAINTAINED

- All records that document the conduct of the laboratory experiments and results obtained, as well as the equipment and chemicals used
- Protocol and any Amendments
- List of any Protocol Deviations
- List of Standard Operating Procedures

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• QAPP and any Amendments • List of any QAPP Deviations		

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Appendix A

Supply List for the Aromatase Assay

Materials

13 x 100 mm test tubes
 Test tube rack
 Pasteur pipets
 1-Dram vials with caps – silylated
 Assorted laboratory glassware – beakers, graduated cylinders, volumetric flasks, bottles, vials
 Pipet tips
 Ice bucket
 Ice
 Assorted glass pipets
 Scintillation vials
 Disposable microcuvettes
 Pipet bulbs

Equipment

Shaking water bath capable of maintaining 37°C
 Vortexer
 Lab timer
 Low speed centrifuge capable of holding test tubes
 Micropipettors – various volumes ranging from 1 µL to 5 mL
 Liquid scintillation spectrometer
 Uv-Vis spectrophotometer
 HPLC (pumps, injector, reversed phase column (suggested: Zorbax SB-C₁₈ column (4.6 x 250 mm)), uv/vis and radiochemical detectors, data collection/analysis system)
 Analytical balance
 pH meter

Chemicals

4-androstene-3,17-dione (ASDN)
 ([1β-³H(N)]-androst-4-ene-3,17-dione ([³H]ASDN)
 Sodium phosphate monobasic (JT Baker, cat # 4011-01, 137.99 g/mol)
 Sodium phosphate dibasic (JT Baker, cat # 4062-01, 141.96 g/mol)
 Propylene glycol (JT Baker, cat # 9402-01, 76.1 g/mol)
 NADPH (β-nicotinamide adenine dinucleotide phosphate, reduced form, tetrasodium salt, Sigma, cat # 1630, 833.4 g/mol)
 4-hydroxyandrostendione (4-OH ASDN) formulation
 Reference chemical formulations
 DC Protein Assay kit - Bio-Rad (Hercules, CA)
 Bovine serum albumin
 Human CYP19 + P450 Reductase SUPERSOMES™, BD Biosciences, catalog number 456260
 Tetrahydrofuran (HPLC grade)
 Methanol (HPLC grade)
 Ultima Gold or other scintillation cocktail
 Absolute ethanol
 Methylene chloride

APPENDIX C

Material Data Sheets



Human CYP19 + P450 Reductase SUPERSOMES™

Catalog Number.....456260
 Lot Number.....67045

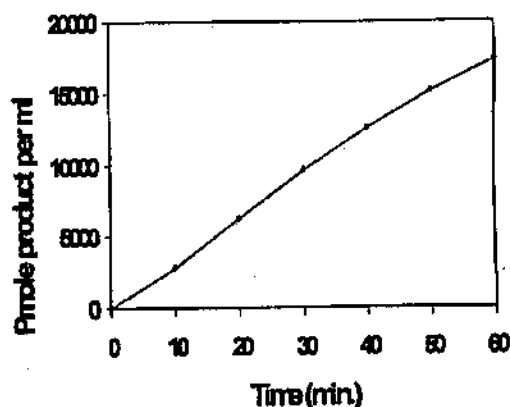
Storage Conditions..STORE AT -80°C
 Date Released 2006 November
 Best Used by.....2009 November

Package Contents.....0.5 nmole cytochrome P450 in 0.5 ml
 Protein Content.....6.5 mg/ml in 100mM potassium phosphate (pH 7.4)
 Cytochrome c Reductase Activity.....290 nmole/(min x mg protein)
 Cytochrome P450 Content.....1000 pmol per ml
 Aromatase Activity.....5.2 pmol product/(min x pmol P450)

This activity is catalyzed by human CYP19 which is expressed from human CYP19 cDNA using a baculovirus expression system. Baculovirus infected insect cells (BTI-TN-5B1-4) were used to prepare these microsomes. These microsomes also contain cDNA-expressed human P450 reductase. A microsome preparation using wild type virus (GENTEST Catalog No. 456200 or 456201) should be used as a control for native activities.

METHOD: A 0.25 ml reaction mixture containing 25 pmole P450, 1.3 mM NADP⁺, 3.3 mM glucose-6-phosphate, 0.4 U/ml glucose-6-phosphate dehydrogenase, 3.3 mM magnesium chloride and 0.05 mM testosterone in 100 mM potassium phosphate (pH 7.4) was incubated at 37°C for 20 min. After incubation, the reaction was stopped by the addition of 125 μ l acetonitrile and centrifuged (10,000 x g) for 3 minutes. 50 μ l of the supernatant was injected into a 4.6 x 250 mm 5 μ C18 HPLC column and eluted isocratically at 45°C with a mobile phase of 60% water and 40% acetonitrile and at a flow rate of 1.5 ml per min. The product was detected by its absorbance at 200 nm and quantitated by comparing the absorbance to a standard curve of (beta)-estradiol.

Time Course of Product Formation



ADVICE

- Thaw rapidly in a 37°C water bath. Keep on ice until use
- Aliquot to minimize freeze-thawing cycles. Less than 20% of the catalytic activity is lost after 6 freeze thaw cycles.
- Metabolite production is linear with respect to enzyme concentration up to at least 50 pmol P450 per ml.
- Metabolite production with testosterone is approximately linear for 40 minutes (see graph above).

THIS PRODUCT IS SUPPLIED FOR LABORATORY RESEARCH USE ONLY.



6 Henshaw St., Woburn, MA 01801 USA
Voice: (781) 935-5115, FAX: (781) 938-8644
info@gentest.com
www.gentest.com

BD Biosciences
Clontech
Discovery Labware
Immunocytometry Systems
Pharmingen



INSECT CELL MICROSOMES SAFETY INFORMATION

HAZARD WARNING:

The product was produced using baculovirus (*Autographa californica*) infected insect cells (BTI-TN-5B1-4). This virus is not known to be pathogenic to humans or other mammals.

SAFETY RECOMMENDATIONS:

When using this product, follow good laboratory safety procedures:

Do not eat, drink or smoke.

Avoid contact with skin or eyes.

Do not inhale aerosols.

Do not pipette by mouth.

Wear suitable protective clothing, gloves and eye protection.

Steam sterilize product or treat product with a 1% solution of sodium hypochlorite prior to disposal.



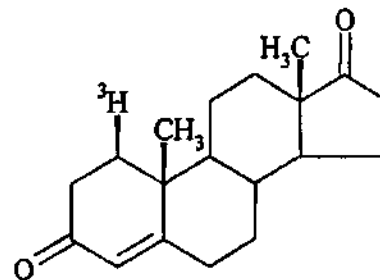
PerkinElmer Life and Analytical Sciences
649 Albany Street, Boston, MA 02118

³H Technical Data
Certificate of Analysis

For Laboratory Use. Analytical chemical for research purposes only.

NET-926 ANDROST-4-ENE-3, 17-DIONE, [1 β -³H(N)]-

Lot Number:	3557306
Specific Activity:	23.5 Ci/mmol
	0.87 TBq/mmol
Production Date:	May 18, 2006



M.W. 286
C₁₉H₂₆O₂

PACKAGING: 1.0 mCi/ml (37 MBq/ml) in ethanol. Shipped on dry ice.

STABILITY AND STORAGE RECOMMENDATIONS:

When androst-4-ene-3, 17-dione, [1 β -³H(N)]- is stored at -20°C in its original solvent and at its original concentration, the rate of decomposition is approximately 1% for 6 months from date of purification. Lot to lot variation may occur, and it is advisable to check purity prior to use.

SPECIFIC ACTIVITY RANGE: 15-30 Ci/mmol (0.55-1.11 TBq/mmol)

RADIOCHEMICAL PURITY: This product was initially found to be greater than 97% when determined by the following methods:

1. High pressure liquid chromatography on a Zorbax ODS column using the following mobile phase:

water : tetrahydrofuran : methanol (40:15:45)

2. Paper chromatography on Whatman No. 1 treated with 30% formamide in acetone using the following solvent system:

hexane saturated with formamide.

3. Thin layer chromatography on silica gel using the following solvent system:

toluene : ethyl acetate, (2:1).

QUALITY CONTROL: The radiochemical purity of androst-4-ene-3, 17-dione, [1 β -³H(N)]- is checked at appropriate intervals using the first listed chromatography method. Current purity data is available upon request.

PREPARATIVE PROCEDURE: Androst-4-ene-3, 17-dione, [1β - $^3\text{H}(\text{N})$]- is prepared by treatment of androst-4-ene-3, 17-dione, [$1\beta,2\beta$ - $^3\text{H}(\text{N})$]- with potassium hydroxide under appropriate conditions (1) Purification is by HPLC.

REFERENCE:

1. R. Gore-Langton, et al., *Endocrinology*, 107, 464 (1980).

HAZARD INFORMATION: WARNING:

WARNING:

Harmful by Contact, Ingestion, Inhalation.

Effects of Exposure: Irritating to Eyes; Slightly Toxic; Flammable.

Target Organs: Central Nervous System.

WARNING: This product contains a chemical known to the state of California to cause cancer.

0186:111596



SIGMA-ALDRICH

Certificate of Analysis

Product Name 4-Androstene-3,17-dione,
≥98%
Product Number A9630
Product Brand Sigma
CAS Number 63-05-8
Molecular Formula C₁₉H₂₆O₂
Molecular Weight 286.41

TEST**APPEARANCE****SOLUBILITY****ULTRAVIOLET/VISIBLE SPECTRUM****PURITY BY HPLC****SHELF LIFE****QC ACCEPTANCE DATE****PRODUCT CROSS REFERENCE
INFORMATION****SPECIFICATION**

WHITE TO OFF-WHITE POWDER

CLEAR, COLORLESS TO FAINT YELLOW
SOLUTION AT 200 MG PLUS 4 ML OF
CHLOROFORMEMM = 15.9 TO 16.5 AT LAMBDA MAX
239 TO 240 NM IN ETHANOL

98% MINIMUM

5 YEARS

LOT 115K12921 RESULTS

OFF-WHITE POWDER

CLEAR VERY FAINT YELLOW

EMM = 16.3 AT LAMBDA MAX 240 NM

99%

DECEMBER 2010

AUGUST 2006

REPLACEMENT FOR ALDRICH #285137

Rodney Burbach, Supervisor
Analytical Services
St. Louis, Missouri USA

EDSP CHAIN OF CUSTODY RECORD

Sherry Black

Battelle Marine Sciences Laboratory
1529 West Sequim Bay Road
Sequim, Washington 98362

PROJECT #: WA 1-1, Task 3b,
TD: 6

EDSP STUDY DIRECTOR: Michael Cobb

PHONE NUMBER: (360) 681-4580

SHIPMENT METHOD: (circle one)

FED Ex

UPS

Other

CONDITIONS: (circle one)

Ice

Dry Ice

Cold Packs

Ambient

RECOMMENDED STORAGE CONDITIONS: (circle one)

RT

4°C

-10°C

-20°C

-80°C

		Receipt Date	# of containers	Observations, Instructions
1	Dimethyl Sulfoxide COMMENTS: 1L, Lot No. 007438D, CAS 67-68-5, 99.94% pure, Mfg. Sigma		1	Store at RT
3	Formestane (a.k.a. 4-Hydroxy ASDN) COMMENTS: 50 mL of 1 mM solution prepared in ethanol, Lot No. 081K2133, CAS 566-48-3, 99.6% pure, Mfg. Sigma	2/13/07 PAT	1	Store @ 2-8°C
4	Ethanol COMMENTS: 1L, Lot No. 06C08PA, CAS 64-17-5, Absolute 200 Proof, Mfg Aaper, Mfd. 03/15/06		1	Store at RT

Assigned ID
12535-10
Store in REF028

See back for signature page

Relinquished By:		
<u>Rhonda K. Karls</u>	<u>2-12-07</u>	<u>1530</u>
Signature	Date	Time
<u>RHONDA K. KARLS</u> <u>Battelle</u>		
Printed Name	Company	
Received By:		
<u>P. Patel</u>	<u>2/13/07</u>	<u>12:55 p.m.</u>
Signature	Date	Time
<u>Purvi Patel</u>	<u>RTI</u>	
Printed Name	Company	

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: 02-20-2007

Sponsor: EPA

Supplier: Battelle Sequim

EPA Contract #: EP-W-06-026

RTI Project #: 0210114.009

Bar Code: CR11601

Vehicle: DMSO

RTI Log No.: 022007-A-01

Formulation Amount: 10 mL

Formulation Date: 2-19-07

Expiration Date: None Given

Formulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
<i>Shannon K. Hahn</i> / 2-20-07	<i>Sherry J. Black</i> / 2/20/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay
Formulation(s) Received: Chemical in DMSO
Date Received: 02-27-2007 Sponsor: EPA
Supplier: Battelle Sequim EPA Contract #: EP-W-06-026
RTI Project #: 0210114.009

Bar Code: CR11602
Vehicle: DMSO
RTI Log No.: 022707-A-01
Formulation Concentration: 0.1 M
Formulation Amount: 10 mL
Formulation Date: 2-26-07
Expiration Date: None Given
Formulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
<i>Shirley H. Hester</i> / 2-27-07	<i>Shirley H. Hester</i> / 2-27-07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant AssayFormulation(s) Received: Chemical in 100% Ethyl AlcoholDate Received: 02-27-2007Sponsor: EPASupplier: Battelle SequimEPA Contract #: EP-W-06-026RTI Project #: 0210114.009Bar Code: CR11603Vehicle: 100% Ethyl AlcoholRTI Log No.: 022707-C-01Formulation Concentration: 0.1 MFormulation Amount: 10 mLFormulation Date: 2-26-07Expiration Date: None GivenFormulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
<i>Shawn Martin</i> / 2-27-07	<i>Shawn Martin</i> / 2/27/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: 02-20-2007

Sponsor: EPA

Supplier: Battelle Sequim

EPA Contract #: EP-W-06-026

RTI Project #: 0210114.009

Bar Code: CR11604

Vehicle: DMSO

RTI Log No.: 022007-B-01

Formulation Amount: 10 mL

Formulation Date: 2-19-07

Expiration Date: None Given

Formulation Storage: 2 - 8°C

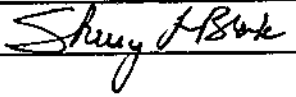
Released By Signature/ Date	Received By Signature/ Date
<i>Sharon R. Martin</i> 12-20-07	<i>Sherry J. Black</i> 12/20/07

RTI International - Materials Handling Facility (MHF)
Sample Custody Sheet
(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received:	Chemical in DMSO	
Date Received:	03-06-2007	Sponsor: EPA
Supplier:	Battelle Sequim	EPA Contract #: EP-W-06-026
RTI Project #:	0210114.009	

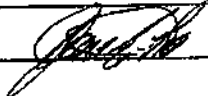
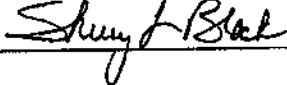
Bar Code:	CR11605
Vehicle:	DMSO
RTI Log No.:	030607-A-01
Formulation Concentration:	0.1 M
Formulation Amount:	10 mL
Formulation Date:	3-5-07
Expiration Date:	None Given
Formulation Storage:	2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
 13-6-07	 13/6/07

RTI International - Materials Handling Facility (MHF)
Sample Custody Sheet
(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay
Formulation(s) Received: Chemical in DMSO
Date Received: 03-06-2007 Sponsor: EPA
Supplier: Battelle Sequim EPA Contract #: EP-W-06-026
RTI Project #: 0210114.009

Bar Code: CR11606
Vehicle: DMSO
RTI Log No.: 030607-B-01
Formulation Concentration: 0.1 M
Formulation Amount: 10 mL
Formulation Date: 3-5-07
Expiration Date: None Given
Formulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
 13-6-07	 13/6/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: <u>03-06-2007</u>	Sponsor: <u>EPA</u>
Supplier: <u>Battelle Sequim</u>	EPA Contract #: <u>EP-W-06-026</u>
RTI Project #: <u>0210114.009</u>	

Bar Code: CR11614

Vehicle: DMSO

RTI Log No.: 030607-C-01

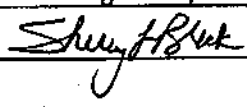
Formulation Concentration: 0.1 M

Formulation Amount: 10 mL

Formulation Date: 3-5-07

Expiration Date: None Given

Formulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
 13-6-07	 12/16/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: 02-27-2007 Sponsor: EPA

Supplier: Battelle Sequim EPA Contract #: EP-W-06-026

RTI Project #: 0210114.009

Bar Code: CR11607

Vehicle: DMSO

RTI Log No.: 022707-B-01

Formulation Concentration: 0.1 M

Formulation Amount: 10 mL

Formulation Date: 2-26-07

Expiration Date: None Given

Formulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
<i>Brian Nelson</i> / 2-27-07	<i>Spuyt Block</i> / 2/27/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: 03-13-2007

Sponsor: EPA

Supplier: Battelle Sequim

EPA Contract #: EP-W-06-026

RTI Project #: 0210114.009

Bar Code: CR11608

Vehicle: DMSO

RTI Log No.: 031307-C-01

Formulation Concentration: 0.1 M

Formulation Amount: 10 mL

Formulation Date: 3-12-07

Expiration Date: None Given

Formulation Storage: 2 - 8°C

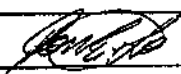
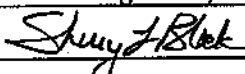
Released By Signature/ Date	Received By Signature/ Date
 1/3/13/07	 1/3/13/07

RTI International - Materials Handling Facility (MHF)
Sample Custody Sheet
(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay
Formulation(s) Received: Chemical in DMSO
Date Received: 03-13-2007 Sponsor: EPA
Supplier: Battelle Sequim EPA Contract #: EP-W-06-026
RTI Project #: 0210114.009

Bar Code: CR11611
Vehicle: DMSO
RTI Log No.: 031307-A-01
Formulation Concentration: 0.1 M
Formulation Amount: 10 mL
Formulation Date: 3-12-07
Expiration Date: None Given
Formulation Storage: -20°C

↑ R2110

Released By Signature/ Date	Received By Signature/ Date
 1/3/13.07	 1/3/13/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: 03-20-2007

Sponsor: EPA

Supplier: Battelle Sequim

EPA Contract #: EP-W-06-026

RTI Project #: 0210114.009

Bar Code: CR11609

Vehicle: DMSO

RTI Log No.: 032007-A-01

Formulation Concentration: 0.1 M

Formulation Amount: 10 mL

Formulation Date: 3-19-07

Expiration Date: None Given

Formulation Storage: 2-8°C

Released By Signature/ Date	Received By Signature/ Date
<i>Sharon Martin</i> / 3-20-07	<i>P. Kates</i> / 3/20/07

RTI International - Materials Handling Facility (MHF)
Sample Custody Sheet
(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay
Formulation(s) Received: Chemical in DMSO
Date Received: 03-20-2007 Sponsor: EPA
Supplier: Battelle Sequim EPA Contract #: EP-W-06-026
RTI Project #: 0210114.009

Bar Code: CR11610
Vehicle: DMSO
RTI Log No.: 032007-B-01
Formulation Concentration: 0.1 M
Formulation Amount: 10 mL
Formulation Date: 3-19-07
Expiration Date: None Given
Formulation Storage: -20°C

Released By Signature/ Date	Received By Signature/ Date
<i>Shane Slater</i> / 3-20-07	<i>P. R. O.</i> / 3/20/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: 03-13-2007

Sponsor: EPA

Supplier: Battelle Sequim

EPA Contract #: EP-W-06-026

RTI Project #: 0210114.009

Bar Code: CR11611

Vehicle: DMSO

RTI Log No.: 031307-A-01

Formulation Concentration: 0.1 M

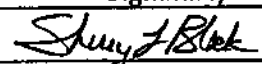
Formulation Amount: 10 mL

Formulation Date: 3-12-07

Expiration Date: None Given

Formulation Storage: -20°C

↑ PR2110

Released By Signature/ Date	Received By Signature/ Date
 13.13.07	 13/13/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received: Chemical in DMSO

Date Received: 03-20-2007

Sponsor: EPA

Supplier: Battelle Sequim

EPA Contract #: EP-W-06-026

RTI Project #: 0210114.009

Bar Code: CR11612

Vehicle: DMSO

RTI Log No.: 032007-C-01

Formulation Concentration: 0.1 M

Formulation Amount: 10 mL

Formulation Date: 3-19-07

Expiration Date: None Given

Formulation Storage: -20°C

Released By Signature/ Date	Received By Signature/ Date
<i>Shawn Nelson</i> / 3-20-07	<i>P. Patel</i> / 3/20/07

RTI International - Materials Handling Facility (MHF)
Sample Custody Sheet
(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay
Formulation(s) Received: Chemical in DMSO
Date Received: 02-20-2007 Sponsor: EPA
Supplier: Battelle Sequim EPA Contract #: EP-W-06-026
RTI Project #: 0210114.009

Bar Code: CR11613
Vehicle: DMSO
RTI Log No.: 022007-C-01
Formulation Amount: 10 mL
Formulation Date: 2-19-07
Expiration Date: None Given
Formulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
<i>Brian Slater</i> / 2-20-07	<i>Shirley Black</i> / 2/20/07

RTI International - Materials Handling Facility (MHF)

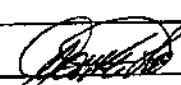
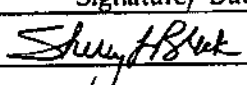
Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay

Formulation(s) Received:	Chemical in DMSO	
Date Received:	03-06-2007	Sponsor: EPA
Supplier:	Battelle Sequim	EPA Contract #: EP-W-06-026
RTI Project #:	0210114.009	

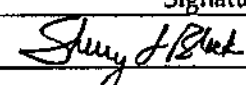
Bar Code:	CR11614
Vehicle:	DMSO
RTI Log No.:	030607-C-01
Formulation Concentration:	0.1 M
Formulation Amount:	10 mL
Formulation Date:	3-5-07
Expiration Date:	None Given
Formulation Storage:	2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
 13-6-07	 13/6/07

RTI International - Materials Handling Facility (MHF)
Sample Custody Sheet
(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay
Formulation(s) Received: Chemical in DMSO
Date Received: 03-13-2007 Sponsor: EPA
Supplier: Battelle Sequim EPA Contract #: EP-W-06-026
RTI Project #: 0210114.009

Bar Code: CR11615
Vehicle: DMSO
RTI Log No.: 031307-B-01
Formulation Concentration: 0.1 M
Formulation Amount: 10 mL
Formulation Date: 3-12-07
Expiration Date: None Given
Formulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
 13.13.07	 13/13/07

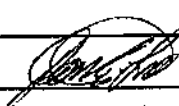
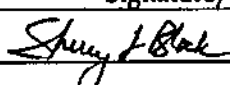
RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant Assay
Formulation(s) Received: Chemical in DMSO
Date Received: 03-13-2007 Sponsor: EPA
Supplier: Battelle Sequim EPA Contract #: EP-W-06-026
RTI Project #: 0210114.009

Bar Code: CR11616
Vehicle: DMSO
RTI Log No.: 031307-D-01
Formulation Concentration: 0.1 M
Formulation Amount: 10 mL
Formulation Date: 3-12-07
Expiration Date: None Given
Formulation Storage: 2 - 8°C

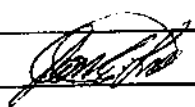
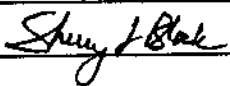
Released By Signature/ Date	Received By Signature/ Date
 13.13.07	 13/13/07

RTI International - Materials Handling Facility (MHF)

Sample Custody Sheet

(Keep with sample(s) or formulation)

Study: Chemicals for Recombinant AssayFormulation(s) Received: Chemical in DMSODate Received: 03-13-2007Sponsor: EPASupplier: Battelle SequimEPA Contract #: EP-W-06-026RTI Project #: 0210114.009Bar Code: CR11616Vehicle: DMSORTI Log No.: 031307-D-01Formulation Concentration: 0.1 MFormulation Amount: 10 mLFormulation Date: 3-12-07Expiration Date: None GivenFormulation Storage: 2 - 8°C

Released By Signature/ Date	Received By Signature/ Date
 13.13.07	 13/13/07

APPENDIX D

Protein Assay Spreadsheets

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	2/20/2007	Test	Chemical NA	# Concentrations tested	NA	Microsome ID	12535-13		
2		NB Ref	12535-30	Counter/delta	0						
3		Technician ID	SLB	Replicate #	NA	Positive Control	4-OH ASDN	Protein stock (mg/10 mL)	Pos Oil Tubes/Conc	Protein stock ID	3
4	Standards:	1.5	0.75	0.5	0.25	0.13	BLK	26.4			
5		0.328	0.277	0.245	0.190	0.143	0.124				
6		0.443	0.298	0.238	0.184	0.159	0.126				
7		0.446	0.273	0.246	0.179	0.159	0.121				
8	Samples:	QC 0.5	QC 0.25	12535-30C	12535-31G	12535-32C	12535-33C				
9		0.332	0.247	0.212	0.223	0.211	0.192				
10		0.337	0.229	0.218	0.214	0.226	0.222				
11				0.181	0.206	0.195	0.205				
12	mg Protein	μL Standard	μL Standard	mg Protein	A _{raw}	A _{std}	Curve	Variables	Regression results		
13	per μL	Used	Measured				Output	m, b			
14	0.00153	25	0.0396	0.442	0.318	0.210	0.0259	s _e , s _y	0.128	-0.001	
15	0.00106	25	0.0264	0.333	0.282	0.158	0.0193	r ² , s _e	0.003	0.001	
16	0.00079	25	0.0198	0.243	0.119	0.043	0.0143	F, df	0.998	0.001	
17	0.00053	25	0.0132	0.184	0.061	0.0068	0.0029	s _{reg} , s _{std}	1656	0.001	4
18	0.00029	25	0.0066	0.154	0.030				0.001	0.000	
19	0.00014	25	0.0034								
20	Blank	0.124		r ² =	0.998				Regression results are calculated using the function		
21				m=	0.128				LINEST		
22				b=	-0.001						
23				μL diluted	Vol usome	Final vol, Diluted		mg protein/μL	average mg/μL	mg/mL	
24	QC 1	A _{raw}	A _{std}	25	1	1	1.03E-03	1.04E-03	1.04		
25	QC 1	0.332	0.208	25	1	1	1.05E-03				
26	QC 1	0.337	0.213	25	1	1					
27	QC 0.5	0.247	0.123	25	1	1	5.94E-04	5.48E-04	0.55		
28	QC 0.5	0.229	0.105	25	1	1	5.02E-04				
29	QC 0.5			25	1	1					
30	QC 0.25	0.202	0.078	25	1	1	3.64E-04	3.28E-04	0.33		
31	QC 0.25	0.188	0.064	25	1	1	2.92E-04				
32	QC 0.25			25	1	1					
33	12535-30C	0.212	0.088	25	120	2400	8.30E-03	7.44E-03	7.44		
34	12535-30C	0.218	0.094	25	120	2400	8.89E-03				
35	12535-30C	0.181	0.067	25	120	2400	6.13E-03				
36	12535-31G	0.223	0.096	25	120	2400	9.42E-03	8.54E-03	8.54		
37	12535-31G	0.214	0.090	25	120	2400	8.50E-03				
38	12535-31G	0.206	0.082	25	120	2400	7.69E-03				
39	12535-32C	0.211	0.087	25	120	2400	8.20E-03	8.16E-03	8.16		
40	12535-32C	0.226	0.102	25	120	2400	9.73E-03				
41	12535-32C	0.195	0.071	25	120	2400	6.56E-03				
42	12535-33C	0.192	0.068	25	120	2400	6.25E-03	7.72E-03	7.72		
43	12535-33C	0.222	0.098	25	120	2400	9.32E-03				
44	12535-33C	0.205	0.081	25	120	2400	7.59E-03				

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	2/22/2007	Test Chemical	CR11601	# Concentrations tested			8	Microsome ID	12535-13
2		NB Ref	12535-46	Counter data filename	23FE0046.P00						
3		Technician ID	SLB	Replicate #	1	Positive Control		4-OH ASDN	Pos Ctl Tubes/Conc	2	
4	Standards:	1.5	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID		
5		0.410	0.276	0.162	0.200	0.134	0.126	26.4			
6		0.416	0.285	0.243	0.209	0.160	0.144				
7		0.420	0.301	0.222	0.171		0.110				
8	Samples:	QC 0.5	QC 0.25	12535-46C	12535-47G	12535-48C					
9		0.333	0.236	0.204	0.229	0.199					
10		0.300	0.183	0.215	0.197	0.226					
11				0.177	0.210	0.195					
12	mg Protein per μ L	μ L Standard		mg Protein Measured	A_{500}		A_{500}	Curve	Variables	Regression results	
13		Used		0.0396	0.415		0.289	Output	m, b	0.138	-0.001
14	25	25		0.0284	0.324		0.197	0.0263	$s_{y x}$, s_y	0.007	0.001
15	0.00106	25		0.0198	0.287		0.161	0.0213	r^2 , $s_{y x}$	0.990	0.002
16	0.00079	25		0.0132	0.216		0.089	0.0114	F , df	377	4
17	0.00053	25		0.0066	0.193		0.067	0.0083	SS_{reg} , SS_{total}	0.001	0.000
18	0.00026	25		0.0034	0.152		0.025	0.0026	Regression results are calculated using the function LINEST		
19	0.00014	25									
20		Blank	0.127	$r^2 =$	0.990						
21				$m =$	0.138						
22				$b =$	-0.001						
23				mg protein measured	Vol usome prep. (μ L)	Final vol. Diluted usomes (μ L)		mg protein/ μ L Prep.	average mg/ μ L	mg/mL	
24	QC 1	A_{500}	0.333	0.208	25	1		1.11E-03	1.01E-03	1.01	
25	QC 1	0.300	0.173	0.028	25	1		9.23E-04			
26	QC 1			0.023	25						
27	QC 0.5	0.238	0.109	0.014	25	1		5.69E-04	5.75E-04	0.57	
28	QC 0.5	0.238	0.111	0.015	25	1		5.80E-04			
29	QC 0.5				25						
30	QC 0.25	0.163	0.036	0.004	25	1		1.65E-04	2.21E-04	0.22	
31	QC 0.25	0.183	0.056	0.007	25	1		2.76E-04			
32	QC 0.25				25						
33	12535-46C	0.204	0.077	0.010	25	2400		7.84E-03	7.25E-03	7.25	
34	12535-46C	0.215	0.086	0.011	25	2400		9.06E-03			
35	12535-46C	0.177	0.050	0.006	25	2400		4.85E-03			
36	12535-47G	0.229	0.102	0.013	25	2400		1.06E-02	8.73E-03	8.73	
37	12535-47G	0.197	0.070	0.009	25	2400		7.07E-03			
38	12535-47G	0.210	0.083	0.011	25	2400		8.50E-03			
39	12535-48C	0.199	0.072	0.009	25	2400		7.29E-03	8.14E-03	8.14	
40	12535-48C	0.228	0.099	0.013	25	2400		1.03E-02			
41	12535-48C	0.195	0.068	0.009	25	2400		6.85E-03			
42					120	2400					
43					120	2400					
44					120	2400					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	2/27/2007	Test	CR11601	# Concentrations tested			8	Microsome ID	12535-13
2		NB Ref	12535-49	Counter data filename	27FE2319.P00						
3		Technician ID	SLB	Replicate #	2	Positive Control		4-OH ASDN	Pos Oli Tubes/Conc	Protein stock ID	2
4	Standards:	1.5	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)			
5		0.412	0.279	0.228	0.156	0.131	0.130	25.6			
6		0.413	0.275	0.238	0.158	0.149	0.112				
7		0.424	0.271	0.205	0.155		0.150				
8	Samples:	QC.1	QC.0.5	12535-49C	12535-50G	12535-51C					
9		0.246	0.164	0.179	0.183	0.192					
10		0.234	0.182	0.187	0.183	0.182					
11				0.172	0.173	0.180					
12	mg Protein	µL Standard	mg Protein	measured	A ₅₀₀	A ₅₀₀	Curve	Variables	Regression results		
13	per µL	Used		0.0384	0.416	0.286	Output	m, b	0.123	0.002	
14	25	25			0.332	0.202	0.0268	se _m , se _b	0.005	0.001	
15	0.00102	25		0.0256	0.275	0.144	0.0198	r ² , se _y	0.984	0.001	
16	0.00077	25		0.0192	0.224	0.093	0.0135	F, df	637	4	
17	0.00051	25		0.0128	0.158	0.028	0.0052	SS _{reg} , SS _{resid}	0.001	0.000	
18	0.00026	25		0.0064	0.140	0.009	0.0032				
19	0.00013	25		0.0033							
20		Blank	0.181	r ² =	0.994						
21				m=	0.123						
22				b=	0.002						
23											
24	QC.1	A ₅₀₀	mg protein measured	µL diluted	Vol usome prep. (µL)	Final vol. Diluted usomes (µL)	mg protein/µL Prep.	average mg/µL	mg/mL		
25	QC.1	0.245	0.016	25	1	1	6.45E-04	6.18E-04	0.62		
26	QC.1	0.234	0.015	25	1	1	5.91E-04				
27	QC.0.5	0.199	0.010	25	1	1	4.19E-04	3.58E-04	0.36		
28	QC.0.5	0.174	0.007	25	1	1	2.97E-04				
29	QC.0.5										
30	QC.0.25	0.164	0.006	25	1	1	2.48E-04	2.92E-04	0.29		
31	QC.0.25	0.182	0.008	25	1	1	3.36E-04				
32	QC.0.25										
33	12535-49C	0.179	0.008	25	120	2400	6.42E-03	6.45E-03	6.45		
34	12535-49C	0.187	0.009	25	120	2400	7.21E-03				
35	12535-49C	0.172	0.007	25	120	2400	5.74E-03				
36	12535-50G	0.183	0.009	25	120	2400	6.81E-03	6.49E-03	6.49		
37	12535-50G	0.163	0.0052	25	120	2400	6.81E-03				
38	12535-50G	0.173	0.007	25	120	2400	5.83E-03				
39	12535-51C	0.192	0.010	25	120	2400	7.70E-03	6.98E-03	6.98		
40	12535-51C	0.182	0.0051	25	120	2400	6.72E-03				
41	12535-51C	0.180	0.008	25	120	2400	6.52E-03				
42											
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Regression results are calculated using the function LINEST

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	2/28/2007	Test Chemical	CR11601	# Concentrations tested		8 Microsome ID	12535-13		
2		NB Ref	12535-52	Counter data filename	28FE2337.P00						
3		Technician ID	SLB	Replicate #	3	Positive Control		4-OH ASDN	Pos Ctl Tubes/Conc	2	
4	Standards:	1.5	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID		
5		0.427	0.237	0.239	0.181	0.137	0.129	26.6			
6		0.420	0.280	0.247	0.185	0.137	0.126				
7		0.423	0.266	0.247	0.214	0.142	0.135				
8	Samples:	QC 0.5	QC 0.25	12535-52C	12535-53G	12535-54C					
9		0.338	0.230	0.208	0.228	0.213					
10		0.313	0.243	0.191	0.211	0.216					
11				0.216	0.200	0.202					
12	mg Protein	μL Standard	μL Standard	mg Protein	A _{raw}	A _{avg}	Curve	Variables	Regression results		
13	per μL	Used	25	Measured			Output	m, b	0.136	0.000	
14	0.00160	25	0.0388	0.0388	0.423	0.293	0.0394		0.007	0.001	
15	0.00106	25	0.0266	0.0266	0.328	0.199	0.0266	s _{avg} , s _{avg}	0.989	0.002	
16	0.00080	25	0.0200	0.0200	0.274	0.144	0.0191	r ² , s _{avg}	364	4	
17	0.00053	25	0.0133	0.0133	0.244	0.114	0.0151	F, df	0.001	0.000	
18	0.00027	25	0.0067	0.0067	0.193	0.063	0.0081	s _{avg} , s _{avg}			
19	0.00014	25	0.0035	0.0035	0.145	0.015	0.0015				
20	Blank	0.130		r ² =	0.989				Regression results are calculated using the function LNEST		
21				m =	0.136						
22				b =	0.000						
23			mg protein measured	μL diluted	Vol usome prep. (μL)	Final vol. Diluted usomes (μL)		mg protein/μL	average mg/μL	mg/mL	
24	QC 1	A _{raw}	0.338	0.208	1	1		1.11E-03	1.04E-03	1.04	
25	QC 1	0.313	0.028	0.024	1	1		9.76E-04			
26	QC 1										
27	QC 0.5	0.230	0.013	0.013	1	1		5.25E-04	5.60E-04	0.56	
28	QC 0.5	0.243	0.015	0.015	1	1		5.96E-04			
29	QC 0.5										
30	QC 0.25	0.180	0.006	0.006	1	1		2.53E-04	2.31E-04	0.23	
31	QC 0.25	0.172	0.005	0.005	1	1		2.10E-04			
32	QC 0.25										
33	12535-52C	0.208	0.010	0.010	120	2400		8.11E-03	7.78E-03	7.78	
34	12535-52C	0.191	0.008	0.008	120	2400		6.26E-03			
35	12535-52C	0.216	0.011	0.011	120	2400		8.98E-03			
36	12535-53G	0.228	0.013	0.013	120	2400		1.03E-02	8.65E-03	8.65	
37	12535-53G	0.211	0.011	0.011	120	2400		8.43E-03			
38	12535-53G	0.200	0.009	0.009	120	2400		7.24E-03			
39	12535-54C	0.213	0.011	0.011	120	2400		8.65E-03	8.36E-03	8.36	
40	12535-54C	0.216	0.006	0.006	120	2400		8.98E-03			
41	12535-54C	0.202	0.009	0.009	120	2400		7.45E-03			
42											
43											
44											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	1/01/00	Test	Chemical	0	# Concentrations tested	0	Microsome ID	0	
2		NB Ref	0	Counter data	0	0					
3		Technician ID	0	Replicate	0	0	Positive Control	4-OH ASDN	Pos Cl Tubes/Conc	2	
4	Standards:	1.5	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID		
5		0.425	0.261	0.234	0.187	0.158	0.113	25.1			
6		0.416	0.253	0.232	0.175	0.167	0.141				
7		0.437	0.278	0.243	0.186	0.159	0.134				
8	Samples:	QC 1	QC 0.5	12535-55C	12535-56G	12535-57C					
9		0.339	0.232	0.211	0.207	0.199					
10		0.320	0.182	0.204	0.207	0.199					
11				0.193	0.196	0.211					
12	mg Protein	μL Standard	mg Protein	mg Protein	A ₅₀₀	A ₅₀₀	A ₅₀₀	Curve	Variables	Regression results	
13	per μL	Used	Measured	Measured				Output	m, b	0.125	0.000
14	0.00151	25	0.0377	0.0377	0.426	0.297	0.0380	se _m , se _b	0.005	0.001	0.001
15	0.00100	25	0.0188	0.0188	0.325	0.196	0.0250	r ² , se _y	0.994	0.001	0.001
16	0.00075	25	0.0126	0.0126	0.264	0.135	0.0172	F, df	719	4	0.001
17	0.00050	25	0.0063	0.0063	0.236	0.107	0.0136	ss _{reg} , ss _{resid}	0.001	0.000	0.000
18	0.00025	25	0.0033	0.0033	0.176	0.047	0.0058				
19	0.00013	25			0.161	0.032	0.0039				
20	Blank	0.128			0.994						
21					m=	0.129					
22					b=	0.000					
23			mg protein measured	μL diluted	Vol usome	Final vol. Diluted		mg protein/μL	average mg/μL	mg/mL	
24	QC 1	A ₅₀₀	0.339	0.210	1	1		1.07E-03	1.02E-03	1.02	
25	QC 1	0.320	0.191	0.25	1	1		9.75E-04			
26	QC 1			25							
27	QC 0.5	0.232	0.103	25	1	1		5.22E-04	5.27E-04	0.53	
28	QC 0.5	0.234	0.105	25	1	1		5.32E-04			
29	QC 0.5			25							
30	QC 0.25	0.181	0.052	25	1	1		2.59E-04	2.62E-04	0.26	
31	QC 0.25	0.182	0.053	25	1	1		2.64E-04			
32	QC 0.25			25							
33	12535-55C	0.211	0.082	25	120	2400		8.27E-03	7.41E-03	7.41	
34	12535-55C	0.204	0.076	25	120	2400		7.55E-03			
35	12535-55C	0.193	0.064	25	120	2400		6.42E-03			
36	12535-56G	0.207	0.078	25	120	2400		7.86E-03	7.48E-03	7.48	
37	12535-56G	0.207	0.078	25	120	2400		7.86E-03			
38	12535-56G	0.196	0.067	25	120	2400		6.75E-03			
39	12535-57C	0.227	0.098	25	120	2400		9.92E-03	8.41E-03	8.41	
40	12535-57C	0.199	0.070	25	120	2400		7.03E-03			
41	12535-57C	0.211	0.082	25	120	2400		8.27E-03			
42											
43											
44											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/6/2007	Test Chemical	CR11502	# Concentrations tested			8 Microsome ID	12535-13	
2		NB Ref	12535-58	Counter dilution	07MR0942.P00						
3		Technician ID	CKN	Replicate #	2	Positive Control		4-OH-ASDN	Pos Ctl Tubes/Conc	2	
4	Standards:	1.5	0.75	0.5	0.25	0.13	Blk	Protein stock (mg/10 mL)	Protein stock ID		
5		0.420	0.279	0.217	0.173	0.130	0.124	26.4			
6		0.423	0.338	0.214	0.199	0.162	0.126				
7		0.420	0.260	0.217	0.185	0.162	0.122				
8	Samples:	QC 1	QC 0.5	QC 0.25	12535-58C	12535-59G	12535-60C				
9		0.325	0.211	0.172	0.186	0.203	0.194				
10		0.279	0.232	0.169	0.191	0.203	0.200				
11					0.197	0.189	0.225				
12	mg Protein	μL Standard	Used	mg Protein Measured	A _{raw}	A _{avg}	Curve	Output	Variables	Regression results	
13	13	0.00158	25	0.0396	0.421	0.297	0.0393	0.134	m, b	0.000	
14	14	0.00106	25	0.0284	0.334	0.210	0.0276	0.005	s _{avg} , s _b	0.001	
15	15	0.00079	25	0.0198	0.270	0.146	0.0190	0.894	r ² , s _{e_y}	0.001	
16	16	0.00053	25	0.0132	0.216	0.092	0.0119	667	F, df	4	
17	17	0.00026	25	0.0066	0.186	0.062	0.0078	0.001	SS _{reg} , SS _{total}	0.000	
18	18	0.00014	25	0.0034	0.152	0.028	0.0034				
19											
20	Blank	0.124		r ² = 0.994							
21				m = 0.134							
22				b = 0.000							
23											
24	QC 1	A _{raw}	0.325	0.201	Vol usome prep. (μL)	Final vol. Diluted usomes (μL)	mg protein/μL Prep.	average mg/μL	mg/mL		
25	QC 1	0.279	0.155	25	1	1	1.06E-03	9.35E-04	0.94		
26	QC 1			25	1	1	8.12E-04				
27	QC 0.5	0.211	0.087	25	1	1	4.49E-04	5.05E-04	0.50		
28	QC 0.5	0.232	0.108	25	1	1	5.61E-04				
29	QC 0.5			25	1	1					
30	QC 0.25	0.172	0.048	25	1	1	2.40E-04	2.32E-04	0.23		
31	QC 0.25	0.169	0.046	25	1	1	2.24E-04				
32	QC 0.25			25	1	1					
33	12535-58C	0.186	0.062	25	120	2400	6.31E-03	6.88E-03	6.88		
34	12535-58C	0.191	0.067	25	120	2400	6.84E-03				
35	12535-58C	0.197	0.073	25	120	2400	7.48E-03				
36	12535-59G	0.203	0.079	25	120	2400	8.12E-03	7.98E-03	7.98		
37	12535-59G	0.203	0.079	25	120	2400	8.12E-03				
38	12535-59G	0.199	0.075	25	120	2400	7.70E-03				
39	12535-60C	0.194	0.070	25	120	2400	7.18E-03	8.48E-03	8.48		
40	12535-60C	0.200	0.076	25	120	2400	7.80E-03				
41	12535-60C	0.225	0.101	25	120	2400	1.05E-02				
42											
43											
44											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L
1			Assay Date	3/9/2007	Test	Chemical	CR11605	# Concentrations tested	8	Microsome ID	12535-13	
2			NB Ref	12535-64	Counter data	13MR0950 P00						
3			Technician ID	SUB	Replicate #	1	Positive Control	4-OH ASDN	Protein stock (mg/10 mL)	Pos Oil Tubes/Conc	2	
4	Standards:	1.5	1	0.75	0.5	0.25	0.13	Blk	25.7	Protein stock ID	12535-64	
5		0.383	0.312	0.252	0.226	0.181	0.135	0.110				
6		0.412	0.304	0.280	0.222	0.190	0.127	0.121				
7		0.371	0.318	0.259	0.227	0.165	0.150	0.109				
8	Samples:	QC.1	QC.0.5	QC.0.25	12535-64C	12535-65G	12535-66C	12535-94C				
9		0.297	0.228	0.178	0.200	0.195	0.199	0.188				
10		0.295	0.235	0.159	0.187	0.206	0.185	0.183				
11					0.199	0.192	0.214	0.193				
12	mg Protein		μL Standard		mg Protein		A _{max}	A _{avg}	Curve	Variables	Regression results	
13	per μL		Used		Measured				Output	m, b		
14	0.00154	25	25	0.0386	0.0386	0.369	0.275	0.275	0.0374	0.143	-0.002	
15	0.00103	25	25	0.0257	0.0257	0.311	0.198	0.198	0.0284	0.007	0.001	
16	0.00077	25	25	0.0193	0.0193	0.264	0.150	0.150	0.0195	0.991	0.001	
17	0.00051	25	25	0.0129	0.0129	0.225	0.112	0.112	0.0140	451	4	
18	0.00026	25	25	0.0064	0.0064	0.179	0.065	0.065	0.0074	0.001	0.000	
19	0.00013	25	25	0.0033	0.0033	0.137	0.024	0.024	0.0015			
20	Blank	0.113		r ² =	0.991					Regression results are calculated using the function		
21				m =	0.143					LINEST		
22				b =	-0.002							
23				mg protein measured	μL diluted μSOMES	Vol usome prep (μL)	Final vol. Diluted usomes (μL)		mg protein/μL Prep.	average mg/μL	mg/mL	
24	QC.1	A _{max}	A _{avg}	0.297	0.184	25	1	1	9.72E-04	9.88E-04	0.97	
25	QC.1	0.295	0.182	0.024	0.024	25	1	1	9.61E-04			
26	QC.1											
27	QC.0.5	0.228	0.115	0.014	0.014	25	1	1	5.77E-04	5.97E-04	0.60	
28	QC.0.5	0.235	0.122	0.015	0.015	25	1	1	6.17E-04			
29	QC.0.5											
30	QC.0.25	0.178	0.065	0.007	0.007	25	1	1	2.91E-04	2.37E-04	0.24	
31	QC.0.25	0.159	0.046	0.005	0.005	25	1	1	1.83E-04			
32	QC.0.25											
33	12535-64C	0.200	0.087	0.010	0.010	25	2400	2400	8.34E-03	7.81E-03	7.81	
34	12535-64C	0.187	0.074	0.009	0.009	25	2400	2400	8.86E-03			
35	12535-64C	0.199	0.088	0.010	0.010	25	2400	2400	8.23E-03			
36	12535-65G	0.195	0.082	0.010	0.010	25	2400	2400	7.77E-03	8.08E-03	8.08	
37	12535-65G	0.206	0.093	0.011	0.011	25	2400	2400	9.03E-03			
38	12535-65G	0.192	0.079	0.009	0.009	25	2400	2400	7.43E-03			
39	12535-66C	0.199	0.088	0.010	0.010	25	2400	2400	8.23E-03	8.27E-03	8.27	
40	12535-66C	0.185	0.072	0.008	0.008	25	2400	2400	6.83E-03			
41	12535-66C	0.214	0.101	0.012	0.012	25	2400	2400	9.94E-03			
42	12535-94C	0.188	0.075	0.009	0.009	25	2400	2400	6.97E-03	7.35E-03	7.35	
43	12535-94C	0.193	0.080	0.009	0.009	25	2400	2400	7.54E-03			
44	12535-94C	0.193	0.080	0.009	0.009	25	2400	2400	7.54E-03			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/13/2007	Test Chemical	CR11605	# Concentrations tested		8	Microsome ID	12535-13	
2		NB Ref	12535-67	Counter data file name	13MR2226 P00						
3		Technician ID	SLB	Replicate #	2	Positive Control		4-OH ASDN	Pos Ctl Tubes/Conc	2	
4	Standards:	1.5	0.75	0.5	0.25	0.13	BLK	Protein stock (mg/10 mL)	Protein stock ID		
5		0.401	0.280	0.214	0.166	0.128	0.128	25.6	12535-67		
6		0.397	0.281	0.214	0.175	0.127	0.132				
7		0.396	0.253	0.215	0.147	0.141	0.128				
8	Samples:	QC 0.5	QC 0.25	12535-67C	12535-68G	12535-68C					
9		0.327	0.226	0.202	0.209	0.194					
10		0.305	0.153	0.189	0.194	0.190					
11				0.195	-0.187	0.187					
12	mg Protein per μ L	μ L Standard		mg Protein Measured	A_{405}	A_{405}	Curve	Variables	Regression results		
13		Used					Output	m, b	0.133	0.001	
14	0.00154	25		0.0384		0.388	0.0373	$s_{a_{avg}}$, s_{a_b}	0.004	0.001	
15	0.00102	25		0.0256		0.317	0.0265	r^2 , s_{e_y}	0.996	0.001	
16	0.00077	25		0.0192		0.271	0.0204	F , df	894	4	
17	0.00051	25		0.0128		0.214	0.0128	$s_{s_{avg}}$, $s_{s_{std}}$	0.001	0.000	
18	0.00026	25		0.0064		0.163	0.0033				
19	0.00013	25		0.0033		0.141	0.012				
20		Blank	0.129	$r^2 =$	0.996						
21				$m =$	0.133						
22				$b =$	0.001						
23				mg protein measured	μ L diluted μ SOMES	Vol usome prep (μ L)	Final vol. Diluted usomes (μ L)	mg protein/ μ L Prep.	average mg/ μ L	mg/mL	
24	QC 1	A_{405}	0.198	0.028	25	1	1	1.11E-03	1.05E-03	1.05	
25	QC 1	0.305	0.176	0.025	25	1	1	9.94E-04			
26	QC 1										
27	QC 0.5	0.226	0.097	0.014	25	1	1	5.73E-04	5.79E-04	0.57	
28	QC 0.5	0.226	0.097	0.014	25	1	1	5.73E-04			
29	QC 0.5										
30	QC 0.25	0.185	0.056	0.009	25	1	1	3.54E-04	2.89E-04	0.27	
31	QC 0.25	0.153	0.024	0.005	25	1	1	1.93E-04			
32	QC 0.25										
33	12535-67C	0.202	0.073	0.011	25	120	2400	6.90E-03	8.19E-03	8.19	
34	12535-67C	0.189	0.060	0.009	25	120	2400	7.51E-03			
35	12535-67C	0.195	0.066	0.010	25	120	2400	8.15E-03			
36	12535-68G	0.209	0.080	0.012	25	120	2400	9.84E-03	8.33E-03	8.33	
37	12535-68G	0.194	0.065	0.010	25	120	2400	8.04E-03			
38	12535-68G	0.187	0.058	0.009	25	120	2400	7.30E-03			
39	12535-69C	0.194	0.065	0.010	25	120	2400	8.04E-03	8.01E-03	8.01	
40	12535-69C	0.190	0.061	0.010	25	120	2400	7.62E-03			
41	12535-69C	0.197	0.068	0.010	25	120	2400	8.36E-03			
42											
43											
44											

APPENDIX E

Aromatase Assay Spreadsheets

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
	Test # Concentrations									
1	Assay Date	2/22/2007	Chemical ID	CR11601	tested	8 Microsome ID 12535-13				
2	NB Ref.	12535-46	Counter data filename	23FE0046.P00						
	Technician								Pos Ctl	
3	ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2		

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/22/2007	Test Chemical ID	CR11601	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-46	Counter data filename	23FE0046.P00					
3	Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0213		39821		1869531			
8	2	0.0197		34576		1755127			
9	3	0.0194		35946		1852887			
10	4	0.0203		39447		1943202			
11	5	0.0193		37397		1937668			
12						Average DPM/g soln		1871683	
13						SD		76505	
14						CV		4.09	
15						µCi/g soln		0.8431	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.04	10		1004.00				
20	Dilution A			100	10.04				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution							8.1364 g	
25	Mass of dilution B used in substrate prep							4.5918 g	
26	Concentration of nonradiolabeled ASDN in substrate soln.							0.5666102 µg/g	
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.010 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8431			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					=	0.5666	+	0.010	
37					=	0.577	µg ASDN/g soln.		
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.461	µCi/µg ASDN			
41									
					9.292.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	2/22/2007	Test Chemical ID	CR11601	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-46	Counter data filename	23FE0046.P00					
3			Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctrl Tubes/Conc	2
4											
5											
6											
7											
8											
9											
10											
11											
12											
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25											
26											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Test # Concentrations Assay: 2/22/2007 Chemical ID CR tested 8 Microsome ID 12535-13										
2	Counter data NB F 12535-46 Hexane 23FE0046.P00										
3	Tec hnic ian ID SLB Replicate # 1 Positive Control 4-OH ASDI Tubes/Conc 2										
4											
5	Vial #	Time	DPM1				ISIE				Round DPM
6	2	5	39820.97	17549.09	0	11.97	483.71				39821
7	3	5	34575.74	15142.22	0	11.89	479.07				34576
8	4	5	35945.61	15714.74	0	11.78	474.83				35946
9	5	5	39447.43	17269.43	0	11.83	476.2				39447
10	6	5	37996.91	15425.12	0	11.83	478.36				37397
11	8	5	15129.13	7124.58	0	12.68	534.26				15129
12	9	5	15357.95	7214.99	0	12.68	534.04				15358
13	10	5	14923.88	6973.33	0	12.85	532.7				14924
14	11	5	15162.88	7102.88	0	12.71	534.14				15163
15	12	5	14304.83	6699.24	0	12.63	531.34				14305
16	13	5	14352.57	6781.99	0	12.63	535.43				14353
17	14	5	14196.5	6649.15	0	12.7	535.48				14197
18	15	5	14017.68	6590.04	0	12.67	533.62				14018
19	16	5	72.97	34.95	0	15.07	533.96				73
20	17	5	73.51	34.55	0	15.26	534.03				74
21	18	5	68.38	33.62	0	13.49	531.71				68
22	19	5	69.45	34.79	0	13.36	532.29				69
23	20	5	115.8	55.58	0	13.61	531.83				116
24	21	5	111.65	55.4	0	13.35	532.8				112
25	22	5	95.73	46.75	0	13.83	537.01				96
26	23	5	101.19	47.88	0	14.98	533.47				101
27	25	5	162.76	83.65	0	12.66	534.09				163
28	26	5	165.22	77.06	0	13.49	533.46				165
29	27	5	172.96	77.92	0	13.89	532.65				173
30	28	5	167.5	78.37	0	13.99	535.86				168
31											
32											
33	29	5	886.47	419.3	0	12.82	534.54				886
34	30	5	837.03	395.02	0	12.72	535.35				837
35	31	5	895.18	410.29	0	12.74	531.76				895
36	32	5	883.68	417.67	0	12.64	532.07				884
37											
38											
39	33	5	1601.31	749.52	0	12.89	532.52				1601
40	34	5	1584.88	742.02	0	12.66	532.67				1585
41	35	5	1513.74	707.88	0	12.63	529.81				1514
42	36	5	1524.12	713.79	0	12.57	533.02				1524
43											
44											
45	37	5	5294.97	2460.52	0	12.8	531.52				5295
46	38	5	5272.85	2477.36	0	12.57	534.51				5273
47	39	5	5383.21	2502.08	0	12.66	531.8				5363
48	40	5	5263.59	2488.53	0	12.6	532.61				5264
49											
50											
51	41	5	7661.1	3583.63	0	12.7	532.12				7661
52	42	5	7822.05	3672.8	0	12.84	532.8				7822
53	43	5	7730.8	3629.33	0	12.57	531.29				7731
54	44	5	7709.78	3634.14	0	12.66	534.01				7710
55											
56											
57	45	5	12199.55	5707.72	0	12.71	532.93				12200
58	46	5	12290.38	5805.76	0	12.65	535.84				12290
59	47	5	12049.95	5643.49	0	12.67	533.73				12050
60	48	5	11811.08	5551.71	0	12.7	534.18				11811
61											
62											
63	49	5	13477.38	6332.22	0	12.69	535.29				13477
64	50	5	13433.71	6317.96	0	12.66	533.14				13434
65	51	5	13725.09	6440.82	0	12.69	533.83				13725
66	52	5	12762.79	6022.68	0	12.64	534.95				12763
67											
68											
69	53	5	13889.53	6529.98	0	12.7	536.01				13890
70	54	5	13907.07	6533.13	0	12.81	532.28				13907
71	55	5	13580.63	6384.41	0	12.65	533.55				13581
72	56	5	13663.84	6394.89	0	12.69	532.51				13664
73											
74											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	12243.64	5733.16	0	12.86	530.23				12244
76	59	5	12359.38	5784.7	0	12.74	533.57				12359
77	60	5	12602.62	5889.11	0	12.71	532.98				12603
78	61	5	12657	5923.35	0	12.7	531.62				12657
79	62	5	12025.82	5657.08	0	12.69	534.78				12026
80	63	5	12081.7	5704.28	0	12.71	537.18				12092
81	64	5	12607.94	5884.83	0	12.68	531.43				12608
82	65	5	12821.7	6009.51	0	12.77	534.44				12822
83	66	5	12649.19	5955.91	0	12.65	535.04				12649
84	67	5	12678.55	5954.09	0	12.68	536.28				12677
85	68	5	13588.06	6386.47	0	12.7	534.08				13588
86	69	5	13616.05	6397.61	0	12.71	534.91				13616
87	70	5	13410.76	6281.55	0	12.89	531.56				13411
88	71	5	13343.17	6268.18	0	12.89	535.81				13343
89	72	5	13771.25	6494.96	0	12.75	536.95				13771
90	73	5	13914.27	6507.84	0	12.88	529.95				13914
91	74	5	14169.83	6627.42	0	12.69	532.98				14170
92	75	5	14028.48	6614.17	0	12.65	535.16				14028
93	76	5	13840.76	6473.19	0	12.68	532.16				13841
94	77	5	13707.35	6430.76	0	12.73	534.62				13707
95	78	5	13889.59	6490.83	0	12.62	531.55				13890
96	79	5	13929.36	6551.22	0	12.73	536.03				13929
97	80	5	14531.16	6810.01	0	12.66	533.95				14531
98	81	5	14615.72	6863.01	0	12.66	531.62				14616
99	83	5	14019.42	6590.15	0	12.66	535.05				14019
100	84	5	13987.81	6611.88	0	12.66	534.73				13988
101	85	5	13904.73	6528.71	0	12.65	533.35				13905
102	86	5	13935.32	6513.64	0	12.65	531.75				13935
103	87	5	13683.8	6426.12	0	12.7	534.21				13684
104	88	5	14530.65	6784.23	0	12.69	531.7				14531
105	89	5	14338.82	6711.21	0	12.68	534.05				14339
106	90	5	14516.9	6806.7	0	12.89	535.05				14517
107	91	5	14450.82	6782.9	0	12.88	532.46				14451
108	92	5	13809.14	6428.54	0	12.73	537.47				13809
109	93	5	14149.18	6636.33	0	12.63	530.5				14149
110	94	5	14408.98	6737.35	0	12.69	532.74				14409
111	95	5	14065.97	6806.2	0	12.72	536.29				14066
112	96	5	14246.24	6669.77	0	12.65	532.54				14246
113	97	5	14128.83	6644.74	0	12.64	534.83				14129
114	98	5	14396.89	6752.74	0	12.7	533.91				14397
115	99	5	13794.27	6461.55	0	12.57	529.83				13794
116	100	5	14018.64	6553.45	0	12.64	530.76				14019
117	101	5	13948.39	6606.45	0	12.84	536.38				13948
118	102	5	14061.42	6614.93	0	12.67	533.5				14061
119	103	5	14209.72	6686.82	0	12.6	531.98				14210
120	104	5	14051.72	6626.88	0	12.71	535.57				14052
121	105	5	13828.13	6496.33	0	12.88	532.83				13828
122	106	5	13855.83	6499.46	0	12.88	533.3				13856
123	107	5	236262.16	95347.5	0	11.59	439.72				236262
124	108	5	9.86	5.68	0	27.08	478.86				10
125											0
126											0
127											0
128											0

Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID		Replicate	Nominal total volume (mL)	Aliq. #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (cpm)	Total DPM corrected for background (background tubes)	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrone formed/mg protein/min)	
1	Full growth control	1	2	0.5	15129	30256	30437	60874	0.1	187168	60874	0.0052	0.005	15	0.438	
2	Full growth control	2	2	0.5	15359	30718	30881	61699	0.1	187168	61699	0.0054	0.005	15	0.459	
3	Full growth control	3	2	0.5	14924	29848	29887	59774	0.1	187168	59774	0.0051	0.005	15	0.409	
4	Full growth control	4	2	0.5	15033	30066	30088	60174	0.1	187168	60174	0.0052	0.005	15	0.419	
5	Full growth control	5	2	0.5	14853	29706	29858	59718	0.1	187168	59718	0.0051	0.005	15	0.402	
6	Background control	1	2	0.5	14192	28384	28215	56430	0.1	187168	56430	0.0043	0.005	15	0.000	
7	Background control	2	2	0.5	14016	28036	28147	56294	0.1	187168	56294	0.0042	0.005	15	0.000	
8	Background control	3	2	0.5	73	146	147	294	0.1	187168	294	-0.0001	0.005	15	-0.001	
9	Background control	4	2	0.5	68	136	137	274	0.1	187168	274	-0.0001	0.005	15	-0.001	
10	Background control	5	2	0.5	69	138	139	278	0.1	187168	278	-0.0001	0.005	15	-0.001	
11	4-OH ASDN	1	2	0.5	112	224	226	452	0.1	187168	452	0.0001	0.005	15	0.001	
12	4-OH ASDN	2	2	0.5	118	236	238	476	0.1	187168	476	0.0001	0.005	15	0.001	
13	4-OH ASDN	3	2	0.5	98	196	197	394	0.1	187168	394	0.0001	0.005	15	0.000	
14	4-OH ASDN	4	2	0.5	101	202	203	406	0.1	187168	406	0.0001	0.005	15	0.000	
15	4-OH ASDN	5	2	0.5	163	326	328	656	0.1	187168	656	0.0001	0.005	15	0.002	
16	4-OH ASDN	1	2	0.5	173	346	348	692	0.1	187168	692	0.0001	0.005	15	0.002	
17	4-OH ASDN	2	2	0.5	168	336	338	676	0.1	187168	676	0.0001	0.005	15	0.002	
18	4-OH ASDN	3	2	0.5	0	0	0	0	0.1	187168	0	-0.0004	0.005	15	-0.003	
19	4-OH ASDN	4	2	0.5	846	1692	1723	3446	0.1	187168	3446	0.0033	0.005	15	0.002	
20	4-OH ASDN	5	2	0.5	837	1674	1674	3348	0.1	187168	3348	0.0032	0.005	15	0.002	
21	4-OH ASDN	1	2	0.5	895	1790	1779	3558	0.1	187168	3558	0.0034	0.005	15	0.023	
22	4-OH ASDN	2	2	0.5	864	1728	1728	3456	0.1	187168	3456	0.0032	0.005	15	0.003	
23	4-OH ASDN	3	2	0.5	0	0	0	0	0.1	187168	0	-0.0004	0.005	15	-0.003	
24	4-OH ASDN	4	2	0.5	1460	2920	2920	5840	0.1	187168	5840	0.0043	0.005	15	0.043	
25	4-OH ASDN	5	2	0.5	1596	3192	3186	6372	0.1	187168	6372	0.0043	0.005	15	0.043	
26	4-OH ASDN	1	2	0.5	1701	3402	3399	6798	0.1	187168	6798	0.0043	0.005	15	0.043	
27	4-OH ASDN	2	2	0.5	1614	3228	3208	6416	0.1	187168	6416	0.0043	0.005	15	0.041	
28	4-OH ASDN	3	2	0.5	0	0	0	0	0.1	187168	0	-0.0004	0.005	15	-0.003	
29	4-OH ASDN	4	2	0.5	5285	10569	10569	21138	0.1	187168	21138	0.0054	0.005	15	0.003	
30	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
31	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
32	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
33	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
34	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
35	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
36	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
37	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
38	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
39	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
40	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
41	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
42	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
43	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
44	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
45	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
46	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
47	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
48	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
49	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
50	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
51	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
52	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
53	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
54	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
55	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
56	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
57	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
58	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
59	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
60	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
61	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
62	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
63	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
64	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
65	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
66	4-OH ASDN	1	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
67	4-OH ASDN	2	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
68	4-OH ASDN	3	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
69	4-OH ASDN	4	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	
70	4-OH ASDN	5	2	0.5	5273	10546	10546	21092	0.1	187168	21092	0.0054	0.005	15	0.003	

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot volumes			
6		Read if value not within mean $\pm$ 10%			
7		0.99			
8		0.99			
9		0.99			
10		1.01			
11		1.00			
12		1.00			
13		1.01			
14		0.99			
15		0.99			
16		1.01			
17		0.99			
18		1.01			
19		1.02			
20		0.99			
21		0.99			
22		1.03			
23		0.99			
24		1.01			
25		1.01			
26		0.99			
27		#DIV/0!			
28		#DIV/0!			
29		1.03			
30		0.97			
31		0.97			
32		0.99			
33		#DIV/0!			
34		#DIV/0!			
35		1.01			
36		0.99			
37		1.00			
38		1.00			
39		#DIV/0!			
40		#DIV/0!			
41		1.00			
42		1.00			
43		0.99			
44		#DIV/0!			
45		#DIV/0!			
46		0.99			
47		1.01			
48		1.00			
49		1.00			
50		1.00			
51		#DIV/0!			
52		#DIV/0!			
53		1.00			
54		1.00			
55		1.00			
56		0.99			
57		#DIV/0!			
58		#DIV/0!			
59		1.00			
60		1.00			
61		1.04			
62		0.96			
63		#DIV/0!			
64		#DIV/0!			
65		1.00			
66		1.00			
67		1.00			
68		1.00			
69		#DIV/0!			
70		#DIV/0!			
71		#DIV/0!			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	2/22/2007	Test Chemical	CR11601	# Concentrations tested		12535-13								
2		N/B Ref.	12535-46	Conc. data	28FE0046.P00			8	Monomer ID							
3		Technician ID	SLB	Replicate #	1	Positive Control		4-OH ASDN	Pos Ctrl Tubes/Conc							
4																
5	Sample ID				Calculate DPM in aqueous portion after extraction											
6	Sample type	Regalipal	Nominal total volume (mL)	Aq. volume (mL)	Aq. #	DPM/mg	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (net)	Total DPM corrected for background (Background Tubes)	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrone formed/mg protein/min)
7	CR11601	1-1	2	0.5	1	12244	24485	24803	49206	0.1	187168	48352	0.0526	0.005	15	0.350
8		1-2	2	0.5	1	12659	24718	50520		0.1	187168	50165	0.0540	0.003	15	0.360
9		1-3	2	0.5	2	12603	25206	25760	48738	0.1	187168	47802	0.0515	0.005	15	0.344
10		2-1	2	0.5	1	12692	24184	29430	50460	0.1	187168	50506	0.0544	0.005	15	0.362
11		2-2	2	0.5	2	12602	25544	26326	50682	0.1	187168	50288	0.0541	0.005	15	0.361
12		2-3	2	0.5	1	13560	27232	27204	54408	0.1	187168	54054	0.0562	0.005	15	0.389
13		3-1	2	0.5	1	13411	26522	26754	53508	0.1	187168	53154	0.0572	0.005	15	0.381
14		3-2	2	0.5	2	13343	26686	27685	55310	0.1	187168	55018	0.0592	0.005	15	0.395
15		3-3	2	0.5	1	13914	27628	28168	55596	0.1	187168	58042	0.0603	0.005	15	0.402
16		4-1	2	0.5	1	13841	27092	27548	55098	0.1	187168	54742	0.0589	0.005	15	0.393
17		4-2	2	0.5	2	13707	27414	27619	55638	0.1	187168	55264	0.0595	0.005	15	0.397
18		4-3	2	0.5	1	13529	27958	28147	56394	0.1	187168	57940	0.0624	0.005	15	0.416
19		5-1	2	0.5	1	14019	28038	28007	56014	0.1	187168	55660	0.0599	0.005	15	0.399
20		5-2	2	0.5	1	13905	27810	27840	55850	0.1	187168	55326	0.0595	0.005	15	0.397
21		5-3	2	0.5	1	13684	27468	28215	55430	0.1	187168	56076	0.0603	0.005	15	0.402
22		6-1	2	0.5	2	14531	29062	29036	57719	0.1	187168	57558	0.0617	0.005	15	0.412
23		6-2	2	0.5	1	14517	29034	29050	58120	0.1	187168	57906	0.0630	0.005	15	0.400
24		6-3	2	0.5	2	14451	28902	28558	57116	0.1	187168	56762	0.0611	0.005	15	0.407
25		7-1	2	0.5	1	14109	28298	28312	56624	0.1	187168	56270	0.0606	0.005	15	0.404
26		7-2	2	0.5	2	14128	28248	28526	57052	0.1	187168	56698	0.0610	0.005	15	0.407
27		7-3	2	0.5	1	13907	28794	27813	55526	0.1	187168	55272	0.0595	0.005	15	0.397
28		8-1	2	0.5	1	13948	27886	28009	55918	0.1	187168	54664	0.0589	0.005	15	0.399
29		8-2	2	0.5	2	14201	28420	28262	55924	0.1	187168	56170	0.0604	0.005	15	0.403
30		8-3	2	0.5	1	13928	27656	27684	55368	0.1	187168	53914	0.0582	0.005	15	0.395

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC check - aliquot variance
5		
6		% RE
7		1.00
8		1.00
9		1.00
10		1.00
11		1.00
12		1.00
13		0.99
14		1.01
15		1.00
16		1.00
17		1.00
18		1.00
19		1.00
20		1.00
21		0.99
22		1.01
23		1.01
24		0.98
25		1.00
26		1.00
27		1.00
28		1.00
29		1.00
30		1.00
31		1.00
32		1.00
33		1.00
34		1.00
35		0.97
36		1.03
37		0.96
38		1.01
39		1.03
40		0.97
41		0.98
42		1.01
43		0.99
44		1.01
45		0.99
46		1.01
47		0.99
48		1.01
49		1.00
50		1.00
51		1.01
52		0.99
53		1.00
54		1.00

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L
1			Assay Date	2/22/2007	Test Chemical ID	CR11601				8 Microsome ID	12535-13	
2			NP Ref	12535-46	Counter data filename	23F-E0046.P00						
3			Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN		Pos Oil Tubes/Conc	2	
4												
5	Control Type	Portion	Average	SD								
6	Full activity	Overall	0.419	0.016		100% % of Full						
7	Background (uncorrected)	Overall	0.003	0.001			0.6 (Rad if >1%)					
8												
9												
10	Positive Control	Level	Replicate	Positive Ctrl M	Log(Positive Ctrl)	Activity	Percent of control values					
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.002	Level	Log(Pos Ctrl)	1	Replicate	2	
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002	1	-5.00	0.5	0.6		
13							2	-6.00	5.3	5.5		
14	4-OH ASDN	2	1	1.00E-06	-6.00	0.022	3	-6.30	10.3	9.8		
15	4-OH ASDN	2	2	1.00E-06	-6.00	0.023	4	-7.00	35.6	35.8		
16							5	-7.30	82.4	82.3		
17	4-OH ASDN	3	1	5.00E-07	-6.30	0.043	6	-8.00	83.3	80.2		
18	4-OH ASDN	3	2	5.00E-07	-6.30	0.041	7	-8.00	91.6	92.7		
19							8	-10.00	94.6			
20	4-OH ASDN	4	1	1.00E-07	-7.00	0.149						
21	4-OH ASDN	4	2	1.00E-07	-7.00	0.150						
22												
23	4-OH ASDN	5	1	5.00E-08	-7.30	0.220						
24	4-OH ASDN	5	2	5.00E-08	-7.30	0.219						
25												
26	4-OH ASDN	6	1	1.00E-08	-8.00	0.349						
27	4-OH ASDN	6	2	1.00E-08	-8.00	0.340						
28												
29	4-OH ASDN	7	1	1.00E-09	-9.00	0.384						
30	4-OH ASDN	7	2	1.00E-09	-9.00	0.378						
31												
32	4-OH ASDN	8	1	1.00E-10	-10.00	0.395						
33	4-OH ASDN	8	2	1.00E-10	-10.00	0.398						
34												
35												
36												
37	Test Chemical	Level	Replicate	Test chemical M	Log(test chemical)	Activity	Percent of control values					
38	CR11601	1	1	1.00E-03	-3.00	0.350	Level	Log(test chemical)	1	Replicate	2	3
39	CR11601	1	2	1.00E-03	-3.00	0.360	1	-3.00	83.7	85.9		
40	CR11601	1	3	1.00E-03	-3.00	0.344	2	-4.00	86.5	92.6		
41	CR11601	2	1	1.00E-04	-4.00	0.362	3	-5.00	91.1	94.3		
42	CR11601	2	2	1.00E-04	-4.00	0.361	4	-6.00	93.8	94.7		
43	CR11601	2	3	1.00E-04	-4.00	0.388	5	-7.00	95.4	94.8		
44	CR11601	3	1	1.00E-05	-5.00	0.381	6	-8.00	96.3	95.5		
45	CR11601	3	2	1.00E-05	-5.00	0.395	7	-9.00	97.1	94.7		
46	CR11601	3	3	1.00E-05	-5.00	0.402	8	-10.00	95.4	96.2		
47	CR11601	4	1	1.00E-06	-6.00	0.393						
48	CR11601	4	2	1.00E-06	-6.00	0.397						
49	CR11601	4	3	1.00E-06	-6.00	0.416						
50	CR11601	5	1	1.00E-07	-7.00	0.399						
51	CR11601	5	2	1.00E-07	-7.00	0.397						
52	CR11601	5	3	1.00E-07	-7.00	0.402						
53	CR11601	6	1	1.00E-08	-8.00	0.412						
54	CR11601	6	2	1.00E-08	-8.00	0.400						
55	CR11601	6	3	1.00E-08	-8.00	0.407						
56	CR11601	7	1	1.00E-09	-9.00	0.404						
57	CR11601	7	2	1.00E-09	-9.00	0.407						
58	CR11601	7	3	1.00E-09	-9.00	0.397						
59	CR11601	8	1	1.00E-10	-10.00	0.399						
60	CR11601	8	2	1.00E-10	-10.00	0.403						
61	CR11601	8	3	1.00E-10	-10.00	0.395						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/27/2007	Test	Chemical ID	CR11601	# Concentrations	tested	8	Microsome ID 12535-13
2	NB Ref.	12535-49	Counter data filename	27FE2319.P00					
3	Technician ID	SLB	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/27/2007	Test Chemical ID	CR11601	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-49	Counter data filename	27FE2319.P00					
3	Technician ID	SLB	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0207		37096		1792077			
8	2	0.0197		33626		1706904			
9	3	0.0171		35988		2104561			
10	4	0.2020		39666		196366			
11	5	0.0197		37751		1916294			
12						Average DPM/g soln		1543241	
13						SD		767630	
14						CV		49.74	
15						µCi/g soln		0.6952	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1085 g			
25	Mass of dilution B used in substrate prep					4.5560 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56131763 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.008 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.6952			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5613	+	0.008		
37				=	0.570	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=			1.220 µCi/µg ASDN					
41				7.757.E+05 dpm/nmol					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	2/27/2007	Test Chemical ID	CR11601	# Concentrations tested		8	Microsome ID	12535-13
2		NB Ref	12535-49	Counter data filename	27FE2319.P00					
3		Technician ID	SLB	Replicate #	2	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4										
5	Microsome Dilution Details									
6										
7	Dilution A	0.12 mL microsome Stock used							Positive Control Concentrations	
8		2.4 mL total volume							Level	
9		20 dilution factor							1	1.00E-05
10									2	1.00E-06
11	Dilution B	1.5 mL microsome Dilution A used							3	5.00E-07
12		60 mL total volume							4	1.00E-07
13		40 dilution factor							5	5.00E-08
14									6	1.00E-08
15	Dilution C (if applicable)	mL microsome Dilution B used							7	1.00E-09
16		mL total volume							8	1.00E-10
17		dilution factor								
18	NA									
19		800 total dilution factor							Test Chemical Concentrations	
20									Level	
21	Protein Concentration (stock microsomes, mg/mL):			8.00					1	1.00E-04
22	Protein Concentration (dilution added to assay, mg/mL):			0.01					2	1.00E-05
23									3	1.00E-06
24									4	5.00E-07
25									5	1.00E-07
26									6	1.00E-08
									7	1.00E-09
									8	1.00E-10

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Assay Date	2/27/2007	Test Chemical ID	CR11601	# Concentrations tested	8	Microsome ID	12535-13		
2	NB Ref.	12535-49	Counter data filename	27FE2319.F00						
3	Technician	SLB	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2		
4										
5	Vial #	Time (min)	DPM1			tSIE				Round DPM
6	2	5	37096.39	16939.46	0	12.23	508.71			37096
7	3	5	33826	14935.48	0	12	488.8			33626
8	4	5	35988.29	18306.9	0	13.83	621			35988
9	5	5	39668.18	20071.18	0	13.77	614.95			39666
10	6	5	37751.3	16550.55	0	11.83	478.45			37751
11	8	5	13710.34	6430.42	0	12.7	535.27			13710
12	9	5	14018.03	8583.71	0	12.67	535.69			14018
13	10	5	13193.25	6222.71	0	12.75	537.43			13193
14	11	5	12999.79	8161.09	0	12.67	537.83			13000
15	12	5	11410.31	5368.67	0	12.64	534.94			11410
16	13	5	11385.3	5372.25	0	12.65	538.73			11385
17	14	5	10343	4908.25	0	12.72	539.44			10343
18	15	5	10527.89	4983.11	0	12.67	537.02			10528
19	16	5	55.84	24.36	0	14.24	535.23			56
20	17	5	76.49	34.29	0	14.03	538.22			76
21	18	5	87.67	40.27	0	13.57	531.51			88
22	19	5	83.76	38.73	0	14.28	535.86			84
23	20	5	62.78	29.73	0	13.28	535.07			63
24	21	5	82.69	29.35	0	14.22	534.56			83
25	22	5	114.67	50.7	0	14.17	533.85			115
26	23	5	56.98	26.83	0	15.02	559.55			57
27	25	5	163.26	72.37	0	14.08	536.14			163
28	26	5	161.85	77.84	0	12.83	534.7			162
29	27	5	823.51	388	0	12.5	535.62			824
30	28	5	832.53	399.51	0	12.57	535.41			833
31	29	5	1567.67	743.14	0	12.82	537.78			1568
32	30	5	1477.48	701.43	0	12.54	536.37			1477
33	31	5	4826.08	2257.48	0	12.78	534.71			4827
34	32	5	4837.59	2288.73	0	12.73	538.68			4838
35	33	5	7418.42	3493	0	12.74	536.1			7418
36	34	5	7468.33	3521.72	0	12.72	538.77			7468
37	35	5	11255.11	5319.83	0	12.75	541.35			11255
38	36	5	11280.22	5362.13	0	12.69	540.72			11280
39	37	5	13131.4	6173.88	0	12.64	532.78			13131
40	38	5	13246.95	6216.36	0	12.74	536.29			13247
41	39	5	12648.99	5971.31	0	12.63	533.25			12649
42	40	5	12783.18	6012.03	0	12.67	535.86			12783
43	41	5	154.74	71.58	0	13.31	535.04			155
44	42	5	152.78	68.51	0	14.11	537.22			153
45	43	5	866.53	409.03	0	12.86	537.02			867
46	44	5	851.57	401.93	0	12.84	539.25			852
47	45	5	1405.41	659.89	0	12.96	535.92			1405
48	46	5	1380.09	650.76	0	12.5	534.13			1380
49	47	5	4636.38	2178.93	0	12.69	538.68			4636
50	48	5	4700.89	2233.84	0	12.66	537.61			4701
51	49	5	6721.11	3164.87	0	12.65	538.08			6721
52	50	5	6794.76	3214.09	0	12.68	536.7			6795
53	51	5	9446.21	4454.93	0	12.68	538.08			9446
54	52	5	9412.87	4452.21	0	12.6	535.04			9413
55	53	5	12282.54	5834.55	0	12.6	538.78			12283
56	54	5	12192.04	5779.08	0	12.61	535.7			12192
57	55	5	12540.38	5927.37	0	12.7	537.82			12540
58	56	5	12661.48	5995.51	0	12.67	538.57			12661
59	58	5	13004.75	6131.85	0	12.73	536.43			13005
60	59	5	12755.36	6032.98	0	12.76	539.25			12755
61	60	5	12637.8	5962.76	0	12.61	535.22			12638
62	61	5	12687.31	5968.12	0	12.76	536.45			12687
63	62	5	10797.83	5108.81	0	12.71	536.22			10798
64	63	5	10712.44	5070.95	0	12.7	535.83			10712
65	64	5	13294.18	6218.72	0	12.71	535.23			13294
66	65	5	13341.95	6318.51	0	12.68	538.2			13342
67	66	5	13486.41	6384.12	0	12.71	537.51			13486
68	67	5	13374.78	6310.83	0	12.57	536.75			13375
69	68	5	11509.82	5409.65	0	12.74	536.48			11510
70	69	5	11459.47	5399.23	0	12.67	536			11459
71	70	5	13172.07	6252.17	0	12.68	539.01			13172
72	71	5	13428.44	6314.94	0	12.73	538.58			13429
73	72	5	12286.51	5770.99	0	12.66	536.41			12287
74	73	5	12316.86	5802.41	0	12.69	535.95			12317
75	74	5	11293.89	5345.2	0	12.72	538.16			11294
76	75	5	11293.27	5317.98	0	12.64	535.16			11299
77	76	5	5234.46	2555.06	0	13.01	567.67			5234
78	77	5	0	0	0	0	610.54			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
78	78	5	11739.09	5529.78	0	12.63	537.11				11739
80	79	5	11785.66	5576.16	0	12.65	536.99				11786
81	80	5	11857.35	5612.07	0	12.74	537.73				11857
82	81	5	11799.4	5554.82	0	12.63	535.77				11799
83	83	5	12862.9	6062.31	0	12.74	537.43				12863
84	84	5	13472.46	6369.17	0	12.69	539.15				13472
85	85	5	11205.12	5291.92	0	12.64	535.07				11205
86	86	5	11688.85	5527.65	0	12.72	536.82				11689
87	87	5	11082.81	5247.52	0	12.68	536.24				11083
88	88	5	11183.45	5246.61	0	12.75	536.72				11183
89	89	5	13133.67	6209.74	0	12.7	538.31				13134
90	90	5	13292.93	6246.05	0	12.67	536.54				13293
91	91	5	11913.31	5629.57	0	12.62	532.93				11913
92	92	5	11924.98	5645.32	0	12.69	536.65				11925
93	93	5	11329.01	5372.15	0	12.59	535.75				11329
94	94	5	11457.73	5397	0	12.66	535.72				11458
95	95	5	13585.57	6378.85	0	12.65	534.02				13586
96	96	5	13660.01	6378.66	0	12.66	531.99				13660
97	97	5	11141.49	5231.6	0	12.7	535.16				11141
98	98	5	11157.86	5227.35	0	12.73	535.54				11158
99	99	5	11634.31	5478.04	0	12.68	533.79				11634
100	100	5	11662.58	5499.44	0	12.59	535.06				11663
101	101	5	12964.19	6107.33	0	12.73	535.17				12964
102	102	5	12943.75	6032.26	0	12.64	534.21				12944
103	103	5	11216.46	5322.72	0	12.72	537.41				11216
104	104	5	11236.7	5327.12	0	12.71	541.04				11237
105	105	5	12933.33	6114.3	0	12.66	535.43				12933
106	106	5	13064.57	6195.6	0	12.64	535.08				13065
107	107	5	236938.41	95146.18	0	11.55	436.84				236938
108	108	5	5.08	0	0	48.05	478.65				5
109											0
110											0
111											0
112											0
113											0
114											0
115											0
116											0
117											0
118											0
119											0
120											0
121											0
122											0
123											0

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P						
Sample ID	Sample type	Fractional solvent volume (mL)	Aliq. #	Test Chemical ID	Assay Date	20272007	12835-49	NIB Ref. Technician ID	# Concentrations tested		8	Micrograms ID	12935-13								
									NIB Ref. Technician ID	SLR							2	Positive Control	4-OH-ASON	Pos Ctrl Tubes/Conc	2
Calculate DPM in aqueous portion after extraction																					
Calculate nmol ³ H ₂ O formed																					
Total DPM conversion factor																					
nmol ³ H ₂ O formed																					
Final [protein] in assay																					
Incubation time (min)																					
Aromatase activity (nmol isoprenol formed per mg protein)																					
0.474																					

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance Rec'd value not within mean $\pm$ 10%			
6					
7		0.99			
8		1.01			
9		0.91			
10		0.99		Background activity uncorrected for background	
11		1.00			
12		1.00			
13		0.99			
14		1.01		netol $^3\text{H}_2\text{O}$ formed	Req activity
15				3.40E-04	0.002
16					
17		1.02		4.43E-04	0.003
18		0.98			
19		1.00		3.75E-04	0.002
20		1.00			
21		1.00		5.90E-04	0.004
22		1.00			
23		1.00			
24		1.00			
25		1.00			
26		1.01			
27		0.99			
28					
29		0.99			
30		1.01			
31		1.01			
32		0.99			
33					
34		1.04			
35					
36		0.98			
37		1.01			
38		0.99			
39					
40					
41		1.00			
42		1.00			
43		0.99			
44		1.01			
45					
46		1.00			
47					
48		1.00			
49		0.99			
50		1.01			
51					
52					
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57					
58		1.00			
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63					
64					
65		0.99			
66		1.01			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Sample type	Assay Date	NB Ref	Test Chemical ID	GR11601	B Concentrations tested		8-Microsome ID	12535-13						
						2772007	277E2319-P00								
1															
2															
3															
4															
5															
Sample ID	Replicates/Level	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	
6	CR11601	1-4	2	0.5	13005	26010	25162	51520	0.1	154324	51191	0.0560	0.005	15	0.440
7	CR11601	1-4	2	0.5	12750	25510	50670	0.1	154324	50341	0.0549	0.005	15	0.433	
8	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
9	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
10	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
11	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
12	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
13	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
14	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
15	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
16	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
17	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
18	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
19	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
20	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
21	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
22	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
23	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
24	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
25	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
26	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
27	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
28	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
29	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
30	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
31	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
32	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
33	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
34	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
35	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
36	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
37	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
38	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
39	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
40	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
41	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
42	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
43	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
44	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
45	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
46	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
47	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
48	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
49	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
50	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
51	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
52	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433
53	CR11601	1-4	2	0.5	12638	25276	25035	50670	0.1	154324	50341	0.0549	0.005	15	0.433

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC Check - aliquot variance
6		% RE
7		1.01
8		0.99
9		1.00
10		1.00
11		1.00
12		1.00
13		1.00
14		1.00
15		1.00
16		1.00
17		1.00
18		1.00
19		0.99
20		1.01
21		1.00
22		1.00
23		1.00
24		1.00
25		1.00
26		#VALUE!
27		1.00
28		1.00
29		1.00
30		1.00
31		0.99
32		1.00
33		0.99
34		1.02
35		1.00
36		1.00
37		0.99
38		1.01
39		1.00
40		1.00
41		0.99
42		1.01
43		1.00
44		1.00
45		1.00
46		1.00
47		1.00
48		1.00
49		1.00
50		1.00
51		1.00
52		1.00
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	2/27/2007	Test Chemical ID	GR11601							
2	NIS Ref	12535-49	Center data filename	27FE2319.P00							
3	Technician ID	SLB	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc			12535-13	
4	Control Type	Portion	SD								
5	Full activity	Overall	0.417		Bkg % of Full						
6	Background (uncorrected)	Overall	0.003			0.7 (Red if >1%)					
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.003						
12	4-OH ASDN	1	2	-5.00	0.002						
13	4-OH ASDN	2	1	-6.00	0.026						
14	4-OH ASDN	2	2	-6.00	0.027						
15	4-OH ASDN	3	1	-6.30	0.050						
16	4-OH ASDN	3	2	-6.30	0.045						
17	4-OH ASDN	4	1	-7.00	0.163						
18	4-OH ASDN	4	2	-7.00	0.156						
19	4-OH ASDN	5	1	-7.30	0.253						
20	4-OH ASDN	5	2	-7.30	0.229						
21	4-OH ASDN	6	1	-8.00	0.385						
22	4-OH ASDN	6	2	-8.00	0.321						
23	4-OH ASDN	7	1	-9.00	0.451						
24	4-OH ASDN	7	2	-9.00	0.418						
25	4-OH ASDN	8	1	-10.00	0.434						
26	4-OH ASDN	8	2	-10.00	0.430						
27											
28											
29											
30											
31											
32											
33											
34											
35											
36	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
37	GR11601	1	1	-4.00	0.440						
38	GR11601	1	2	-4.00	0.433						
39	GR11601	2	1	-5.00	0.367						
40	GR11601	2	2	-5.00	0.455						
41	GR11601	3	1	-5.00	0.459						
42	GR11601	3	2	-5.00	0.392						
43	GR11601	4	1	-6.00	0.454						
44	GR11601	4	2	-6.00	0.420						
45	GR11601	5	1	-6.00	0.386						
46	GR11601	5	2	-6.00	0.402						
47	GR11601	6	1	-6.30	0.447						
48	GR11601	6	2	-6.30	0.404						
49	GR11601	7	1	-7.00	0.391						
50	GR11601	7	2	-7.00	0.450						
51	GR11601	8	1	-7.00	0.380						
52	GR11601	8	2	-7.00	0.451						
53	GR11601	9	1	-8.00	0.407						
54	GR11601	9	2	-8.00	0.389						
55	GR11601	10	1	-9.00	0.465						
56	GR11601	10	2	-9.00	0.390						
57	GR11601	11	1	-9.00	0.388						
58	GR11601	11	2	-9.00	0.441						
59	GR11601	12	1	-10.00	0.383						
60	GR11601	12	2	-10.00	0.444						
61	GR11601	13	1	-10.00	0.444						
62	GR11601	13	2	-10.00	0.444						
63	GR11601	14	1	-10.00	0.444						
64	GR11601	14	2	-10.00	0.444						
65	GR11601	15	1	-10.00	0.444						
66	GR11601	15	2	-10.00	0.444						
67	GR11601	16	1	-10.00	0.444						
68	GR11601	16	2	-10.00	0.444						
69	GR11601	17	1	-10.00	0.444						
70	GR11601	17	2	-10.00	0.444						
71	GR11601	18	1	-10.00	0.444						
72	GR11601	18	2	-10.00	0.444						
73	GR11601	19	1	-10.00	0.444						
74	GR11601	19	2	-10.00	0.444						
75	GR11601	20	1	-10.00	0.444						
76	GR11601	20	2	-10.00	0.444						
77	GR11601	21	1	-10.00	0.444						
78	GR11601	21	2	-10.00	0.444						
79	GR11601	22	1	-10.00	0.444						
80	GR11601	22	2	-10.00	0.444						
81	GR11601	23	1	-10.00	0.444						
82	GR11601	23	2	-10.00	0.444						
83	GR11601	24	1	-10.00	0.444						
84	GR11601	24	2	-10.00	0.444						
85	GR11601	25	1	-10.00	0.444						
86	GR11601	25	2	-10.00	0.444						
87	GR11601	26	1	-10.00	0.444						
88	GR11601	26	2	-10.00	0.444						
89	GR11601	27	1	-10.00	0.444						
90	GR11601	27	2	-10.00	0.444						
91	GR11601	28	1	-10.00	0.444						
92	GR11601	28	2	-10.00	0.444						
93	GR11601	29	1	-10.00	0.444						
94	GR11601	29	2	-10.00	0.444						
95	GR11601	30	1	-10.00	0.444						
96	GR11601	30	2	-10.00	0.444						
97	GR11601	31	1	-10.00	0.444						
98	GR11601	31	2	-10.00	0.444						
99	GR11601	32	1	-10.00	0.444						
100	GR11601	32	2	-10.00	0.444						
101	GR11601	33	1	-10.00	0.444						
102	GR11601	33	2	-10.00	0.444						
103	GR11601	34	1	-10.00	0.444						
104	GR11601	34	2	-10.00	0.444						
105	GR11601	35	1	-10.00	0.444						
106	GR11601	35	2	-10.00	0.444						
107	GR11601	36	1	-10.00	0.444						
108	GR11601	36	2	-10.00	0.444						
109	GR11601	37	1	-10.00	0.444						
110	GR11601	37	2	-10.00	0.444						
111	GR11601	38	1	-10.00	0.444						
112	GR11601	38	2	-10.00	0.444						
113	GR11601	39	1	-10.00	0.444						
114	GR11601	39	2	-10.00	0.444						
115	GR11601	40	1	-10.00	0.444						
116	GR11601	40	2	-10.00	0.444						
117	GR11601	41	1	-10.00	0.444						
118	GR11601	41	2	-10.00	0.444						
119	GR11601	42	1	-10.00	0.444						
120	GR11601	42	2	-10.00	0.444						
121	GR11601	43	1	-10.00	0.444						
122	GR11601	43	2	-10.00	0.444						
123	GR11601	44	1	-10.00	0.444						
124	GR11601	44	2	-10.00	0.444						
125	GR11601	45	1	-10.00	0.444						
126	GR11601	45	2	-10.00	0.444						
127	GR11601	46	1	-10.00	0.444						
128	GR11601	46	2	-10.00	0.444						
129	GR11601	47	1	-10.00	0.444						
130	GR11601	47	2	-10.00	0.444						
131	GR11601	48	1	-10.00	0.444						
132	GR11601	48	2	-10.00	0.444						
133	GR11601	49	1	-10.00	0.444						
134	GR11601	49	2	-10.00	0.444						
135	GR11601	50	1	-10.00	0.444						
136	GR11601	50	2	-10.00	0.444						
137	GR11601	51	1	-10.00	0.444						
138	GR11601	51	2	-10.00	0.444						
139	GR11601	52	1	-10.00	0.444						
140	GR11601	52	2	-10.00	0.444						
141	GR11601	53	1	-10.00	0.444						
142	GR11601	53	2	-10.00	0.444						
143	GR11601	54	1	-10.00	0.444						
144	GR11601	54	2	-10.00	0.444						
145	GR11601	55	1	-10.00	0.444						
146	GR11601	55	2	-10.00	0.444						
147	GR11601	56	1	-10.00	0.444						
148	GR11601	56	2	-10.00	0.444						
149	GR11601	57	1	-10.00	0.444						
150	GR11601	57	2	-10.00	0.444						
151	GR11601	58	1	-10.00	0.444						
152	GR11601	58	2	-10.00	0.444						
153	GR11601	59	1	-10.00	0.444						
154	GR11601	59	2	-10.00	0.444						
155	GR11601	60	1	-10.00	0.444						
156	GR11601	60	2								

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
	Test # Concentrations								
1	Assay Date	2/28/2007	Chemical ID	CR11601		tested	8 Microsome ID 12535-13		
2	NB Ref.	12535-52	Counter data filename	28FE2337.P00					
	Technician		Pos Ctl						
3	ID	SLB	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/28/2007	Test Chemical ID	CR11601	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-52	Counter data filename	28FE2337.P00					
3	Technician ID	SLB	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0204		39020		1912745			
8	2	0.0200		35254		1762700			
9	3	0.0201		38321		1906517			
10	4	0.0202		39135		1937376			
11	5	0.0193		41765		2163990			
12						Average DPM/g soln		1936666	
13						SD		144414	
14						CV		7.46	
15						µCi/g soln		0.8724	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.00	10		1000.00				
20	Dilution A			100	10.00				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1386 g			
25	Mass of dilution B used in substrate prep					4.5818 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56297152 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8724			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					=	0.5630	+	0.011	
37					=	0.574	µg ASDN/g soln.		
38	3) Calculate Solution Specific Activity								
39					=	(µCi/g soln.)/(µg ASDN/g soln.)			
40					=	1.521	µCi/µg ASDN		
41						9.670.E+05	dpm/nmol		

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	2/28/2007	Test Chemical ID	CR11601	# Concentrations tested			8 Microsome ID	12535-13
2		NB Ref	12535-52	Counter data filename	28FE2337.P00					
3		Technician ID	SLB	Replicate #	3	Positive Control	4-OH ASDN		Pos Ctrl Tubes/Conc	2
4										
5	Microsome Dilution Details									
6										
7	Dilution A		0.12 mL microsome Stock used				Positive Control Concentrations			
8			2.4 mL total volume				Level			
9			20 dilution factor				1	1.00E-05		
10							2	1.00E-06		
11	Dilution B		1.5 mL microsome Dilution A used				3	5.00E-07		
12			60 mL total volume				4	1.00E-07		
13			40 dilution factor				5	5.00E-08		
14							6	1.00E-08		
15	Dilution C (if applicable)		mL microsome Dilution B used				7	1.00E-09		
16			mL total volume				8	1.00E-10		
17	NA		dilution factor							
18							Test Chemical Concentrations			
19			800 total dilution factor				Level			
20							1	1.00E-04		
21	Protein Concentration (stock microsomes, mg/mL):			8.00			2	1.00E-05		
22	Protein Concentration (dilution added to assay, mg/mL):			0.01			3	1.00E-06		
23							4	5.00E-07		
24							5	1.00E-07		
25							6	1.00E-08		
26							7	1.00E-09		
							8	1.00E-10		

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Test # Concentrations Assay: 2/28/2007 Chemical ID: CF tested: 8 Microsome ID: 12535-13										
2	Counter data NB: 12535-52 filename: 28FE2337.P00										
3	Tec hnic ian ID: SLB Replicate #: 3 Positive Control: 4-OH-ASD Tubes/Conc: 2										
4											
5	Vial #	Time	DPM1	ISIE				Round DPM			
6	2	5	39020.43	17462.52	0	12.06	493.24	39020			
7	3	5	35253.89	15654.29	0	11.96	486.68	35254			
8	4	5	38321.35	16698.49	0	11.92	478.9	38321			
9	5	5	39134.99	17170.71	0	11.94	482.22	39135			
10	6	5	41765.24	18255.33	0	11.88	478.08	41765			
11	8	5	13491.1	6357.58	0	12.69	533.54	13491			
12	9	5	13508.69	6360.43	0	12.7	536.33	13507			
13	10	5	13199.97	6235.87	0	12.75	538.05	13200			
14	11	5	14147.07	6633.08	0	12.7	533.68	14147			
15	12	5	12265.89	5819.7	0	12.64	536.08	12266			
16	13	5	12130.52	5741.55	0	12.63	535.52	12131			
17	14	5	12606.48	5987.85	0	12.68	531.52	12806			
18	15	5	12643.65	5992.88	0	12.64	531.26	12644			
19	16	5	84.14	33.81	0	10.82	528.38	64			
20	17	5	66.65	34.14	0	11.91	529.37	67			
21	18	5	62.91	32.56	0	11.64	528.37	63			
22	19	5	68.93	30.19	0	11.91	531.1	59			
23	20	5	59.63	28.99	0	12.56	531.97	60			
24	21	5	53.21	27.22	0	11.75	531.92	53			
25	22	5	45.06	25.23	0	11.02	531.78	45			
26	23	5	44.46	25.84	0	11.93	533.43	44			
27	25	5	149.77	76.28	0	11.81	533.41	150			
28	26	5	157.11	76.09	0	11.37	530.97	157			
29	27	5	125.38	69.02	0	11.33	531.5	125			
30	28	5	135.62	70.91	0	12.31	534.16	138			
31											
32											
33	29	5	746.43	366.37	0	12.4	534.07	746			
34	30	5	830.06	396.49	0	12.83	529.46	830			
35	31	5	788.69	377.94	0	12.57	535.36	787			
36	32	5	775.64	374.4	0	12.51	533.62	776			
37											
38											
39	33	5	1385.39	652.17	0	12.6	533.56	1385			
40	34	5	1424.37	675.11	0	12.7	535.2	1424			
41	35	5	1497.98	688.42	0	12.93	532.93	1498			
42	36	5	1479.14	701.07	0	12.32	534.49	1479			
43											
44											
45	37	5	5195.46	2441.77	0	12.7	533.45	5195			
46	38	5	5119.54	2413.48	0	12.71	535	5120			
47	39	5	4954.09	2323.03	0	12.69	532.22	4954			
48	40	5	4926.35	2345.32	0	12.62	532.71	4926			
49											
50											
51	41	5	7822.75	3668.8	0	12.72	533.77	7823			
52	42	5	7555.61	3551.42	0	12.62	534.97	7556			
53	43	5	7279.47	3420.81	0	12.62	532.2	7279			
54	44	5	7221.87	3390.53	0	12.81	532.04	7222			
55											
56											
57	45	5	11648.67	5500.68	0	12.63	533.93	11649			
58	46	5	11649.11	5477.13	0	12.65	534.15	11649			
59	47	5	11503.99	5408.53	0	12.68	534.31	11504			
60	48	5	11247.41	5255.55	0	12.67	532.31	11247			
61											
62											
63	49	5	12859.34	6070.99	0	12.65	537.11	12859			
64	50	5	13244.35	6251.99	0	12.61	533.57	13244			
65	51	5	12634.65	5901.85	0	12.72	531.39	12635			
66	52	5	12880.96	6025.17	0	12.71	532.85	12881			
67											
68											
69	53	5	13401.76	6287.97	0	12.62	533.12	13402			
70	54	5	13872.02	6485.03	0	12.87	532.54	13872			
71	55	5	13015.26	6094.86	0	12.74	534.29	13015			
72	56	5	12680.34	5955.65	0	12.65	534.54	12680			
73											
74											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	12598.97	5934.07	0	12.7	534.38				12599
76	59	5	13058.44	6120.7	0	12.58	530.46				13058
77	60	5	12850.31	6030.99	0	12.7	535.35				12850
78	61	5	12757.62	5977.39	0	12.76	534.14				12758
79	62	5	13258.39	6206.25	0	12.74	532.58				13258
80	63	5	13051.88	6133.19	0	12.66	533.79				13052
81	64	5	14036.51	6566.52	0	12.6	531.62				14037
82	65	5	14201.79	6823.13	0	12.63	531.45				14202
83	66	5	13893.68	6488.92	0	12.66	532.59				13894
84	67	5	13642.55	6421.4	0	12.68	534.33				13643
85	68	5	13832.18	6476.63	0	12.61	528.94				13832
86	69	5	13640.29	6441.87	0	12.72	531.51				13640
87	70	5	13169.25	6245.38	0	12.68	536.37				13169
88	71	5	13971.95	6557.41	0	12.63	532.88				13972
89	72	5	13462.99	6286.98	0	12.7	530.78				13463
90	73	5	13365.33	6254.93	0	12.7	533.25				13365
91	74	5	14230.85	6642.45	0	12.68	530.43				14231
92	75	5	14224.23	6658.55	0	12.71	532.54				14224
93	76	5	14146	6655.63	0	12.61	534.73				14146
94	77	5	14175.96	6669.41	0	12.65	534.01				14176
95	78	5	13937.18	6539.21	0	12.59	533.73				13937
96	79	5	13813.56	6471.83	0	12.66	534.23				13814
97	80	5	14253.85	6690.97	0	12.66	533.41				14254
98	81	5	14094.81	6623.3	0	12.57	533.66				14095
99	83	5	14120.03	6628.6	0	12.68	532.58				14120
100	84	5	13873.47	6560.71	0	12.7	535.72				13873
101	85	5	13496.29	6328.44	0	12.69	532.3				13496
102	86	5	13915.79	6527.31	0	12.64	534.24				13916
103	87	5	12066.04	5658.73	0	12.65	531.22				12066
104	88	5	11993.94	5617.84	0	12.88	531.25				11994
105	89	5	13708.13	6434.42	0	12.73	535.6				13708
106	90	5	14152.47	6614.98	0	12.59	531.33				14152
107	91	5	14094.59	6589.13	0	12.86	531.3				14095
108	92	5	14067.16	6593.87	0	12.66	531.41				14067
109	93	5	12711.19	5949.8	0	12.88	531.23				12711
110	94	5	12631.72	5863.95	0	12.61	536.07				12632
111	95	5	13697.98	6433.72	0	12.62	531.96				13698
112	96	5	13911.96	6527.73	0	12.65	534.08				13912
113	97	5	14685.28	6890.82	0	12.58	531.1				14685
114	98	5	14643.16	6881.38	0	12.65	532.22				14643
115	99	5	12249.9	5735.19	0	12.56	530.49				12250
116	100	5	12270.86	5715.57	0	12.64	530.69				12271
117	101	5	13355.79	6236.97	0	12.65	531.1				13356
118	102	5	13242.17	6218.1	0	12.68	533.49				13242
119	103	5	14275.97	6658.35	0	12.65	530.07				14276
120	104	5	13547.79	6345.82	0	12.66	532.94				13548
121	105	5	12480.25	5821.06	0	12.67	530.28				12480
122	106	5	12515.04	5915.17	0	12.64	533.37				12515
123	107	5	236824.58	94746.04	0	11.57	435.37				236825
124	108	5	0	1.15	0	0	440.46				0
125											0
126											0
127											0
128											0
129											0
130											0
131											0
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134											0
135											0
136											0
137											0
138											0
139											0

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1					Test Chemical ID	CR11601	# Concentrations tested			8 Microsome ID	12535-13					
2					Course data	2867-2337-100										
3					Replicate #	3	Positive Control			4 OH-ASON	Pos Ctrl Tubes/Conc					
4					SLB											
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Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		QC check - aliquot			
5		variance			
6		Read if value not			
7		within mean +/-			
8		10%			
9		1.00			
10		0.97			
11		1.03			
12		0.96			
13		1.00			
14		1.00			
15		0.98			
16		1.02			
17		1.03			
18		0.97			
19		1.06			
20		0.94			
21		1.01			
22		0.99			
23		1.02			
24		0.96			
25		1.04			
26		0.94			
27		1.00			
28		0.95			
29		1.05			
30		1.01			
31		0.98			
32		1.02			
33		0.99			
34		1.03			
35		0.97			
36		1.01			
37		0.99			
38		1.04			
39		0.96			
40		1.00			
41		0.95			
42		1.05			
43		1.01			
44		0.98			
45		1.02			
46		0.99			
47		1.03			
48		0.97			
49		1.01			
50		0.99			
51		1.04			
52		0.96			
53		1.00			
54		0.95			
55		1.05			
56		1.01			
57		0.98			
58		1.02			
59		0.99			
60		1.03			
61		0.97			
62		1.01			
63		0.99			
64		1.04			
65		0.96			
66		1.00			
67		0.95			
68		1.05			
69		1.01			
70		0.98			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date
2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007	2/28/2007
Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID	Chemical ID
GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801	GR11801
Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data	Conc. data
280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000	280.000000
12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52	12535-52
Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control	Positive Control
4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD	4-OH-ASD
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34
35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37
38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39
40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44
45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46
47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47
48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48
49	49	49	49	49	49	49	49	49	49	49	49	49	49	49	49	49
50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
51	51	51	51	51	51	51	51	51	51	51	51	51	51	51	51	51
52	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52
53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53
54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		0.98
8		1.02
9		1.00
10		1.00
11		1.01
12		0.99
13		0.99
14		1.01
15		1.01
16		0.99
17		1.00
18		0.97
19		1.03
20		1.00
21		1.00
22		1.00
23		1.00
24		1.00
25		1.00
26		1.00
27		1.00
28		1.00
29		1.01
30		0.99
31		1.01
32		0.99
33		0.98
34		1.02
35		1.00
36		1.00
37		0.99
38		1.02
39		1.00
40		1.00
41		1.00
42		1.00
43		0.99
44		1.01
45		1.00
46		1.00
47		1.00
48		1.00
49		1.00
50		1.00
51		1.00
52		0.97
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	2/28/2007	Test Chemical ID	CR11601	28FE2337 P00	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13	
2	NB Ref	12535-52	Counter data filename	Replicate #							
3	Technician ID	SLB									
4	Control Type	Portion	SD								
5	Full activity	Average	0.358								
6	Background (uncorrected)	Overall	0.002								
7											
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	2	1.00E-05	-5.00	0.002						
13	4-OH ASDN	1	1.00E-06	-6.00	0.020						
14	4-OH ASDN	2	1.00E-06	-6.00	0.020						
15	4-OH ASDN	1	1.00E-07	-7.00	0.037						
16	4-OH ASDN	2	1.00E-07	-7.00	0.039						
17	4-OH ASDN	1	1.00E-08	-8.00	0.320						
18	4-OH ASDN	2	1.00E-08	-8.00	0.312						
19	4-OH ASDN	1	1.00E-09	-9.00	0.358						
20	4-OH ASDN	2	1.00E-09	-9.00	0.350						
21	4-OH ASDN	1	1.00E-10	-10.00	0.375						
22	4-OH ASDN	2	1.00E-10	-10.00	0.355						
23	4-OH ASDN	1	1.00E-10	-10.00	0.352						
24	4-OH ASDN	2	1.00E-10	-10.00	0.352						
25	4-OH ASDN	1	1.00E-04	-4.00	0.361						
26	4-OH ASDN	2	1.00E-04	-4.00	0.368						
27	4-OH ASDN	1	1.00E-05	-5.00	0.378						
28	4-OH ASDN	2	1.00E-05	-5.00	0.380						
29	4-OH ASDN	1	1.00E-06	-6.00	0.373						
30	4-OH ASDN	2	1.00E-06	-6.00	0.368						
31	4-OH ASDN	1	1.00E-07	-7.00	0.391						
32	4-OH ASDN	2	1.00E-07	-7.00	0.389						
33	4-OH ASDN	1	1.00E-08	-8.00	0.381						
34	4-OH ASDN	2	1.00E-08	-8.00	0.389						
35	4-OH ASDN	1	1.00E-09	-9.00	0.394						
36	4-OH ASDN	2	1.00E-09	-9.00	0.376						
37	4-OH ASDN	1	1.00E-10	-10.00	0.390						
38	4-OH ASDN	2	1.00E-10	-10.00	0.381						
39	4-OH ASDN	1	1.00E-04	-4.00	0.381						
40	4-OH ASDN	2	1.00E-04	-4.00	0.389						
41	4-OH ASDN	1	1.00E-05	-5.00	0.378						
42	4-OH ASDN	2	1.00E-05	-5.00	0.380						
43	4-OH ASDN	1	1.00E-06	-6.00	0.373						
44	4-OH ASDN	2	1.00E-06	-6.00	0.368						
45	4-OH ASDN	1	1.00E-07	-7.00	0.391						
46	4-OH ASDN	2	1.00E-07	-7.00	0.389						
47	4-OH ASDN	1	1.00E-08	-8.00	0.381						
48	4-OH ASDN	2	1.00E-08	-8.00	0.389						
49	4-OH ASDN	1	1.00E-09	-9.00	0.394						
50	4-OH ASDN	2	1.00E-09	-9.00	0.376						
51	4-OH ASDN	1	1.00E-10	-10.00	0.390						
52	4-OH ASDN	2	1.00E-10	-10.00	0.381						
53	4-OH ASDN	1	1.00E-04	-4.00	0.381						
54	4-OH ASDN	2	1.00E-04	-4.00	0.389						
55	4-OH ASDN	1	1.00E-05	-5.00	0.378						
56	4-OH ASDN	2	1.00E-05	-5.00	0.380						
57	4-OH ASDN	1	1.00E-06	-6.00	0.373						
58	4-OH ASDN	2	1.00E-06	-6.00	0.368						
59	4-OH ASDN	1	1.00E-07	-7.00	0.391						
60	4-OH ASDN	2	1.00E-07	-7.00	0.389						
61	4-OH ASDN	1	1.00E-08	-8.00	0.381						
62	4-OH ASDN	2	1.00E-08	-8.00	0.389						
63	4-OH ASDN	1	1.00E-09	-9.00	0.394						
64	4-OH ASDN	2	1.00E-09	-9.00	0.376						
65	4-OH ASDN	1	1.00E-10	-10.00	0.390						
66	4-OH ASDN	2	1.00E-10	-10.00	0.381						
67	4-OH ASDN	1	1.00E-04	-4.00	0.381						
68	4-OH ASDN	2	1.00E-04	-4.00	0.389						
69	4-OH ASDN	1	1.00E-05	-5.00	0.378						
70	4-OH ASDN	2	1.00E-05	-5.00	0.380						
71	4-OH ASDN	1	1.00E-06	-6.00	0.373						
72	4-OH ASDN	2	1.00E-06	-6.00	0.368						
73	4-OH ASDN	1	1.00E-07	-7.00	0.391						
74	4-OH ASDN	2	1.00E-07	-7.00	0.389						
75	4-OH ASDN	1	1.00E-08	-8.00	0.381						
76	4-OH ASDN	2	1.00E-08	-8.00	0.389						
77	4-OH ASDN	1	1.00E-09	-9.00	0.394						
78	4-OH ASDN	2	1.00E-09	-9.00	0.376						
79	4-OH ASDN	1	1.00E-10	-10.00	0.390						
80	4-OH ASDN	2	1.00E-10	-10.00	0.381						
81	4-OH ASDN	1	1.00E-04	-4.00	0.381						
82	4-OH ASDN	2	1.00E-04	-4.00	0.389						
83	4-OH ASDN	1	1.00E-05	-5.00	0.378						
84	4-OH ASDN	2	1.00E-05	-5.00	0.380						
85	4-OH ASDN	1	1.00E-06	-6.00	0.373						
86	4-OH ASDN	2	1.00E-06	-6.00	0.368						
87	4-OH ASDN	1	1.00E-07	-7.00	0.391						
88	4-OH ASDN	2	1.00E-07	-7.00	0.389						
89	4-OH ASDN	1	1.00E-08	-8.00	0.381						
90	4-OH ASDN	2	1.00E-08	-8.00	0.389						
91	4-OH ASDN	1	1.00E-09	-9.00	0.394						
92	4-OH ASDN	2	1.00E-09	-9.00	0.376						
93	4-OH ASDN	1	1.00E-10	-10.00	0.390						
94	4-OH ASDN	2	1.00E-10	-10.00	0.381						
95	4-OH ASDN	1	1.00E-04	-4.00	0.381						
96	4-OH ASDN	2	1.00E-04	-4.00	0.389						
97	4-OH ASDN	1	1.00E-05	-5.00	0.378						
98	4-OH ASDN	2	1.00E-05	-5.00	0.380						
99	4-OH ASDN	1	1.00E-06	-6.00	0.373						
100	4-OH ASDN	2	1.00E-06	-6.00	0.368						
101	4-OH ASDN	1	1.00E-07	-7.00	0.391						
102	4-OH ASDN	2	1.00E-07	-7.00	0.389						
103	4-OH ASDN	1	1.00E-08	-8.00	0.381						
104	4-OH ASDN	2	1.00E-08	-8.00	0.389						
105	4-OH ASDN	1	1.00E-09	-9.00	0.394						
106	4-OH ASDN	2	1.00E-09	-9.00	0.376						
107	4-OH ASDN	1	1.00E-10	-10.00	0.390						
108	4-OH ASDN	2	1.00E-10	-10.00	0.381						
109	4-OH ASDN	1	1.00E-04	-4.00	0.381						
110	4-OH ASDN	2	1.00E-04	-4.00	0.389						
111	4-OH ASDN	1	1.00E-05	-5.00	0.378						
112	4-OH ASDN	2	1.00E-05	-5.00	0.380						
113	4-OH ASDN	1	1.00E-06	-6.00	0.373						
114	4-OH ASDN	2	1.00E-06	-6.00	0.368						
115	4-OH ASDN	1	1.00E-07	-7.00	0.391						
116	4-OH ASDN	2	1.00E-07	-7.00	0.389						
117	4-OH ASDN	1	1.00E-08	-8.00	0.381						
118	4-OH ASDN	2	1.00E-08	-8.00	0.389						
119	4-OH ASDN	1	1.00E-09	-9.00	0.394						
120	4-OH ASDN	2	1.00E-09	-9.00	0.376						
121	4-OH ASDN	1	1.00E-10	-10.00	0.390						
122	4-OH ASDN	2	1.00E-10	-10.00	0.381						
123	4-OH ASDN	1	1.00E-04	-4.00	0.381						
124	4-OH ASDN	2	1.00E-04	-4.00	0.389						
125	4-OH ASDN	1	1.00E-05	-5.00	0.378						
126	4-OH ASDN	2	1.00E-05	-5.00	0.380						
127	4-OH ASDN	1	1.00E-06	-6.00	0.373						
128	4-OH ASDN	2	1.00E-06	-6.00	0.368						
129	4-OH ASDN	1	1.00E-07	-7.00	0.391						
130	4-OH ASDN	2	1.00E-07	-7.00	0.389						
131	4-OH ASDN	1	1.00E-08	-8.00	0.381						
132	4-OH ASDN	2	1.00E-08	-8.00	0.389						
133	4-OH ASDN	1	1.00E-09	-9.00	0.394						
134	4-OH ASDN	2	1.00E-09	-9.00	0.376						
135	4-OH ASDN	1	1.00E-10	-10.00	0.390						
136	4-OH ASDN	2	1.00E-10	-10.00	0.381						
137	4-OH ASDN	1	1.00E-04	-4.00	0.381						
138	4-OH ASDN	2	1.00E-04	-4.00	0.389						
139	4-OH ASDN	1	1.00E-05	-5.00	0.378						
140	4-OH ASDN	2	1.00E-05	-5.00	0.380						
141	4-OH ASDN	1	1.00E-06	-6.00	0.373						
142	4-OH ASDN	2	1.00E-06	-6.00	0.368						
143	4-OH ASDN	1	1.00E-07	-7.00	0.391						
144	4-OH ASDN	2	1.00E-07	-7.00	0.389						
145	4-OH ASDN	1	1.00E-08	-8.00	0.381						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/1/2007	Test Chemical ID	CR11602	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-55	Counter data filename	15MR2024.P00					
3	Technician ID	CKN	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/1/2007	Test Chemical ID	CR11602	# Concentrations tested			8; Microsome ID	12535-13
2	NB Ref.	12535-55	Counter data filename	15MR2024.P00					
3	Technician ID	CKN	Replicate #	1	Positive Control			Pos Ctl	
4								4-OH ASDN Tubes/Conc	2
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0172		33988		1976047			
8	2	0.0169		33661		1991775			
9	3	0.0166		35519		2139699			
10	4	0.0159		34138		2147044			
11	5	0.0173		34368		1986590			
12						Average DPM/g soln		2048231	
13						SD		87075	
14						CV		4.25	
15						µCi/g soln		0.9226	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution								8.1049 g
25	Mass of dilution B used in substrate prep								4.5799 g
26	Concentration of nonradiolabeled ASDN in substrate soln.							0.56564863 µg/g	
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =		0.011 µg/g soln.						
29			µg/g soln.						
30	a. µCi/g soln		0.9226						
31	b. Specific activity of [³ H]ASDN (µCi/mmol)		23500000						
32	c. Molecular wt of ASDN (mg/mmol)		286.4						
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36	= 0.5656 + 0.011								
37	= 0.577 µg ASDN/g soln.								
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.599 µCi/µg ASDN								
41	1.017.E+06 dpm/nmol								

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K																				
1			Assay Date	3/1/2007	Test Chemical ID	CR11602	# Concentrations tested	8	Microsome ID	12535-13																					
2			NB Ref	12535-55	Counter data filename	15MR2024_P00																									
3			Technician ID	CKN	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2																					
4	Microsome Dilution Details																														
5	0.12 mL microsome Stock used																														
6	2.4 mL total volume																														
7	20 dilution factor																														
8																															
9																															
10																															
11	1.5 mL microsome Dilution A used																														
12	60 mL total volume																														
13	40 dilution factor																														
14																															
15	mL microsome Dilution B used																														
16	mL total volume																														
17	dilution factor																														
18	NA																														
19	800 total dilution factor																														
20																															
21	Protein Concentration (stock microsomes, mg/mL):																														
22	Protein Concentration (dilution added to assay, mg/mL):																														
23	8.00																														
24	0.01																														
25																															
26																															
<table border="1"> <thead> <tr> <th colspan="2">Positive Control Concentrations</th> </tr> <tr> <th>Level</th> <th>Final Concentration (M)</th> </tr> </thead> <tbody> <tr><td>1</td><td>1.00E-05</td></tr> <tr><td>2</td><td>1.00E-06</td></tr> <tr><td>3</td><td>5.00E-07</td></tr> <tr><td>4</td><td>1.00E-07</td></tr> <tr><td>5</td><td>5.00E-08</td></tr> <tr><td>6</td><td>1.00E-08</td></tr> <tr><td>7</td><td>1.00E-09</td></tr> <tr><td>8</td><td>1.00E-10</td></tr> </tbody> </table>												Positive Control Concentrations		Level	Final Concentration (M)	1	1.00E-05	2	1.00E-06	3	5.00E-07	4	1.00E-07	5	5.00E-08	6	1.00E-08	7	1.00E-09	8	1.00E-10
Positive Control Concentrations																															
Level	Final Concentration (M)																														
1	1.00E-05																														
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6	1.00E-08																														
7	1.00E-09																														
8	1.00E-10																														
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Test Chemical Concentrations																															
Level	Final Concentration (M)																														
1	1.00E-03																														
2	1.00E-04																														
3	1.00E-05																														
4	1.00E-06																														
5	1.00E-07																														
6	1.00E-08																														
7	1.00E-09																														
8	1.00E-10																														

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
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Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	53	5	9308.27	4356.34	0	12.83	539.52				9308
72	54	5	11082.81	5144.44	0	12.71	533.17				11083
73	55	5	10344.48	4813.36	0	12.71	535.87				10344
74	56	5	10021.45	4651.73	0	12.66	533.78				10021
75											
76	58	5	510.98	243.59	0	12.75	535.81				511
77	59	5	510.8	239.62	0	12.84	533.91				511
78	60	5	455.6	220.47	0	12.68	536.48				456
79	61	5	518.74	235.24	0	13.01	537.13				519
80	62	5	415.39	192.51	0	12.85	533.93				415
81	63	5	422.41	201.78	0	12.53	536.24				422
82	64	5	6808.71	3205.73	0	12.68	538.55				6809
83	65	5	6644.66	3115.69	0	12.75	539				6645
84	66	5	6700.59	3106.71	0	12.84	538.48				6701
85	67	5	6539.68	3038.2	0	12.82	537.13				6539
86	68	5	6883.31	3233.22	0	12.76	538.68				6883
87	69	5	6691.31	3138.59	0	12.68	536.87				6691
88	70	5	10542.4	4942.45	0	12.71	540.26				10542
89	71	5	10168.07	4780.4	0	12.72	538.22				10168
90	72	5	9858.5	4823.4	0	12.72	535.94				9857
91	73	5	9888.58	4813.82	0	12.75	535.98				9889
92	74	5	9212.74	4342.28	0	12.61	537.75				9213
93	75	5	9629	4473.04	0	12.75	533.42				9629
94	76	5	9586.37	4463.01	0	12.69	537.54				9586
95	77	5	9915.17	4618.96	0	12.69	533.49				9915
96	78	5	10835.48	5051.24	0	12.77	536.22				10835
97	79	5	10491.35	4865.28	0	12.79	535.25				10491
98	80	5	9654.62	4536.74	0	12.69	537.83				9655
99	81	5	10470.67	4912.96	0	12.77	538.41				10471
100	83	5	10169.99	4749.98	0	12.77	538.39				10170
101	84	5	10027.11	4703.05	0	12.7	537.39				10027
102	85	5	10822.7	5060.71	0	12.72	537.3				10823
103	86	5	10829.9	5084.33	0	12.76	538.94				10830
104	87	5	10334.19	4808.56	0	12.75	534.97				10334
105	88	5	10194.76	4777.3	0	12.69	537.84				10195
106	89	5	10719.27	4979.98	0	12.76	534.75				10719
107	90	5	10514.24	4952.79	0	12.64	538.5				10514
108	91	5	10957.62	5101.04	0	12.79	535.19				10958
109	92	5	10611.76	4968.75	0	12.71	534.89				10612
110	93	5	9879.48	4628.88	0	12.74	538.52				9879
111	94	5	9915.84	4600.46	0	12.75	538.89				9916
112	95	5	10678.61	4959.66	0	12.74	538.47				10679
113	96	5	10448.94	4829.93	0	12.78	534.76				10449
114	97	5	10445.1	4927.76	0	12.7	537.89				10445
115	98	5	10300.65	4940.37	0	12.68	535.76				10301
116	99	5	10776.16	5019.91	0	12.79	538.29				10776
117	100	5	10487.98	4915.71	0	12.69	537.02				10488
118	101	5	10408.65	4863.25	0	12.78	537.67				10409
119	102	5	11026.47	5145.8	0	12.76	537.72				11026
120	103	5	5021.35	2413.95	0	13.13	566.36				5021
121	104	5	5114.68	2433.79	0	13.11	557.96				5115
122	105	5	5372.88	2570.35	0	13.17	562.77				5373
123	106	5	5232.73	2513.07	0	13.15	562.78				5233
124											
125	107	5	231086.09	36112.47	0	7.74	170.23				231086

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1			Assay Date	3/1/2007	Test Chemical	CR11602	# Concentrations tested	12435-13	R Microsome ID							
2			MB Ref. Technician	12435-56	Counter data	15MAR2024 P00										
3			ID	CKN	1	Positive Control	4-OH ANDONIPos Ctl Tubes(Dmc)	2								
5	Sample ID															
6	Sample type	Replicate	Volume (mL)	Aliq #	DPH/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (final)	Calculated nmol ³ H ₂ O formed (Background subtracted)	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)		
7	Full activity control	1	2	0.5	11837	23874	47056	0.1	204823	46502	0.0469	0.006	15	0.300		
8		2	0.5	11891	23102	23081	47462	0.1	204823	46908	0.0461	0.006	15	0.306		
9		3	0.5	11866	23570	23681	47462	0.1	204823	46908	0.0461	0.006	15	0.306		
10		4	0.5	11786	23570	23670	47462	0.1	204823	46908	0.0461	0.006	15	0.306		
11		5	0.5	10879	21890	21818	43636	0.1	204823	43182	0.0429	0.006	15	0.283		
12		6	0.5	10843	21866	21843	43636	0.1	204823	43182	0.0429	0.006	15	0.283		
13		7	0.5	10648	21236	21167	42214	0.1	204823	41850	0.0412	0.006	15	0.274		
14		8	0.5	10599	21018	20973	41850	0.1	204823	41850	0.0412	0.006	15	0.274		
15	Background control	1	2	0.5	113	226	448	0.1	204823	14	0.0000	0.006	15	0.000		
16		2	0.5	121	242	234	468	0.1	204823	-139	-0.0001	0.006	15	-0.001		
17		3	0.5	79	156	158	316	0.1	204823	-1	0.0000	0.006	15	0.000		
18		4	0.5	103	206	207	412	0.1	204823	126	0.0031	0.006	15	0.001		
19		5	0.5	124	248	250	496	0.1	204823	490	0.0094	0.006	15	0.003		
20		6	0.5	144	288	288	576	0.1	204823	570	0.0093	0.006	15	0.002		
21	4-OH ANDON	1-1	2	0.5	224	448	896	0.1	204823	280	0.0033	0.006	15	0.002		
22		1-2	2	0.5	185	370	734	0.1	204823	280	0.0033	0.006	15	0.002		
23		1-3	2	0.5	182	364	728	0.1	204823	280	0.0033	0.006	15	0.002		
24		2-1	2	0.5	824	1648	3292	0.1	204823	2843	0.0028	0.006	15	0.019		
25		2-2	2	0.5	827	1654	3292	0.1	204823	2843	0.0028	0.006	15	0.019		
26		2-3	2	0.5	858	1716	3433	0.1	204823	2843	0.0028	0.006	15	0.019		
27		3-1	2	0.5	1545	3090	6226	0.1	204823	5772	0.0067	0.006	15	0.004		
28		3-2	2	0.5	1568	3136	6276	0.1	204823	5772	0.0067	0.006	15	0.004		
29		3-3	2	0.5	1230	2460	4940	0.1	204823	4486	0.0044	0.006	15	0.029		
30		4-1	2	0.5	1240	2480	4960	0.1	204823	4486	0.0044	0.006	15	0.029		
31		4-2	2	0.5	5836	11670	22886	0.1	204823	22432	0.0221	0.006	15	0.147		
32		4-3	2	0.5	5808	11216	22432	0.1	204823	22432	0.0221	0.006	15	0.147		
33		5-1	2	0.5	3552	7104	15206	0.1	204823	14752	0.0145	0.006	15	0.097		
34		5-2	2	0.5	3721	7502	15206	0.1	204823	14752	0.0145	0.006	15	0.097		
35		5-3	2	0.5	6825	13650	26848	0.1	204823	26394	0.0260	0.006	15	0.173		
36		6-1	2	0.5	6589	13188	26448	0.1	204823	26394	0.0260	0.006	15	0.173		
37		6-2	2	0.5	5800	11620	23486	0.1	204823	23032	0.0226	0.006	15	0.151		
38		6-3	2	0.5	5783	11598	23486	0.1	204823	23032	0.0226	0.006	15	0.151		
39		7-1	2	0.5	10944	20166	40548	0.1	204823	40904	0.0394	0.006	15	0.263		
40		7-2	2	0.5	10160	20380	40548	0.1	204823	40904	0.0394	0.006	15	0.263		
41		7-3	2	0.5	9480	18912	37472	0.1	204823	37018	0.0364	0.006	15	0.243		
42		8-1	2	0.5	10384	20688	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
43		8-2	2	0.5	10187	20374	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
44		8-3	2	0.5	11714	23426	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
45		9-1	2	0.5	11673	23348	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
46		9-2	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
47		9-3	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
48		10-1	2	0.5	10344	20688	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
49		10-2	2	0.5	10187	20374	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
50		10-3	2	0.5	11714	23426	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
51		11-1	2	0.5	11673	23348	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
52		11-2	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
53		11-3	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
54		12-1	2	0.5	10344	20688	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
55		12-2	2	0.5	10187	20374	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
56		12-3	2	0.5	11714	23426	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
57		13-1	2	0.5	11673	23348	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
58		13-2	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
59		13-3	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
60		14-1	2	0.5	10344	20688	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
61		14-2	2	0.5	10187	20374	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
62		14-3	2	0.5	11714	23426	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
63		15-1	2	0.5	11673	23348	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
64		15-2	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
65		15-3	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
66		16-1	2	0.5	10344	20688	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
67		16-2	2	0.5	10187	20374	40922	0.1	204823	40904	0.0394	0.006	15	0.263		
68		16-3	2	0.5	11714	23426	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
69		17-1	2	0.5	11673	23348	46774	0.1	204823	46320	0.0456	0.006	15	0.304		
70		17-2	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		
71		17-3	2	0.5	8386	16772	33544	0.1	204823	33544	0.0329	0.006	15	0.219		

Aronatase Assay Spreadsheet

	R	S	T	U	V
1					
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5		OD check - aliquot variance Rod if value not within mean $\pm$ 40%			
6					
7		1.01			
8		0.99			
9		1.00			
10		1.00			
11		1.01			
12		0.99			
13		1.01			
14		0.99			
15		0.97			
16		1.03			
17		1.00			
18		1.00			
19		0.91			
20		1.08			
21		1.01			
22		0.99			
23		0.99			
24		1.01			
25		1.01			
26		0.99			
27					
28					
29		1.00			
30		1.00			
31		0.98			
32		1.02			
33					
34		0.99			
35		1.01			
36		1.00			
37		1.00			
38		1.00			
39					
40					
41		1.02			
42		0.98			
43		1.00			
44		0.99			
45					
46					
47		1.02			
48		0.98			
49		1.02			
50		0.98			
51					
52					
53		1.00			
54		1.00			
55		0.99			
56		1.01			
57					
58					
59		1.01			
60		0.99			
61		1.00			
62		1.00			
63					
64					
65		0.91			
66		1.08			
67		1.00			
68		0.98			
69		0.98			
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Level	Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used (assay tube) (mL)	total DPM in assay tube (initial)	Calculate nmoL H ₂ O formed	nmol H ₂ O formed	Final (percent) at assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
1	CR11602	1-1	2	0.5	1	511	1022	2044	0.1	204823	1550	0.0016	0.005	15	0.010
2	CR11602	1-2	2	0.5	1	456	912	1824	0.1	204823	1486	0.0015	0.005	15	0.010
3	CR11602	1-3	2	0.5	1	519	1038	2076	0.1	204823	1570	0.0016	0.005	15	0.010
4	CR11602	2-1	2	0.5	1	427	854	1708	0.1	204823	1454	0.0015	0.005	15	0.010
5	CR11602	2-2	2	0.5	1	609	1218	2436	0.1	204823	1654	0.0017	0.005	15	0.010
6	CR11602	2-3	2	0.5	1	645	1290	2580	0.1	204823	1694	0.0017	0.005	15	0.010
7	CR11602	3-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
8	CR11602	3-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
9	CR11602	3-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
10	CR11602	4-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
11	CR11602	4-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
12	CR11602	4-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
13	CR11602	5-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
14	CR11602	5-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
15	CR11602	5-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
16	CR11602	6-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
17	CR11602	6-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
18	CR11602	6-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
19	CR11602	7-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
20	CR11602	7-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
21	CR11602	7-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
22	CR11602	8-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
23	CR11602	8-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
24	CR11602	8-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
25	CR11602	9-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
26	CR11602	9-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
27	CR11602	9-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
28	CR11602	10-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
29	CR11602	10-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
30	CR11602	10-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
31	CR11602	11-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
32	CR11602	11-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
33	CR11602	11-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
34	CR11602	12-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
35	CR11602	12-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
36	CR11602	12-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
37	CR11602	13-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
38	CR11602	13-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
39	CR11602	13-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
40	CR11602	14-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
41	CR11602	14-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
42	CR11602	14-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
43	CR11602	15-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
44	CR11602	15-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
45	CR11602	15-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
46	CR11602	16-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
47	CR11602	16-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
48	CR11602	16-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
49	CR11602	17-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
50	CR11602	17-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
51	CR11602	17-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
52	CR11602	18-1	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
53	CR11602	18-2	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010
54	CR11602	18-3	2	0.5	1	653	1306	2612	0.1	204823	1706	0.0017	0.005	15	0.010

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		
6		QC check - aliquot variance
7		% RE
8		1.00
9		1.00
10		0.94
11		1.06
12		0.99
13		1.01
14		1.01
15		0.99
16		1.01
17		0.99
18		0.99
19		1.02
20		0.98
21		1.00
22		1.00
23		0.98
24		1.02
25		0.98
26		1.02
27		1.02
28		0.98
29		0.98
30		1.04
31		1.01
32		0.99
33		1.00
34		1.00
35		1.01
36		0.99
37		1.01
38		0.99
39		1.02
40		0.99
41		1.00
42		1.00
43		1.01
44		0.99
45		1.01
46		0.99
47		1.00
48		0.98
49		0.97
50		1.00
51		0.99
52		1.01
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/1/2007	Test Chemical ID	CR11602	15MR2024.P00	1	Positive Control	4-OH ASDN	Pos CII Tubes/Conc	2	
2	NB Ref.	12535-55	Center data file name	15MR2024.P00	Replicate #						
3	Technician ID	CKN									
4											
5	Control Type	Portion	Average	SD							
6	Full activity	Overall	0.293	0.016							
7	Background (uncorrected)	Overall	0.003	0.001							
8											
9											
10	Positive Control	Level	Replicate	Positive CII M	Log(Positive CII)	Activity					
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.019					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.015					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.038					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.029					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.147					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.097					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.173					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.151					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.283					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.243					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.265					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.304					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.264					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.284					
27											
28											
29											
30											
31											
32											
33											
34											
35											
36	Test Chemical	Level	Replicate	Test chemical M	Log(test chemical)	Activity					
37	CR11602	1	1	1.00E-03	-3.00	0.010					
38	CR11602	1	2	1.00E-03	-3.00	0.010					
39	CR11602	2	1	1.00E-03	-3.00	0.009					
40	CR11602	2	2	1.00E-04	-4.00	0.173					
41	CR11602	2	3	1.00E-04	-4.00	0.171					
42	CR11602	3	1	1.00E-05	-5.00	0.175					
43	CR11602	3	2	1.00E-05	-5.00	0.269					
44	CR11602	3	3	1.00E-05	-5.00	0.266					
45	CR11602	4	1	1.00E-06	-6.00	0.244					
46	CR11602	4	2	1.00E-06	-6.00	0.253					
47	CR11602	4	3	1.00E-06	-6.00	0.277					
48	CR11602	5	1	1.00E-07	-7.00	0.262					
49	CR11602	5	2	1.00E-07	-7.00	0.281					
50	CR11602	5	3	1.00E-07	-7.00	0.266					
51	CR11602	6	1	1.00E-08	-8.00	0.275					
52	CR11602	6	2	1.00E-08	-8.00	0.280					
53	CR11602	6	3	1.00E-08	-8.00	0.255					
54	CR11602	7	1	1.00E-09	-9.00	0.274					
55	CR11602	7	2	1.00E-09	-9.00	0.269					
56	CR11602	7	3	1.00E-09	-9.00	0.276					
57	CR11602	8	1	1.00E-10	-10.00	0.278					
58	CR11602	8	2	1.00E-10	-10.00	0.263					
59	CR11602	8	3	1.00E-10	-10.00	0.275					
60											
61											

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/9/2007	Test Chemical ID	CR11602	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-98	Counter data filename	14MR0944.P00					
3	Technician		Replicate #	4	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/9/2007	Test Chemical ID	CR11602	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-98	Counter data filename	14MR0944.P00					
3	Technician ID	CKN	Replicate #	4	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0201		38690		1924876			
8	2	0.0200		39109		1955450			
9	3	0.0200		38615		1930750			
10	4	0.0199		39350		1977387			
11	5	0.0200		38041		1902050			
12						Average DPM/g soln		1938103	
13						SD		29039	
14						CV		1.50	
15						µCi/g soln		0.8730	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution				7.9546 g				
25	Mass of dilution B used in substrate prep				4.5260 g				
26	Concentration of nonradiolabeled ASDN in substrate soln.				0.57011691 µg/g				
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011	µg/g soln.			
29						µg/g soln.			
30	a. µCi/g soln					0.8730			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					=	0.5701	+	0.011	
37					=	0.581 µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.503 µCi/µg ASDN								
41	9.558.E+05 dpm/nmol								

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/9/2007	Test Chemical ID	CR11602	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-98	Counter data filename	14MR0944.P00					
3			Technician ID	CKN	Replicate #	4	Positive Control	4-OH ASDN	Tubes/Conc	Pos Ctrl	2
4											
5	Microsome Dilution Details										
6											
7	Dilution A										
8		0.12 mL microsome Stock used									
9		2.4 mL total volume									
10		20 dilution factor									
11	Dilution B										
12		1.5 mL microsome Dilution A used									
13		60 mL total volume									
14		40 dilution factor									
15	Dilution C (if applicable)										
16		mL microsome Dilution B used									
17		mL total volume									
18		dilution factor									
19		800 total dilution factor									
20											
21	Protein Concentration (stock microsomes, ng/mL):										
22	Protein Concentration (dilution added to assay, mg/mL):										
23											
24											
25											
26											

Aromalase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Test # Chemical Concentrations As: 3/9/2007 ID C tested 8 Microsome ID 12535-13									
2	Counter data NB 12535-98 filename 14MR0944.P00									
3	Technician ID CKN Replicate # 4 Positive Control 4-OH ASD Tubes/Conc 2									
4										
5	Vial	Tin	DPM1	tSIE						Round DPM
6										
7	2	5	38690.16	19440.5	0	13.54	592.54			38690
8	3	5	39109.11	19674.89	0	13.52	591.12			39109
9	4	5	38614.99	19479	0	13.53	593.35			38615
10	5	5	39349.6	19637.13	0	13.55	588.28			39350
11	6	5	38040.53	19130.65	0	13.64	597.02			38041
12										
13	8	5	7350.7	3475.66	0	12.58	523.51			7351
14	9	5	7428.49	3525.85	0	12.62	526.53			7428
15	10	5	5876.78	2788.58	0	12.56	526.33			5877
16	11	5	5822.31	2748.73	0	12.61	525.34			5822
17	12	5	6837.57	3130.75	0	12.59	525.94			6638
18	13	5	6688.44	3143.11	0	12.66	525.82			6688
19	14	5	4967.1	2337.7	0	12.54	523.76			4967
20	15	5	4892.61	2279.28	0	12.7	524.41			4893
21	16	5	56.87	23.75	0	13.13	525.14			57
22	17	5	54.17	27.01	0	11.9	526.99			54
23	18	5	55.63	25.41	0	8.99	526.4			56
24	19	5	67.89	33.78	0	11.87	526.43			68
25	20	5	54.83	26.36	0	10.41	524.82			55
26	21	5	60.71	25.29	0	11.74	525.49			61
27	22	5	137.11	58.26	0	13	527.38			137
28	23	5	123.98	57.14	0	12.06	524.6			124
29	24	5								
30	25	5	127.38	61.73	0	12.44	524.71			127
31	26	5	155.29	67.99	0	12.18	526.65			155
32	27	5	140.56	66.67	0	11.7	526.02			141
33	28	5	134.38	61	0	12.07	526.04			134
34										
35										
36	29	5	737.99	353.97	0	12.25	523.75			738
37	30	5	764.21	353.71	0	12.58	524.07			764
38	31	5	686.53	323.71	0	12.29	530.87			687
39	32	5	703.13	321.77	0	12.35	523.92			703
40										
41										
42	33	5	1252.21	583.34	0	12.66	525.64			1252
43	34	5	1212.43	571.99	0	12.56	524.99			1212
44	35	5	1136.54	528.86	0	12.68	530.86			1137
45	36	5	1174.12	551.74	0	12.66	527.63			1174
46										
47										
48	37	5	3566.42	1688.7	0	12.52	524.71			3566
49	38	5	3527.32	1653	0	12.5	523.17			3527
50	39	5	3358.2	1595.53	0	12.62	528.26			3358
51	40	5	3252.2	1528.25	0	12.61	525.1			3252
52										
53										
54	41	5	4529.14	2136.5	0	12.61	527.55			4529
55	42	5	4626.06	2174.84	0	12.57	527.64			4626
56	43	5	4883.36	2296.4	0	12.83	524.67			4883
57	44	5	4786.38	2280.61	0	12.52	524.02			4786
58										
59										
60	45	5	6047.2	2848.95	0	12.65	528.3			6047
61	46	5	6088.79	2863.99	0	12.68	525.86			6089
62	47	5	5821.64	2753.83	0	12.54	524.09			5822
63	48	5	5672.49	2697.75	0	12.57	525.56			5672
64										
65										
66	49	5	4852.23	2304.78	0	12.54	527.09			4852
67	50	5	4886.18	2328.06	0	12.54	523.81			4886
68	51	5	6508.42	3071.69	0	12.66	523.99			6508
69	52	5	6536.08	3076.24	0	12.66	525.53			6536
70										

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71											
72	53	5	4910.19	2307.32	0	12.67	527.23				4910
73	54	5	4923.36	2313.71	0	12.57	524.13				4923
74	55	5	6834.5	3242.64	0	12.63	528.88				6835
75	56	5	6875.42	3269.48	0	12.59	527.31				6875
76											
77	58	5	391.16	183.91	0	12.46	526.81				391
78	59	5	409.65	186.88	0	12.26	524.26				410
79	60	5	298.1	137.93	0	12.43	526.56				298
80	61	5	270.28	126.67	0	11.76	523.07				270
81	62	5	406.73	187.1	0	12.64	522.79				407
82	63	5	391.97	185.65	0	12.56	527.17				392
83	64	5	970.57	455.38	0	12.19	524.19				971
84	65	5	965.7	453.46	0	12.25	526.13				966
85	66	5	1513.49	730.39	0	12.45	528.61				1513
86	67	5	1513.42	723.9	0	12.44	526.65				1513
87	68	5	1317.74	633.43	0	12.45	528.4				1318
88	69	5	1306.64	615.42	0	12.64	525.39				1307
89	70	5	2417.72	1135.96	0	12.7	524.77				2418
90	71	5	2375.16	1117.05	0	12.75	525.93				2375
91	72	5	2770.88	1321.13	0	12.55	527.66				2771
92	73	5	2808.06	1335.64	0	12.42	524.36				2808
93	74	5	2845.68	1341.47	0	12.6	527.04				2846
94	75	5	2609.28	1333.01	0	12.39	525.92				2609
95	76	5	4071.4	1931.16	0	12.49	525.87				4071
96	77	5	4015.21	1931.02	0	12.57	529.51				4015
97	78	5	4330.53	2048.2	0	12.65	525.34				4331
98	79	5	4250.67	2007.56	0	12.63	526.88				4251
99	80	5	3904.71	1840.28	0	12.71	526.66				3905
100	81	5	3938.8	1865.94	0	12.61	528.01				3939
101	82	5	4584.09	2144.11	0	12.59	524.79				4584
102	83	5	4649.68	2181.68	0	12.64	524.79				4650
103	84	5	6201.74	2940.44	0	12.57	526.83				6202
104	85	5	6149.85	2919.35	0	12.54	525.15				6150
105	86	5	5880.12	2764.05	0	12.57	524.94				5880
106	87	5	5747.34	2727.67	0	12.56	527.28				5747
107	88	5	5816.62	2755.41	0	12.54	523.66				5817
108	89	5	5894.68	2785.68	0	12.56	525.49				5895
109	90	5	3937.98	1842.74	0	12.71	525.42				3938
110	91	5	3956.4	1866.78	0	12.61	525.15				3956
111	92	5	5550.73	2629.33	0	12.5	528.52				5551
112	93	5	6508.07	2821.04	0	12.57	526.83				6508
113	94	5	6280.18	2954.77	0	12.73	526.57				6280
114	95	5	6230.16	2953.79	0	12.57	526.06				6230
115	96	5	6790.92	3199.33	0	12.85	526.15				6791
116	97	5	6712.17	3191.97	0	12.68	527.36				6712
117	98	5	5209.24	2469.14	0	12.47	524.96				5209
118	99	5	5211.05	2456.66	0	12.64	527.39				5211
119	100	5	5887.69	2773.14	0	12.64	524.81				5888
120	101	5	5985.35	2808.56	0	12.61	523.07				5985
121	102	5	4692.79	2217.29	0	12.61	527.13				4693
122	103	5	4616.15	2171.84	0	12.72	523				4616
123	104	5	5685.54	2679.61	0	12.67	525.63				5686
124	105	5	5747.02	2703.09	0	12.57	525.71				5747
125	106	5	235967.72	57239.71	0	8.75	239.34				235968
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	3/9/2007	Test Chemical ID	CR11602	# Concentrations tested	12535-13								
2		NE Ref. Technician ID	12535-98	Counter date	14MR0944 P100										
3		Replicate #	4	Positive Control	LOH ASDN	Pos Chl Tubes/Conc	2								
4															
5	Sample ID	Replicate	Nominal test volume (mL)	Aliq. #	Calculate DPM in aqueous portion after extraction	DPM/assay	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM to assay tube (dpm)	Calculate nmol ³ H-OH formed Total DPM corrected for background (Background 1 tube)	nmol ³ H-OH formed	Final (pmol) in assay (ng/mL)	Incubation time (min)	Aromatase activity (pmol estrogen formed/pmol protein/min)
6	7 (all activity control)	1	2	0.5	1	7261	14702	14778	29558	0.1	193810	29558	0.006	15	0.204
7	8	2	2	0.5	2	7426	14856	23388	23388	0.1	193810	23388	0.006	15	0.161
8	9	3	2	0.5	2	5622	11544	11544	11544	0.1	193810	11544	0.006	15	0.184
9	10	4	2	0.5	2	6636	13276	13276	13276	0.1	193810	13276	0.006	15	0.135
10	11	5	2	0.5	2	4967	9934	9934	9934	0.1	193810	9934	0.006	15	0.001
11	12	6	2	0.5	2	4893	9786	9786	9786	0.1	193810	9786	0.006	15	0.000
12	13	7	2	0.5	2	54	108	111	222	0.1	193810	111	0.006	15	0.000
13	14	8	2	0.5	2	56	112	124	248	0.1	193810	124	0.006	15	0.000
14	15	9	2	0.5	2	68	136	148	296	0.1	193810	148	0.006	15	0.000
15	16	10	2	0.5	2	75	150	162	324	0.1	193810	162	0.006	15	0.000
16	17	11	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
17	18	12	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
18	19	13	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
19	20	14	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
20	21	15	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
21	22	16	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
22	23	17	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
23	24	18	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
24	25	19	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
25	26	20	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
26	27	21	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
27	28	22	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
28	29	23	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
29	30	24	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
30	31	25	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
31	32	26	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
32	33	27	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
33	34	28	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
34	35	29	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
35	36	30	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
36	37	31	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
37	38	32	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
38	39	33	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
39	40	34	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
40	41	35	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
41	42	36	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
42	43	37	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
43	44	38	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
44	45	39	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
45	46	40	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
46	47	41	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
47	48	42	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
48	49	43	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
49	50	44	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
50	51	45	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
51	52	46	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
52	53	47	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
53	54	48	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
54	55	49	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
55	56	50	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
56	57	51	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
57	58	52	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
58	59	53	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
59	60	54	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
60	61	55	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
61	62	56	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
62	63	57	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
63	64	58	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
64	65	59	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
65	66	60	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
66	67	61	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
67	68	62	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
68	69	63	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
69	70	64	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002
70	71	65	2	0.5	2	134	268	276	552	0.1	193810	276	0.006	15	0.002

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12535-98, CR11602, CKN.xls
Control Activity Calculation

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Sample ID	Assay Date	3/10/2007	Chemical ID	CR111602	# Concentrations tested	12595-13								
2	Replicate	12595-96	14MR0044_P00	Counter data filename											
3	Technician ID, CN	Replicate #	4	Positive Control	4-OH Andro P04 Ctl Tubes/Conc	2									
4															
5	Calculate DPM in aqueous portion after extraction														
6	Calculate DPM in assay tube														
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Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/2/2007	Test Chemical ID	CR11602	8 Microsome ID	12535-13					
2	NB Ref.	12535-98	Chemical data file name	14MR0044.P00	4. Positive Control	4-OH ASDN	Pos Ch Tube/Conc				
3	Technician ID	CKN	Replicate #								
4	Control Type	Average	SD								
5	Full activity	0.171									
6	Background (unconnected)	0.002									
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.002						
12	4-OH ASDN	1	2	-5.00	0.002						
13	4-OH ASDN	2	1	-6.00	0.019						
14	4-OH ASDN	2	2	-6.00	0.017						
15	4-OH ASDN	3	1	-6.30	0.032						
16	4-OH ASDN	3	2	-6.30	0.030						
17	4-OH ASDN	4	1	-7.00	0.097						
18	4-OH ASDN	4	2	-7.00	0.090						
19	4-OH ASDN	5	1	-7.30	0.126						
20	4-OH ASDN	5	2	-7.30	0.130						
21	4-OH ASDN	6	1	-8.00	0.167						
22	4-OH ASDN	6	2	-8.00	0.158						
23	4-OH ASDN	7	1	-9.00	0.134						
24	4-OH ASDN	7	2	-9.00	0.180						
25	4-OH ASDN	8	1	-10.00	0.135						
26	4-OH ASDN	8	2	-10.00	0.199						
27	4-OH ASDN										
28	4-OH ASDN										
29	4-OH ASDN										
30	4-OH ASDN										
31	4-OH ASDN										
32	4-OH ASDN										
33	4-OH ASDN										
34	4-OH ASDN										
35	4-OH ASDN										
36	4-OH ASDN										
37	Test Chemical	Level	Replicate	Log[Test chemical]	Activity						
38	CR11602	1	1	-3.00	0.009						
39	CR11602	1	2	-3.00	0.006						
40	CR11602	1	3	-3.00	0.009						
41	CR11602	2	1	-3.00	0.025						
42	CR11602	2	2	-3.30	0.040						
43	CR11602	2	3	-3.30	0.034						
44	CR11602	3	1	-3.60	0.065						
45	CR11602	3	2	-3.60	0.076						
46	CR11602	3	3	-3.60	0.077						
47	CR11602	4	1	-4.00	0.111						
48	CR11602	4	2	-4.00	0.118						
49	CR11602	4	3	-4.00	0.107						
50	CR11602	5	1	-4.30	0.127						
51	CR11602	5	2	-4.30	0.170						
52	CR11602	5	3	-4.30	0.160						
53	CR11602	6	1	-4.60	0.161						
54	CR11602	6	2	-4.60	0.109						
55	CR11602	6	3	-4.60	0.152						
56	CR11602	7	1	-5.00	0.172						
57	CR11602	7	2	-5.00	0.186						
58	CR11602	7	3	-5.00	0.143						
59	CR11602	8	1	-6.00	0.163						
60	CR11602	8	2	-6.00	0.126						
61	CR11602	8	3	-6.00	0.157						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	3/13/2007	Test	Chemical ID	CR11602	# Concentrations	tested	8	Microsome ID	12535-13
2	NB Ref.	12535-99	Counter data	filename	13MR2313.P00					
3	ID	CKN	Replicate #	5	Positive Control	4-OH ASDN	Tubes/Conc			2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/13/2007	Test Chemical ID	CR11602	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-99	Counter data filename	13MR2313.P00					
3	Technician ID	CKN	Replicate #	5	Positive Control	4-OH ASDN	Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0208		42128		2025385			
8	2	0.0206		41417		2010534			
9	3	0.0200		43172		2158600			
10	4	0.0204		43130		2114216			
11	5	0.0200		40349		2017450			
12						Average DPM/g soln		2065237	
13						SD		67044	
14						CV		3.25	
15						µCi/g soln		0.9303	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					7.9393 g			
25	Mass of dilution B used in substrate prep					4.5116 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56882995 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.9303				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					= 0.5688 + 0.011				
37					= 0.580 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39					= (µCi/g soln.)/(µg ASDN/g soln.)				
40					= 1.603 µCi/µg ASDN				
41					1.020.E+06 dpm/nmol				

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/13/2007	Test Chemical ID	CR11602	# Concentrations tested			8	Microsome ID 12535-13
2		NB Ref	12535-99	Counter data filename	13MR2313.P00					
3		Technician ID	CKN	Replicate #	5	Positive Control	4-OH ASDN	Tubes/Conc	2	Pos Ctl
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
			Test #							
1		As: 3/13/2007	Chemical ID C	Concentrations tested		8	Microsome ID 12535-13			
2		NB 12535-99	Counter data filename 13MR2313.P00							
3		Te	Replicate # 5	Positive Control		Pos Cil	4-OH ASD Tubes/Conc		2	
4		ID CKN								
5	Vial	Tin	DPM1		tSIE					Round DPM
6										
7	2	5	42127.5	21208.86	0	13.56	590.42			42128
8	3	5	41416.54	20826.13	0	13.49	590.92			41417
9	4	5	43172.32	21641.12	0	13.56	592.42			43172
10	5	5	43129.89	21442.53	0	13.44	582.32			43130
11	6	5	40349.25	20183.64	0	13.47	585.84			40349
12										
13	8	5	6905.53	3242.43	0	12.56	521.25			6906
14	9	5	6717.6	3169.84	0	12.47	522.4			6718
15	10	5	7025.77	3332.38	0	12.57	522.57			7026
16	11	5	6981.14	3299.17	0	12.44	521.34			6981
17	12	5	7019.27	3307.83	0	12.57	524.3			7019
18	13	5	7106.2	3342.19	0	12.5	522.4			7106
19	14	5	6045.41	2873.53	0	12.44	523.87			6045
20	15	5	5987.04	2833.74	0	12.5	524.47			5987
21	16	5	74.47	36.95	0	10.4	520.6			74
22	17	5	88.74	40.56	0	11.19	523.71			89
23	18	5	107.69	47.82	0	12.16	523.01			108
24	19	5	92.8	38.95	0	14.58	525.93			93
25	20	5	168.69	81.55	0	11.59	521.62			169
26	21	5	180.93	87.27	0	11.87	522.74			181
27	22	5	96.01	44.73	0	11.54	520.21			96
28	23	5	93.67	42.14	0	12.16	519.53			94
29	24	5	152.83	66.75	0	12.69	520.38			
30	24	5	152.83	66.75	0	12.69	520.38			153
31	25	5	169.23	77.44	0	12.58	523.69			169
32	26	5	185.88	82.69	0	13.27	517.36			186
33	27	5	190.96	84.9	0	12.82	524.01			191
34										
35										
36	28	5	751.47	351.57	0	12.35	521.27			751
37	29	5	750.9	345.91	0	12.65	522.75			751
38	30	5	813.32	373.96	0	12.84	521.71			813
39	31	5	782.05	370.57	0	12.31	520.55			782
40										
41										
42	32	5	1480.85	695.19	0	12.56	523.57			1481
43	33	5	1460.5	710.39	0	12.22	522.08			1461
44	34	5	1312.95	612.52	0	12.59	523.02			1313
45	35	5	1349.09	634.32	0	12.58	520.9			1349
46										
47										
48	37	5	3721.46	1756.06	0	12.49	521.66			3721
49	38	5	3719.19	1752.41	0	12.57	521.82			3719
50	39	5	3783.69	1774.51	0	12.56	521.68			3784
51	40	5	3723.98	1751.61	0	12.6	520.39			3724
52										
53										
54	41	5	5639.54	2664.66	0	12.53	521.59			5640
55	42	5	5693.78	2662.16	0	12.51	519.78			5694
56	43	5	4464	2097.88	0	12.53	519.8			4464
57	44	5	4523.59	2122.2	0	12.51	521.47			4524
58										
59										
60	45	5	7124.81	3327.55	0	12.52	522.11			7125
61	46	5	7012.22	3298.32	0	12.52	522.41			7012
62	47	5	6230.88	2966.97	0	12.4	522.05			6231
63	48	5	6183.87	2927.3	0	12.49	522.01			6184
64										0
65										0
66	49	5	7627.39	3624.5	0	12.39	522.2			7627
67	50	5	7616.96	3590.32	0	12.6	522.29			7617
68	51	5	5710.09	2678.15	0	12.51	519.27			5710
69	52	5	5627.29	2646.94	0	12.51	522.46			5627
70										

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71											
72	53	5	6643.99	3127.13	0	12.54	523.7				6644
73	54	5	6476.78	3061.41	0	12.59	525.77				6477
74	55	5	7830.56	3713.4	0	12.48	521.73				7831
75	56	5	7593.95	3585.03	0	12.6	524.09				7594
76											
77	58	5	411.92	189.67	0	12.53	521.03				412
78	59	5	385.22	185.09	0	12.25	520.68				385
79	60	5	426.03	204.56	0	12.05	524.05				426
80	61	5	441.5	212.4	0	12.3	522.33				442
81	62	5	415.79	192.99	0	12.41	523.04				416
82	63	5	415.52	191.54	0	12.56	521.51				416
83	64	5	1499.58	695.03	0	12.62	522.76				1500
84	65	5	1450.54	686.66	0	12.44	519.58				1451
85	66	5	1552.57	729.66	0	12.5	520.65				1553
86	67	5	1508.94	706.17	0	12.37	520.14				1509
87	68	5	1418.95	680.56	0	12.52	521.42				1419
88	69	5	1513.7	714.74	0	12.58	522.95				1514
89	70	5	3013.46	1410.21	0	12.64	523.82				3013
90	71	5	2986.49	1414.73	0	12.45	523.99				2986
91	72	5	2946.55	1383.91	0	12.53	522.87				2947
92	73	5	2900.71	1365.59	0	12.68	523.92				2901
93	74	5	2983.91	1397.99	0	12.41	521.87				2984
94	75	5	3100.2	1469.76	0	12.56	523.64				3100
95	76	5	4971.35	2331.93	0	12.52	522				4971
96	77	5	4908.64	2309.54	0	12.41	519.33				4909
97	78	5	5206.76	2429.95	0	12.55	518.95				5207
98	79	5	5128.03	2409.28	0	12.47	519.47				5128
99	80	5	4675.17	2212.24	0	12.49	520.04				4675
100	81	5	4798.48	2252.44	0	12.49	519.13				4788
101	82	5	6552.95	3073.93	0	12.46	519.52				6553
102	83	5	6590.79	3078.76	0	12.51	519.94				6591
103	84	5	5723.67	2692.67	0	12.55	520.5				5724
104	85	5	5564.96	2618.69	0	12.57	523.93				5585
105	86	5	6139.31	2880.36	0	12.56	522.14				6139
106	87	5	6079.3	2873.9	0	12.46	521.42				6079
107	88	5	6659.75	3131.45	0	12.55	522.86				6660
108	89	5	6560	3079.92	0	12.64	524.91				6560
109	90	5	6538.67	3098.74	0	12.4	523.43				6539
110	91	5	6303.88	2961.95	0	12.58	523.62				6304
111	92	5	6556.2	3086.97	0	12.5	521.58				6556
112	93	5	6625.55	3083.9	0	12.52	518.43				6626
113	94	5	8056.71	3778.66	0	12.57	522.01				8057
114	95	5	7795.31	3663.37	0	12.52	519.58				7795
115	96	5	6387.27	3015.96	0	12.53	525.11				6387
116	97	5	6205.75	2928.83	0	12.5	522.91				6206
117	98	5	6779.53	3178.02	0	12.72	524.04				6780
118	99	5	6771.67	3188.79	0	12.51	522.56				6772
119	100	5	4709.04	2271.52	0	12.66	538.64				4709
120	101	5	4654.07	2231.32	0	12.71	542.79				4654
121	102	5	7559.06	3558.4	0	12.53	521				7559
122	103	5	7442.59	3527.68	0	12.47	522.92				7443
123	104	5	7603.73	3576.23	0	12.58	524.49				7604
124	105	5	7711.19	3611.95	0	12.5	520.44				7711
125	106	5	237263.63	96038.4	0	11.5	425.55				237284
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	3/13/2007	Chemical	CR11602	# Concentrations tested	12535-13								
2		NIB Ref.	12535-00	Conc. data	13MR2313 P100										
3		Technician		Replicate	4-OM ASDN	Pos Ctrl Tubes/Conc.	2								
4		ID	CKN	#		5	Positive Control								
5	Sample ID	Replicate	Volume (mL)	Aliq. #	CPM/Assay	DPH/mL	Ave DPH/mL	Total DPM	Volume of substrate solution (mL)	total DPM in assay tube (initial)	Calculate and ³ H ₂ O formed background (background Tubes)	Final ³ H ₂ O formed (mg/L)	Final protein in assay (mg/L)	Incubation time (min)	Aromatase activity (fmol estrogen formed/pmol/min)
6	Full activity control	1	2	0.5	1	6905	13312	27448	0.1	206524	26798	0.0263	0.005	15	0.175
7		2	0.5	2	13481	13481	13481	26914	0.1	206524	27582	0.0270	0.005	15	0.180
8		3	0.5	1	7026	14052	14052	28114	0.1	206524	27582	0.0270	0.005	15	0.180
9		4	0.5	2	7019	14038	14126	28220	0.1	206524	27768	0.0273	0.005	15	0.182
10		5	0.5	1	7106	14212	14212	28424	0.1	206524	28112	0.0272	0.005	15	0.184
11		6	0.5	2	6946	13892	13892	27784	0.1	206524	27784	0.0270	0.005	15	0.180
12		7	0.5	1	5987	11974	11974	23948	0.1	206524	23948	0.0248	0.005	15	0.160
13	Background control	1	2	0.5	1	74	148	326	0.1	206524	126	-0.0001	0.005	15	-0.001
14		2	0.5	2	89	178	178	356	0.1	206524	126	-0.0001	0.005	15	-0.001
15		3	0.5	1	106	212	212	424	0.1	206524	50	0.0004	0.005	15	0.000
16		4	0.5	2	98	196	196	392	0.1	206524	50	0.0004	0.005	15	0.000
17		5	0.5	1	169	338	338	676	0.1	206524	248	0.0002	0.005	15	0.002
18		6	0.5	2	151	302	302	604	0.1	206524	248	0.0002	0.005	15	0.002
19		7	0.5	1	94	188	188	376	0.1	206524	126	-0.0001	0.005	15	-0.001
20		8	0.5	2	103	206	206	412	0.1	206524	126	-0.0001	0.005	15	-0.001
21	4-OM ASDN	1	2	0.5	1	186	372	744	0.1	206524	302	0.0003	0.005	15	0.002
22		2	0.5	2	181	362	362	724	0.1	206524	302	0.0003	0.005	15	0.002
23		3	0.5	1	1481	2962	2962	5924	0.1	206524	5432	0.0053	0.005	15	0.036
24		4	0.5	2	1481	2962	2962	5924	0.1	206524	5432	0.0053	0.005	15	0.036
25		5	0.5	1	1313	2626	2626	5252	0.1	206524	4972	0.0048	0.005	15	0.032
26		6	0.5	2	1359	2718	2718	5436	0.1	206524	4972	0.0048	0.005	15	0.032
27		7	0.5	1	3721	7442	7442	14884	0.1	206524	14428	0.0142	0.005	15	0.094
28		8	0.5	2	3719	7436	7436	14872	0.1	206524	14428	0.0142	0.005	15	0.094
29		9	0.5	1	3784	7568	7568	15136	0.1	206524	14584	0.0143	0.005	15	0.095
30		10	0.5	2	3724	7448	7448	14896	0.1	206524	14584	0.0143	0.005	15	0.095
31		11	0.5	1	5840	11680	11680	23360	0.1	206524	22216	0.0218	0.005	15	0.145
32		12	0.5	2	5840	11680	11680	23360	0.1	206524	22216	0.0218	0.005	15	0.145
33		13	0.5	1	4664	9328	9328	18656	0.1	206524	17584	0.0172	0.005	15	0.115
34		14	0.5	2	4624	9048	9048	18096	0.1	206524	17584	0.0172	0.005	15	0.115
35		15	0.5	1	7125	14250	14250	28500	0.1	206524	27822	0.0273	0.005	15	0.182
36		16	0.5	2	7012	14024	14024	28048	0.1	206524	27822	0.0273	0.005	15	0.182
37		17	0.5	1	6231	12462	12462	24924	0.1	206524	24378	0.0239	0.005	15	0.159
38		18	0.5	2	6184	12368	12368	24736	0.1	206524	24378	0.0239	0.005	15	0.159
39		19	0.5	1	7627	15254	15254	30508	0.1	206524	30338	0.0308	0.005	15	0.199
40		20	0.5	2	7617	15234	15234	30468	0.1	206524	30338	0.0308	0.005	15	0.199
41		21	0.5	1	5710	11420	11420	22840	0.1	206524	22222	0.0218	0.005	15	0.145
42		22	0.5	2	5627	11254	11254	22508	0.1	206524	22222	0.0218	0.005	15	0.145
43		23	0.5	1	8844	17688	17688	35376	0.1	206524	25790	0.0253	0.005	15	0.160
44		24	0.5	2	8477	16954	16954	33908	0.1	206524	25790	0.0253	0.005	15	0.160
45		25	0.5	1	7594	15188	15188	30376	0.1	206524	30338	0.0308	0.005	15	0.199
46		26	0.5	2	7594	15188	15188	30376	0.1	206524	30338	0.0308	0.005	15	0.199
47		27	0.5	1					0.1	206524			0.005	15	
48		28	0.5	2					0.1	206524			0.005	15	
49		29	0.5	1					0.1	206524			0.005	15	
50		30	0.5	2					0.1	206524			0.005	15	
51		31	0.5	1					0.1	206524			0.005	15	
52		32	0.5	2					0.1	206524			0.005	15	
53		33	0.5	1					0.1	206524			0.005	15	
54		34	0.5	2					0.1	206524			0.005	15	
55		35	0.5	1					0.1	206524			0.005	15	
56		36	0.5	2					0.1	206524			0.005	15	
57		37	0.5	1					0.1	206524			0.005	15	
58		38	0.5	2					0.1	206524			0.005	15	
59		39	0.5	1					0.1	206524			0.005	15	
60		40	0.5	2					0.1	206524			0.005	15	
61		41	0.5	1					0.1	206524			0.005	15	
62		42	0.5	2					0.1	206524			0.005	15	
63		43	0.5	1					0.1	206524			0.005	15	
64		44	0.5	2					0.1	206524			0.005	15	
65		45	0.5	1					0.1	206524			0.005	15	
66		46	0.5	2					0.1	206524			0.005	15	
67		47	0.5	1					0.1	206524			0.005	15	
68		48	0.5	2					0.1	206524			0.005	15	
69		49	0.5	1					0.1	206524			0.005	15	
70		50	0.5	2					0.1	206524			0.005	15	

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Sample ID	Sample type	Replicate Level	Assay Date	Chemical ID	Test ID	Chemical Name	# Concentrations tested	Microplate ID	12545-13							
1			3/13/2007	CR11802		31MR2313.P00	5	Positive Control	4.0H A500 Pos Cd Tubes Conc.	2						
2			12545-98													
3			Technician ID: CN													
Sample ID	Sample type	Replicate Level	Nominal total volume (mL)	Aliq. #	Aliq. volume (mL)	DPW/aliquot	DPW/mL	Ave DPW/mL	Total DPW	Volume of substrate solution used/assay tube (mL)	total DPW in assay tube (initial)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Inoculation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
5	CR11802	1-1	2	0.5	2	412	824	797	1594	0.1	206524	1142	0.0011	0.005	15	0.007
6		1-2	2	0.5	2	351	702	768	1536	0.1	206524	1294	0.0013	0.005	15	0.008
7		1-3	2	0.5	2	426	852	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
8		1-4	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
9		1-5	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
10		1-6	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
11		1-7	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
12		1-8	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
13		1-9	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
14		1-10	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
15		1-11	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
16		1-12	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
17		1-13	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
18		1-14	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
19		1-15	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
20		1-16	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
21		1-17	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
22		1-18	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
23		1-19	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
24		1-20	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
25		1-21	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
26		1-22	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
27		1-23	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
28		1-24	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
29		1-25	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
30		1-26	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
31		1-27	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
32		1-28	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
33		1-29	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
34		1-30	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
35		1-31	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
36		1-32	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
37		1-33	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
38		1-34	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
39		1-35	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
40		1-36	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
41		1-37	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
42		1-38	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
43		1-39	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
44		1-40	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
45		1-41	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
46		1-42	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
47		1-43	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
48		1-44	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
49		1-45	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
50		1-46	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
51		1-47	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
52		1-48	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
53		1-49	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008
54		1-50	2	0.5	2	416	832	832	1664	0.1	206524	1212	0.0012	0.005	15	0.008

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		
6		QC check - aliquot variance
7		% RE
8		1.03
9		0.97
10		0.99
11		1.02
12		1.00
13		1.00
14		1.02
15		0.98
16		1.01
17		0.99
18		0.97
19		1.02
20		1.00
21		1.01
22		0.99
23		0.98
24		1.02
25		1.01
26		0.99
27		1.01
28		0.99
29		0.99
30		1.01
31		1.00
32		1.02
33		1.00
34		0.98
35		1.00
36		1.00
37		1.01
38		0.99
39		1.02
40		0.99
41		0.99
42		1.01
43		1.02
44		0.98
45		1.01
46		0.99
47		1.00
48		1.00
49		0.99
50		1.01
51		1.01
52		0.99
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/13/2007	Test Chemical ID	CR11602	# Concentrations	8	Microsome ID	12535-13			
2	NB Ref.	12535-99	Counter data filename	13MR2313.P00							
3	Technician ID	CKN	Replicate #		6	Positive Control	4-OH ASDN	Pos. Ctrl Tubes/Conc		2	
4	Control Type										
5	Portion	Average	SD								
6	Full activity	0.173	0.013								
7	Background (unconnected)										
8	Overall	0.003	0.001								
9											
10	Positive Control										
11	4-OH ASDN	1	1.00E-05	-5.00	Activity	0.001					
12	4-OH ASDN	2	1.00E-05	-5.00		0.002					
13	4-OH ASDN	1	1.00E-05	-6.00		0.017					
14	4-OH ASDN	2	1.00E-05	-6.00		0.018					
15	4-OH ASDN	3	1.00E-05	-6.00		0.036					
16	4-OH ASDN	4	1.00E-05	-6.00		0.032					
17	4-OH ASDN	1	5.00E-07	-6.30		0.094					
18	4-OH ASDN	2	5.00E-07	-6.30		0.095					
19	4-OH ASDN	3	1.00E-07	-7.00		0.145					
20	4-OH ASDN	4	1.00E-07	-7.00		0.115					
21	4-OH ASDN	5	5.00E-08	-7.30		0.162					
22	4-OH ASDN	6	5.00E-08	-8.00		0.159					
23	4-OH ASDN	7	1.00E-09	-9.00		0.195					
24	4-OH ASDN	8	1.00E-09	-9.00		0.145					
25	4-OH ASDN	1	1.00E-10	-10.00		0.169					
26	4-OH ASDN	2	1.00E-10	-10.00		0.199					
27	4-OH ASDN	3									
28	4-OH ASDN	4									
29	4-OH ASDN	5									
30	4-OH ASDN	6									
31	4-OH ASDN	7									
32	4-OH ASDN	8									
33	4-OH ASDN	1									
34	4-OH ASDN	2									
35	4-OH ASDN	3									
36	4-OH ASDN	4									
37	Test Chemical										
38	CR11602	1	1.00E-03	-3.00	Activity	0.007					
39	CR11602	2	1.00E-03	-3.00		0.008					
40	CR11602	3	1.00E-03	-3.00		0.008					
41	CR11602	4	1.00E-04	-3.30		0.036					
42	CR11602	5	5.00E-04	-3.30		0.037					
43	CR11602	6	5.00E-04	-3.30		0.035					
44	CR11602	7	2.50E-04	-3.60		0.076					
45	CR11602	8	2.50E-04	-3.60		0.074					
46	CR11602	1	1.00E-04	-4.00		0.077					
47	CR11602	2	1.00E-04	-4.00		0.126					
48	CR11602	3	1.00E-04	-4.00		0.132					
49	CR11602	4	1.00E-04	-4.00		0.121					
50	CR11602	5	5.00E-05	-4.30		0.169					
51	CR11602	6	5.00E-05	-4.30		0.145					
52	CR11602	7	2.50E-05	-4.60		0.157					
53	CR11602	8	2.50E-05	-4.60		0.170					
54	CR11602	1	1.00E-05	-5.00		0.165					
55	CR11602	2	1.00E-05	-5.00		0.169					
56	CR11602	3	1.00E-05	-5.00		0.204					
57	CR11602	4	1.00E-05	-5.00		0.162					
58	CR11602	5	1.00E-05	-5.00		0.174					
59	CR11602	6	1.00E-06	-6.00		0.198					
60	CR11602	7	1.00E-06	-6.00		0.163					
61	CR11602	8	1.00E-06	-6.00		0.187					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/1/2007	Test Chemical ID	CR11603	# Concentrations tested			8 Microsome ID	12535-13
2	NB Ref.	12535-56	Counter data filename	01MR2205.P00				Pos Ctl	
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/1/2007	Test Chemical ID	CR11603	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-56	Counter data filename	01MR2205.P00					
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0193		35636		1846425			
8	2	0.0192		32500		1692708			
9	3	0.0187		39832		2130053			
10	4	0.0194		43655		2250258			
11	5	0.0199		41529		2086884			
12						Average DPM/g soln	2001266		
13						SD	226438		
14						CV	11.31		
15						µCi/g soln	0.9015		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution				8.1341 g				
25	Mass of dilution B used in substrate prep				4.5763 g				
26	Concentration of nonradiolabeled ASDN in substrate soln.				0.56316941 µg/g				
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011	µg/g soln.			
29						µg/g soln.			
30	a. µCi/g soln				0.9015				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5632	+	10.011		
37				=	0.574 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40				=	1.570 µCi/µg ASDN				
41	9.983.E+05 dpm/nmol								

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/1/2007	Test Chemical ID	CR11603	# Concentrations tested	8	Microsome ID	12535-13	
2		NB Ref	12535-56	Counter data filename	01MR2205.P00					
3		Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4										
5	Microsome Dilution Details									
6										
7	Dilution A									
8		0.12 mL microsome Stock used								
9		2.4 mL total volume								
10		20 dilution factor								
11	Dilution B									
12		1.5 mL microsome Dilution A used								
13		60 mL total volume								
14		40 dilution factor								
15	Dilution C (if applicable)									
16		mL microsome Dilution B used								
17		mL total volume								
18		dilution factor								
19	NA									
20		800 total dilution factor								
21	Protein Concentration (stock microsomes, mg/mL):			8.00						
22	Protein Concentration (dilution added to assay, mg/mL):			0.01						
23										
24										
25										
26										

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Test Chemical # Concentrations Assay: 3/1/2007 ID CF tested 8 Microsome ID 12535-13										
2	Counter data NB I 12535-56 filename 01MR2205.P00										
3	Tec hnic lan Pos Ctl										
4	ID PRP Replicate # 1 Positive Control 4-OH ASD Tubes/Conc 2										
5	Vial # Time DPM1 ISIE Round DPM										
6	2	5	35635.64	14329.3	0	12.76	491.57				35836
7	3	5	32500.46	13147.14	0	12.8	494.95				32500
8	4	5	39831.75	15954.42	0	12.79	490.8				39832
9	5	5	43654.88	17483.06	0	12.69	488.15				43655
10	6	5	41528.58	16616.34	0	12.77	489.34				41529
11	8	5	10985.59	4795.3	0	13.61	552.21				10986
12	9	5	10963.08	4756.42	0	13.71	553.05				10963
13	10	5	10990.58	4763.54	0	13.61	547.82				10991
14	11	5	10762.34	4690.54	0	13.65	555.2				10762
15	12	5	9766.66	4247.78	0	13.7	553.03				9767
16	13	5	9646.01	4196.14	0	13.72	555.35				9646
17	14	5	10514.96	4557.82	0	13.68	550.9				10515
18	15	5	10368.89	4522.9	0	13.69	555.79				10369
19	16	5	61.46	24.94	0	12.62	551.82				61
20	17	5	56.33	26.62	0	14.63	556.15				56
21	18	5	61.92	26.54	0	13.17	552.88				62
22	19	5	61.39	26.98	0	13.82	553.86				61
23	20	5	44.79	20.54	0	10.62	552.09				45
24	21	5	84.78	33.18	0	16.32	552.86				85
25	22	5	38.56	17.74	0	12.24	554.51				39
26	23	5	48.51	22.5	0	13.1	552.75				49
27	25	5	127.66	55.5	0	14.08	553.51				128
28	26	5	166.55	71.94	0	13.52	551.14				167
29	27	5	131.15	59.14	0	12.46	553.2				131
30	28	5	127.15	55.58	0	12.85	554.43				127
31	29	5	758.81	328.22	0	13.84	555				759
32	30	5	746.27	318.94	0	14.1	555.66				746
33	31	5	688.74	304.34	0	13.55	555.21				689
34	32	5	683.45	295.34	0	13.65	552.75				683
35	33	5	1201.3	527.94	0	13.59	553.7				1201
36	34	5	1247.85	546.14	0	13.56	553.05				1248
37	35	5	1165.31	499.38	0	13.82	553.44				1165
38	36	5	1128.25	489.7	0	13.76	553.61				1128
39	37	5	4329.61	1873.82	0	13.64	551.96				4330
40	38	5	4244.11	1868.3	0	13.69	557.39				4244
41	39	5	3897.3	1672.74	0	13.81	552.59				3897
42	40	5	3798.88	1654.1	0	13.6	554.32				3800
43	41	5	5549.06	2391.54	0	13.71	552.14				5549
44	42	5	5485.5	2372.3	0	13.85	554.67				5486
45	43	5	5768.14	2495.3	0	13.94	553.66				5768
46	44	5	5759.65	2491.1	0	13.79	552.23				5760
47	45	5	9545.34	4158.9	0	13.77	555.71				9545
48	46	5	9657.23	4204.7	0	13.7	553.59				9657
49	47	5	9239.59	4019.74	0	13.66	552.33				9240
50	48	5	9255.02	4024.38	0	13.65	551.72				9255
51	49	5	10229.89	4458.58	0	13.74	554.49				10230
52	50	5	10194.68	4432.5	0	13.81	557.43				10195
53	51	5	10272.92	4455.9	0	13.71	552.59				10273
54	52	5	9825.42	4294.18	0	13.74	555.91				9825
55	53	5	10384.46	4494.3	0	13.71	552.62				10384
56	54	5	10204.99	4452.22	0	13.6	555.7				10205
57	55	5	10335.77	4485.9	0	13.77	554.65				10336
58	56	5	10109.77	4416.98	0	13.75	558.66				10110
59	58	5	8695.16	3802.1	0	13.73	554.44				8695
60	59	5	8664.27	3791.34	0	13.67	556.91				8664
61	60	5	7692.03	3386.22	0	13.64	554.85				7692
62	61	5	7760.75	3369.5	0	13.68	553.92				7761
63	62	5	8228.56	3588.9	0	13.68	555.75				8227
64	63	5	8192.28	3551.54	0	13.74	554.97				8192
65	64	5	8473.23	3696.18	0	13.77	556.77				8473
66	65	5	8519.15	3764.34	0	13.74	557.52				8518
67	66	5	9016.13	3907.7	0	13.78	553.62				9016
68	67	5	8984.25	3906.3	0	13.75	554.66				8984
69	68	5	8629.89	3756.78	0	13.82	553.75				8630
70	69	5	8784.47	3781.82	0	13.64	551.58				8784
71	70	5	10438.75	4557.22	0	13.64	555.13				10439

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
72	71	5	10556.96	4589.9	0	13.76	553.74				10557
73	72	5	9688.58	4211.38	0	13.7	557.21				9689
74	73	5	9185.95	4024.5	0	13.68	558.88				9186
75	74	5	9343.15	4074.54	0	13.68	553.97				9343
76	75	5	9364.63	4072.14	0	13.77	552.51				9365
77	76	5	10283.96	4475.02	0	13.64	553.73				10284
78	77	5	10211.15	4434.98	0	13.74	554				10211
79	78	5	10676.27	4614.5	0	13.79	553.97				10676
80	79	5	10509	4570.1	0	13.74	555.33				10509
81	80	5	10322.98	4490.42	0	13.72	554.97				10323
82	81	5	10280.05	4456.1	0	13.71	551.41				10280
83	82	5	10533.32	4572.34	0	13.79	554.82				10533
84	83	5	10559.25	4600.1	0	13.73	553.77				10559
85	84	5	10430.48	4532.14	0	13.68	553.07				10430
86	85	5	9884.59	4285.54	0	13.75	555.39				9885
87	86	5	9445.11	4099.1	0	13.78	556.7				9445
88	87	5	9320.94	4057.14	0	13.66	553.57				9321
89	88	5	10223.16	4435.54	0	13.66	552.07				10223
90	89	5	10077.41	4397.1	0	13.7	554.81				10077

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
91	90	5	9820.33	4233.94	0	13.79	553.26				9820
92	91	5	9695.75	4212.18	0	13.81	554.68				9696
93	92	5	9746.59	4245.54	0	13.68	555.11				9747
94	93	5	9611.12	4167.14	0	13.74	552.65				9611
95	94	5	9755.05	4232.18	0	13.84	553.7				9755
96	95	5	9620.14	4159.1	0	13.71	549.95				9620
97	96	5	9238.31	4010.58	0	13.62	550.99				9238
98	97	5	9298.74	4039.98	0	13.71	555.18				9299
99	98	5	10195.94	4421.9	0	13.7	551.91				10196
100	99	5	10068.67	4359.58	0	13.78	552.35				10069
101	100	5	10220.9	4429.34	0	13.72	553.71				10221
102	101	5	10107.77	4373.5	0	13.78	551.08				10108
103	102	5	9226.48	3996.78	0	13.72	553.06				9226
104	103	5	8991.98	3911.14	0	13.71	555.78				8992
105	104	5	10042.44	4334.3	0	13.76	553.94				10042
106	105	5	10149.73	4382.3	0	13.72	549.64				10150
107	107	5	234515.56	108714.98	0	14.98	626.52				234516
108											0
109											0
110											0
111											0
112											0
113											0
114											0
115											0
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Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Level	Volume (mL)	Aliq #	DPW/mL	DPW/mL	Ave DPM/mL	Total DPM	Volume of substrate solution (used/assay tube) (mL)	total DPM in assay tube (initial)	Calculate nmoL ³ H ₂ O formed	nmol ³ H ₂ O formed	Final (protein) (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
1	CR11603	1-1	2	0.5	1	8695	17390	34718	0.1	200127	34409	0.0345	0.005	15	0.230
2	CR11603	1-2	2	0.5	1	8694	17328	34596	0.1	200127	34677	0.0307	0.005	15	0.205
3	CR11603	1-3	2	0.5	1	7692	15384	15453	0.1	200127	30677	0.0327	0.005	15	0.218
4	CR11603	2-1	2	0.5	1	8727	17454	18419	0.1	200127	32839	0.0340	0.005	15	0.227
5	CR11603	2-2	2	0.5	1	8473	16945	17091	0.1	200127	34182	0.0358	0.005	15	0.239
6	CR11603	2-3	2	0.5	1	8618	17236	18000	0.1	200127	34600	0.0347	0.005	15	0.231
7	CR11603	3-1	2	0.5	1	8994	17988	17414	0.1	200127	34828	0.0416	0.005	15	0.279
8	CR11603	3-2	2	0.5	1	8530	17060	17500	0.1	200127	34599	0.0376	0.005	15	0.251
9	CR11603	3-3	2	0.5	1	10438	20876	20995	0.1	200127	41763	0.0372	0.005	15	0.248
10	CR11603	4-1	2	0.5	1	9698	19378	18475	0.1	200127	37521	0.0408	0.005	15	0.272
11	CR11603	4-2	2	0.5	1	9343	18686	18708	0.1	200127	37187	0.0422	0.005	15	0.281
12	CR11603	4-3	2	0.5	1	10284	20568	20485	0.1	200127	40781	0.0440	0.005	15	0.274
13	CR11603	5-1	2	0.5	1	10676	21352	21185	0.1	200127	42141	0.0420	0.005	15	0.260
14	CR11603	5-2	2	0.5	1	10280	20560	20639	0.1	200127	41206	0.0405	0.005	15	0.270
15	CR11603	5-3	2	0.5	1	10533	21066	21082	0.1	200127	42184	0.0374	0.005	15	0.249
16	CR11603	6-1	2	0.5	1	9445	18890	18766	0.1	200127	37532	0.0404	0.005	15	0.269
17	CR11603	6-2	2	0.5	1	10723	21446	20300	0.1	200127	40900	0.0405	0.005	15	0.270
18	CR11603	6-3	2	0.5	1	10077	20154	19516	0.1	200127	40371	0.0386	0.005	15	0.257
19	CR11603	7-1	2	0.5	1	9606	19212	19375	0.1	200127	39716	0.0388	0.005	15	0.257
20	CR11603	7-2	2	0.5	1	9511	19022	18537	0.1	200127	38521	0.0369	0.005	15	0.246
21	CR11603	7-3	2	0.5	1	9520	19040	18537	0.1	200127	38521	0.0404	0.005	15	0.269
22	CR11603	8-1	2	0.5	1	10708	21416	20329	0.1	200127	40558	0.0405	0.005	15	0.270
23	CR11603	8-2	2	0.5	1	9276	18552	18718	0.1	200127	38507	0.0363	0.005	15	0.242
24	CR11603	8-3	2	0.5	1	10442	20884	20192	0.1	200127	40155	0.0402	0.005	15	0.268

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC check - aligned variance
5		
6		% RE
7		1.00
8		1.00
9		1.00
10		1.00
11		1.00
12		1.00
13		0.99
14		1.01
15		1.00
16		1.00
17		1.01
18		0.99
19		1.01
20		1.01
21		1.03
22		0.97
23		1.00
24		1.00
25		1.00
26		1.00
27		1.01
28		0.99
29		1.00
30		1.00
31		1.00
32		1.03
33		0.97
34		1.01
35		0.99
36		1.01
37		0.99
38		1.01
39		0.99
40		1.01
41		0.99
42		1.01
43		0.99
44		1.01
45		1.00
46		1.01
47		0.99
48		1.01
49		0.99
50		1.01
51		0.99
52		1.01
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L						
Assay Date		NB Ref	Technician ID	PRP	Test Chemical ID	CR11603	# Concentrations	8	Microsome ID	12535-13	2							
3/1/2007													12535-56	01M22/05.P00	Replicate #	1	Positive Control	4-OH ASDN
12535-56																		
1	Control Type	Portion	Average	SD	Bg % of Full													
2	Full activity	Overall	0.279	0.015	0.5 (Red if >1%)													
3	Background (unconnected)	Overall	0.002	0.000														
4																		
5																		
6																		
7																		
8																		
9																		
10	Positive Control	Level	Replicate	[Positive Ctrl] M	Log(Positive Ctrl)	Activity	Percent of control values											
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.002	Level	Log(test chemical)	Replicate									
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002	1	-5.00	1	0.9	2	0.7						
13							2	-6.00		6.7	6.0							
14	4-OH ASDN	2	1	1.00E-06	-6.00	0.019	3	-6.00		11.2	10.4							
15	4-OH ASDN	2	2	1.00E-06	-6.00	0.017	4	-7.00		40.5	36.3							
16							5	-7.30		52.3	54.8							
17	4-OH ASDN	3	1	5.00E-07	-6.30	0.031	6	-8.00		91.4	88.0							
18	4-OH ASDN	3	2	5.00E-07	-6.30	0.029	7	-9.00		97.2	95.7							
19							8	-10.00		98.0	97.3							
20	4-OH ASDN	4	1	1.00E-07	-7.00	0.113												
21	4-OH ASDN	4	2	1.00E-07	-7.00	0.101												
22																		
23	4-OH ASDN	5	1	5.00E-08	-7.30	0.146												
24	4-OH ASDN	5	2	5.00E-08	-7.30	0.152												
25																		
26	4-OH ASDN	6	1	1.00E-08	-8.00	0.255												
27	4-OH ASDN	6	2	1.00E-08	-8.00	0.245												
28																		
29	4-OH ASDN	7	1	1.00E-09	-9.00	0.271												
30	4-OH ASDN	7	2	1.00E-09	-9.00	0.267												
31																		
32	4-OH ASDN	8	1	1.00E-10	-10.00	0.273												
33	4-OH ASDN	8	2	1.00E-10	-10.00	0.272												
34																		
35																		
36																		
37	Test Chemical	Level	Replicate	[test chemical] M	Log(test chemical)	Activity	Percent of control values											
38	CR11603	1	1	1.00E-03	-3.00	0.230	Level	Log(test chemical)	Replicate									
39	CR11603	1	2	1.00E-03	-3.00	0.205	1	-3.00	1	82.6	73.4	3						
40	CR11603	2	1	1.00E-03	-3.00	0.218	2	-4.00		81.3	85.6							
41	CR11603	2	2	1.00E-04	-4.00	0.227	3	-5.00		100.0	89.8							
42	CR11603	2	1	1.00E-04	-4.00	0.239	4	-6.00		97.6	100.9							
43	CR11603	3	3	1.00E-04	-4.00	0.231	5	-7.00		100.4	96.7							
44	CR11603	3	1	1.00E-05	-5.00	0.279	6	-8.00		96.6	89.2							
45	CR11603	3	2	1.00E-05	-5.00	0.251	7	-9.00		96.5	95.5							
46	CR11603	3	3	1.00E-05	-5.00	0.248	8	-10.00		92.2	86.7							
47	CR11603	4	1	1.00E-06	-6.00	0.272												
48	CR11603	4	2	1.00E-06	-6.00	0.281												
49	CR11603	4	3	1.00E-06	-6.00	0.274												
50	CR11603	5	1	1.00E-07	-7.00	0.290												
51	CR11603	5	2	1.00E-07	-7.00	0.270												
52	CR11603	5	3	1.00E-07	-7.00	0.248												
53	CR11603	6	1	1.00E-08	-8.00	0.270												
54	CR11603	6	2	1.00E-08	-8.00	0.259												
55	CR11603	6	3	1.00E-08	-8.00	0.257												
56	CR11603	7	1	1.00E-09	-9.00	0.257												
57	CR11603	7	2	1.00E-09	-9.00	0.246												
58	CR11603	7	3	1.00E-09	-9.00	0.269												
59	CR11603	8	1	1.00E-10	-10.00	0.276												
60	CR11603	8	2	1.00E-10	-10.00	0.242												
61	CR11603	8	3	1.00E-10	-10.00	0.268												

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/6/2007	Test Chemical ID	CR11603	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-59	Counter data filename	06MR2145.P00					
	Technician		Pos Ctl						
3	ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/6/2007	Test Chemical ID	CR11603	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-59	Counter data filename	06MR2145.P00					
3	Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0193		35849		1857461			
8	2	0.0191		34121		1786440			
9	3	0.0199		38411		1930201			
10	4	0.0196		39113		1995561			
11	5	0.0194		35458		1827732			
12						Average DPM/g soln		1879479	
13						SD		83467	
14						CV		4.44	
15						µCi/g soln		0.8466	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.00	10		1000.00				
20	Dilution A			100	10.00				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1007 g			
25	Mass of dilution B used in substrate prep					4.5505 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56174158 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.010 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8466			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5617	+	0.010		
37				=	0.572	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=		1.480	µCi/µg ASDN					
41			9.410.E+05	dpm/nmol					

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/6/2007	Test Chemical ID	CR11603	# Concentrations tested			8: Microsome ID	12535-13
2			NB Ref	12535-59	Counter data filename	06MR2145.P00					
3			Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
5			Microsome Dilution Details								
6											
7				0.12 mL microsome Stock used							
8				2.4 mL total volume							
9				20 dilution factor							
10											
11				1.5 mL microsome Dilution A used							
12				60 mL total volume							
13				40 dilution factor							
14											
15				mL microsome Dilution B used							
16				mL total volume							
17				dilution factor							
18				800 total dilution factor							
19											
20											
21				Protein Concentration (stock microsomes, mg/mL):	8.00						
22				Protein Concentration (dilution added to assay, mg/mL):	0.01						
23											
24											
25											
26											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
			Test #							
1	As: 3/6/2007		Chemical ID C	Concentrations tested				8	Microsome ID 12535-13	
2			Counter data filename	D6MR2145.P00						
3	Te									
4	ch									
	nic									
	ian									
3	ID PRP		Replicate # 2	Positive Control		Pos Ctl	4-OH ASD Tubes/Conc		2	
5	Vial Tin DPM1									Round DPM
6	2	5	35849.29	14541.92	0	13.09	503.08			35849
7	3	5	34120.5	13762.44	0	13.06	498.92			34121
8	4	5	38410.62	15358.08	0	12.94	492.61			38411
9	5	5	39112.68	15462.68	0	12.81	485.73			39113
10	6	5	35457.98	14009.36	0	12.81	484.7			35458
11	8	5	12451.17	5325.76	0	13.77	543.91			12451
12	9	5	12214.54	5221.32	0	13.87	547.78			12215
13	10	5	11755.78	5039.52	0	13.8	547.99			11756
14	11	5	11782.67	5089.76	0	13.83	551.99			11783
15	12	5	10526.39	4516.64	0	13.8	546.43			10526
16	13	5	10329.99	4430.12	0	13.82	548.42			10330
17	14	5	10992.41	4741.56	0	13.75	548.04			10992
18	15	5	10945.7	4714.98	0	13.76	549.28			10946
19	16	5	57.67	25.2	0	14.67	549.32			58
20	17	5	59.31	23.36	0	17.2	548.42			59
21	18	5	48.52	18.32	0	16.42	549.49			49
22	19	5	59.28	26.56	0	15.4	549.04			59
23	20	5	60.99	27.52	0	13.25	547.1			61
24	21	5	64.63	27.36	0	16.18	551.16			65
25	22	5	57.61	22.96	0	18.29	548.74			58
26	23	5	70.88	31.76	0	14.71	549.88			71
27	25	5	139.91	64.12	0	14.69	552.07			140
28	26	5	151.38	62.8	0	14.65	546.41			151
29	27	5	146.99	65.2	0	14.35	551.15			147
30	28	5	138.54	58.92	0	15.63	547.86			139
31	29	5	785.88	341.16	0	13.77	550.25			786
32	30	5	784.18	338.36	0	13.97	550.77			784
33	31	5	714.45	313.4	0	14.18	546.84			714
34	32	5	720.91	309.12	0	13.98	550.55			721
35	33	5	1333.56	580.12	0	13.78	550.7			1334
36	34	5	1351.32	582	0	14.12	548.86			1351
37	35	5	1315.6	565.36	0	13.86	546.81			1316
38	36	5	1309.45	566.72	0	13.96	548.19			1309
39	37	5	4759.83	2062.28	0	13.72	550.02			4760
40	38	5	4731.82	2041.8	0	13.91	550.41			4732
41	39	5	4557.91	1957.16	0	13.89	549.61			4558
42	40	5	4394.71	1897.92	0	13.81	549.25			4395
43	41	5	6730.52	2888.4	0	13.8	547.33			6731
44	42	5	6602.32	2833	0	13.82	549.08			6602
45	43	5	6769.32	2912.4	0	13.83	550.95			6769
46	44	5	6789.95	2911.12	0	13.88	550.75			6790
47	45	5	10436.22	4471.72	0	13.86	549.72			10436
48	46	5	10338.66	4497.32	0	13.99	556.43			10339
49	47	5	9997.83	4300.32	0	13.83	548.78			9998
50	48	5	9974.8	4298.36	0	13.86	551.46			9975
51	49	5	11914.49	5120	0	13.78	548.09			11914
52	50	5	11846.62	5101.36	0	13.83	551.66			11847
53	51	5	11442.29	4913.96	0	13.85	550			11442
54	52	5	10880.41	4704.76	0	13.87	552.6			10880
55	53	5	11371.12	4920.76	0	13.86	553.55			11371
56	54	5	11288.34	4861.12	0	13.82	550.35			11288
57	55	5	12145.35	5213.36	0	13.78	548.72			12145
58	56	5	11941.83	5108.36	0	13.84	545.93			11942
59	58	5	10322.83	4458.72	0	13.82	551.69			10323
60	59	5	10193.03	4410.36	0	13.81	551.43			10193
61	60	5	8909.29	3842	0	13.79	551.09			8909
62	61	5	8830.34	3787.32	0	13.78	547.94			8830
63	62	5	8584.19	3676.12	0	13.82	550.19			8584
64	63	5	8479.43	3647.76	0	13.79	548.6			8479
65	64	5	9649.67	4121.56	0	13.92	548.17			9650
66	65	5	9581.29	4128.16	0	13.87	550.33			9581
67	66	5	9129.67	3943.36	0	13.87	552.56			9130
68	67	5	9295.3	4010.56	0	13.79	551.19			9295
69	68	5	9134.52	3945.52	0	13.97	556.58			9135
70	69	5	9054.89	3932.32	0	13.98	558.83			9055

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	10488.51	4520.76	0	13.83	549.69				10489
72	71	5	10378.14	4494.68	0	13.85	552.25				10378
73	72	5	11061.94	4761.8	0	13.87	551.05				11062
74	73	5	10879.45	4682.36	0	13.89	551.79				10879
75	74	5	10975.28	4716.76	0	13.82	551.42				10975
76	75	5	10966.81	4735.12	0	13.85	550.77				10967
77	76	5	11408.78	4905.72	0	13.89	549.4				11409
78	77	5	11339.26	4898.68	0	13.88	551.07				11339
79	78	5	11279.46	4865.92	0	13.81	551.77				11279
80	79	5	11183.69	4808.12	0	13.87	549.98				11184
81	80	5	11722.99	5030.32	0	13.82	549.76				11723
82	81	5	11825.77	5091.16	0	13.8	549.08				11826
83	82	5	9917.32	4256.16	0	13.87	547.5				9917
84	83	5	9604.91	4137.16	0	13.92	550.94				9605
85	84	5	11386.36	4892	0	13.87	551.19				11386
86	85	5	11107.7	4757.6	0	13.82	547.69				11108
87	86	5	11413.03	4898.32	0	13.81	545.57				11413
88	87	5	11211.54	4828.8	0	13.92	552.3				11212
89	88	5	10627.79	4557.36	0	13.82	547.69				10628
90	89	5	10364.8	4476.72	0	13.8	551.52				10365
91	90	5	11337.34	4897.52	0	13.86	548.94				11337
92	91	5	11389.33	4889.92	0	13.81	548.1				11389
93	92	5	10136.61	4393.48	0	13.87	552.07				10137
94	93	5	10405.63	4471.44	0	13.87	547.66				10406
95	94	5	11148.15	4779.96	0	13.79	546.72				11148
96	95	5	11004.6	4723	0	13.8	548.05				11005
97	96	5	11264.48	4805.6	0	13.79	546.99				11264
98	97	5	11179.78	4788.92	0	13.79	547.91				11180
99	98	5	11039.58	4735	0	13.78	545.5				11040
100	99	5	11089.3	4761.32	0	13.86	547.61				11089
101	100	5	10922.42	4692	0	13.88	548.24				10922
102	101	5	10883.34	4653.76	0	13.71	545.69				10883
103	102	5	11177.48	4805.52	0	13.77	547.44				11177

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
104	103	5	11200.58	4803.12	0	13.76	548.34				11201
105	104	5	11725.51	5063.72	0	13.77	546.8				11726
106	105	5	11825.46	5071.4	0	13.82	548.55				11825
107	107	5	236001.19	108526.39	0	15.05	621.75				236001
108											0
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1				Assay Date	3/8/2007	Chemical ID	CP11603	# Concentrations tested	1252b-13						
2				NE Ref.	12535-59	Source data									
3				Replicate	IRP	Pre-Run Control	4-OH ASDN	Pos CUI Tubes/Conc	2						
4															
5	Sample ID	Replicate	Nominal test volume (mL)	Aliq Volume (mL)	Aliq #	DPM (avg)	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate added (mL)	total DPM in assay tube (initial)	nmol ³ H ₂ O formed	Final (pmol) in assay (fmol/mL)	Incubation time (min)	Aromatase activity (nmol ³ H ₂ O formed per nmol protein/min)
6	Full Biotin control	1	2	0.5	1	12631	26092	24556	49332	0.1	187948	49392	0.005	15	0.348
7		2	2	0.5	2	11762	23512	23559	47076	0.1	187948	49398	0.005	15	0.332
8		3	2	0.5	1	11762	23512	23559	47076	0.1	187948	49398	0.005	15	0.332
9		4	2	0.5	2	10226	20452	20856	41712	0.1	187948	49398	0.005	15	0.332
10		5	2	0.5	1	10226	20452	20856	41712	0.1	187948	49398	0.005	15	0.332
11		6	2	0.5	2	10226	20452	20856	41712	0.1	187948	49398	0.005	15	0.332
12		7	2	0.5	1	10226	20452	20856	41712	0.1	187948	49398	0.005	15	0.332
13		8	2	0.5	2	10226	20452	20856	41712	0.1	187948	49398	0.005	15	0.332
14		9	2	0.5	1	10226	20452	20856	41712	0.1	187948	49398	0.005	15	0.332
15	Background control	1	2	0.5	1	58	116	117	234	0.1	187948	49398	0.005	15	0.000
16		2	2	0.5	2	58	116	117	234	0.1	187948	49398	0.005	15	0.000
17		3	2	0.5	1	58	116	117	234	0.1	187948	49398	0.005	15	0.000
18		4	2	0.5	2	58	116	117	234	0.1	187948	49398	0.005	15	0.000
19		5	2	0.5	1	58	116	117	234	0.1	187948	49398	0.005	15	0.000
20		6	2	0.5	2	58	116	117	234	0.1	187948	49398	0.005	15	0.000
21		7	2	0.5	1	58	116	117	234	0.1	187948	49398	0.005	15	0.000
22		8	2	0.5	2	58	116	117	234	0.1	187948	49398	0.005	15	0.000
23	4-OH ASDN	1-1	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
24		2-1	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
25		3-1	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
26		4-1	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
27		5-1	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
28		6-1	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
29		7-1	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
30		8-1	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
31		9-1	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
32		1-2	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
33		2-2	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
34		3-2	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
35		4-2	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
36		5-2	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
37		6-2	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
38		7-2	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
39		8-2	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
40		9-2	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
41		1-3	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
42		2-3	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
43		3-3	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
44		4-3	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
45		5-3	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
46		6-3	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
47		7-3	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
48		8-3	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
49		9-3	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
50		1-4	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
51		2-4	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
52		3-4	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
53		4-4	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
54		5-4	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
55		6-4	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
56		7-4	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
57		8-4	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
58		9-4	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
59		1-5	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
60		2-5	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
61		3-5	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
62		4-5	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
63		5-5	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
64		6-5	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
65		7-5	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
66		8-5	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
67		9-5	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
68		1-6	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000
69		2-6	2	0.5	1	140	280	291	582	0.1	187948	49398	0.005	15	0.000
70		3-6	2	0.5	2	140	280	291	582	0.1	187948	49398	0.005	15	0.000

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1			Assay Date	3/6/2007	Test Chemical	CR11003	# Concentrations tested			8. Microsome ID	12535-13					
2			NB Ref.	12535-59	Concentration	0.68MR2145 P100										
3			Technician	DBP	Replicate #	2	Positive Control	4.0H A50N	Pos Cts Tubes/Conc							
4																
5	Sample ID															
6	Sample Replicate/Level	1-1	2	0.5	1	10321	20645	17739	35476	0.1	187948	40792	0.034	0.005	15	0.289
7	CR11003	1-1	2	0.5	2	10321	20398	17739	35476	0.1	187948	35239	0.0374	0.005	15	0.250
8	CR11003	1-2	2	0.5	1	68009	17818	17739	35476	0.1	187948	35239	0.0374	0.005	15	0.250
9	CR11003	1-2	2	0.5	2	68009	17818	17739	35476	0.1	187948	35239	0.0374	0.005	15	0.250
10	CR11003	1-3	2	0.5	1	8594	17188	17483	34126	0.1	187948	35239	0.0374	0.005	15	0.250
11	CR11003	1-3	2	0.5	2	8594	16938	17483	34126	0.1	187948	35239	0.0374	0.005	15	0.250
12	CR11003	2-1	2	0.5	1	8594	17188	17483	34126	0.1	187948	35239	0.0374	0.005	15	0.250
13	CR11003	2-1	2	0.5	2	8594	16938	17483	34126	0.1	187948	35239	0.0374	0.005	15	0.250
14	CR11003	2-2	2	0.5	1	9120	18260	18425	36950	0.1	187948	35239	0.0374	0.005	15	0.250
15	CR11003	2-2	2	0.5	2	9120	18260	18425	36950	0.1	187948	35239	0.0374	0.005	15	0.250
16	CR11003	2-3	2	0.5	1	9120	18260	18425	36950	0.1	187948	35239	0.0374	0.005	15	0.250
17	CR11003	2-3	2	0.5	2	9120	18260	18425	36950	0.1	187948	35239	0.0374	0.005	15	0.250
18	CR11003	3-1	2	0.5	1	10489	20978	20867	41734	0.1	187948	41494	0.0412	0.005	15	0.284
19	CR11003	3-1	2	0.5	2	10489	20978	20867	41734	0.1	187948	41494	0.0412	0.005	15	0.284
20	CR11003	3-2	2	0.5	1	11692	22124	21941	43982	0.1	187948	43642	0.0464	0.005	15	0.309
21	CR11003	3-2	2	0.5	2	11692	22124	21941	43982	0.1	187948	43642	0.0464	0.005	15	0.309
22	CR11003	3-3	2	0.5	1	10875	21950	21942	43984	0.1	187948	43642	0.0464	0.005	15	0.309
23	CR11003	3-3	2	0.5	2	10875	21950	21942	43984	0.1	187948	43642	0.0464	0.005	15	0.309
24	CR11003	4-1	2	0.5	1	11409	22818	22748	45096	0.1	187948	45255	0.0481	0.005	15	0.321
25	CR11003	4-1	2	0.5	2	11409	22818	22748	45096	0.1	187948	45255	0.0481	0.005	15	0.321
26	CR11003	4-2	2	0.5	1	11723	23558	23463	44926	0.1	187948	44985	0.0475	0.005	15	0.317
27	CR11003	4-2	2	0.5	2	11723	23558	23463	44926	0.1	187948	44985	0.0475	0.005	15	0.317
28	CR11003	4-3	2	0.5	1	11723	23558	23463	44926	0.1	187948	44985	0.0475	0.005	15	0.317
29	CR11003	4-3	2	0.5	2	11723	23558	23463	44926	0.1	187948	44985	0.0475	0.005	15	0.317
30	CR11003	5-1	2	0.5	1	9817	19834	19522	39044	0.1	187948	39004	0.0412	0.005	15	0.275
31	CR11003	5-1	2	0.5	2	9817	19834	19522	39044	0.1	187948	39004	0.0412	0.005	15	0.275
32	CR11003	5-2	2	0.5	1	11386	22772	22484	44988	0.1	187948	44748	0.0478	0.005	15	0.317
33	CR11003	5-2	2	0.5	2	11386	22772	22484	44988	0.1	187948	44748	0.0478	0.005	15	0.317
34	CR11003	5-3	2	0.5	1	11413	22826	22625	45250	0.1	187948	45010	0.0478	0.005	15	0.319
35	CR11003	5-3	2	0.5	2	11413	22826	22625	45250	0.1	187948	45010	0.0478	0.005	15	0.319
36	CR11003	6-1	2	0.5	1	10628	21256	20993	41988	0.1	187948	41746	0.0444	0.005	15	0.296
37	CR11003	6-1	2	0.5	2	10628	21256	20993	41988	0.1	187948	41746	0.0444	0.005	15	0.296
38	CR11003	6-2	2	0.5	1	11337	22674	22726	45452	0.1	187948	45212	0.0480	0.005	15	0.320
39	CR11003	6-2	2	0.5	2	11337	22674	22726	45452	0.1	187948	45212	0.0480	0.005	15	0.320
40	CR11003	7-1	2	0.5	1	10436	20812	20853	41988	0.1	187948	40948	0.0434	0.005	15	0.289
41	CR11003	7-1	2	0.5	2	10436	20812	20853	41988	0.1	187948	40948	0.0434	0.005	15	0.289
42	CR11003	7-2	2	0.5	1	11005	22010	22153	44306	0.1	187948	44068	0.0468	0.005	15	0.312
43	CR11003	7-2	2	0.5	2	11005	22010	22153	44306	0.1	187948	44068	0.0468	0.005	15	0.312
44	CR11003	7-3	2	0.5	1	11264	22528	22444	44888	0.1	187948	44648	0.0474	0.005	15	0.315
45	CR11003	7-3	2	0.5	2	11264	22528	22444	44888	0.1	187948	44648	0.0474	0.005	15	0.315
46	CR11003	8-1	2	0.5	1	11040	22090	22129	44256	0.1	187948	44018	0.0468	0.005	15	0.312
47	CR11003	8-1	2	0.5	2	11040	22090	22129	44256	0.1	187948	44018	0.0468	0.005	15	0.312
48	CR11003	8-2	2	0.5	1	10922	21844	21805	43810	0.1	187948	43370	0.0461	0.005	15	0.307
49	CR11003	8-2	2	0.5	2	10922	21844	21805	43810	0.1	187948	43370	0.0461	0.005	15	0.307
50	CR11003	8-3	2	0.5	1	11177	22354	22378	44756	0.1	187948	44515	0.0473	0.005	15	0.315
51	CR11003	8-3	2	0.5	2	11177	22354	22378	44756	0.1	187948	44515	0.0473	0.005	15	0.315
52	CR11003	9-1	2	0.5	1	11726	23452	23551	47102	0.1	187948	46862	0.0488	0.005	15	0.332
53	CR11003	9-1	2	0.5	2	11726	23452	23551	47102	0.1	187948	46862	0.0488	0.005	15	0.332

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/6/2007	Test Chemical ID	CR11603	# Concentrations	8	Microsome ID	12535-13			
2	NB Ref	12535-59	Control (as blank)	06MP2145.P00							
3	Technician ID	PRP	Replicate #								
4											
5	Control Type	Portion	SD								
6	Full activity	Average	0.321								
7	Background (uncorrected)	Overall	0.002								
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.002						
12	4-OH ASDN	1	2	-5.00	0.002						
13	4-OH ASDN	2	1	-6.00	0.021						
14	4-OH ASDN	2	2	-6.00	0.019						
15	4-OH ASDN	3	1	-6.30	0.036						
16	4-OH ASDN	3	2	-6.30	0.035						
17	4-OH ASDN	4	1	-7.00	0.193						
18	4-OH ASDN	4	2	-7.00	0.125						
19	4-OH ASDN	5	1	-7.30	0.187						
20	4-OH ASDN	5	2	-7.30	0.190						
21	4-OH ASDN	6	1	-8.00	0.293						
22	4-OH ASDN	6	2	-8.00	0.281						
23	4-OH ASDN	7	1	-9.00	0.335						
24	4-OH ASDN	7	2	-9.00	0.315						
25	4-OH ASDN	8	1	-10.00	0.319						
26	4-OH ASDN	8	2	-10.00	0.340						
27	4-OH ASDN										
28	4-OH ASDN										
29	4-OH ASDN										
30	4-OH ASDN										
31	4-OH ASDN										
32	4-OH ASDN										
33	4-OH ASDN										
34	4-OH ASDN										
35	4-OH ASDN										
36	4-OH ASDN										
37	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
38	CR11603	1	1	-3.00	0.288						
39	CR11603	1	2	-3.00	0.250						
40	CR11603	2	1	-3.00	0.240						
41	CR11603	2	2	-4.00	0.271						
42	CR11603	2	3	-4.00	0.259						
43	CR11603	3	1	-4.00	0.266						
44	CR11603	3	2	-5.00	0.294						
45	CR11603	3	3	-5.00	0.306						
46	CR11603	4	1	-6.00	0.309						
47	CR11603	4	2	-6.00	0.321						
48	CR11603	4	3	-6.00	0.317						
49	CR11603	5	1	-7.00	0.332						
50	CR11603	5	2	-7.00	0.275						
51	CR11603	5	3	-7.00	0.317						
52	CR11603	6	1	-7.00	0.319						
53	CR11603	6	2	-8.00	0.296						
54	CR11603	6	3	-8.00	0.320						
55	CR11603	7	1	-8.00	0.289						
56	CR11603	7	2	-9.00	0.312						
57	CR11603	7	3	-9.00	0.316						
58	CR11603	8	1	-9.00	0.312						
59	CR11603	8	2	-10.00	0.307						
60	CR11603	8	3	-10.00	0.315						
61	CR11603	8		-10.00	0.332						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/7/2007	Test Chemical ID	CR11603	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-62	Counter data filename	08MR1556.P00			Pos Ctl		
3	Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/7/2007	Test Chemical ID	CR11603	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-62	Counter data filename	08MR1556.P00					
3	Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0191		33590		1758639			
8	2	0.0196		37208		1898367			
9	3	0.0196		38270		1952551			
10	4	0.0192		37191		1937031			
11	5	0.0198		36626		1849798			
12						Average DPM/g soln		1879277	
13						SD		78249	
14						CV		4.16	
15						µCi/g soln		0.8465	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution				8.1275 g				
25	Mass of dilution B used in substrate prep				4.5756 g				
26	Concentration of nonradiolabeled ASDN in substrate soln.				0.56241457 µg/g				
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.010 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.8465				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					= 0.5624 + 0.010				
37					= 0.573 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.478 µCi/µg ASDN								
41		9.398.E+05 dpm/nmol							

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/7/2007	Test Chemical ID	CR11603	# Concentrations tested	8	Microsome ID	12535-13	
2			NB Ref	12535-62	Counter data filename	06MR1556.P00					
3			Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc	Pos Ctl	2
4											
5			Microsome Dilution Details								
6											
7			Dilution A								
8				0.12 mL microsome Stock used							
9				2.4 mL total volume							
10				20 dilution factor							
11			Dilution B								
12				1.5 mL microsome Dilution A used							
13				60 mL total volume							
14				40 dilution factor							
15			Dilution C (if applicable)								
16				mL microsome Dilution B used							
17				mL total volume							
18				dilution factor							
19				800 total dilution factor							
20											
21			Protein Concentration (stock microsomes, mg/mL):		8.00						
22			Protein Concentration (dilution added to assay, mg/mL):		0.01						
23											
24											
25											
26											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Test # Chemical Concentrations As: 3/7/2007 ID C tested 8 Microsome ID 12535-13									
2		Counter data NB 12535-62 filename 08MR1556.P00									
3		Te ch nic lan ID PRP Replicate # 3 Positive Control 4-OH ASD Tubes/Conc 2									
4											
5		Vial	Tin	DPM1			ISIE				Round DPM
6	2	5	33590.27	13693.12	0	12.94	501.37				33590
7	3	5	37208.18	14744.88	0	12.75	485.78				37208
8	4	5	38269.75	15220.44	0	12.81	488.33				38270
9	5	5	37191.28	14676.24	0	12.8	484.03				37191
10	6	5	36626.38	14510.16	0	12.81	487.66				36626
11	8	5	9973.39	4281.12	0	13.67	545.83				9973
12	9	5	9804.11	4220.12	0	13.69	547.65				9804
13	10	5	10020.61	4323.84	0	13.65	548.33				10021
14	11	5	9887.71	4278.88	0	13.72	548.23				9888
15	12	5	8737.46	3781.44	0	13.69	548.74				8737
16	13	5	8817.99	3820.84	0	13.62	549.37				8818
17	14	5	9069.87	3828.28	0	13.67	548.66				9070
18	15	5	8800.83	3811.24	0	13.72	551.91				8801
19	16	5	67.98	29.44	0	13.49	550.46				68
20	17	5	64.67	28.68	0	14.71	551.34				65
21	18	5	51.63	23.64	0	13.25	545.94				52
22	19	5	67.46	27.64	0	13.24	550.08				67
23	20	5	55.04	23.12	0	11.72	548.05				55
24	21	5	58.63	25.88	0	14.7	550.44				59
25	22	5	57.07	25.68	0	12.91	549.16				57
26	23	5	54.02	23.64	0	13.98	551.05				54
27	25	5	133.23	57.76	0	12.67	548.49				133
28	26	5	142.02	61.48	0	12.7	553.4				142
29	27	5	141.78	59.84	0	12.52	548.67				142
30	28	5	149.57	62.44	0	13.85	551.28				150
31	29	5	784.24	335.92	0	13.77	549.89				784
32	30	5	799.22	344.44	0	13.63	548.79				799
33	31	5	734.95	318.24	0	13.8	550.99				735
34	32	5	716.3	304.24	0	13.98	552.88				716
35	33	5	1344.43	590.24	0	13.48	550.86				1344
36	34	5	1382.97	598.08	0	13.87	552.2				1383
37	35	5	1304.11	558.04	0	13.75	549.85				1304
38	36	5	1290	559.72	0	13.63	551.83				1290
39	37	5	3998.31	1718.64	0	13.84	553.29				3998
40	38	5	3879.49	1687.04	0	13.79	553.43				3879
41	39	5	4108.85	1771.44	0	13.89	552.22				4109
42	40	5	4051.21	1748.44	0	13.64	547.99				4051
43	41	5	6152.6	2630.44	0	13.93	552.44				6153
44	42	5	6144.1	2656.32	0	13.72	550.69				6144
45	43	5	5496.88	2355.64	0	13.89	553.56				5497
46	44	5	5614.63	2455.04	0	13.67	552.1				5615
47	45	5	8274.87	3573.04	0	13.74	550.92				8275
48	46	5	8225.31	3542.84	0	13.8	552.96				8225
49	47	5	8578.64	3693.68	0	13.76	549.36				8579
50	48	5	8569.19	3710.52	0	13.75	550.56				8569
51	49	5	9775.62	4230.88	0	13.73	551.84				9776
52	50	5	9655.88	4177.28	0	13.72	550.09				9656
53	51	5	10407.01	4498.88	0	13.74	551.46				10407
54	52	5	10183.26	4354.12	0	13.58	539.27				10183
55	53	5	10273.78	4457.04	0	13.79	552.49				10274
56	54	5	10101.65	4359.36	0	13.75	550.68				10102
57	55	5	9730.61	4182.76	0	13.78	546.4				9731
58	56	5	9413.45	4099.88	0	13.7	552.18				9413
59	58	5	8244.59	3588.96	0	13.78	552.12				8245
60	59	5	8382.38	3631.68	0	13.82	554.69				8382
61	60	5	7035.93	3041.12	0	13.71	553.14				7036
62	61	5	6934.35	3007.64	0	13.81	551.43				6934
63	62	5	7887.14	3411.24	0	13.76	550.51				7887
64	63	5	7829.47	3399.24	0	13.79	552.63				7829
65	64	5	7232.67	3120.84	0	13.76	551.96				7233
66	65	5	7254.41	3140.44	0	13.74	550.8				7254
67	66	5	7756.79	3329.12	0	13.78	549.47				7757
68	67	5	7636.78	3300.6	0	13.68	551.05				7637
69	68	5	8795.82	3776.84	0	13.72	547.98				8796
70	69	5	8636.63	3771.48	0	13.67	553.54				8637

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	9018.98	3881.12	0	13.73	551.8				9019
72	71	5	8914.3	3854.24	0	13.76	550.14				8914
73	72	5	8642.88	3736.48	0	13.79	551.06				8643
74	73	5	8437.58	3631.04	0	13.74	550.44				8438
75	74	5	7991.47	3438.84	0	13.78	551.2				7991
76	75	5	7919.03	3417.32	0	13.78	550.37				7919
77	76	5	9789.7	4220.52	0	13.68	548.53				9770
78	77	5	9583.08	4172.32	0	13.71	554.82				9583
79	78	5	9314.76	4030.84	0	13.82	552.41				9315
80	79	5	9328.2	4035.24	0	13.76	552.32				9328
81	80	5	9150.16	3987.24	0	13.8	552.69				9150
82	81	5	9115.27	3928.12	0	13.8	550.66				9115
83	82	5	8531.28	3699.64	0	13.7	549.66				8531
84	83	5	8451.23	3658.44	0	13.67	550.46				8451
85	84	5	7846.83	3384	0	13.83	551.78				7847
86	85	5	7834.22	3363.28	0	13.88	550.82				7834
87	86	5	9018.34	3876.44	0	13.74	551.96				9018
88	87	5	9175.3	3966.84	0	13.78	552.67				9175
89	88	5	8722.49	3733.84	0	13.7	548.52				8722
90	89	5	8668.98	3758.88	0	13.68	549.91				8667
91	90	5	9356.93	4044.68	0	13.81	552.29				9357
92	91	5	9480.81	4097.12	0	13.87	555.21				9481
93	92	5	9852.44	4252.88	0	13.89	555.85				9852
94	93	5	9570.93	4124.68	0	13.78	550.04				9571
95	94	5	9701.64	4196.48	0	13.75	551.89				9702
96	95	5	9528.94	4115.52	0	13.8	551.62				9529
97	96	5	8431.63	3650.08	0	13.79	554.68				8432
98	97	5	8418.2	3640.48	0	13.78	550.71				8418
99	98	5	8861.05	3842.04	0	13.76	551.89				8861

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
100	99	5	8769.95	3775.72	0	13.79	549.87				8770
101	100	5	8628.63	3730.44	0	13.8	551.87				8629
102	101	5	8576.26	3700.84	0	13.77	551.09				8576
103	102	5	8503.4	3698.68	0	13.66	553.84				8503
104	103	5	8625.99	3712.24	0	13.74	551.03				8626
105	104	5	9040.36	3904.44	0	13.67	549.37				9040
106	105	5	9026.87	3904.48	0	13.8	552.2				9027
107	107	5	235358.19	108663.68	0	15.1	625.74				235358
108											0
109											0
110											0
111											0
112											0
113											0
114											0
115											0
116											0
117											0
118											0
119											0
120											0
121											0
122											0
123											0
124											0
125											0
126											0
127											0
128											0
129											0
130											0
131											0
132											0
133											0
134											0
135											0
136											0
137											0
138											0
139											0
140											0
141											0
142											0
143											0

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	Assay Date	Chemical ID	Test ID	Concentrations tested	Microscopy ID	12535-13										
1	3/7/2007	CR11603	12535-40	Conc. daily	08MR1556.P00	12535-13										
2	NE Ref. Technician ID	12535-40	PRP	3	Positive Control	4-CH ASDN										
3																
4																
Sample ID	Replicate	Volume (µl)	Aliq #	DPH/mL	DPH/mL	Ave DPM/mL	Total DPM	Volume of substrate solution (µL)	total DPM in assay tube (initial)	Calculate nmoL ³ H ₂ O formed	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrone formed/mg/min)		
5	1	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
6	2	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
7	3	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
8	4	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
9	5	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
10	6	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
11	7	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
12	8	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
13	9	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
14	10	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
15	11	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
16	12	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
17	13	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
18	14	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
19	15	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
20	16	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
21	17	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
22	18	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
23	19	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
24	20	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
25	21	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
26	22	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
27	23	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
28	24	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
29	25	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
30	26	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
31	27	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
32	28	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
33	29	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
34	30	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
35	31	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
36	32	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
37	33	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
38	34	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
39	35	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
40	36	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
41	37	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
42	38	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
43	39	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
44	40	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
45	41	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
46	42	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
47	43	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
48	44	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
49	45	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
50	46	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
51	47	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
52	48	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
53	49	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
54	50	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
55	51	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
56	52	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
57	53	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
58	54	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
59	55	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
60	56	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
61	57	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
62	58	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
63	59	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
64	60	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
65	61	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
66	62	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
67	63	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
68	64	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
69	65	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
70	66	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
71	67	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
72	68	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
73	69	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
74	70	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
75	71	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
76	72	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
77	73	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
78	74	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
79	75	2	0.5	1	9737	17474	17595	35110	0.1	137928	34872	0.0371	0.005	15	0.247	
80	76	2	0.5	1	9970	18169	17871	35242	0.1	137928	35504	0.0378	0.005	15	0.252	
81	77	2	0.5	1	9804	19009	13777	39554	0.1	137928	39315	0.0418	0.005	15	0.279	
82	78	2	0.5	1	10021	20042	19909	39416	0.1	137928	39569	0.0421	0.005	15	0.281	
83	79	2	0.5													

Aromatase Assay Spreadsheet

	R	S	T	U	V
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Aromatase Assay Spreadsheet

Assay Date: 3/7/2007		Chemical ID: CR11603		# Concentrations: Untest		8 Macroscopic ID: 12535-13													
NE Ref: 12435-62		Gross file number: 00MR1556_P00																	
Technician ID: PRSP		Replicates: 3		Positive Control		4-OH ASDON Pos Ctrl Tubes/Conc		Z											
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q			
Sample	Replicates	Normal total	Aliq	Aliq	DPH/aliq	DPH/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used (assay tube (mL))	total DPM in assay tube (initial)	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)					
7	CR11603	1-1	2	0.5	8245	16490	16827	33254	0.1	187928	33918	0.005	15	0.234					
8		1-2	2	0.5	8362	16724	13970	27940	0.1	187928	27702	0.005	15	0.197					
9		1-3	2	0.5	7936	15868	15719	31432	0.1	187928	31184	0.005	15	0.221					
10		2-1	2	0.5	7829	15658	14487	28974	0.1	187928	28736	0.005	15	0.204					
11		2-2	2	0.5	7253	14508	15394	30746	0.1	187928	30550	0.005	15	0.217					
12		2-3	2	0.5	7157	14314	17433	34968	0.1	187928	34528	0.005	15	0.246					
13		3-1	2	0.5	8019	16038	17933	35865	0.1	187928	35628	0.005	15	0.253					
14		3-2	2	0.5	8435	16870	17081	34162	0.1	187928	33824	0.005	15	0.241					
15		3-3	2	0.5	7931	15982	14910	31620	0.1	187928	31582	0.005	15	0.224					
16		4-1	2	0.5	9770	19540	19553	38706	0.1	187928	38468	0.005	15	0.273					
17		4-2	2	0.5	9315	18630	18643	37266	0.1	187928	37048	0.005	15	0.263					
18		4-3	2	0.5	8150	16300	18265	36530	0.1	187928	36392	0.005	15	0.257					
19		5-1	2	0.5	8451	16902	16982	33964	0.1	187928	33726	0.005	15	0.239					
20		5-2	2	0.5	7847	15694	15981	31382	0.1	187928	31124	0.005	15	0.221					
21		5-3	2	0.5	9018	18036	18193	36386	0.1	187928	36148	0.005	15	0.256					
22		6-1	2	0.5	8722	17444	17989	34778	0.1	187928	34540	0.005	15	0.245					
23		6-2	2	0.5	8067	16134	18818	37859	0.1	187928	37598	0.005	15	0.265					
24		6-3	2	0.5	9521	19042	19423	38946	0.1	187928	38608	0.005	15	0.274					
25		7-1	2	0.5	8702	17404	19231	38462	0.1	187928	38224	0.005	15	0.271					
26		7-2	2	0.5	8418	16836	18850	33700	0.1	187928	33462	0.005	15	0.237					
27		7-3	2	0.5	8831	17662	17631	35262	0.1	187928	35024	0.005	15	0.248					
28		8-1	2	0.5	9629	19258	17205	34410	0.1	187928	34172	0.005	15	0.242					
29		8-2	2	0.5	8503	17006	17129	34058	0.1	187928	34020	0.005	15	0.241					
30		8-3	2	0.5	9040	18080	18087	36134	0.1	187928	35808	0.005	15	0.255					

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		OC attack - aligned
5		variance
6		% RE
7		0.98
8		1.01
9		1.01
10		0.99
11		1.00
12		1.00
13		1.00
14		1.00
15		1.01
16		0.99
17		1.01
18		0.98
19		1.01
20		0.99
21		1.01
22		0.99
23		1.00
24		1.00
25		1.01
26		0.99
27		1.00
28		1.00
29		1.00
30		1.00
31		1.00
32		1.00
33		1.00
34		1.00
35		0.99
36		1.01
37		1.00
38		1.00
39		0.99
40		1.01
41		1.01
42		0.99
43		1.01
44		0.99
45		1.00
46		1.01
47		0.99
48		1.00
49		1.00
50		1.00
51		0.99
52		1.01
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/7/2007	Test Chemical ID	CR11603	# Concentrations					
2		NB Ref.	12535-62	Counter dil. formula	DMRT556-P00						
3		Technician ID	PRP	Replicate #		3	Positive Control	4-OH ASDN	Pos CH Tubes/Conc		2
4											
5	Control Type	Average	SD								
6	Full activity	0.265	0.018								
7	Background (unconnected)										
8	Overall	0.002	0.000								
9											
10	Positive Control	Level	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity					
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.002					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002					
13											
14	4-OH ASDN	2	1	1.00E-06	-6.00	0.021					
15	4-OH ASDN	2	2	1.00E-06	-6.00	0.019					
16											
17	4-OH ASDN	3	1	5.00E-07	-6.30	0.037					
18	4-OH ASDN	3	2	5.00E-07	-6.30	0.035					
19											
20	4-OH ASDN	4	1	1.00E-07	-7.00	0.110					
21	4-OH ASDN	4	2	1.00E-07	-7.00	0.114					
22											
23	4-OH ASDN	5	1	5.00E-08	-7.30	0.173					
24	4-OH ASDN	5	2	5.00E-08	-7.30	0.156					
25											
26	4-OH ASDN	6	1	1.00E-08	-8.00	0.232					
27	4-OH ASDN	6	2	1.00E-08	-8.00	0.242					
28											
29	4-OH ASDN	7	1	1.00E-09	-9.00	0.274					
30	4-OH ASDN	7	2	1.00E-09	-9.00	0.280					
31											
32	4-OH ASDN	8	1	1.00E-10	-10.00	0.287					
33	4-OH ASDN	8	2	1.00E-10	-10.00	0.270					
34											
35											
36											
37	Test Chemical	Level	Replicate	Test chemical M	Log(test chemical)	Activity					
38	CR11603	1	1	1.00E-03	-3.00	0.234					
39	CR11603	1	2	1.00E-03	-3.00	0.197					
40	CR11603	1	3	1.00E-03	-3.00	0.221					
41	CR11603	2	1	1.00E-04	-4.00	0.204					
42	CR11603	2	2	1.00E-04	-4.00	0.217					
43	CR11603	2	3	1.00E-04	-4.00	0.246					
44	CR11603	3	1	1.00E-05	-5.00	0.263					
45	CR11603	3	2	1.00E-05	-5.00	0.241					
46	CR11603	3	3	1.00E-05	-5.00	0.224					
47	CR11603	4	1	1.00E-06	-6.00	0.273					
48	CR11603	4	2	1.00E-06	-6.00	0.263					
49	CR11603	4	3	1.00E-06	-6.00	0.257					
50	CR11603	5	1	1.00E-07	-7.00	0.239					
51	CR11603	5	2	1.00E-07	-7.00	0.221					
52	CR11603	5	3	1.00E-07	-7.00	0.256					
53	CR11603	6	1	1.00E-08	-8.00	0.245					
54	CR11603	6	2	1.00E-08	-8.00	0.265					
55	CR11603	6	3	1.00E-08	-8.00	0.271					
56	CR11603	7	1	1.00E-09	-9.00	0.237					
57	CR11603	7	2	1.00E-09	-9.00	0.242					
58	CR11603	7	3	1.00E-09	-9.00	0.242					
59	CR11603	8	1	1.00E-10	-10.00	0.241					
60	CR11603	8	2	1.00E-10	-10.00	0.255					
61	CR11603	8	3	1.00E-10	-10.00	0.255					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
	Test # Concentrations								
1	Assay Date	2/22/2007	Chemical ID	CR11604	tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-47	Counter data filename	22FE2315.P00					
	Technician						Pos Ctl		
3	ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/22/2007	Test Chemical ID	CR11604	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-47	Counter data filename	22FE2315.P00					
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0198		35691		1802576			
8	2	0.0195		34039		1745590			
9	3	0.0199		38680		1943719			
10	4	0.0199		37647		1891809			
11	5	0.0202		40011		1980743			
12						Average DPM/g soln		1872887	
13						SD		97668	
14						CV		5.21	
15						µCi/g soln		0.8436	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.04	10		1004.00				
20	Dilution A			100	10.04				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution								8.0565 g
25	Mass of dilution B used in substrate prep								4.5722 g
26	Concentration of nonradiolabeled ASDN in substrate soln.								0.56978698 µg/g
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.010 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln								0.8436
31	b. Specific activity of [³ H]ASDN (µCi/mmol)								23500000
32	c. Molecular wt of ASDN (mg/mmol)								286.4
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36		=							0.5698 + 0.010
37		=							0.580 µg ASDN/g soln.
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40		=							1.454 µCi/µg ASDN
41	9.247.E+05 dpm/nmol								

Aromatase Assay Spreadsheet

[illegible]

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Ass:	2/22/2007	Test Chemical ID	CF	# Concentrations tested	8	Microsome ID	12535-13			
2	NBI	12535-47	Counter data filename	22FE2315.P00							
3	Tec										
4	hnic										
5	ID	PRP	Replicate #	1	Positive Control	4-OH ASD	Tubes/Conc	2			
6	Vial #	Time	DPM1								Round DPM
7	2	5	35090.76	14189.64	0	12.86	488.6				35691
8	3	5	34039.26	13686.8	0	12.99	495.63				34039
9	4	5	38679.89	15398.64	0	12.87	488.53				38680
10	5	5	37647.18	15004.28	0	12.8	488.38				37647
11	6	5	40010.62	15895.88	0	12.84	489.38				40011
12	8	5	10992.35	4746.84	0	13.72	548.99				10992
13	9	5	10919.73	4719.2	0	13.59	546.43				10920
14	10	5	11605.1	5007.48	0	13.76	546.41				11605
15	11	5	11628.13	5006.08	0	13.76	545.43				11628
16	12	5	9563.81	4087.88	0	13.71	546.4				9564
17	13	5	9395.53	4051.48	0	13.61	545.22				9395
18	14	5	9304.86	3993.24	0	13.79	541.64				9305
19	15	5	9444.22	4040.64	0	13.73	545.66				9444
20	16	5	54.45	22.4	0	13.05	547.83				54
21	17	5	65.89	26.04	0	13.03	546.12				66
22	18	5	82.48	34.4	0	13.03	546.88				82
23	19	5	56.18	23.24	0	12.42	548.55				56
24	20	5	70.55	28.6	0	12.6	548.72				71
25	21	5	55.75	24.24	0	13.49	546.88				56
26	22	5	66.29	27.84	0	13.05	547.81				66
27	23	5	61.3	26	0	12.37	546.16				61
28	25	5	163.87	69.88	0	13.41	546.57				164
29	26	5	140.25	58	0	13.64	548.78				140
30	27	5	131.7	59.88	0	13.41	545.16				132
31	28	5	154	66.84	0	13.97	547.66				154
32	29	5	686.04	296.84	0	13.82	548.5				686
33	30	5	697.03	295.24	0	13.79	546.48				697
34	31	5	649.08	281.24	0	13.39	551.57				649
35	32	5	687.6	296.52	0	13.74	549.93				688
36	33	5	1318.49	557.2	0	13.73	544.81				1318
37	34	5	1298.58	559.24	0	13.53	548.22				1299
38	35	5	1186.96	515.68	0	13.6	546.04				1187
39	36	5	1145.72	493.4	0	13.45	546.79				1146
40	37	5	4093.18	1749.8	0	13.83	547.54				4093
41	38	5	4089.77	1760.2	0	13.78	549.59				4090
42	39	5	2573.75	1115.24	0	13.66	545.46				2574
43	40	5	2578.7	1107.68	0	13.82	548.74				2579
44	41	5	5368.54	2287.2	0	13.87	545.58				5369
45	42	5	5286.79	2277.6	0	13.81	548.86				5287
46	43	5	5926.49	2556.64	0	13.71	547.33				5926
47	44	5	5822.63	2507.68	0	13.73	548.3				5823
48	45	5	9441.74	4069	0	13.78	546.95				9442
49	46	5	9384.87	4049.32	0	13.71	546.64				9385
50	47	5	9128.08	3934.04	0	13.72	548				9128
51	48	5	6014.55	3688.64	0	13.7	547.34				6015
52	49	5	9949.41	4271.4	0	13.74	547.22				9949
53	50	5	9937.54	4269.44	0	13.78	548.19				9938
54	51	5	9262.04	3992.88	0	13.83	548.21				9262
55	52	5	9221.73	3942	0	13.74	546.73				9222
56	53	5	9929.54	4275.68	0	13.7	547.76				9930
57	54	5	9818.69	4237.6	0	13.66	546.56				9819
58	55	5	9610.77	4129.12	0	13.72	545.47				9611
59	56	5	9514.79	4086.32	0	13.74	546.75				9515
60	58	5	11183.86	4822.24	0	13.77	547.55				11184
61	59	5	11205.16	4827.84	0	13.7	548.42				11206
62	60	5	10899.37	4675.64	0	13.8	546.97				10899
63	61	5	10735.83	4650.88	0	13.7	548.65				10736
64	62	5	11326.47	4866.88	0	13.76	547.35				11326
65	63	5	11176.63	4832.4	0	13.73	548.76				11177
66	64	5	11090.48	4784.8	0	13.72	547.48				11090
67	65	5	11223.17	4811.64	0	13.75	547.91				11223
68	66	5	11371.96	4893.8	0	13.69	546.52				11372
69	67	5	11247.68	4817.48	0	13.81	547.11				11248
70	68	5	11406.79	4891.4	0	13.68	549.17				11407
71	69	5	11489.5	4920.12	0	13.83	546.67				11490
72	70	5	11788.3	5051.4	0	13.77	546.81				11788

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
72	71	5	11655.9	5015.64	0	13.75	547.36				11656
73	72	5	10853.57	4676.64	0	13.81	550.11				10854
74	73	5	10774.66	4592.04	0	13.78	545.46				10775
75	74	5	11532.91	4926	0	13.68	542.77				11533
76	75	5	11216.38	4845.64	0	13.71	549.84				11216
77	76	5	9632.35	4146.04	0	13.81	549.92				9632
78	77	5	9706.05	4150.12	0	13.74	544.88				9706

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
79	78	5	10616.85	4581.76	0	13.76	549.03				10617
80	79	5	10646.22	4567.12	0	13.72	546.94				10646
81	80	5	10084.74	4299.4	0	13.76	544.59				10085
82	81	5	10120.98	4325.88	0	13.76	547.09				10121
83	83	5	10785.89	4621.4	0	13.73	545.31				10786
84	84	5	10779.88	4623.68	0	13.69	545.38				10780
85	85	5	10252.48	4444.68	0	13.7	548.38				10252
86	86	5	10398.57	4456	0	13.75	545.34				10399
87	87	5	9428.51	4039.08	0	13.7	544.99				9429
88	88	5	9325.46	4012.84	0	13.74	548.75				9325
89	89	5	10062.64	4358.64	0	13.65	547.89				10063
90	90	5	10231.23	4362	0	13.73	545.03				10231
91	91	5	10638.62	4607	0	13.62	548.65				10639
92	92	5	10557.99	4538.8	0	13.75	546.55				10558
93	93	5	9317.24	4008.88	0	13.76	546.96				9317
94	94	5	9260.8	3985.84	0	13.74	545.72				9261
95	95	5	10336.61	4408.28	0	13.77	546.29				10337
96	96	5	10092.71	4351.08	0	13.78	548.61				10093
97	97	5	10459.38	4512.6	0	13.7	548.3				10459
98	98	5	10318.74	4409.4	0	13.74	546.17				10319
99	99	5	10505.45	4523.8	0	13.64	548.28				10506
100	100	5	10570.56	4552.68	0	13.8	547.69				10571
101	101	5	10465.48	4489	0	13.66	544.21				10465
102	102	5	10238.73	4418.6	0	13.77	546.35				10239
103	103	5	10141.83	4334.8	0	13.71	545.42				10142
104	104	5	10132.14	4331.4	0	13.68	543.49				10132
105	105	5	9327.19	4003.52	0	13.69	544.8				9327
106	106	5	9328.92	3986	0	13.76	543.74				9329
107	107	5	237539.42	109214.92	0	15.02	621.25				237539
108											0
109											0
110											0
111											0
112											0
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Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		Assay Date	2/22/2007	Test ID	CR11604	# Concentrations tested	12535-13									
2		NB Ref	12535-47	Conc. Range	22FE2315 P00											
3		Technician ID	PPP	Replicate #	1	Positive Control	4-OH ANDN Pos Ctl Tubes Conc									
4																
5	Sample ID															
6	Sample type	Replicate/Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	nmol ³ H ₂ O formed	Falal [protein] in assay (mg/mL)	incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	
7	CR11604	1-1	2	0.5	1	11194	22368	22368	44778	0.1	187289	46522	0.0481	15	0.321	
8																
9		1-2	2	0.5	2	11205	22410	21835	43270	0.1	187289	43014	0.0465	15	0.310	
10																
11		1-3	2	0.5	2	10999	21798	21535	43270	0.1	187289	44750	0.0484	15	0.323	
12																
13		2-1	2	0.5	2	11336	22562	22503	45006	0.1	187289	44370	0.0460	15	0.320	
14																
15		2-2	2	0.5	2	11771	22954	22813	44626	0.1	187289	44884	0.0466	15	0.324	
16																
17		2-3	2	0.5	2	11220	22445	22820	45240	0.1	187289	45538	0.0492	15	0.328	
18																
19		3-1	2	0.5	2	11268	22496	22897	45794	0.1	187289	45532	0.0494	15	0.326	
20																
21		3-2	2	0.5	2	11407	22814	23444	46888	0.1	187289	45302	0.0485	15	0.310	
22																
23		3-3	2	0.5	2	11566	23312	21629	43285	0.1	187289	45342	0.0489	15	0.325	
24																
25		4-1	2	0.5	2	10954	21706	22749	45688	0.1	187289	38420	0.0415	15	0.277	
26																
27		4-2	2	0.5	2	11246	22432	18338	36076	0.1	187289	42270	0.0457	15	0.305	
28																
29		4-3	2	0.5	2	9706	19412	21263	42526	0.1	187289	40156	0.0434	15	0.290	
30																
31		5-1	2	0.5	2	10571	21234	20206	40412	0.1	187289	42876	0.0464	15	0.309	
32																
33		5-2	2	0.5	2	10485	20976	20651	41302	0.1	187289	41046	0.0444	15	0.286	
34																
35		5-3	2	0.5	2	10760	21560	18754	37508	0.1	187289	37252	0.0403	15	0.269	
36																
37		6-1	2	0.5	2	10399	20798	20294	40598	0.1	187289	40332	0.0436	15	0.291	
38																
39		6-2	2	0.5	2	8275	16550	21197	42394	0.1	187289	42138	0.0456	15	0.304	
40																
41		6-3	2	0.5	2	10639	21278	16578	37156	0.1	187289	36900	0.0399	15	0.268	
42																
43		7-1	2	0.5	2	9317	18634	20430	40860	0.1	187289	40604	0.0439	15	0.293	
44																
45		7-2	2	0.5	2	8531	17062	20778	41556	0.1	187289	41300	0.0447	15	0.298	
46																
47		7-3	2	0.5	2	10393	20798	21076	42162	0.1	187289	41895	0.0463	15	0.302	
48																
49		8-1	2	0.5	2	9525	21010	20704	41408	0.1	187289	41152	0.0445	15	0.297	
50																
51		8-2	2	0.5	2	10485	20976	20274	40548	0.1	187289	40262	0.0436	15	0.290	
52																
53		8-3	2	0.5	2	10132	20284	18656	37312	0.1	187289	37056	0.0401	15	0.287	
54																

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC check - all good
5		Variance
6		% RE
7		1.00
8		1.00
9		1.01
10		0.99
11		1.01
12		1.01
13		0.99
14		1.01
15		1.01
16		0.99
17		1.00
18		1.00
19		1.01
20		0.99
21		1.00
22		1.01
23		0.99
24		1.00
25		1.00
26		1.00
27		1.00
28		1.00
29		1.00
30		1.00
31		1.00
32		1.00
33		0.99
34		1.01
35		1.01
36		0.99
37		1.01
38		1.00
39		1.00
40		1.00
41		1.00
42		1.01
43		1.01
44		0.99
45		1.01
46		0.99
47		1.00
48		1.00
49		1.00
50		0.99
51		1.00
52		1.00
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A		B		C	D	E	F	G	H	I	J	K	L
1	Assay Date	2/22/2007		CR11604	CR11604	22FE2315.P00	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13		
2	NB Ref	12535-47		PRP	PRP	22FE2315.P00	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13		
3	Technician ID	PRP		PRP	PRP	22FE2315.P00	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13		
4	Control Type	Average		SD	SD	SD	SD	SD	SD	SD	SD	SD	SD
5	Full activity	0.297		0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032	0.032
6	Background	0.002		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
7	(uncorrected)												
8													
9													
10	Positive Control	Level	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity							
11	4-OH ASDN	1	1	1.00E-06	-5.00	0.003							
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002							
13	4-OH ASDN	2	1	1.00E-08	-6.00	0.018							
14	4-OH ASDN	2	2	1.00E-08	-6.00	0.017							
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.036							
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.032							
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.116							
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.072							
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.152							
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.168							
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.270							
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.260							
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.285							
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.265							
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.283							
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.274							
27	4-OH ASDN												
28	4-OH ASDN												
29	4-OH ASDN												
30	4-OH ASDN												
31	4-OH ASDN												
32	4-OH ASDN												
33	4-OH ASDN												
34	4-OH ASDN												
35	4-OH ASDN												
36	4-OH ASDN												
37	Test Chemical	Level	Replicate	Test chemical M	Log(test chemical)	Activity							
38	CR11604	1	1	1.00E-03	-3.00	0.321							
39	CR11604	1	2	1.00E-03	-3.00	0.310							
40	CR11604	2	1	1.00E-04	-4.00	0.323							
41	CR11604	2	2	1.00E-04	-4.00	0.320							
42	CR11604	3	1	1.00E-04	-4.00	0.324							
43	CR11604	3	2	1.00E-04	-4.00	0.328							
44	CR11604	4	1	1.00E-05	-5.00	0.336							
45	CR11604	4	2	1.00E-05	-5.00	0.310							
46	CR11604	5	1	1.00E-06	-6.00	0.325							
47	CR11604	5	2	1.00E-06	-6.00	0.277							
48	CR11604	6	1	1.00E-06	-6.00	0.305							
49	CR11604	6	2	1.00E-06	-6.00	0.290							
50	CR11604	7	1	1.00E-07	-7.00	0.303							
51	CR11604	7	2	1.00E-07	-7.00	0.293							
52	CR11604	8	1	1.00E-07	-7.00	0.268							
53	CR11604	8	2	1.00E-07	-7.00	0.291							
54	CR11604	9	1	1.00E-08	-8.00	0.304							
55	CR11604	9	2	1.00E-08	-8.00	0.265							
56	CR11604	10	1	1.00E-08	-8.00	0.283							
57	CR11604	10	2	1.00E-08	-8.00	0.288							
58	CR11604	11	1	1.00E-09	-9.00	0.302							
59	CR11604	11	2	1.00E-09	-9.00	0.297							
60	CR11604	12	1	1.00E-10	-10.00	0.290							
61	CR11604	12	2	1.00E-10	-10.00	0.287							

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/27/2007	Test Chemical ID	CR11604	# Concentrations tested			8 Microsome ID	12535-13
2	NB Ref.	12535-50	Counter data filename	27FE2332.P00				Pos Ctl	
3	ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/27/2007	Test Chemical ID	CR11604	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-50	Counter data filename	27FE2332.P00				Pos Ctl	
3	Technician ID	PRP	Replicate #	2	Positive Control		4-OH ASDN	Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0192		33212		1729792			
8	2	0.0196		34486		1759490			
9	3	0.0200		38749		1937450			
10	4	0.0195		38656		1982359			
11	5	0.0191		38778		2030262			
12						Average DPM/g soln		1887870	
13						SD		135215	
14						CV		7.16	
15						µCi/g soln		0.8504	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0033 g			
25	Mass of dilution B used in substrate prep					4.4316 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.55316787 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.010 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8504			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					=	0.5532	+	0.010	
37					=	0.564	µg ASDN/g soln.		
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.509	µCi/µg ASDN			
41						9.595.E+05	dpm/nmol		

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	2/27/2007	Test Chemical ID	CR11604	# Concentrations tested	8	Microsome ID	12535-13	
2		NB Ref	12535-50	Counter data filename	27FE2332.P00					
3		Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	
4										
5	Microsome Dilution Details									
6										
7	Dilution A									
8		0.12 mL microsome Stock used								
9		2.4 mL total volume								
10		20 dilution factor								
11	Dilution B									
12		1.5 mL microsome Dilution A used								
13		60 mL total volume								
14		40 dilution factor								
15	Dilution C (if applicable)									
16		mL microsome Dilution B used								
17		mL total volume								
18		dilution factor								
19		800 total dilution factor								
20										
21	Protein Concentration (stock microsomes, mg/mL):									
22	Protein Concentration (dilution added to assay, mg/mL):									
23				8.00						
24				0.01						
25										
26										

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Assay	2/27/2007	Test Chemical ID	CF	# Concentrations tested	8	Microsome ID	12535-13		
2		NB f	12535-50	Counter data filename	27FE2332.P00						
3		Tec						Pos Ctl			
4		hnic									
5		ian									
6		ID	PRP	Replicate #	2	Positive Control	4-OH ASD	Tubes/Conc	2		
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Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
72	71	5	10889.5	4688.12	0	13.7	549.23				10890
73	72	5	11018.84	4758.28	0	13.69	549.22				11019
74	73	5	11032.46	4758.72	0	13.65	550.04				11032
75	74	5	10132.25	4378.92	0	13.58	546.38				10132
76	75	5	10108.4	4357.68	0	13.67	549.17				10108
77	76	5	11538.05	4968.68	0	13.79	548.86				11536
78	77	5	11630.38	5035.96	0	13.67	548.8				11630

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
79	78	5	11094.17	4802.88	0	13.63	549.52				11094
80	79	5	11108.65	4842.88	0	13.59	551.37				11109
81	80	5	10728.8	4627.2	0	13.63	546.54				10729
82	81	5	10466.79	4534.52	0	13.63	550.16				10467
83	82	5	10286.57	4447.96	0	13.63	549.45				10286
84	83	5	10417.51	4478.48	0	13.62	545.98				10418
85	84	5	10975.4	4730.48	0	13.63	545.94				10975
86	85	5	10597.52	4600.88	0	13.64	551.01				10598
87	86	5	10385.91	4473.48	0	13.72	548.26				10386
88	87	5	10307.04	4457.92	0	13.67	549.31				10307
89	88	5	10579.29	4592.32	0	13.67	548.75				10579
90	89	5	10627.45	4573.88	0	13.69	547.77				10627
91	90	5	10382.04	4473.68	0	13.74	550.27				10382
92	91	5	10267.69	4414.28	0	13.71	545.67				10268
93	92	5	10034.63	4339.48	0	13.62	549.64				10035
94	93	5	9783.2	4225.52	0	13.75	548.77				9783
95	94	5	10408.8	4473.92	0	13.68	544.7				10409
96	95	5	10276.68	4454.72	0	13.68	548.81				10277
97	96	5	10536.32	4536.72	0	13.62	547.13				10536
98	97	5	9020.6	3907.56	0	13.81	551.29				9021
99	98	5	10649.73	4588.68	0	13.62	544.61				10650
100	99	5	10658.83	4587.88	0	13.54	544.99				10659
101	100	5	11203.83	4814.12	0	13.61	544.84				11204
102	101	5	11215.23	4820.92	0	13.77	546.81				11215
103	102	5	10876.66	4729.96	0	13.56	545.63				10877
104	103	5	10954.04	4708.32	0	13.73	545.36				10954
105	104	5	10670.38	4605.32	0	13.64	547.25				10670
106	105	5	10695.19	4614.92	0	13.73	547.94				10695
107	107	5	238897.39	108652.4	0	14.9	616.47				236897
108											0
109											0
110											0
111											0
112											0
113											0
114											0
115											0
116											0
117											0
118											0
119											0
120											0
121											0
122											0
123											0

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot			
6		variance			
7		Read if value not			
8		within mean +/-			
9		10%			
10		1.00			
11		1.02			
12		0.98			
13		1.00			
14		1.01			
15		0.99			
16					
17		0.99			
18		1.01			
19		1.01			
20		0.98			
21		1.00			
22		1.00			
23		0.99			
24		1.02			
25		1.02			
26		0.98			
27		0.98			
28		#DIV/0!			
29		1.01			
30		0.98			
31		0.98			
32		1.01			
33		#DIV/0!			
34		#DIV/0!			
35		1.03			
36		0.97			
37		1.00			
38		1.00			
39		#DIV/0!			
40		#DIV/0!			
41		1.00			
42		1.00			
43		1.00			
44		0.98			
45		#DIV/0!			
46		#DIV/0!			
47		1.02			
48		0.98			
49		1.02			
50		0.98			
51		#DIV/0!			
52		#DIV/0!			
53		1.01			
54		0.98			
55		1.00			
56		1.00			
57		#DIV/0!			
58		#DIV/0!			
59		1.01			
60		0.98			
61		1.00			
62		1.00			
63		#DIV/0!			
64		#DIV/0!			
65		1.00			
66		1.00			
67		1.00			
68		1.00			
69		#DIV/0!			
70		#DIV/0!			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		Assay Date : 7/27/2007		CR11804	# Concentrations tested	8	Microsome ID	12535-13								
2		NU Ref : 12535-80		Counter and	27FE2332 P00											
3		Technician ID: PRP		Replicate #	2	Positive Control	4-OH Andro	For Ctl Tubes/Conc								
4																
5	Sample ID															
6	Sample type	Replicate Level	Normal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/aliq	OPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	Calculate nmol H_2O formed Total DPM corrected for background (Background Tubes)	nmol H_2O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
7	CR11804	1-1	2	0.5	2	11750	23500	23479	46958	0.1	188787	46732	0.0487	0.005	15	0.325
8																
9		1-2	2	0.5	1	10897	21794	22016	44032	0.1	188787	43605	0.0457	0.005	15	0.304
10																
11		1-3	2	0.5	1	11119	22235	21156	42304	0.1	188787	41773	0.0435	0.005	15	0.280
12																
13		2-1	2	0.5	1	10423	20845	21273	42546	0.1	188787	48020	0.0500	0.005	15	0.334
14																
15		2-2	2	0.5	1	11324	22648	22397	44794	0.1	188787	44588	0.0465	0.005	15	0.310
16																
17		2-3	2	0.5	1	10731	21462	23547	47094	0.1	188787	45868	0.0488	0.005	15	0.326
18																
19		3-1	2	0.5	1	10731	21462	21663	43326	0.1	188787	43120	0.0449	0.005	15	0.289
20																
21		3-2	2	0.5	1	10119	20238	22051	44102	0.1	188787	43876	0.0457	0.005	15	0.305
22																
23		3-3	2	0.5	1	10132	20264	20940	41880	0.1	188787	40254	0.0420	0.005	15	0.280
24																
25		4-1	2	0.5	1	11536	23072	23166	46332	0.1	188787	46106	0.0481	0.005	15	0.330
26																
27		4-2	2	0.5	1	11094	22188	22203	44406	0.1	188787	44190	0.0460	0.005	15	0.307
28																
29		4-3	2	0.5	1	10729	21458	21195	42392	0.1	188787	42166	0.0439	0.005	15	0.293
30																
31		5-1	2	0.5	1	10266	20532	20704	41408	0.1	188787	41182	0.0429	0.005	15	0.286
32																
33		5-2	2	0.5	1	10975	21950	21573	43146	0.1	188787	42620	0.0447	0.005	15	0.298
34																
35		5-3	2	0.5	1	10380	20772	20893	41886	0.1	188787	41150	0.0428	0.005	15	0.286
36																
37		6-1	2	0.5	1	10578	21158	21205	42412	0.1	188787	42166	0.0440	0.005	15	0.283
38																
39		6-2	2	0.5	1	10392	20764	20650	41300	0.1	188787	41074	0.0428	0.005	15	0.285
40																
41		6-3	2	0.5	1	10355	20710	19816	39636	0.1	188787	39410	0.0411	0.005	15	0.274
42																
43		7-1	2	0.5	1	10409	20818	20686	41372	0.1	188787	41146	0.0429	0.005	15	0.286
44																
45		7-2	2	0.5	1	10551	21102	19557	39114	0.1	188787	39088	0.0405	0.005	15	0.270
46																
47		7-3	2	0.5	1	10650	21318	21309	42618	0.1	188787	42392	0.0442	0.005	15	0.295
48																
49		8-1	2	0.5	1	11284	22468	22419	44838	0.1	188787	44612	0.0485	0.005	15	0.310
50																
51		8-2	2	0.5	1	10977	21954	21931	43862	0.1	188787	43636	0.0455	0.005	15	0.303
52																
53		8-3	2	0.5	1	10670	21340	21365	42750	0.1	188787	42504	0.0443	0.005	15	0.295
54																

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		1.00
8		1.00
9		0.99
10		1.01
11		1.01
12		0.99
13		1.01
14		0.99
15		1.01
16		0.99
17		0.99
18		1.01
19		0.99
20		1.01
21		1.00
22		1.00
23		1.00
24		1.00
25		1.00
26		1.00
27		1.00
28		1.00
29		1.01
30		0.99
31		0.99
32		1.01
33		1.02
34		0.98
35		1.00
36		1.00
37		1.00
38		1.00
39		1.01
40		0.99
41		1.01
42		0.99
43		1.00
44		0.99
45		1.00
46		0.99
47		1.00
48		1.00
49		1.00
50		1.00
51		1.00
52		1.00
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	2/27/2007	Test Chemical ID	CR11604	8 Microsome ID	12535-13					
2	NB Ref	12535-50	Chemical class file name	27FE2332.P00	2 Positive Control	4-OH ASDN					
3	Technician ID	PRP	Replicate #								
4	Control Type	Portion	Average								
5	Full activity	Overall	0.304								
6	Background	Overall	0.002								
7	(uncorrected)										
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.002						
12	4-OH ASDN	1	2	-5.00	0.003						
13	4-OH ASDN	2	1	-6.00	0.020						
14	4-OH ASDN	2	2	-6.00	0.020						
15	4-OH ASDN	3	1	-7.30	0.036						
16	4-OH ASDN	3	2	-6.30	0.034						
17	4-OH ASDN	4	1	-7.00	0.132						
18	4-OH ASDN	4	2	-7.00	0.121						
19	4-OH ASDN	5	1	-7.30	0.165						
20	4-OH ASDN	5	2	-7.30	0.160						
21	4-OH ASDN	6	1	-8.00	0.272						
22	4-OH ASDN	6	2	-8.00	0.232						
23	4-OH ASDN	7	1	-8.00	0.273						
24	4-OH ASDN	7	2	-9.00	0.278						
25	4-OH ASDN	8	1	-10.00	0.293						
26	4-OH ASDN	8	2	-10.00	0.285						
27	4-OH ASDN										
28	4-OH ASDN										
29	4-OH ASDN										
30	4-OH ASDN										
31	4-OH ASDN										
32	4-OH ASDN										
33	4-OH ASDN										
34	4-OH ASDN										
35	4-OH ASDN										
36	4-OH ASDN										
37	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
38	CR11604	1	1	-4.00	0.325						
39	CR11604	1	2	-4.00	0.304						
40	CR11604	2	1	-5.00	0.290						
41	CR11604	2	2	-5.00	0.334						
42	CR11604	3	1	-5.00	0.310						
43	CR11604	3	2	-5.00	0.326						
44	CR11604	4	1	-6.00	0.298						
45	CR11604	4	2	-6.00	0.305						
46	CR11604	5	1	-6.30	0.280						
47	CR11604	5	2	-6.30	0.320						
48	CR11604	6	1	-7.00	0.307						
49	CR11604	6	2	-7.00	0.293						
50	CR11604	7	1	-7.00	0.286						
51	CR11604	7	2	-7.00	0.298						
52	CR11604	8	1	-7.00	0.286						
53	CR11604	8	2	-8.00	0.293						
54	CR11604	9	1	-8.00	0.285						
55	CR11604	9	2	-8.00	0.274						
56	CR11604	10	1	-9.00	0.286						
57	CR11604	10	2	-9.00	0.270						
58	CR11604	11	1	-9.00	0.295						
59	CR11604	11	2	-10.00	0.310						
60	CR11604	12	1	-10.00	0.303						
61	CR11604	12	2	-10.00	0.299						
62	CR11604	13	1	-10.00	0.325						
63	CR11604	13	2	-10.00	0.304						
64	CR11604	14	1	-10.00	0.290						
65	CR11604	14	2	-10.00	0.334						
66	CR11604	15	1	-10.00	0.310						
67	CR11604	15	2	-10.00	0.326						
68	CR11604	16	1	-10.00	0.298						
69	CR11604	16	2	-10.00	0.305						
70	CR11604	17	1	-10.00	0.280						
71	CR11604	17	2	-10.00	0.320						
72	CR11604	18	1	-10.00	0.307						
73	CR11604	18	2	-10.00	0.293						
74	CR11604	19	1	-10.00	0.286						
75	CR11604	19	2	-10.00	0.298						
76	CR11604	20	1	-10.00	0.286						
77	CR11604	20	2	-10.00	0.293						
78	CR11604	21	1	-10.00	0.280						
79	CR11604	21	2	-10.00	0.320						
80	CR11604	22	1	-10.00	0.307						
81	CR11604	22	2	-10.00	0.293						
82	CR11604	23	1	-10.00	0.286						
83	CR11604	23	2	-10.00	0.298						
84	CR11604	24	1	-10.00	0.286						
85	CR11604	24	2	-10.00	0.293						
86	CR11604	25	1	-10.00	0.280						
87	CR11604	25	2	-10.00	0.320						
88	CR11604	26	1	-10.00	0.307						
89	CR11604	26	2	-10.00	0.293						
90	CR11604	27	1	-10.00	0.286						
91	CR11604	27	2	-10.00	0.298						
92	CR11604	28	1	-10.00	0.286						
93	CR11604	28	2	-10.00	0.293						
94	CR11604	29	1	-10.00	0.280						
95	CR11604	29	2	-10.00	0.320						
96	CR11604	30	1	-10.00	0.307						
97	CR11604	30	2	-10.00	0.293						
98	CR11604	31	1	-10.00	0.286						
99	CR11604	31	2	-10.00	0.298						
100	CR11604	32	1	-10.00	0.286						
101	CR11604	32	2	-10.00	0.293						
102	CR11604	33	1	-10.00	0.280						
103	CR11604	33	2	-10.00	0.320						
104	CR11604	34	1	-10.00	0.307						
105	CR11604	34	2	-10.00	0.293						
106	CR11604	35	1	-10.00	0.286						
107	CR11604	35	2	-10.00	0.298						
108	CR11604	36	1	-10.00	0.286						
109	CR11604	36	2	-10.00	0.293						
110	CR11604	37	1	-10.00	0.280						
111	CR11604	37	2	-10.00	0.320						
112	CR11604	38	1	-10.00	0.307						
113	CR11604	38	2	-10.00	0.293						
114	CR11604	39	1	-10.00	0.286						
115	CR11604	39	2	-10.00	0.298						
116	CR11604	40	1	-10.00	0.286						
117	CR11604	40	2	-10.00	0.293						
118	CR11604	41	1	-10.00	0.280						
119	CR11604	41	2	-10.00	0.320						
120	CR11604	42	1	-10.00	0.307						
121	CR11604	42	2	-10.00	0.293						
122	CR11604	43	1	-10.00	0.286						
123	CR11604	43	2	-10.00	0.298						
124	CR11604	44	1	-10.00	0.286						
125	CR11604	44	2	-10.00	0.293						
126	CR11604	45	1	-10.00	0.280						
127	CR11604	45	2	-10.00	0.320						
128	CR11604	46	1	-10.00	0.307						
129	CR11604	46	2	-10.00	0.293						
130	CR11604	47	1	-10.00	0.286						
131	CR11604	47	2	-10.00	0.298						
132	CR11604	48	1	-10.00	0.286						
133	CR11604	48	2	-10.00	0.293						
134	CR11604	49	1	-10.00	0.280						
135	CR11604	49	2	-10.00	0.320						
136	CR11604	50	1	-10.00	0.307						
137	CR11604	50	2	-10.00	0.293						
138	CR11604	51	1	-10.00	0.286						
139	CR11604	51	2	-10.00	0.298						
140	CR11604	52	1	-10.00	0.286						
141	CR11604	52	2	-10.00	0.293						
142	CR11604	53	1	-10.00	0.280						
143	CR11604	53	2	-10.00	0.320						
144	CR11604	54	1	-10.00	0.307						
145	CR11604	54	2	-10.00	0.293						
146	CR11604	55	1	-10.00	0.286						
147	CR11604	55	2	-10.00	0.298						
148	CR11604	56	1	-10.00	0.286						
149	CR11604	56	2	-10.00	0.293						
150	CR11604	57	1	-10.00	0.280						
151	CR11604	57	2	-10.00	0.320						
152	CR11604	58	1	-10.00	0.307						
153	CR11604	58	2	-10.00	0.293						
154	CR11604	59	1	-10.00	0.286						
155	CR11604	59	2	-10.00	0.298						
156	CR1160										

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
			Test		# Concentrations				
1	Assay Date	2/28/2007	Chemical ID	CR11604	tested		8	Microsome ID	12535-13
2	NB Ref.	12535-53	Counter data filename	28FE2210.P00					
	Technician						Pos Ctl		
3	ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/28/2007	Test Chemical ID	CR11604	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-53	Counter data filename	28FE2210.P00				Pos Ctl Tubes/Conc	
3	Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN			2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0195		37181		1906718			
8	2	0.0195		36030		1847692			
9	3	0.0194		39598		2041134			
10	4	0.0194		41404		2134227			
11	5	0.0197		42816		2173401			
12						Average DPM/g soln		2020634	
13						SD		141020	
14						CV		6.98	
15						µCi/g soln		0.9102	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.00	10		1000.00				
20	Dilution A			100	10.00				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0257 g			
25	Mass of dilution B used in substrate prep					4.4793 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.55811954 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.9102			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36	=			0.5581	+	0.011			
37	=			0.569	µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=			1.599 µCi/µg ASDN					
41				1.017.E+06 dpm/nmol					

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	2/28/2007	Test Chemical ID	CR11604	# Concentrations tested	8	Microsome ID	12535-13	
2			NB Ref	12535-53	Counter data filename	28FE2210.P00					
3			Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl		2
4											
5	Microsome Dilution Details										
6											
7	Dilution A										
8			0.12 mL microsome Stock used								
9			2.4 mL total volume								
10			20 dilution factor								
11	Dilution B										
12			1.5 mL microsome Dilution A used								
13			60 mL total volume								
14			40 dilution factor								
15	Dilution C (if applicable)										
16			mL microsome Dilution B used								
17			mL total volume								
18			dilution factor								
19			800 total dilution factor								
20											
21	Protein Concentration (stock microsomes, mg/mL):										
22	Protein Concentration (dilution added to assay, mg/mL):										
23					8.00						
24					0.01						
25											
26											

Positive Control Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	5.00E-07
4	1.00E-07
5	5.00E-08
6	1.00E-08
7	1.00E-09
8	1.00E-10

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-04
2	1.00E-05
3	1.00E-06
4	5.00E-07
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Test # Concentrations										
2	Assay 2/28/2007 Chemical ID CF tested 8 Microsome ID 12535-13										
3	NB 12535-53 Counter data filename 28FE2210.P00 Tec hnic Pos Ctl ID PRP Replicate # 3 Positive Control 4-OH ASD Tubes/Conc 2										
4											
5	Vial #	Time	DPM1				tS/E				Round DPM
6	2	5	37180.62	14763.78	0	12.69	484.89				37181
7	3	5	36030.43	14298.22	0	12.75	485.01				36030
8	4	5	39598.38	15685.98	0	12.74	483.54				39598
9	5	5	41404.15	16518.78	0	12.74	487.17				41404
10	6	5	42816.48	17051.94	0	12.79	487.35				42816
11	8	5	12148.18	5221.14	0	13.83	547.17				12148
12	9	5	12180.02	5270.94	0	13.66	546.33				12180
13	10	5	11799.91	5080.22	0	13.66	546.54				11800
14	11	5	11618.44	5028.26	0	13.68	547.93				11618
15	12	5	10928.5	4711.74	0	13.65	545.92				10929
16	13	5	10731.12	4633.74	0	13.73	548.36				10731
17	14	5	10809.6	4675.62	0	13.64	546.09				10810
18	15	5	10883.82	4664.94	0	13.68	546.47				10884
19	16	5	63.52	27.22	0	11.5	549.95				64
20	17	5	53.16	25.78	0	12.82	545.79				53
21	18	5	71.74	28.94	0	13.34	549.1				72
22	19	5	58.63	26.78	0	17.11	547.23				59
23	20	5	72.09	30.34	0	14.84	545.74				72
24	21	5	73.26	30.58	0	14.46	546.37				73
25	22	5	54.62	22.74	0	15.59	547.11				55
26	23	5	60.19	25.98	0	11.83	548.53				60
27	25	5	156.61	66.18	0	13.22	548.96				157
28	26	5	144.47	60.18	0	13.43	545.71				144
29	27	5	148.9	63.74	0	13.01	545.45				149
30	28	5	146.31	60.82	0	13.3	548.11				146
31	29	5	862.81	367.58	0	13.83	545.29				863
32	30	5	842.33	381.34	0	13.81	545.73				842
33	31	5	713.28	308.98	0	13.4	546.57				713
34	32	5	760.15	327.54	0	13.45	548.08				760
35	33	5	1267.99	557.54	0	13.47	549.06				1268
36	34	5	1304.02	552.38	0	13.95	548.9				1304
37	35	5	1369	586.98	0	13.57	546.84				1369
38	36	5	1338.58	574.14	0	13.85	548.59				1339
39	37	5	4821.67	2079.74	0	13.68	548.26				4822
40	38	5	4636.38	2006.62	0	13.67	547.32				4636
41	39	5	4507.46	1947.42	0	13.59	547.37				4507
42	40	5	4485.84	1927.94	0	13.65	546.81				4486
43	41	5	6730.41	2892.14	0	13.77	547.86				6730
44	42	5	6662.23	2872.42	0	13.7	548.63				6662
45	43	5	6195.74	2663.82	0	13.67	546.32				6196
46	44	5	6099.36	2637.02	0	13.59	547.7				6099
47	45	5	9923.06	4277.54	0	13.64	548.04				9923
48	46	5	9871.47	4251.42	0	13.69	547.8				9871
49	47	5	9274.59	4006.86	0	13.6	548.45				9275
50	48	5	9183.63	3948.74	0	13.63	544.23				9184
51	49	5	12208.24	5231.34	0	13.69	543.6				12208
52	50	5	12188.46	5288.58	0	13.65	548.98				12188
53	51	5	11668.92	5004.26	0	13.69	546.46				11669
54	52	5	11302.25	4863.74	0	13.65	547.99				11302
55	53	5	11809.89	5065.74	0	13.66	544.66				11810
56	54	5	11625.69	5009.98	0	13.67	545.57				11626
57	55	5	11350.13	4893.38	0	13.59	545.6				11350
58	56	5	10752.92	4634.54	0	13.73	547.84				10753
59	58	5	13196.24	5671.38	0	13.75	545.95				13196
60	59	5	13202.88	5636.18	0	13.74	545.04				13203
61	60	5	12656.49	5474.94	0	13.66	551.27				12656
62	61	5	11895.59	5182.58	0	13.83	547.71				11896
63	62	5	12325.45	5336.82	0	13.69	550.1				12325
64	63	5	12097.95	5235.74	0	13.71	549.17				12098
65	64	5	12003.92	5137.94	0	13.87	545.22				12004
66	65	5	12034.63	5191.22	0	13.66	545.05				12035
67	66	5	12798.98	5492.98	0	13.67	542.39				12799
68	67	5	12702.57	5489.22	0	13.61	546.5				12703
69	68	5	12901.14	5549.58	0	13.76	547.06				12901
70	69	5	13001.57	5580.58	0	13.63	543.9				13002
71	70	5	10998.31	4765.78	0	13.65	547.63				10998

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
72	71	5	10996.17	4678.54	0	13.69	545.72				10996
73	72	5	12258.43	5281.14	0	13.7	547.07				12258
74	73	5	12009.03	5208.58	0	13.64	549.01				12009
75	74	5	11863.24	5142.34	0	13.69	550.71				11863
76	75	5	11851.52	5089.14	0	13.66	545.92				11852
77	76	5	11306.99	4847.54	0	13.72	545.49				11307
78	77	5	11305.61	4857.18	0	13.67	545.95				11306

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
79	78	5	12422.59	5350.18	0	13.7	546.45				12423
80	79	5	12156.98	5238.14	0	13.7	546.96				12157
81	80	5	11728.88	5047.42	0	13.66	547.09				11729
82	81	5	11598.81	5001.38	0	13.58	546.85				11599
83	82	5	12268.38	5295.34	0	13.71	547.45				12268
84	83	5	12028.19	5194.94	0	13.7	545.75				12028
85	84	5	12184.95	5266.02	0	13.89	547.97				12185
86	85	5	12675.76	5484.18	0	13.63	549.52				12676
87	86	5	11410.95	4937.14	0	13.59	547.23				11411
88	87	5	11321.31	4884.38	0	13.75	550.84				11321
89	88	5	11447.52	4927.82	0	13.64	547.03				11448
90	89	5	11054.36	4771.82	0	13.72	547.62				11054
91	90	5	11537.27	4975.98	0	13.73	548.03				11537
92	91	5	11224.34	4836.22	0	13.72	547.48				11224
93	92	5	11297.08	4868.18	0	13.62	546.8				11297
94	93	5	11197.22	4807.34	0	13.69	543.74				11197
95	94	5	12120.15	5228.02	0	13.58	546.66				12120
96	95	5	11459.52	4970.82	0	13.7	549.87				11460
97	96	5	11752.61	5085.02	0	13.68	547.98				11753
98	97	5	12049.68	5176.58	0	13.71	547				12050
99	98	5	11136.98	4793.82	0	13.67	545.42				11137
100	99	5	11123.62	4747.54	0	13.65	543.6				11124
101	100	5	11546.9	4957.38	0	13.64	545.33				11547
102	101	5	11273.89	4862.18	0	13.67	547.01				11274
103	102	5	11751.55	5079.5	0	13.63	548.15				11752
104	103	5	11484.77	4918.74	0	13.69	544.04				11485
105	104	5	12012.44	5174.34	0	13.76	546.28				12012
106	105	5	11875.09	5092.38	0	13.67	543.22				11875
107	107	5	236697.25	106773.5	0	14.96	618.93				236697
108											0
109											0
110											0
111											0
112											0
113											0
114											0
115											0
116											0
117											0
118											0
119											0
120											0
121											0
122											0
123											0

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	2/28/2007	Test Chemical ID	CR11604	# Concentrations tested	8	Microsome ID	2535-13						
2		NP Ref. Technician	12535-53	Replicate #	28EE2740.F00	4 OH ASDNI Pos Ctl Tubes/Conc	2								
3															
4															
5	Sample ID	Calculate DPM in aqueous portion after extraction													
6	Sample type	Replicate #	Normal final volume (mL)	Aliq Volume (mL)	Aliq #	DPH/mL	DPH/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (total)	Calculate and ³ H ₂ O formed background (background) Tubes	Final [protein] in assay (mg/mL)	Inoculation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
7	Full assay control	1	2	0.5	1	12148	24396	24378	48956	0.1	202063	48402	0.0076	15	0.317
8		2	2	0.5	2	12188	24360	23418	46956	0.1	202063	46582	0.005	15	0.305
9		3	2	0.5	3	11800	23800	21600	43320	0.1	202063	43086	0.005	15	0.282
10		4	2	0.5	4	10928	21856	21462	43368	0.1	202063	43134	0.005	15	0.283
11		5	2	0.5	5	10810	21620	21684	43368	0.1	202063	43134	0.005	15	0.283
12		6	2	0.5	6	10824	21768	21684	43368	0.1	202063	43134	0.005	15	0.283
13	Background control	1	2	0.5	1	64	128	117	234	0.1	202063	-20	0.000	15	0.000
14		2	2	0.5	2	53	106	131	262	0.1	202063	-8	0.000	15	0.000
15		3	2	0.5	3	72	144	145	289	0.1	202063	36	0.000	15	0.000
16		4	2	0.5	4	55	110	115	230	0.1	202063	-24	0.000	15	0.000
17		5	2	0.5	5	60	120	115	230	0.1	202063	-24	0.000	15	0.000
18		6	2	0.5	6	157	314	301	602	0.1	202063	346	0.003	15	0.002
19	4 OH ASDNI	1	2	0.5	1	144	288	295	590	0.1	202063	336	0.003	15	0.002
20		2	2	0.5	2	149	298	295	590	0.1	202063	336	0.003	15	0.002
21		3	2	0.5	3	146	292	0	0	0.1	202063	-254	-0.002	15	-0.002
22		4	2	0.5	4	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
23		5	2	0.5	5	863	1726	1705	3410	0.1	202063	3158	0.005	15	0.021
24		6	2	0.5	6	842	1684	1673	3346	0.1	202063	2692	0.005	15	0.018
25		7	2	0.5	7	713	1426	1473	2946	0.1	202063	2692	0.005	15	0.018
26		8	2	0.5	8	760	1520	0	0	0.1	202063	-254	-0.002	15	-0.002
27		9	2	0.5	9	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
28		10	2	0.5	10	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
29		11	2	0.5	11	1968	3936	2572	5144	0.1	202063	4680	0.005	15	0.032
30		12	2	0.5	12	1904	3808	2708	5416	0.1	202063	5182	0.005	15	0.034
31		13	2	0.5	13	1883	3766	2708	5416	0.1	202063	5182	0.005	15	0.034
32		14	2	0.5	14	1376	2752	0	0	0.1	202063	-254	-0.002	15	-0.002
33		15	2	0.5	15	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
34		16	2	0.5	16	4822	9644	9453	18916	0.1	202063	18662	0.005	15	0.122
35		17	2	0.5	17	4636	9272	9272	18544	0.1	202063	18662	0.005	15	0.122
36		18	2	0.5	18	4507	9014	8993	17986	0.1	202063	17732	0.005	15	0.116
37		19	2	0.5	19	4486	8972	8972	17944	0.1	202063	17732	0.005	15	0.116
38		20	2	0.5	20	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
39		21	2	0.5	21	6730	13460	13392	26784	0.1	202063	26530	0.005	15	0.174
40		22	2	0.5	22	6582	13164	12285	24570	0.1	202063	24398	0.005	15	0.160
41		23	2	0.5	23	6196	12392	12285	24570	0.1	202063	24398	0.005	15	0.160
42		24	2	0.5	24	5199	10398	10398	20796	0.1	202063	20796	0.005	15	0.122
43		25	2	0.5	25	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
44		26	2	0.5	26	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
45		27	2	0.5	27	9873	19746	19746	39492	0.1	202063	39334	0.005	15	0.258
46		28	2	0.5	28	9873	19746	19746	39492	0.1	202063	39334	0.005	15	0.258
47		29	2	0.5	29	8275	16550	16459	32918	0.1	202063	36654	0.005	15	0.240
48		30	2	0.5	30	8275	16550	16459	32918	0.1	202063	36654	0.005	15	0.240
49		31	2	0.5	31	9184	18368	18368	36736	0.1	202063	36654	0.005	15	0.240
50		32	2	0.5	32	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
51		33	2	0.5	33	12208	24416	24396	48792	0.1	202063	48638	0.005	15	0.318
52		34	2	0.5	34	12188	24376	24396	48792	0.1	202063	48638	0.005	15	0.318
53		35	2	0.5	35	11665	23330	22971	45942	0.1	202063	45948	0.005	15	0.300
54		36	2	0.5	36	11302	22604	22604	45208	0.1	202063	45948	0.005	15	0.300
55		37	2	0.5	37	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
56		38	2	0.5	38	11810	23620	23438	46872	0.1	202063	46872	0.005	15	0.306
57		39	2	0.5	39	11810	23620	23438	46872	0.1	202063	46872	0.005	15	0.306
58		40	2	0.5	40	11850	23700	22103	44206	0.1	202063	43852	0.005	15	0.288
59		41	2	0.5	41	10733	21466	21466	42932	0.1	202063	42932	0.005	15	0.268
60		42	2	0.5	42	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002
61		43	2	0.5	43	0	0	0	0	0.1	202063	-254	-0.002	15	-0.002

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance			
6		Read if value not within 1% +/- 10%			
7		1.01			
8		1.01			
9		1.01			
10		0.99			
11		1.01			
12		0.99			
13		1.00			
14		1.00			
15		1.00			
16		0.91			
17		1.10			
18		0.90			
19		0.99			
20		1.01			
21		1.01			
22		1.04			
23		0.96			
24		1.01			
25		1.01			
26		0.99			
27		#DIV/0!			
28		#DIV/0!			
29		1.01			
30		0.99			
31		0.97			
32		1.03			
33		#DIV/0!			
34		#DIV/0!			
35		0.99			
36		1.01			
37		1.01			
38		0.99			
39		#DIV/0!			
40		#DIV/0!			
41		1.02			
42		0.98			
43		1.00			
44		1.00			
45		#DIV/0!			
46		#DIV/0!			
47		1.01			
48		0.99			
49		1.01			
50		0.99			
51		#DIV/0!			
52		#DIV/0!			
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57		1.00			
58		1.00			
59		1.00			
60		1.00			
61		1.02			
62		0.98			
63		#DIV/0!			
64		#DIV/0!			
65		1.01			
66		1.00			
67		1.03			
68		0.97			
69		#DIV/0!			
70		#DIV/0!			

Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date		2/28/2017	Chemical	CR11604	# Concentrations tested	12535-13										
NB Ref.		12535-53	Incubation time	28FE2210.P00	4-OH ASDN Pos Ctrl Tubes/Conc											
Technician ID		PRP	Replicate #	3	Primitive Control											
Calculate DPM in aqueous portion after extraction																
Sample ID	Replicates	Level	Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	nmol ³ H ₂ O formed	Final [³ H ₂ O] formed (ng/mL)	Incubation time (min)	Aromatase activity (nmol estrone/estrogen formed/protein/min)		
7	CR11604	1-1	2	0.5	1	13198	26392	26389	52798	202063	52944	0.005	15	0.345		
8		1-2	2	0.5	2	13203	26409	49304	202063	49050	0.0482	0.005	15	0.322		
9		1-3	2	0.5	1	12656	25312	24652	202063	46592	0.0478	0.005	15	0.319		
10		1-4	2	0.5	2	11396	22892	24423	202063	47694	0.0470	0.005	15	0.314		
11		1-5	2	0.5	1	12353	24950	48646	202063	48646	0.0489	0.005	15	0.333		
12		1-6	2	0.5	2	12098	24196	24039	202063	48078	0.0505	0.005	15	0.314		
13		1-7	2	0.5	1	12053	24076	48078	202063	48078	0.0505	0.005	15	0.333		
14		1-8	2	0.5	2	12769	25598	25502	202063	51004	0.0507	0.005	15	0.339		
15		1-9	2	0.5	2	12763	25405	51004	202063	51004	0.0507	0.005	15	0.339		
16		1-10	2	0.5	1	12901	25802	25903	202063	51606	0.0507	0.005	15	0.339		
17		1-11	2	0.5	2	13002	26004	43838	202063	43838	0.0507	0.005	15	0.339		
18		1-12	2	0.5	1	10988	21965	21964	202063	43734	0.0507	0.005	15	0.339		
19		1-13	2	0.5	2	10988	21965	21964	202063	43734	0.0507	0.005	15	0.339		
20		1-14	2	0.5	1	12258	24515	24267	202063	48344	0.0507	0.005	15	0.339		
21		1-15	2	0.5	2	12098	24076	48078	202063	48078	0.0507	0.005	15	0.339		
22		1-16	2	0.5	1	11853	23728	23715	202063	47490	0.0507	0.005	15	0.339		
23		1-17	2	0.5	2	11853	23728	23715	202063	47490	0.0507	0.005	15	0.339		
24		1-18	2	0.5	1	11853	23728	23715	202063	47490	0.0507	0.005	15	0.339		
25		1-19	2	0.5	2	11853	23728	23715	202063	47490	0.0507	0.005	15	0.339		
26		1-20	2	0.5	1	11853	23728	23715	202063	47490	0.0507	0.005	15	0.339		
27		1-21	2	0.5	2	11853	23728	23715	202063	47490	0.0507	0.005	15	0.339		
28		1-22	2	0.5	1	11853	23728	23715	202063	47490	0.0507	0.005	15	0.339		
29		1-23	2	0.5	2	11729	23458	23128	202063	46595	0.0466	0.005	15	0.304		
30		1-24	2	0.5	1	11590	23198	23026	202063	45838	0.0475	0.005	15	0.317		
31		1-25	2	0.5	2	12268	24538	24295	202063	48392	0.0487	0.005	15	0.324		
32		1-26	2	0.5	1	12165	24370	24861	202063	49468	0.0445	0.005	15	0.293		
33		1-27	2	0.5	2	12676	25352	27732	202063	55654	0.0440	0.005	15	0.296		
34		1-28	2	0.5	1	11411	22932	22752	202063	45004	0.0445	0.005	15	0.293		
35		1-29	2	0.5	2	11321	22642	22611	202063	44522	0.0440	0.005	15	0.293		
36		1-30	2	0.5	1	11448	22896	22502	202063	44522	0.0440	0.005	15	0.293		
37		1-31	2	0.5	2	11654	24108	22761	202063	45522	0.0440	0.005	15	0.293		
38		1-32	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
39		1-33	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
40		1-34	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
41		1-35	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
42		1-36	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
43		1-37	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
44		1-38	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
45		1-39	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
46		1-40	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
47		1-41	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
48		1-42	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
49		1-43	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
50		1-44	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
51		1-45	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
52		1-46	2	0.5	1	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		
53		1-47	2	0.5	2	11537	23074	22644	202063	44522	0.0440	0.005	15	0.293		

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		
	QC check - all input variables	
		% RE
6		1.00
7		1.00
8		1.00
9		1.03
10		0.97
11		1.01
12		0.99
13		1.00
14		1.00
15		1.00
16		1.00
17		1.00
18		1.00
19		1.00
20		1.01
21		0.99
22		1.00
23		1.00
24		1.00
25		1.00
26		1.00
27		1.01
28		0.99
29		1.01
30		0.99
31		1.01
32		0.99
33		1.00
34		1.00
35		1.00
36		1.00
37		1.02
38		0.99
39		1.01
40		0.99
41		1.00
42		1.00
43		1.03
44		0.97
45		0.99
46		1.01
47		1.00
48		1.00
49		0.99
50		1.01
51		0.99
52		1.01
53		0.99
54		0.99

Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L
			Assay Date	2/25/2007	Test Chemical ID	CR11604	# Concentrations			Microsome ID	12535-13	
			NB Ref	12535-53	Counter data filename	29FE2210.P00	3					
			Technician ID	PRP	Replicate #					Pos Ctl Tubes/Conc	2	
Control Type		Portion	Average	SD								
Full activity		Overall	0.297	0.017								
Background (uncorrected)		Overall	0.002	0.000								
					Bkg % of Full							
					0.6 (Red # >1%)							

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	3/9/2007	Test	Chemical ID	CR11605	# Concentrations	tested	8	Microsome ID	12535-13
2	NB Ref.	12535-64	Counter data	filename	13MR0950.P00					
3	Technician							Pos Ctl		
3	ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc			2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/9/2007	Test Chemical ID	CR11605	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-64	Counter data filename	13MR0950.P00					
3	Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0201		37857		1883433			
8	2	0.0197		37242		1890457			
9	3	0.0189		37129		1964497			
10	4	0.0188		36850		1960106			
11	5	0.0180		36329		2018278			
12						Average DPM/g soln		1943354	
13						SD		58413	
14						CV		2.90	
15						µCi/g soln		0.8754	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution								8.0939 g
25	Mass of dilution B used in substrate prep								4.5413 g
26	Concentration of nonradiolabeled ASDN in substrate soln.								0.56219901 µg/g
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.8754				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					0.5622	+	0.011		
37					0.573	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.528 µCi/µg ASDN								
41	9.716.E+05 dpm/nmol								

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/9/2007	Test Chemical ID	CR11605	# Concentrations tested	8	Microsome ID	12535-13	
2		NB Ref	12535-64	Counter data filename	13MR0950.P00					
3		Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2	
4										
5	Microsome Dilution Details									
6										
7	Dilution A									
8		0.12 mL microsome Stock used								
9		2.4 mL total volume								
10		20 dilution factor								
11	Dilution B									
12		1.5 mL microsome Dilution A used								
13		60 mL total volume								
14		40 dilution factor								
15	Dilution C (if applicable)									
16		mL microsome Dilution B used								
17		mL total volume								
18		dilution factor								
19										
20		800 total dilution factor								
21	Protein Concentration (stock microsomes, mg/mL):									
22	Protein Concentration (dilution added to assay, mg/mL):									
23										
24										
25										
26										

Aromalase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Assay Date	3/9/2007	Test Chemical ID	CR11605	# Concentrations tested	8	Microsome ID	12535-13		
2	NB Ref.	12535-64	Counter data filename	13MR0950.P00						
3	Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2		
4										
5	Vial	Time (min)	DPM1			ISIE				Round DPM
6	2	5	37857.23	16753.72	0	11.94	485.65			37857
7	3	5	37242.43	16119.14	0	11.88	477.72			37242
8	4	5	37129.27	16152.22	0	11.83	475.68			37129
9	5	5	36849.82	15981.66	0	11.91	477.55			36850
10	6	5	36329.39	15678.13	0	11.78	471.66			36329
11	8	5	11085.36	5159.15	0	12.72	534.79			11085
12	9	5	11317.64	5297.65	0	12.76	542.83			11318
13	10	5	8992.75	4190.66	0	12.7	535.82			8993
14	11	5	8912.13	4153.57	0	12.82	536.28			8912
15	12	5	10288.67	4829.19	0	12.64	534.94			10289
16	13	5	10219.08	4802.31	0	12.79	539.37			10219
17	14	5	9902.13	4626.67	0	12.76	537.15			9902
18	15	5	10346.63	4818.63	0	12.72	535.88			10347
19	16	5	69.07	36.09	0	13.04	534.91			69
20	17	5	66	29.49	0	13.73	535.67			66
21	18	5	66.73	30.53	0	14.89	535.04			67
22	19	5	61.88	30.91	0	12.48	538.33			62
23	20	5	57.86	27.58	0	12.2	536.45			58
24	21	5	65.82	30.62	0	15.96	538.39			66
25	22	5	68.59	33.02	0	14.36	535.21			69
26	23	5	62.81	28.97	0	15.09	535.24			63
27	25	5	167.42	78.08	0	12.51	535.02			167
28	26	5	150.17	72.79	0	12.91	536.19			150
29	27	5	159.76	69.14	0	13.62	536.83			160
30	28	5	163.06	74.44	0	12.98	534.72			163
31										0
32										0
33	29	5	898.37	410.26	0	13.14	535.52			898
34	30	5	895.04	417.81	0	12.79	536.58			895
35	31	5	809.63	384.63	0	12.51	534.66			810
36	32	5	838.99	392.45	0	12.9	537.96			839
37										0
38										0
39	33	5	1423	665.78	0	12.77	537.18			1423
40	34	5	1440.49	657.66	0	13.18	535.95			1440
41	35	5	1450.58	679.24	0	12.97	537.38			1451
42	36	5	1479.02	691.47	0	12.83	536.74			1479
43										0
44										0
45	37	5	4345.48	2033.39	0	12.69	535.77			4345
46	38	5	4419.3	2069.22	0	12.75	538.62			4419
47	39	5	4574.32	2153.84	0	12.65	535.24			4574
48	40	5	4642.62	2161.68	0	12.63	534.51			4643
49										0
50										0
51	41	5	6362.77	2980.51	0	12.72	537.1			6363
52	42	5	6238.2	2888.69	0	12.74	533.31			6236
53	43	5	5455.49	2521.73	0	12.83	534.8			5455
54	44	5	5476.04	2546.21	0	12.71	532.64			5476
55										0
56										0
57	45	5	9365.95	4359.58	0	12.78	535.77			9366
58	46	5	9432.48	4403.59	0	12.8	537.41			9432
59	47	5	9211.08	4283.35	0	12.7	536.04			9211
60	48	5	9177.13	4295.33	0	12.71	536.89			9177
61										0
62										0
63	49	5	10831.64	5082.09	0	12.67	536.09			10832
64	50	5	10872.62	5078.95	0	12.7	538.46			10873
65	51	5	9430.22	4401.28	0	12.61	534.5			9430
66	52	5	9433.75	4417.6	0	12.73	536.78			9434
67										0
68										0
69	53	5	11063.32	5176.69	0	12.77	538.86			11063
70	54	5	11197.87	5229.11	0	12.77	537.48			11198
71	55	5	8374.35	3909.72	0	12.71	537.91			8374
72	56	5	9002.09	4220.73	0	12.75	537.59			9002
73										0
74										0

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	7940.44	3712.89		0	12.76	534.43			7940
76	59	5	7767.84	3639.86		0	12.68	537.9			7768
77	60	5	8741.18	4037.83		0	12.75	533.18			8741
78	61	5	8287.62	3872.16		0	12.67	536.81			8288
79	62	5	8254.38	3856.61		0	12.78	536.66			8254
80	63	5	8428.16	3917.14		0	12.72	533.83			8428
81	64	5	8918.75	4131.97		0	12.65	532.48			8919
82	65	5	8683.93	4073.11		0	12.71	534.52			8684
83	66	5	9758.66	4577.98		0	12.72	535.31			9759
84	67	5	9916.36	4618.69		0	12.76	535.2			9916
85	68	5	9882.98	4574		0	12.75	530.82			9883
86	69	5	9816.56	4570.36		0	12.7	534.52			9817
87	70	5	9367.64	4336.28		0	12.72	532.78			9368
88	71	5	9159.88	4268.22		0	12.69	535			9160
89	72	5	10047.74	4682.6		0	12.75	534.75			10048
90	73	5	9966.3	4651.9		0	12.69	535.26			9966
91	74	5	10030.95	4690.74		0	12.71	538.23			10031
92	75	5	9965.5	4636.39		0	12.76	533.07			9966
93	77	5	9214.49	4319.83		0	12.64	533.58			9214
94	78	5	9151.22	4287.8		0	12.71	537.21			9151
95	79	5	10072.53	4677.04		0	12.73	533.78			10073
96	80	5	10220.85	4804.49		0	12.72	537.65			10221
97	81	5	10449.33	4870.99		0	12.72	535.73			10449
98	82	5	10286.16	4787.71		0	12.7	535.29			10286
99	83	5	10204.47	4758.01		0	12.72	535.72			10204
100	84	5	10157.79	4762.95		0	12.68	536.99			10158
101	85	5	8104.75	3784.33		0	12.76	535.05			8105
102	86	5	7997.22	3731.07		0	12.74	534.72			7997
103	87	5	9327.95	4351.7		0	12.72	534.44			9328
104	88	5	9742.72	4557.21		0	12.81	536.68			9743
105	89	5	9956.72	4651.01		0	12.79	534.15			9957
106	90	5	9933.75	4648		0	12.74	535.96			9934
107	91	5	9849.21	4629.44		0	12.61	536.96			9849
108	92	5	9831.87	4622.79		0	12.74	539.14			9832
109	93	5	10736.26	4974.24		0	12.83	536.46			10736
110	94	5	10672.73	5024.02		0	12.68	538.86			10673
111	95	5	10898.47	5110.49		0	12.68	538.99			10898
112	96	5	11101.42	5168.12		0	12.77	534.58			11101
113	97	5	10487.96	4944.84		0	12.64	537.91			10488
114	98	5	10806.2	5041.84		0	12.71	536.6			10806
115	99	5	10300.7	4808.4		0	12.77	534.79			10301
116	100	5	10471.54	4890.31		0	12.74	535.28			10472
117	101	5	10421.13	4869.63		0	12.71	533.89			10421
118	102	5	10409.29	4865.73		0	12.75	536.12			10409
119	103	5	10329.56	4829.67		0	12.77	537.08			10330
120	104	5	10154.85	4727.88		0	12.77	535.67			10155
121	105	5	10393.47	4853.35		0	12.72	534.86			10393
122	106	5	10533.99	4938.52		0	12.71	536.79			10534
123	107	5	0	0		0	0	475.23			0
124											0

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC Check - aliquot variance Read 11 values not within mean $\pm$ 10%			
6					
7		0.99			
8		1.01			
9		1.00			
10		1.00			
11		1.00			
12		1.00			
13		0.98			
14		1.02			
15		0.99			
16		1.01			
17		1.00			
18		0.99			
19		1.00			
20		1.00			
21		0.99			
22		1.00			
23		0.99			
24		1.00			
25		0.99			
26		1.01			
27		#DIV/0!			
28		#DIV/0!			
29		1.00			
30		0.98			
31		1.02			
32		#DIV/0!			
33		0.99			
34		1.01			
35		0.99			
36		1.01			
37		0.99			
38		1.01			
39		#DIV/0!			
40		#DIV/0!			
41		0.99			
42		1.01			
43		0.98			
44		1.01			
45		#DIV/0!			
46		#DIV/0!			
47		1.01			
48		0.99			
49		1.00			
50		1.00			
51		#DIV/0!			
52		#DIV/0!			
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57		#DIV/0!			
58		#DIV/0!			
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63		#DIV/0!			
64		#DIV/0!			
65		0.99			
66		1.01			
67		0.98			
68		1.04			
69		#DIV/0!			
70		#DIV/0!			

Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date		Assay ID	Chemical	CR11605	# Concentrations tested	8	Microsome ID	12535-13								
NEB Ref.		12535-54	13MR0850_P100													
Technician ID		SLB	1	Positive Control												
Sample ID		Calculate DPM in aqueous portion after extraction														
Sample type	Replicate Level	Normal total volume (mL)	Aliq volume (mL)	Aliq #	DPW/aliquot	DPW/mL	Ave DPW/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	nmol ³ H ₂ O formed (background Tubes)	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)		
7	CR11605	1-1	2	0.5	1	7940	15880	15708	31416	194335	31158	0.0321	15	0.214		
8		1-2	2	0.5	2	7768	15536	15709	31416	194335	31768	0.0348	15	0.232		
9		1-3	2	0.5	1	8288	16576	17029	34058	194335	33104	0.0341	15	0.277		
10		2-1	2	0.5	2	8428	16856	16882	33864	194335	34346	0.0360	15	0.240		
11		2-2	2	0.5	2	8919	17838	17893	35606	194335	38090	0.0402	15	0.268		
12		2-3	2	0.5	1	8994	17988	18075	35950	194335	38140	0.0403	15	0.269		
13		3-1	2	0.5	1	9398	18796	18526	37056	194335	38266	0.0379	15	0.252		
14		3-2	2	0.5	2	10146	20294	20014	40028	194335	39768	0.0409	15	0.273		
15		3-3	2	0.5	2	9956	19912	19897	39894	194335	39734	0.0409	15	0.273		
16		4-1	2	0.5	2	10221	20442	20284	40588	194335	40328	0.0415	15	0.277		
17		4-2	2	0.5	1	10449	20898	20735	41470	194335	41210	0.0424	15	0.283		
18		4-3	2	0.5	1	10264	20528	20362	40724	194335	40464	0.0416	15	0.278		
19		5-1	2	0.5	1	8106	16210	16102	32204	194335	31944	0.0329	15	0.219		
20		5-2	2	0.5	2	7997	15994	15871	31842	194335	32882	0.0390	15	0.260		
21		5-3	2	0.5	1	8328	16656	16681	33364	194335	33104	0.0341	15	0.277		
22		6-1	2	0.5	2	9937	19874	19881	39762	194335	39522	0.0407	15	0.271		
23		6-2	2	0.5	2	9934	19868	19881	39762	194335	39102	0.0402	15	0.268		
24		6-3	2	0.5	2	9932	19864	19881	39762	194335	42668	0.0438	15	0.292		
25		7-1	2	0.5	1	10673	21346	21599	43898	194335	43738	0.0450	15	0.300		
26		7-2	2	0.5	1	10686	21372	21284	42588	194335	42328	0.0438	15	0.290		
27		7-3	2	0.5	2	10421	20842	20773	41546	194335	41286	0.0425	15	0.283		
28		8-1	2	0.5	2	10409	20818	20830	41660	194335	41400	0.0426	15	0.284		
29		8-2	2	0.5	1	10330	20660	20485	40670	194335	40710	0.0419	15	0.279		
30		8-3	2	0.5	2	10353	20706	20827	41854	194335	41594	0.0428	15	0.285		

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		1.01
8		0.89
9		1.03
10		0.97
11		0.99
12		1.01
13		0.99
14		1.01
15		0.99
16		1.01
17		1.01
18		1.01
19		0.99
20		1.01
21		1.01
22		1.00
23		1.00
24		1.00
25		1.00
26		1.00
27		0.99
28		1.01
29		1.01
30		0.99
31		1.00
32		1.00
33		1.01
34		0.99
35		0.98
36		1.02
37		1.00
38		1.00
39		1.00
40		1.00
41		1.00
42		1.00
43		0.99
44		1.01
45		0.99
46		1.01
47		0.99
48		1.01
49		1.00
50		1.01
51		0.99
52		0.99
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/9/2007	Test Chemical ID	CR11605	# Concentrations	8	Microsome ID	12535-13			
2	NB Ref.	12535-64	Counter date filename	13MR0950.P00	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2		
3	Technician ID	SLB	Replicate #								
4	Control Type	Portion	SD								
5	Full activity	Overall	0.276								
6	Background (uncorrected)	Overall	0.002								
7											
8											
9											
10	Positive Control	Level	Replicate	[Positive Ctl] M	Log[Positive Ctl]	Activity					
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.003					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.023					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.021					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.036					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.036					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.116					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.125					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.171					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.148					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.266					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.251					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.296					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.257					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.304					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.237					
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	[Test chemical] M	Log[Test chemical]	Activity					
38	CR11605	1	1	1.00E-03	-3.00	0.214					
39	CR11605	1	2	1.00E-03	-3.00	0.232					
40	CR11605	1	3	1.00E-03	-3.00	0.227					
41	CR11605	2	1	1.00E-04	-4.00	0.240					
42	CR11605	2	2	1.00E-04	-4.00	0.269					
43	CR11605	2	3	1.00E-04	-4.00	0.269					
44	CR11605	3	1	1.00E-05	-5.00	0.252					
45	CR11605	3	2	1.00E-05	-5.00	0.273					
46	CR11605	3	3	1.00E-05	-5.00	0.273					
47	CR11605	4	1	1.00E-06	-6.00	0.250					
48	CR11605	4	2	1.00E-06	-6.00	0.277					
49	CR11605	4	3	1.00E-06	-6.00	0.283					
50	CR11605	5	1	1.00E-07	-7.00	0.278					
51	CR11605	5	2	1.00E-07	-7.00	0.219					
52	CR11605	5	3	1.00E-07	-7.00	0.260					
53	CR11605	6	1	1.00E-08	-8.00	0.271					
54	CR11605	6	2	1.00E-08	-8.00	0.268					
55	CR11605	6	3	1.00E-08	-8.00	0.292					
56	CR11605	7	1	1.00E-09	-9.00	0.300					
57	CR11605	7	2	1.00E-09	-9.00	0.290					
58	CR11605	7	3	1.00E-09	-9.00	0.283					
59	CR11605	8	1	1.00E-10	-10.00	0.264					
60	CR11605	8	2	1.00E-10	-10.00	0.279					
61	CR11605	8	3	1.00E-10	-10.00	0.285					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
	Test # Concentrations								
1	Assay Date	3/13/2007	Chemical ID	CR11605	tested		8 Microsome ID 12535-13		
2	NB Ref.	12535-67	Counter data filename	13MR2226.P00					
	Technician		Pos Clt						
3	ID	SLB	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/13/2007	Test Chemical ID CR11605		# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-67	Counter data filename 13MR2226.P00					Pos Ctl	
3	Technician ID	SLB	Replicate #		2	Positive Control	4-OH ASDN	Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)	DPM/Aliq.		DPM/g soln.				
7	1	0.0211	40996		1942938				
8	2	0.0202	40418		2000891				
9	3	0.0202	40510		2005446				
10	4	0.0203	41283		2033645				
11	5	0.0201	42423		2110597				
12	Average DPM/g soln						2018703		
13	SD						61035		
14	CV						3.02		
15	µCi/g soln						0.9093		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution				8.1021 g				
25	Mass of dilution B used in substrate prep				4.5551 g				
26	Concentration of nonradiolabeled ASDN in substrate soln.				0.56277448 µg/g				
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.9093				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					= 0.5628 + 0.011				
37					= 0.574 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.585 µCi/µg ASDN				
41					1.007.E+06 dpm/nmol				

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/13/2007	Test Chemical ID	CR11605	# Concentrations tested			8 Microsome ID	12535-13
2		NB Ref	12535-67	Counter data filename	13MR2226.P00					
3		Technician ID	SLB	Replicate #	2	Positive Control	2	4-OH ASDN	Pos Ctl	2
4										
5		Microsome Dilution Details								
6										
7		0.12 mL microsome Stock used								
8		2.4 mL total volume								
9		20 dilution factor								
10										
11		1.5 mL microsome Dilution A used								
12		60 mL total volume								
13		40 dilution factor								
14										
15		mL microsome Dilution B used								
16		mL total volume								
17		dilution factor								
18		NA								
19		800 total dilution factor								
20										
21		Protein Concentration (stock microsomes, mg/mL):								
22		Protein Concentration (dilution added to assay, mg/mL):								
23										
24										
25										
26										

Positive Control Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	5.00E-07
4	1.00E-07
5	5.00E-08
6	1.00E-08
7	1.00E-09
8	1.00E-10

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	1.00E-07
4	5.00E-08
5	1.00E-08
6	5.00E-09
7	1.00E-09
8	1.00E-10

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Assay Date	3/13/2007	Test Chemical ID	CR11605	# Concentrations tested	8	Microsome ID	12535-13			
2	NB Ref.	12535-67	Counter data filename	13MR2226.P00							
3	Technician	SLB	Replicate #	2	Positive Control	4-OH ASDI Pos Ctl Tubes/Conc	2				
4											
5	Vial	Time (min)	DPM1			IS/E				Round DPM	
6	2	5	40995.73	17528.62	0	11.89	465.32			40996	
7	3	5	40417.59	17200.42	0	11.67	461.62			40418	
8	4	5	40509.62	17409.93	0	11.69	466.76			40510	
9	5	5	41282.8	17695.52	0	11.71	468.35			41283	
10	6	5	42423.32	18148.49	0	11.67	465.55			42423	
11	8	5	8498.79	3944.19	0	12.63	530.53			8499	
12	9	5	8604.98	4021.26	0	12.67	532.58			8605	
13	10	5	7601.37	3537.92	0	12.67	531.63			7601	
14	11	5	7646.95	3554.34	0	12.64	531.21			7647	
15	12	5	8695.69	3149.45	0	12.48	530.86			8696	
16	13	5	8880.13	3201.15	0	12.72	532.51			8880	
17	14	5	8755.05	3154.09	0	12.67	532.41			8755	
18	15	5	6773.35	3152.1	0	12.77	534.38			6773	
19	16	5	67.43	27.76	0	14.66	535.94			67	
20	17	5	58.6	30.59	0	13.24	535.78			59	
21	18	5	74.07	31.8	0	15.79	533.92			74	
22	19	5	50.26	30.14	0	10.71	532.58			50	
23	20	5	65.67	29.75	0	13.24	531.04			66	
24	21	5	64.9	29.33	0	13.7	533.54			65	
25	22	5	57.22	29.22	0	11.99	528.99			57	
26	23	5	85.6	34.12	0	14.45	533.79			86	
27	25	5	180.56	77.94	0	13.46	532.42			161	
28	26	5	161.07	68.12	0	13.47	532.02			161	
29	27	5	132.76	85.87	0	12.5	530.55			133	
30	28	5	144.79	87.37	0	12.63	533.17			145	
31										0	
32										0	
33	29	5	815.46	380.05	0	12.73	527.69			815	
34	30	5	806.05	367.65	0	12.79	529.14			806	
35	31	5	755.18	340.99	0	12.79	526.88			755	
36	32	5	797.08	333.45	0	12.73	528.96			797	
37										0	
38										0	
39	33	5	1354.52	622.25	0	12.75	528.97			1355	
40	34	5	1375.68	647.67	0	12.41	529.96			1376	
41	35	5	1320.27	615.51	0	12.72	531.93			1320	
42	36	5	1391.41	650.44	0	12.74	528.52			1391	
43										0	
44										0	
45	37	5	3572.14	1660.67	0	12.66	528.9			3572	
46	38	5	3606.92	1675.89	0	12.65	529.4			3607	
47	39	5	3924.16	1817.76	0	12.65	525.95			3924	
48	40	5	3762.42	1728.5	0	12.7	529.9			3762	
49										0	
50										0	
51	41	5	4902.18	2262.68	0	12.7	530.21			4902	
52	42	5	4876.35	2253.84	0	12.66	531.35			4876	
53	43	5	5308.42	2455.2	0	12.69	530.76			5308	
54	44	5	5270.43	2449.63	0	12.65	530.85			5270	
55										0	
56										0	
57	45	5	6975.99	3247.58	0	12.57	530.85			6976	
58	46	5	6960.23	3235.09	0	12.67	533.76			6960	
59	47	5	6274.79	2935.78	0	12.67	533.9			6275	
60	48	5	6293.45	2934.89	0	12.68	532.08			6293	
61										0	
62										0	
63	49	5	7236.96	3364.05	0	12.62	530.64			7237	
64	50	5	7270.73	3388.63	0	12.61	529.48			7271	
65	51	5	8548.88	3027.38	0	12.69	529.38			8549	
66	52	5	8511.48	3011.65	0	12.72	531.01			8511	
67										0	
68										0	
69	53	5	7353.39	3421.29	0	12.56	530.47			7353	
70	54	5	7516.35	3477.61	0	12.66	532.93			7516	
71	55	5	7826.8	3656.43	0	12.53	533.03			7827	
72	56	5	7928.42	3693.69	0	12.67	532.06			7928	
73										0	
74										0	

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	8137.8	3811.35	0	12.68	533.54				8138
76	59	5	8282.37	3843.84	0	12.76	531.59				8292
77	60	5	7968.65	3743.04	0	12.57	533.25				7969
78	61	5	8042.3	3752.41	0	12.6	531.36				8042
79	62	5	8296.05	3880.75	0	12.54	530.39				8296
80	63	5	7979.82	3727.07	0	12.56	531.76				7980
81	64	5	7805.31	3612.85	0	12.59	529.15				7805
82	65	5	8029.8	3736.7	0	12.61	531.12				8030
83	66	5	8062.66	3747.35	0	12.63	530.19				8063
84	67	5	7999.46	3740.06	0	12.87	533.83				7999
85	68	5	8290.6	3876.95	0	12.55	530.5				8291
86	69	5	8421.88	3922.76	0	12.73	532.28				8422
87	70	5	8528.07	3897.91	0	12.71	534.58				8528
88	71	5	8498	3999	0	12.61	534.86				8498
89	72	5	8576.43	4010.3	0	12.64	533.28				8576
90	73	5	8785.62	4079.36	0	12.66	536.21				8786
91	74	5	8670.03	4039.44	0	12.64	530.68				8670
92	75	5	8891.48	4111.09	0	12.68	531.95				8891
93	76	5	6938.94	3229.28	0	12.61	528.67				6939
94	77	5	7023.39	3282.1	0	12.74	530.62				7023
95	78	5	8638.11	4004.85	0	12.89	531.43				8638
96	79	5	8621.73	4033.78	0	12.69	535.64				8622
97	80	5	8289.78	3855.78	0	12.67	533.7				8290
98	81	5	8170.48	3804.65	0	12.64	532.09				8170
99	83	5	8633.14	3993.35	0	12.68	531.28				8633
100	84	5	8667.11	4033.63	0	12.65	530.9				8667
101	85	5	8490.71	3930.26	0	12.66	531.15				8491
102	86	5	8483.46	3969.24	0	12.65	534.15				8483
103	87	5	7964.29	3713.19	0	12.62	532.45				7964
104	88	5	7992.36	3708.68	0	12.64	530.82				7992
105	89	5	7330.05	3393.96	0	12.68	531.03				7330
106	90	5	7181.07	3365.21	0	12.6	532.94				7181
107	91	5	8863.67	4180.27	0	12.67	535.21				8864
108	92	5	8899.1	4154.59	0	12.63	532.63				8899
109	93	5	8388.46	3893.4	0	12.68	531.89				8388
110	94	5	8622.08	4015.95	0	12.75	533.25				8622
111	95	5	8636.13	4034.53	0	12.64	532.94				8636
112	96	5	8614.49	4008.35	0	12.67	534.09				8614
113	97	5	9096.23	4252.92	0	12.64	533.19				9096
114	98	5	8898.1	4113.99	0	12.71	530.97				8898
115	99	5	8610.7	3990.47	0	12.6	534.76				8611
116	100	5	8613.62	3996.8	0	12.74	533.51				8614
117	101	5	8251.8	3843.42	0	12.64	532.54				8252
118	102	5	8514.87	3892.01	0	12.58	532.96				8515
119	103	5	8491.36	3871.82	0	12.67	534.24				8491
120	104	5	8808.79	4102.44	0	12.51	531.45				8809
121	105	5	8790.39	4096.42	0	12.65	532.49				8790
122	106	5	8880.4	4121.81	0	12.72	531.82				8880
123	107	5	227930.11	36369.11	0	7.73	172.21				227930
124	108	5	3.21	0	0	0	476.82				3
125											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	3/13/2007	Chemical ID	CR14605	# Concentrations tested	8	Microsome ID	9255-13						
2		NB Ref. Technician	12535-87	Counter use	13MR2226-P00										
3		ID	SI	E	2	Positive Control	2	4-OH-ANDN	Pre-Cl. Inhibitor Conc						
4															
5	Sample ID														
6	Sample type	Replicate	Nominal total volume (mL)	Aliq. #	DPH/mL	DPH/mL	Ave. DPH/mL	Total DPM	Volume of substrate solution necessary (mL)	Total DPM in assay tube (dpm)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final (product) in assay (mg/mL)	Inhibition (nmol ³ H ₂ O formed)	Aromatase activity (nmol ³ H ₂ O formed/mg protein/min)
7	Full activity control	1	2	0.5	1	8496	17104	34208	0.1	201870	-3946	0.0037	0.005	15	0.25
8		2	2	0.5	2	8096	15248	30496	0.1	201870	30294	0.0090	0.005	15	0.000
9		3	2	0.5	3	7831	15562	30924	0.1	201870	26890	0.0067	0.005	15	0.000
10		4	2	0.5	4	6691	13382	26764	0.1	201870	26890	0.0067	0.005	15	0.000
11		5	2	0.5	5	6691	13382	26764	0.1	201870	26890	0.0067	0.005	15	0.000
12		6	2	0.5	6	6691	13382	26764	0.1	201870	26890	0.0067	0.005	15	0.000
13		7	2	0.5	7	6755	13510	27020	0.1	201870	26794	0.0066	0.005	15	0.177
14		8	2	0.5	8	6713	13446	26892	0.1	201870	-10	0.0000	0.005	15	0.000
15	Background control	1	2	0.5	1	67	134	268	0.1	201870	-14	0.0000	0.005	15	0.000
16		2	2	0.5	2	59	118	236	0.1	201870	0	0.0000	0.005	15	0.000
17		3	2	0.5	3	74	148	296	0.1	201870	24	0.0004	0.005	15	0.000
18		4	2	0.5	4	50	100	200	0.1	201870	280	0.0000	0.005	15	0.000
19		5	2	0.5	5	66	132	264	0.1	201870	280	0.0000	0.005	15	0.000
20		6	2	0.5	6	65	130	260	0.1	201870	280	0.0000	0.005	15	0.000
21		7	2	0.5	7	57	114	228	0.1	201870	280	0.0000	0.005	15	0.000
22		8	2	0.5	8	181	362	724	0.1	201870	280	0.0000	0.005	15	0.000
23	4-OH-ANDN	1-1	2	0.5	1	133	266	532	0.1	201870	280	0.0000	0.005	15	0.000
24		1-2	2	0.5	2	145	290	580	0.1	201870	280	0.0000	0.005	15	0.000
25		1-3	2	0.5	3	0	0	0	0.1	201870	-292	-0.0003	0.005	15	-0.002
26		1-4	2	0.5	4	819	1638	3276	0.1	201870	280	0.0000	0.005	15	0.000
27		1-5	2	0.5	5	806	1612	3224	0.1	201870	280	0.0000	0.005	15	0.000
28		1-6	2	0.5	6	750	1500	3000	0.1	201870	280	0.0000	0.005	15	0.000
29		1-7	2	0.5	7	707	1414	2828	0.1	201870	280	0.0000	0.005	15	0.000
30		1-8	2	0.5	8	0	0	0	0.1	201870	-262	-0.0003	0.005	15	-0.002
31		2-1	2	0.5	1	1355	2710	5420	0.1	201870	5200	0.0022	0.005	15	0.034
32		2-2	2	0.5	2	1376	2752	5504	0.1	201870	5160	0.0021	0.005	15	0.034
33		2-3	2	0.5	3	1391	2782	5564	0.1	201870	5160	0.0021	0.005	15	0.034
34		2-4	2	0.5	4	0	0	0	0.1	201870	-292	-0.0003	0.005	15	-0.002
35		2-5	2	0.5	5	0	0	0	0.1	201870	10966	0.0140	0.005	15	0.069
36		2-6	2	0.5	6	3672	7344	14688	0.1	201870	15110	0.0150	0.005	15	0.160
37		2-7	2	0.5	7	3742	7484	14968	0.1	201870	280	0.0000	0.005	15	0.000
38		2-8	2	0.5	8	0	0	0	0.1	201870	-262	-0.0003	0.005	15	-0.002
39		3-1	2	0.5	1	4802	9604	19208	0.1	201870	19264	0.0192	0.005	15	0.128
40		3-2	2	0.5	2	4876	9752	19504	0.1	201870	20964	0.0209	0.005	15	0.139
41		3-3	2	0.5	3	5398	10796	21592	0.1	201870	20964	0.0209	0.005	15	0.139
42		3-4	2	0.5	4	5270	10540	21080	0.1	201870	-262	-0.0003	0.005	15	-0.002
43		3-5	2	0.5	5	0	0	0	0.1	201870	27610	0.0274	0.005	15	0.183
44		3-6	2	0.5	6	6876	13752	27504	0.1	201870	24874	0.0247	0.005	15	0.165
45		3-7	2	0.5	7	6840	13680	27360	0.1	201870	-262	-0.0003	0.005	15	-0.002
46		3-8	2	0.5	8	7237	14474	28948	0.1	201870	28754	0.0285	0.005	15	0.199
47		4-1	2	0.5	1	7271	14542	29084	0.1	201870	28754	0.0285	0.005	15	0.199
48		4-2	2	0.5	2	6549	13098	26196	0.1	201870	28754	0.0285	0.005	15	0.199
49		4-3	2	0.5	3	6511	13022	26044	0.1	201870	-262	-0.0003	0.005	15	-0.002
50		4-4	2	0.5	4	0	0	0	0.1	201870	28754	0.0285	0.005	15	0.199
51		4-5	2	0.5	5	0	0	0	0.1	201870	28754	0.0285	0.005	15	0.199
52		4-6	2	0.5	6	0	0	0	0.1	201870	28754	0.0285	0.005	15	0.199
53		4-7	2	0.5	7	0	0	0	0.1	201870	28754	0.0285	0.005	15	0.199
54		4-8	2	0.5	8	0	0	0	0.1	201870	28754	0.0285	0.005	15	0.199
55		5-1	2	0.5	1	8273	16546	33092	0.1	201870	31248	0.0310	0.005	15	0.207
56		5-2	2	0.5	2	8240	16480	32960	0.1	201870	-262	-0.0003	0.005	15	-0.002
57		5-3	2	0.5	3	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
58		5-4	2	0.5	4	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
59		5-5	2	0.5	5	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
60		5-6	2	0.5	6	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
61		5-7	2	0.5	7	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
62		5-8	2	0.5	8	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
63		6-1	2	0.5	1	7353	14706	29412	0.1	201870	29476	0.0293	0.005	15	0.185
64		6-2	2	0.5	2	7316	14632	29264	0.1	201870	31248	0.0310	0.005	15	0.207
65		6-3	2	0.5	3	7326	14652	29304	0.1	201870	-262	-0.0003	0.005	15	-0.002
66		6-4	2	0.5	4	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
67		6-5	2	0.5	5	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
68		6-6	2	0.5	6	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
69		6-7	2	0.5	7	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207
70		6-8	2	0.5	8	0	0	0	0.1	201870	31248	0.0310	0.005	15	0.207

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

Assay Date		Chemical		Test		Concentrations tested		Microsome ID		12535-57		NB Ref		Technician ID, S, R		Replicate #		Positive Control		4-OH ASDN Pos Ctrl Tubes/Conc		2		
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y
Sample	Replicate	Normal label	Aliq	Aliq	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate	Total DPM in assay tube	Calculable nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final (protein) in assay	Incubation	Aromatase activity (nmol									
type	Level	volume (mL)	(mL)	#					solution used/assay tube (mL)	(initial)	(background Tubes)		(ng/mL)	time (min)	estrogen formed/ protein/min									
1	CR11605	1-1	2	0.5	1	8138	16276	32890	0.1	201870	32558	0.0324	0.005	15	0.216									
2	CR11605	1-2	2	0.5	2	8282	16564	32722	0.1	201870	31760	0.0315	0.005	15	0.210									
3	CR11605	1-3	2	0.5	1	7969	15938	31676	0.1	201870	32280	0.0320	0.005	15	0.214									
4	CR11605	2-1	2	0.5	1	8296	16592	32722	0.1	201870	31408	0.0312	0.005	15	0.208									
5	CR11605	2-2	2	0.5	2	7980	15960	31676	0.1	201870	31862	0.0318	0.005	15	0.211									
6	CR11605	2-3	2	0.5	1	8033	16066	32124	0.1	201870	33164	0.0329	0.005	15	0.219									
7	CR11605	3-1	2	0.5	1	8528	17056	34052	0.1	201870	33750	0.0335	0.005	15	0.224									
8	CR11605	3-2	2	0.5	2	8438	16876	33722	0.1	201870	34462	0.0342	0.005	15	0.228									
9	CR11605	3-3	2	0.5	1	8780	17560	35122	0.1	201870	34860	0.0345	0.005	15	0.231									
10	CR11605	4-1	2	0.5	1	8891	17782	35526	0.1	201870	34520	0.0340	0.005	15	0.227									
11	CR11605	4-2	2	0.5	2	7023	14046	27924	0.1	201870	32658	0.0324	0.005	15	0.216									
12	CR11605	4-3	2	0.5	1	8636	17272	34520	0.1	201870	34338	0.0341	0.005	15	0.227									
13	CR11605	5-1	2	0.5	1	8170	16340	32620	0.1	201870	32658	0.0324	0.005	15	0.216									
14	CR11605	5-2	2	0.5	2	8633	17266	34600	0.1	201870	34338	0.0341	0.005	15	0.227									
15	CR11605	5-3	2	0.5	1	8491	16982	33948	0.1	201870	33896	0.0334	0.005	15	0.223									
16	CR11605	6-1	2	0.5	1	7964	15928	31676	0.1	201870	31560	0.0314	0.005	15	0.209									
17	CR11605	6-2	2	0.5	2	7330	14660	28622	0.1	201870	29760	0.0285	0.005	15	0.190									
18	CR11605	6-3	2	0.5	1	8864	17728	35526	0.1	201870	35294	0.0350	0.005	15	0.233									
19	CR11605	7-1	2	0.5	2	8386	16776	33620	0.1	201870	33758	0.0335	0.005	15	0.223									
20	CR11605	7-2	2	0.5	1	8622	17244	34420	0.1	201870	34239	0.0340	0.005	15	0.227									
21	CR11605	7-3	2	0.5	2	8511	17022	34052	0.1	201870	33726	0.0335	0.005	15	0.225									
22	CR11605	8-1	2	0.5	1	8282	16564	32722	0.1	201870	32772	0.0330	0.005	15	0.220									
23	CR11605	8-2	2	0.5	2	8491	16982	33904	0.1	201870	34338	0.0341	0.005	15	0.227									
24	CR11605	8-3	2	0.5	1	8609	17218	34520	0.1	201870	35078	0.0348	0.005	15	0.232									

Aromatase Assay Spreadsheet

	R	S
1		
2		
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5		
6		DC Check - aliquot variance
7		% RE
8		0.99
9		1.01
10		1.00
11		1.02
12		0.98
13		0.99
14		1.01
15		1.00
16		0.98
17		1.01
18		1.00
19		1.00
20		1.00
21		0.98
22		1.01
23		0.99
24		1.01
25		0.99
26		1.01
27		1.00
28		1.00
29		1.01
30		0.99
31		1.00
32		1.00
33		1.00
34		1.00
35		1.00
36		1.00
37		1.01
38		0.98
39		1.00
40		1.00
41		0.99
42		1.01
43		1.00
44		1.00
45		1.01
46		0.98
47		0.98
48		1.01
49		0.98
50		1.02
51		0.98
52		1.02
53		0.98
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/13/2007	Test Chemical ID	CR11605		# Concentrations					
2	NE Ref.	12535-67	Counter data filename	13MR2226.P00		2					
3	Technician ID	SLB	Replicate #			2					
4	Control Type	Portion	SD								
5	Full activity	Overall	0.195	0.022							
6	Background (unconnected)	Overall	0.002	0.000							
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctrl)	Activity						
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.020					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.018					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.034					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.034					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.093					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.100					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.128					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.138					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.183					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.185					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.190					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.171					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.195					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.207					
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical) M	Log(test chemical)	Activity					
38	CR11605	1	1	1.00E-05	-5.00	0.216					
39	CR11605	1	2	1.00E-05	-5.00	0.210					
40	CR11605	1	3	1.00E-05	-5.00	0.214					
41	CR11605	2	1	1.00E-06	-6.00	0.208					
42	CR11605	2	2	1.00E-06	-6.00	0.211					
43	CR11605	2	3	1.00E-06	-6.00	0.219					
44	CR11605	3	1	1.00E-07	-7.00	0.224					
45	CR11605	3	2	1.00E-07	-7.00	0.228					
46	CR11605	3	3	1.00E-07	-7.00	0.231					
47	CR11605	4	1	5.00E-08	-7.30	0.183					
48	CR11605	4	2	5.00E-08	-7.30	0.221					
49	CR11605	4	3	5.00E-08	-7.30	0.216					
50	CR11605	5	1	1.00E-08	-8.00	0.227					
51	CR11605	5	2	1.00E-08	-8.00	0.223					
52	CR11605	5	3	1.00E-08	-8.00	0.209					
53	CR11605	6	1	5.00E-09	-8.30	0.190					
54	CR11605	6	2	5.00E-09	-8.30	0.233					
55	CR11605	6	3	5.00E-09	-8.30	0.223					
56	CR11605	7	1	1.00E-09	-9.00	0.227					
57	CR11605	7	2	1.00E-09	-9.00	0.236					
58	CR11605	7	3	1.00E-09	-9.00	0.225					
59	CR11605	8	1	1.00E-10	-10.00	0.220					
60	CR11605	8	2	1.00E-10	-10.00	0.227					
61	CR11605	8	3	1.00E-10	-10.00	0.232					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/14/2007	Test Chemical ID	CR11605	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-70	Counter data filename	14MR2314.P00				Pos Ctl	
3	Technician ID	SLB	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/14/2007	Test Chemical ID	CR11605	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-70	Counter data filename	14MR2314.P00				Pos Ctl	
3	Technician ID	SLB	Replicate #	3	Positive Control		4-OH ASDN	Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0212		38607		1821085			
8	2	0.0208		37543		1804952			
9	3	0.0208		38610		1856250			
10	4	0.0207		39775		1921498			
11	5	0.0204		40359		1978382			
12						Average DPM/g soln		1876433	
13						SD		72465	
14						CV		3.86	
15						µCi/g soln		0.8452	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume: (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1157 g			
25	Mass of dilution B used in substrate prep					4.5706 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56374319 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.010	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8452			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36						0.5637	+	0.010	
37						0.574	µg ASDN/g soln.		
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40						1.472	µCi/µg ASDN		
41						9.362 E+05	dpm/nmol		

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Assay Date	3/14/2007	Test	Chemical ID	CR11605	# Concentrations	tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-70	Counter data	filename	14MR2314.P00						
3	Technician	SLB	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc	2			
4											
5	Vial	Time (min)	DPM1			ISIE					Round DPM
6	2	5	38607.44	16905.74	0	11.9	481.25				38607
7	3	5	37542.53	16309.5	0	11.82	474.44				37543
8	4	5	38610.31	16790.86	0	11.81	475.63				38610
9	5	5	39774.8	17076.24	0	11.85	470.85				39775
10	6	5	40359.39	17337.88	0	11.81	470.83				40359
11	8	5	8416.12	3872.18	0	12.75	528.19				8416
12	9	5	8401.26	3900.91	0	12.72	531.81				8401
13	10	5	7414.26	3450.86	0	12.65	532.82				7414
14	11	5	7532.99	3508.36	0	12.72	532.59				7533
15	12	5	7832.89	3632.72	0	12.66	527.65				7833
16	13	5	7847.89	3639.67	0	12.6	529.54				7848
17	14	5	7719.21	3564.15	0	12.65	529.57				7719
18	15	5	7768.52	3595.8	0	12.65	531.25				7769
19	16	5	59.48	27.3	0	14.41	528.23				59
20	17	5	56.2	26.97	0	12.37	529.14				58
21	18	5	75.21	37.95	0	13.49	528.7				75
22	19	5	58.31	25.44	0	12.45	529.18				58
23	20	5	53.19	30.13	0	13.62	529.5				53
24	21	5	63.6	31.77	0	14.47	531.82				64
25	22	5	69.48	32.54	0	14.41	528.03				69
26	23	5	47.56	24.28	0	11.83	531.04				48
27	25	5	123.22	59.03	0	13.32	528.97				123
28	26	5	135.52	57.61	0	13.48	528.91				136
29	27	5	126.43	58.21	0	12.88	530.22				126
30	28	5	131.27	63.03	0	13.4	531.32				131
31											0
32											0
33	29	5	685.49	316.07	0	12.74	525.4				685
34	30	5	854.61	306.54	0	12.71	530.81				855
35	31	5	641.1	297.97	0	12.59	527.64				641
36	32	5	632.35	299.6	0	12.83	527.66				632
37											0
38											0
39	33	5	1139.42	526.89	0	12.88	530.44				1139
40	34	5	1155.78	526.97	0	12.77	528.96				1156
41	35	5	1119.71	523.27	0	12.87	531.57				1120
42	36	5	1027.32	481.89	0	12.5	531.06				1027
43											0
44											0
45	37	5	3711.55	1734.76	0	12.77	538.07				3712
46	38	5	3642.38	1702.72	0	12.68	530.16				3642
47	39	5	3529.18	1625.1	0	12.84	527.1				3529
48	40	5	3438.45	1599.02	0	12.68	532.08				3438
49											0
50											0
51	41	5	5096	2368.07	0	12.65	529.52				5096
52	42	5	4989.84	2356.02	0	12.56	535.2				4990
53	43	5	4904.14	2278.79	0	12.61	529.43				4904
54	44	5	4887.35	2277.67	0	12.53	531.33				4887
55											0
56											0
57	45	5	7246.57	3355.08	0	12.76	530.91				7247
58	46	5	7295.08	3406.03	0	12.69	533.4				7295
59	47	5	7269.52	3384.44	0	12.68	531.67				7270
60	48	5	7209.37	3357.11	0	12.7	531.84				7209
61											0
62											0
63	49	5	8259.87	3863.55	0	12.69	531.42				8260
64	50	5	8190.58	3821.95	0	12.58	532.48				8191
65	51	5	7565.36	3514.28	0	12.7	531.79				7565
66	52	5	7724.24	3503.35	0	12.62	530.63				7724
67											0
68											0
69	53	5	7455.22	3440.43	0	12.67	528.44				7455
70	54	5	7404.03	3437.51	0	12.69	530.55				7404
71	55	5	7985.16	3720.73	0	12.69	531.73				7985
72	56	5	7980.17	3721.86	0	12.62	531.61				7980
73											0
74											0

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	6587.19	3076.44		0	12.67	532.96			6587
76	59	5	6750.11	3134.16		0	12.76	532.41			6750
77	60	5	8178.05	3804.18		0	12.63	531.13			8178
78	61	5	8250.66	3828.42		0	12.61	530.46			8251
79	62	5	7285.65	3403.14		0	12.7	534.36			7286
80	63	5	7341.91	3386.97		0	12.75	529.95			7342
81	64	5	8775.27	4059.67		0	12.72	530.44			8775
82	65	5	8567.29	3973.05		0	12.7	529.67			8567
83	66	5	8113.92	3816.02		0	12.61	536.67			8114
84	67	5	8331.01	3871.22		0	12.64	530.8			8331
85	68	5	7924.72	3716.01		0	12.57	532.33			7925
86	69	5	7897.21	3709.14		0	12.6	533.84			7897
87	70	5	8040.48	3721.64		0	12.76	530.45			8040
88	71	5	7731.96	3599.63		0	12.72	531.72			7732
89	72	5	8064.88	3749.45		0	12.65	532.01			8065
90	73	5	7949.08	3705.54		0	12.58	530.87			7949
91	74	5	8549.5	3959.84		0	12.63	531.06			8550
92	75	5	8637.25	3996.06		0	12.65	531.54			8637
93	76	5	6121.19	2857.99		0	12.58	534.05			6121
94	77	5	6231.68	2921.76		0	12.68	534.3			6232
95	78	5	7988.26	3700.1		0	12.72	533.7			7988
96	79	5	7763.18	3614.03		0	12.72	531.95			7763
97	80	5	8193.45	3845.68		0	12.68	537.92			8193
98	81	5	8275.75	3872.2		0	12.61	530.15			8276
99	83	5	7549.47	3513.4		0	12.62	531.34			7549
100	84	5	7497.85	3499.05		0	12.59	532.23			7498
101	85	5	7190.41	3338.27		0	12.73	532.63			7190
102	86	5	7284.64	3402.78		0	12.72	534.31			7285
103	87	5	8642.99	4036.33		0	12.69	531.65			8643
104	88	5	8763.73	4069.91		0	12.68	533.17			8764
105	89	5	8113.66	3788.28		0	12.84	533.52			8114
106	90	5	8239.61	3816.56		0	12.78	532.95			8240
107	91	5	8059	3768.78		0	12.68	533.08			8059
108	92	5	8074.3	3774.51		0	12.67	532.96			8074
109	93	5	8164.67	3813.97		0	12.68	534.46			8165
110	94	5	8109.64	3783.6		0	12.61	532.37			8110
111	95	5	8771.19	4104.53		0	12.95	542.65			8771
112	96	5	8619.52	4050.79		0	12.82	537.48			8620
113	97	5	7220.91	3361.74		0	12.79	537.33			7221
114	98	5	7169.67	3349.03		0	12.72	537.63			7170
115	99	5	8026.62	3773.91		0	12.65	535.96			8027
116	100	5	8445.63	3933.84		0	12.76	534.15			8446
117	101	5	8218.02	3805.93		0	12.69	532.23			8218
118	102	5	8174.16	3822.86		0	12.7	533.73			8174
119	103	5	7950.5	3646.34		0	12.88	533.89			7951
120	104	5	7432.58	3456.89		0	12.68	532.64			7433
121	105	5	8386.1	3917.85		0	12.61	534.39			8386
122	106	5	8283.11	3839.99		0	12.62	530.87			8283
123	107	5	229511.92	36169.06		0	7.74	171.2			229512
124	108	5	0	3.59		0	8.27	474.69			0
125											0

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Assay Date	3/14/2007	Chemical ID	CR11695	# Concentrations tested	17335-13										
2	Assay Technician	12535-70	Count data	14MR2314_P00												
3	Replicate	SR	3	Positive Control	4-OMASDN Pre C11 Tube/Gene	2										
4																
5	Sample ID															
6	Sample type	Replicate	Volume (mL)	Aliq. #	DPM/aliq	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM corrected for background (background tubes)	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Inclusion time (min)	Aromatase activity (nmol serotonin forming protein/min)			
7	Full activity control	1	2	0.5	18332	16817	33634	0.1	187643	0.037	0.005	15	0.238			
8		2	0.5	2	8401	18002	36004	0.1	187643	0.037	0.006	15	0.211			
9		3	0.5	2	7414	14828	29656	0.1	187643	0.037	0.005	15	0.222			
10		4	0.5	2	7533	15066	30132	0.1	187643	0.037	0.005	15	0.219			
11		5	0.5	2	7848	15696	31392	0.1	187643	0.037	0.005	15	0.200			
12		6	0.5	2	7719	15438	30876	0.1	187643	0.037	0.005	15	0.200			
13		7	0.5	2	7789	15538	31076	0.1	187643	0.037	0.005	15	0.200			
14	Background control	1	2	0.5	96	112	230	0.1	187643	0.037	0.005	15	0.200			
15		2	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
16		3	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
17		4	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
18		5	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
19		6	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
20		7	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
21		8	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
22		9	0.5	2	96	112	230	0.1	187643	0.037	0.005	15	0.200			
23	4-OMASDN	1	2	0.5	123	246	492	0.1	187643	0.037	0.005	15	0.002			
24		2	0.5	2	136	272	544	0.1	187643	0.037	0.005	15	0.002			
25		3	0.5	2	120	240	480	0.1	187643	0.037	0.005	15	0.002			
26		4	0.5	2	131	262	524	0.1	187643	0.037	0.005	15	0.002			
27		5	0.5	2	0	0	0	0.1	187643	0.037	0.005	15	0.002			
28		6	0.5	2	0	0	0	0.1	187643	0.037	0.005	15	0.002			
29		7	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
30		8	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
31		9	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
32		10	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
33		11	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
34		12	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
35		13	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
36		14	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
37		15	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
38		16	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
39		17	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
40		18	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
41		19	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
42		20	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
43		21	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
44		22	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
45		23	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
46		24	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
47		25	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
48		26	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
49		27	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
50		28	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
51		29	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
52		30	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
53		31	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
54		32	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
55		33	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
56		34	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
57		35	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
58		36	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
59		37	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
60		38	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
61		39	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
62		40	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
63		41	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
64		42	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
65		43	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
66		44	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
67		45	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
68		46	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
69		47	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			
70		48	0.5	2	885	1770	3540	0.1	187643	0.037	0.005	15	0.002			

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		100 check - sequential variance			
6		Replicates not within mean +/- 10%			
7		1.00			
8		1.00			
9		0.99			
10		1.01			
11		1.00			
12		1.00			
13		1.00			
14		1.00			
15		1.00			
16		0.97			
17					
18					
19		0.97			
20		0.97			
21		1.00			
22		0.95			
23		1.05			
24		0.98			
25		1.02			
26					
27		#DIV/0!			
28					
29		1.02			
30		0.98			
31		1.00			
32		0.99			
33		#DIV/0!			
34		#DIV/0!			
35		0.99			
36		1.01			
37		1.04			
38		0.95			
39		#DIV/0!			
40		#DIV/0!			
41		1.01			
42		0.98			
43		0.97			
44		0.99			
45		#DIV/0!			
46		#DIV/0!			
47		1.01			
48		0.99			
49		1.00			
50					
51		#DIV/0!			
52		#DIV/0!			
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57		#DIV/0!			
58		#DIV/0!			
59		1.00			
60		1.00			
61		0.99			
62		1.01			
63		#DIV/0!			
64		#DIV/0!			
65		1.00			
66		1.00			
67		1.00			
68		1.00			
69		1.00			
70		#DIV/0!			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		Assay Date	3/14/2007	Chemical ID	CR11605	# Concentrations tested	5	Microsome ID	12535-13							
2		NS Ref.	12535-20	Cellular Source	44MR2314 P00											
3		Technician	D.S.B.	Replicate #	3	Positive Control	4-OH ASDN Pos. Ctrl Tubes/Conc									
4																
5	Sample ID	Calculate DPM in aqueous portion after extraction														
6	Sample type	Replicate/Level	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (cpm)	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Inhibition time (min)	Aromatase activity (nmol estrone formed per mg protein per min)		
7	CR11605	1-1	2	0.5	1	6957	13714	28574	0.1	187643	28433	0.005	15	0.188		
8		1-2	2	0.5	2	8178	16356	32838	0.1	187643	32617	0.005	15	0.232		
9		1-3	2	0.5	1	7286	14572	2915	0.1	187643	29015	0.005	15	0.207		
10		2-1	2	0.5	1	8775	17550	34554	0.1	187643	34443	0.005	15	0.245		
11		2-2	2	0.5	2	8567	17134	32890	0.1	187643	32649	0.005	15	0.232		
12		2-3	2	0.5	1	8334	16668	31644	0.1	187643	31403	0.005	15	0.224		
13		3-1	2	0.5	1	7925	15850	31644	0.1	187643	31403	0.005	15	0.224		
14		3-2	2	0.5	2	8040	16080	31544	0.1	187643	31303	0.005	15	0.223		
15		3-3	2	0.5	1	8045	16090	32028	0.1	187643	31767	0.005	15	0.226		
16		4-1	2	0.5	1	8537	17074	34374	0.1	187643	34133	0.005	15	0.243		
17		4-2	2	0.5	2	8532	17064	34706	0.1	187643	34485	0.005	15	0.247		
18		4-3	2	0.5	1	7888	15776	31502	0.1	187643	31281	0.005	15	0.233		
19		5-1	2	0.5	1	8163	16326	32838	0.1	187643	32697	0.005	15	0.233		
20		5-2	2	0.5	2	7549	15098	30084	0.1	187643	28853	0.005	15	0.213		
21		5-3	2	0.5	1	7190	14380	28950	0.1	187643	28709	0.005	15	0.204		
22		6-1	2	0.5	1	8643	17286	34814	0.1	187643	34573	0.005	15	0.246		
23		6-2	2	0.5	2	8240	16480	32708	0.1	187643	32467	0.005	15	0.231		
24		6-3	2	0.5	1	8059	16118	32268	0.1	187643	32023	0.005	15	0.228		
25		7-1	2	0.5	1	8110	16220	32350	0.1	187643	32300	0.005	15	0.230		
26		7-2	2	0.5	2	8250	16500	34782	0.1	187643	34641	0.005	15	0.246		
27		7-3	2	0.5	1	7721	15442	28762	0.1	187643	28541	0.005	15	0.203		
28		8-1	2	0.5	1	8027	16054	32146	0.1	187643	32705	0.005	15	0.233		
29		8-2	2	0.5	2	8218	16436	32784	0.1	187643	32543	0.005	15	0.232		
30		8-3	2	0.5	1	7951	15902	30768	0.1	187643	30527	0.005	15	0.217		
31		9-1	2	0.5	1	7433	14866	29338	0.1	187643	28997	0.005	15	0.205		
32		9-2	2	0.5	2	8283	16566	33338	0.1	187643	33097	0.005	15	0.205		

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		
6	QC check - aliquot variance	
7	% RE	
8		0.99
9		1.01
10		1.00
11		1.00
12		1.00
13		1.01
14		0.99
15		1.01
16		1.01
17		1.00
18		1.00
19		1.02
20		0.99
21		1.01
22		0.99
23		0.99
24		1.01
25		0.99
26		1.01
27		1.01
28		0.99
29		0.99
30		1.01
31		1.00
32		1.00
33		0.99
34		1.01
35		0.99
36		1.01
37		0.99
38		1.01
39		1.00
40		1.00
41		1.00
42		1.00
43		1.01
44		0.99
45		1.00
46		1.00
47		0.97
48		1.03
49		1.00
50		1.00
51		1.03
52		0.97
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/14/2007	Test Chemical ID	CR11605	# Concentrations					
2		NR Ref	12535-70	Counter data filename	14MP2314.P00						
3		Technician ID	SLB	Replicate #		3	Positive Control	4-OH ASDN			2
4											
5	Control Type	Portion	SD								
6	Full activity	Overall	0.011								
7	Background (uncorrected)	Overall	0.000								
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctrl)	Activity						
11	4-OH ASDN	1	1	1.00E-06	-5.00						
12	4-OH ASDN	1	2	1.00E-06	-5.00						
13	4-OH ASDN	2	1	1.00E-06	-6.00						
14	4-OH ASDN	2	2	1.00E-06	-6.00						
15	4-OH ASDN	3	1	5.00E-07	-6.30						
16	4-OH ASDN	3	2	5.00E-07	-8.30						
17	4-OH ASDN	4	1	1.00E-07	-7.00						
18	4-OH ASDN	4	2	1.00E-07	-7.00						
19	4-OH ASDN	5	1	5.00E-08	-7.30						
20	4-OH ASDN	5	2	5.00E-08	-7.30						
21	4-OH ASDN	6	1	1.00E-08	-8.00						
22	4-OH ASDN	6	2	1.00E-08	-9.00						
23	4-OH ASDN	7	1	1.00E-09	-9.00						
24	4-OH ASDN	7	2	1.00E-09	-9.00						
25	4-OH ASDN	8	1	1.00E-10	-10.00						
26	4-OH ASDN	8	2	1.00E-10	-10.00						
27	4-OH ASDN										
28	4-OH ASDN										
29	4-OH ASDN										
30	4-OH ASDN										
31	4-OH ASDN										
32	4-OH ASDN										
33	4-OH ASDN										
34	4-OH ASDN										
35	4-OH ASDN										
36	4-OH ASDN										
37	Test Chemical	Level	Replicate	Log(test chemical) M	Log(test chemical)	Activity					
38	CR11605	1	1	1.00E-06	-5.00	0.188					
39	CR11605	1	2	1.00E-06	-5.00	0.232					
40	CR11605	2	1	1.00E-06	-5.00	0.207					
41	CR11605	2	2	1.00E-06	-6.00	0.245					
42	CR11605	3	1	1.00E-06	-6.00	0.232					
43	CR11605	3	2	1.00E-06	-6.00	0.224					
44	CR11605	4	1	1.00E-07	-7.00	0.223					
45	CR11605	4	2	1.00E-07	-7.00	0.226					
46	CR11605	5	1	1.00E-07	-7.00	0.243					
47	CR11605	5	2	1.00E-07	-7.00	0.174					
48	CR11605	6	1	5.00E-08	-7.30	0.223					
49	CR11605	6	2	5.00E-08	-7.30	0.233					
50	CR11605	7	1	1.00E-08	-8.00	0.213					
51	CR11605	7	2	1.00E-08	-8.00	0.204					
52	CR11605	8	1	1.00E-08	-8.00	0.246					
53	CR11605	8	2	1.00E-08	-8.00	0.231					
54	CR11605					0.228					
55	CR11605					0.230					
56	CR11605					0.246					
57	CR11605					0.203					
58	CR11605					0.233					
59	CR11605					0.232					
60	CR11605					0.217					
61	CR11605					0.236					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/9/2007	Test Chemical ID	CR11606	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-65	Counter data filename	10MR0224.P00					
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/9/2007	Test Chemical ID	CR11606	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-65	Counter data filename	10MR0224.P00					
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0194		35505		1830155			
8	2	0.0199		33108		1663719			
9	3	0.0191		39253		2055131			
10	4	0.0196		40236		2052857			
11	5	0.0194		40599		2092732			
12						Average DPM/g soln		1938919	
13						SD		185547	
14						CV		9.57	
15						µCi/g soln		0.8734	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					7.9385 g			
25	Mass of dilution B used in substrate prep					4.4851 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56611075 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8734			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln:								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5661	+	0.011		
37				=	0.577 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=	1.514	µCi/µg ASDN						
41		9.628.E+05	dpm/nmol						

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	
1			Assay Date	3/9/2007	Test Chemical ID	CR11606	# Concentrations tested			8	Microsome ID	12535-13
2			NB Ref	12535-65	Counter data filename	10MR0224.P00						
3			Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc		2
4												
5			Microsome Dilution Details									
6												
7			Dilution A	0.12 mL microsome Stock used								
8				2.4 mL total volume								
9				20 dilution factor								
10												
11			Dilution B	1.5 mL microsome Dilution A used								
12				60 mL total volume								
13				40 dilution factor								
14												
15			Dilution C (if applicable)	mL microsome Dilution B used								
16				mL total volume								
17				dilution factor								
18												
19				800 total dilution factor								
20												
21			Protein Concentration (stock microsomes, mg/mL):		8.00							
22			Protein Concentration (dilution added to assay, mg/mL):		0.01							
23												
24												
25												
26												

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Test # Chemical Concentrations As: 3/9/2007 ID C tested 8 Microsome ID 12535-13									
2		Counter data NB 12535-65 filename 10MR0224.P00									
3		Te ch nic ian ID PRP Replicate # 1 Positive Control 4-OH ASD Tubes/Conc 2									
4											
5	Vial	Tin	DPM1		ISIE						Round DPM
6	2	5	35504.7	13932.3	0	12.79	484.65				35505
7	3	5	33108.19	12907.46	0	12.76	481.22				33108
8	4	5	39253.35	15421.22	0	12.84	486.24				39253
9	5	5	40236.46	15762.26	0	12.73	482.59				40236
10	6	5	40599.39	15992.7	0	12.75	485.06				40599
11	8	5	9717.5	4123.28	0	13.76	547.52				9718
12	9	5	9643.32	4136.06	0	13.78	551.65				9643
13	10	5	11640.72	4985.1	0	13.68	549.3				11641
14	11	5	11637.88	5001.28	0	13.67	552.8				11638
15	12	5	9920.66	4248.3	0	13.74	550.13				9921
16	13	5	9785.08	4212.7	0	13.69	550.92				9785
17	14	5	9406.73	4020.1	0	13.72	547.32				9407
18	15	5	9253.54	3965.86	0	13.69	550.89				9254
19	16	5	48.39	22.9	0	10.75	552.03				48
20	17	5	50.67	23.1	0	12.52	551.46				51
21	18	5	57.41	23.26	0	12.62	549.95				57
22	19	5	53.56	24.1	0	12.05	550.34				54
23	20	5	42.51	19.7	0	9.83	547.92				43
24	21	5	46.97	19.98	0	14.82	551.44				47
25	22	5	63.52	27.9	0	14.71	546.08				64
26	23	5	42.57	20.18	0	10.5	550.35				43
27	25	5	165.83	68.74	0	14.23	550.38				166
28	26	5	145	64.86	0	12.96	552.22				145
29	27	5	165.75	69.06	0	13.53	547.68				166
30	28	5	154.66	66.3	0	14.09	547.43				155
31	29	5	917.9	389.74	0	13.75	551.15				918
32	30	5	948.99	404.7	0	13.92	549.38				949
33	31	5	814.83	350.5	0	13.8	549.75				815
34	32	5	778.3	334.5	0	13.85	550.71				778
35	33	5	1654.46	703.5	0	13.52	547.89				1654
36	34	5	1665.23	700.3	0	13.91	547.22				1665
37	35	5	1539.4	655.26	0	13.8	549.31				1539
38	36	5	1359.93	584.3	0	13.73	551.59				1360
39	37	5	4639.8	1989.26	0	13.63	549.31				4640
40	38	5	4688.07	2035.9	0	13.66	548.95				4688
41	39	5	4799.6	2069.86	0	13.68	551.28				4800
42	40	5	4688.91	2079.38	0	13.62	546.95				4689
43	41	5	5749.2	2464.9	0	13.7	547.47				5749
44	42	5	5653.65	2427.9	0	13.77	549.4				5654
45	43	5	6697.64	2875.9	0	13.67	548.88				6698
46	44	5	6617.31	2843.86	0	13.71	548.01				6617
47	45	5	9934.03	4230.34	0	13.72	547.38				9934
48	46	5	9968.03	4199.86	0	13.79	544.61				9968
49	47	5	9704.04	4149.86	0	13.65	547.77				9704
50	48	5	9638.41	4149.02	0	13.71	549.08				9638
51	49	5	10522.46	4509.26	0	13.72	550.06				10522
52	50	5	10497.68	4498.3	0	13.68	548.89				10498
53	51	5	9802.01	4228.1	0	13.65	550.36				9802
54	52	5	9732.4	4183.46	0	13.69	549.41				9732
55	53	5	9922.3	4223.26	0	13.76	549.51				9922
56	54	5	9636.67	4127.9	0	13.68	547.25				9637
57	55	5	10362.76	4438.5	0	13.77	550.36				10363
58	56	5	10366.06	4458.5	0	13.68	549.03				10366
59	58	5	9261.94	3975.46	0	13.62	547.47				9262
60	59	5	9080.7	3880.98	0	13.79	548.18				9081
61	60	5	9452.55	4053.14	0	13.72	551.24				9453
62	61	5	9292.5	3980.94	0	13.71	549.58				9293
63	62	5	7700.37	3289.5	0	13.69	546.88				7700
64	63	5	7679.3	3272.26	0	13.6	545.81				7679
65	64	5	9880.4	4215.9	0	13.69	545.55				9880
66	65	5	9824	4207.86	0	13.72	549.98				9824
67	66	5	9415.29	4024.1	0	13.68	547.73				9415
68	67	5	9382.82	3997.34	0	13.71	546.95				9383
69	68	5	9275.83	3967.86	0	13.65	547.9				9276
70	69	5	9398.88	3981.26	0	13.76	546.33				9399

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	9830.63	4210.74	0	13.66	548.48				9831
72	71	5	9708.5	4120.06	0	13.68	545.83				9709
73	72	5	9113.88	3898.74	0	13.68	548.33				9114
74	73	5	9021.43	3827.3	0	13.68	547.16				9021
75	74	5	9765.25	4158.54	0	13.71	545.29				9765
76	75	5	9594.4	4113.98	0	13.76	550.79				9594
77	76	5	7646.85	3347.14	0	13.58	545.48				7647
78	77	5	7927.83	3380.5	0	13.65	546.31				7928
79	78	5	9980.91	4248.86	0	13.66	545.11				9981
80	79	5	9819.73	4203.14	0	13.68	547.23				9820
81	80	5	7643.32	3280.1	0	13.65	548.03				7643
82	81	5	7657.13	3281.14	0	13.64	548.44				7657
83	82	5	9639.58	4116.3	0	13.61	545				9640
84	83	5	9794.24	4154.9	0	13.59	543.42				9794
85	84	5	8728.92	3720.5	0	13.65	547.72				8729
86	85	5	8847.58	3768.3	0	13.72	546.85				8848
87	86	5	9863.01	4194.7	0	13.71	546.4				9863
88	87	5	9655.17	4143.34	0	13.68	548.74				9655
89	88	5	9175.1	3933.1	0	13.71	548.33				9175
90	89	5	8875.06	3788.34	0	13.7	548.15				8875

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
91	90	5	9944.14	4230.5	0	13.68	545.66				9944
92	91	5	9189.74	3986.5	0	13.72	551.87				9190
93	92	5	9539.12	4086.66	0	13.7	545.09				9539
94	93	5	9671.55	4157.86	0	13.63	545.08				9672
95	94	5	10223.86	4376.7	0	13.67	547.25				10224
96	95	5	10149.6	4320.5	0	13.7	546.41				10150
97	96	5	9545.88	4083.3	0	13.61	546.36				9546
98	97	5	9587.53	4100.08	0	13.7	546.54				9588
99	98	5	9937.84	4233.66	0	13.59	543.91				9938
100	99	5	9908.67	4236.3	0	13.58	544.07				9909
101	100	5	9138.74	3886.46	0	13.65	540.63				9139
102	101	5	9047.49	3851.3	0	13.66	544.83				9047
103	102	5	7361.66	3117.38	0	13.65	544.64				7362
104	103	5	7228.59	3083.46	0	13.59	543.44				7229
105	104	5	8717.13	3717.5	0	13.61	542.67				8717
106	105	5	8659.07	3674.66	0	13.67	546.4				8659
107	107	5	235409.16	108152.58	0	14.92	619.33				235409
108											0
109											0
110											0
111											0
112											0
113											0
114											0
115											0
116											0
117											0
118											0
119											0
120											0
121											0
122											0
123											0
124											0
125											0
126											0
127											0
128											0
129											0
130											0
131											0
132											0
133											0
134											0

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		QC check - aliquot variance			
5		Reel if value not within mean +/- 10%			
6					
7		1.00			
8		1.00			
9		1.00			
10		1.00			
11		1.01			
12		0.99			
13		1.01			
14		0.99			
15		0.97			
16		1.03			
17		1.03			
18		0.97			
19		0.96			
20		1.04			
21					
22					
23		1.07			
24		0.93			
25		1.03			
26		0.97			
27		#DIV/0!			
28		#DIV/0!			
29		0.99			
30		1.02			
31		1.02			
32		0.95			
33		#DIV/0!			
34		#DIV/0!			
35		1.00			
36		1.00			
37		1.06			
38		0.94			
39		#DIV/0!			
40		#DIV/0!			
41		0.98			
42		1.01			
43		0.93			
44		1.01			
45		#DIV/0!			
46		#DIV/0!			
47		1.01			
48		0.96			
49		1.01			
50		0.99			
51		#DIV/0!			
52		#DIV/0!			
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57		#DIV/0!			
58		#DIV/0!			
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63		#DIV/0!			
64		#DIV/0!			
65		1.01			
66		0.90			
67		1.00			
68		1.00			
69		1.00			
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		Assay Date: 3/20/2007	Chemical ID: CR11606	Test Concentration: 12535461	8	Microcomp ID: 12535461										
2		NB Ref: 12535461														
3		Technician: JDRP														
4																
5	Sample ID															
6	Sample type	Replicate	Nominal total volume (mL)	Aliq. #	DPH/mL	Ave DPH/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate nmoL ³ H ₂ O formed	nmol ³ H ₂ O formed	Final [aromatase] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)		
7	CR11606	1-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
8		1-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
9		1-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
10		2-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
11		2-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
12		2-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
13		3-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
14		3-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
15		3-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
16		4-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
17		4-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
18		4-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
19		5-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
20		5-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
21		5-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
22		6-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
23		6-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
24		6-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
25		7-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
26		7-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
27		7-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
28		8-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
29		8-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
30		8-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
31		9-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
32		9-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
33		9-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
34		10-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
35		10-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
36		10-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
37		11-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
38		11-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
39		11-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
40		12-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
41		12-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
42		12-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
43		13-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
44		13-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
45		13-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
46		14-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
47		14-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
48		14-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
49		15-1	2	0.5	9262	18524	36686	0.1	193892	36686	0.0379	0.005	15	0.253		
50		15-2	2	0.5	9081	18162	37492	0.1	193892	37492	0.0387	0.005	15	0.258		
51		15-3	2	0.5	9453	18806	38758	0.1	193892	38758	0.0417	0.005	15	0.212		
52		16-1	2	0.5	7679	15598	39428	0.1	193892	39428	0.0407	0.005	15	0.272		
53		16-2	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		
54		16-3	2	0.5	8224	16438	37566	0.1	193892	37566	0.0388	0.005	15	0.258		

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		1.01
8		0.99
9		1.01
10		0.99
11		1.00
12		1.00
13		1.00
14		1.00
15		1.00
16		0.99
17		1.01
18		1.01
19		0.99
20		1.01
21		0.99
22		1.01
23		0.99
24		1.01
25		0.99
26		1.01
27		1.01
28		0.99
29		1.00
30		1.00
31		0.99
32		1.01
33		0.99
34		1.01
35		1.01
36		0.99
37		1.02
38		0.99
39		1.04
40		0.96
41		0.99
42		1.01
43		1.00
44		1.00
45		1.00
46		1.00
47		1.00
48		1.00
49		1.01
50		0.99
51		1.01
52		0.99
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/9/2007	Test Chemical ID	CR11606	Counter date Reconc	10MR0224-P00	1	Positive Control	4-OH ASDN	1	Microsome ID	12535-13
2	Assay Ref	12535-65	PRP	Replicate #								2
3	Technician ID											
4												
5	Control Type	Portion	Average	SD								
6	Full activity	Overall	0.279	0.029								
7	Background (uncorrected)	Overall	0.001	0.000								
8												
9												
10	Positive Control	Level	Replicate	Log(Positive Ctrl)	Activity							
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.003						
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.024						
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.021						
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.045						
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.039						
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.128						
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.132						
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.157						
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.183						
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.274						
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.266						
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.280						
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.269						
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.269						
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.266						
27	4-OH ASDN											
28	4-OH ASDN											
29	4-OH ASDN											
30	4-OH ASDN											
31	4-OH ASDN											
32	4-OH ASDN											
33	4-OH ASDN											
34	4-OH ASDN											
35	4-OH ASDN											
36	Test Chemical	Level	Replicate	Log(test chemical) M	Activity							
37	CR11606	1	1	1.00E-03	-3.00	0.253						
38	CR11606	1	2	1.00E-03	-3.00	0.258						
39	CR11606	2	1	1.00E-03	-3.00	0.212						
40	CR11606	2	2	1.00E-04	-4.00	0.272						
41	CR11606	2	3	1.00E-04	-4.00	0.259						
42	CR11606	3	1	1.00E-04	-4.00	0.257						
43	CR11606	3	2	1.00E-05	-5.00	0.299						
44	CR11606	3	3	1.00E-05	-5.00	0.260						
45	CR11606	4	1	1.00E-05	-5.00	0.267						
46	CR11606	4	2	1.00E-06	-6.00	0.217						
47	CR11606	4	3	1.00E-06	-6.00	0.273						
48	CR11606	5	1	1.00E-07	-7.00	0.210						
49	CR11606	5	2	1.00E-07	-7.00	0.268						
50	CR11606	5	3	1.00E-07	-7.00	0.242						
51	CR11606	6	1	1.00E-07	-7.00	0.269						
52	CR11606	6	2	1.00E-08	-8.00	0.249						
53	CR11606	6	3	1.00E-08	-8.00	0.264						
54	CR11606	7	1	1.00E-09	-9.00	0.281						
55	CR11606	7	2	1.00E-09	-9.00	0.264						
56	CR11606	7	3	1.00E-09	-9.00	0.273						
57	CR11606	8	1	1.00E-10	-10.00	0.250						
58	CR11606	8	2	1.00E-10	-10.00	0.201						
59	CR11606	8	3	1.00E-10	-10.00	0.239						
60	CR11606											
61	CR11606											

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/13/2007	Test Chemical ID	CR11606	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-68	Counter data filename	13MR2049.P00				Pos Ctl	
3	Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/13/2007	Test Chemical ID	CR11606	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-68	Counter data filename	13MR2049.P00				Pos Ctl	
3	Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0184		36669		1992880			
8	2	0.0193		36879		1910829			
9	3	0.0196		42015		2143622			
10	4	0.0192		39519		2058281			
11	5	0.0200		44032		2201600			
12						Average DPM/g soln			2061443
13						SD			115966
14						CV			5.63
15						µCi/g soln			0.9286
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor		[ASDN] in solution (µg/mL)			
19	Stock	10.01	10			1001.00			
20	Dilution A			100		10.01			
21	Dilution B			10		1.00			
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					7.9469 g			
25	Mass of dilution B used in substrate prep					4.5823 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.5771914 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.9286			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					=	0.5772 + 0.011			
37					=	0.589 µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=					1.578 µCi/µg ASDN			
41						1.003.E+06 dpm/nmol			

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
			Test #							
1			Chemical Concentrations							
	As: 3/13/2007	ID C	tested			8	Microsome ID	12535-13		
2			Counter data filename	13MR2048.P00						
	Te									
	ch									
	nic									
	lan									
3			Replicate	Positive		Pos Ctl				
	ID PRP	# 2	Control	4-OH ASD Tubes/Conc		2				
4										
5	Vial	Tin	DPM1			ISIE				Round DPM
6	2	5	36669.14	14314.94	0	12.88	484.89			36669
7	3	5	36879.01	14435.22	0	12.9	486.28			36879
8	4	5	42015.03	16423.18	0	12.87	484.67			42015
9	5	5	39519.18	15477.82	0	12.88	485.12			39519
10	6	5	44031.63	17190.14	0	12.93	485.97			44032
11	8	5	8886.76	3797.58	0	13.81	551.78			8887
12	9	5	8802.18	3789.58	0	13.78	551.63			8802
13	10	5	8078.79	2605.94	0	13.79	552.21			6077
14	11	5	6112.54	2814.78	0	13.87	549.13			6113
15	12	5	7595.81	3238.34	0	13.82	552.15			7596
16	13	5	7350.58	3128.54	0	13.83	552.76			7351
17	14	5	8569.06	3840.18	0	13.77	547.98			8569
18	15	5	8429.74	3593.7	0	13.79	549.3			8430
19	16	5	64.96	26.7	0	15.48	548.58			65
20	17	5	55.96	25.22	0	11.2	552.36			56
21	18	5	49.19	21.94	0	11.83	550.28			49
22	19	5	58.05	25.7	0	12.17	550.29			58
23	20	5	65.53	27.74	0	15.19	549.62			66
24	21	5	62.69	28.34	0	14.27	549.89			63
25	22	5	64.46	27.54	0	11.44	549.06			64
26	23	5	45.14	19.7	0	12.81	550.41			45
27	25	5	167.27	69.54	0	13.7	547.07			167
28	26	5	166.76	70.1	0	13.53	548.57			167
29	27	5	186.82	80.3	0	14.07	547.29			187
30	28	5	154.65	65.5	0	13.72	552.08			155
31	29	5	939.89	404.3	0	13.6	550.22			940
32	30	5	923.69	394.74	0	13.75	547.16			924
33	31	5	844.54	359.94	0	13.45	547.34			845
34	32	5	804.58	341.94	0	13.92	547.74			805
35	33	5	1630.38	691.14	0	13.89	549.19			1630
36	34	5	1562.77	673.54	0	13.88	549.04			1563
37	35	5	1493.7	628.7	0	13.71	547.3			1494
38	36	5	1496.15	631.14	0	14.13	550.36			1496
39	37	5	4548.15	1939.94	0	13.67	545.13			4548
40	38	5	4432.87	1894.34	0	13.83	549.21			4433
41	39	5	4713.01	2007.34	0	13.73	548.1			4713
42	40	5	4488.07	1909.9	0	13.76	548.78			4488
43	41	5	6141.08	2610.58	0	13.7	545.32			6141
44	42	5	6205.37	2649.94	0	13.88	547.5			6205
45	43	5	5948.32	2525.98	0	13.81	543.53			5948
46	44	5	5930.77	2501.58	0	13.83	544.31			5931
47	45	5	8104	3432.18	0	13.79	546.89			8104
48	46	5	8058.08	3417.94	0	13.7	544.65			8058
49	47	5	8077.69	3426.34	0	13.78	545.99			8078
50	48	5	7981.46	3386.1	0	13.83	543.29			7981
51	49	5	8247.97	3499.54	0	13.7	545.45			8248
52	50	5	8243.64	3498.18	0	13.77	544.33			8244
53	51	5	9198.74	3898.86	0	13.58	540.45			9197
54	52	5	9069.09	3869.98	0	13.65	544.64			9069
55	53	5	9301.89	3966.14	0	13.67	546.65			9302
56	54	5	9332.8	3970.18	0	13.67	543.7			9333
57	55	5	8157.28	3459.14	0	13.72	545.99			8157
58	56	5	8241.55	3477.98	0	13.82	544.01			8242
59	58	5	8387.8	3537.9	0	13.82	542.21			8388
60	59	5	8336.81	3544.14	0	13.81	546.54			8336
61	60	5	7807.43	3303.38	0	13.8	542.84			7807
62	61	5	7886.5	3328.34	0	13.79	543.98			7886
63	62	5	8456.94	3610.3	0	13.65	546.46			8457
64	63	5	8393.71	3588.1	0	13.74	546.63			8394
65	64	5	9292.79	3939.94	0	13.87	545.81			9293
66	65	5	9295.6	3941.94	0	13.86	547.11			9296
67	66	5	9512.58	4057.7	0	13.7	546.77			9513
68	67	5	9600.52	4081.98	0	13.82	547.27			9601
69	68	5	8054.2	3444.78	0	13.78	551.33			8054
70	69	5	8272.29	3494.7	0	13.98	547.11			8272

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	8045.59	3427.3	0	13.85	550.4				9046
72	71	5	8124.04	3474.94	0	13.79	550.76				9124
73	72	5	9029.46	3854.9	0	13.83	549.08				9029
74	73	5	8828.08	3772.9	0	13.94	550.75				8828
75	74	5	9234.66	3950.34	0	13.87	551.59				9235
76	75	5	9226.71	3941.34	0	13.84	548.7				9227
77	76	5	9673.58	4151.1	0	13.81	552.06				9674
78	77	5	9594.09	4115.1	0	13.79	552.56				9594
79	78	5	7841.6	3349.9	0	13.89	551.84				7842
80	79	5	7816.45	3317.54	0	13.84	550.48				7816
81	80	5	8910	3824.5	0	13.98	556.41				8910
82	81	5	8690.42	3685.5	0	13.86	550.24				8690
83	82	5	9312.62	3967.94	0	13.91	552.16				9313
84	83	5	9134.42	3901.74	0	13.81	550.66				9134
85	84	5	9212.81	3959.34	0	13.92	554.39				9213
86	85	5	9106.99	3904.5	0	13.87	551.74				9107
87	86	5	9897.35	4213.5	0	13.95	550.33				9897
88	87	5	9743.04	4172.02	0	13.87	552.66				9743
89	88	5	8955.46	3833.54	0	13.9	551.74				8955
90	89	5	8850.76	3784.02	0	13.89	552.37				8851
91	90	5	8754.35	3746.02	0	13.86	552.18				8754
92	91	5	8760.56	3767.9	0	13.86	555.1				8761
93	92	5	9104.15	3876.7	0	13.93	551				9104
94	93	5	9149.5	3898.7	0	13.9	552.02				9150
95	94	5	8939.6	3808.34	0	13.76	549.68				8940
96	95	5	8881.89	3802.78	0	13.98	554.31				8882
97	96	5	7710.28	3328.94	0	14	560.27				7710
98	97	5	8451.12	3598.94	0	13.86	551.12				8451
99	98	5	9639.35	4130.3	0	13.9	553.11				9639
100	99	5	9429.04	4030.5	0	13.79	551.31				9429
101	100	5	9498.81	4052.14	0	13.92	552.42				9499

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
102	101	5	9603	4140.9	0	13.88	556.17				9603
103	102	5	8568.71	3656.1	0	13.82	550.74				8569
104	103	5	8765.11	3807.84	0	14.05	567.3				8765
105	104	5	9042.36	3890.74	0	13.79	555.1				9042
106	105	5	9106.48	3886.38	0	13.83	550.42				9106
107	107	5	236391.61	108728.1	0	15.22	625.91				236392
108											0
109											0
110											0
111											0
112											0
113											0
114											0
115											0
116											0
117											0
118											0
119											0
120											0
121											0
122											0
123											0
124											0
125											0
126											0
127											0
128											0
129											0
130											0
131											0
132											0
133											0
134											0
135											0
136											0
137											0
138											0
139											0
140											0
141											0
142											0
143											0
144											0

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Assay Date	Chemical ID	Test ID	Chemical ID	# Concentrations tested	# Microsomes ID								
1		3/12/2007	CR11606				12535-13								
2															
3															
4															
5															
6	Sample type	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/assay	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay (mL)	total DPM in assay tubes (total)	nmol ³ H ₂ O formed	Final protein (mg/mL)	Inhibition (nmol ³ H ₂ O formed/mg protein)	Aromatase activity (nmol estrogen formed/mg protein/h)
7	Full activity control	1	2	0.5	1	26871	17774	35548	35548	0.1	208144	0.050	0.005	15	0.160
8		2	2	0.5	2	6077	12154	24310	24310	0.1	208144	0.0241	0.005	15	0.197
9		3	2	0.5	2	6113	12226	24452	24452	0.1	208144	0.0241	0.005	15	0.197
10		4	2	0.5	2	7598	15192	30384	30384	0.1	208144	0.0241	0.005	15	0.197
11		5	2	0.5	2	7951	15902	31804	31804	0.1	208144	0.0241	0.005	15	0.197
12		6	2	0.5	2	8599	17138	34276	34276	0.1	208144	0.0241	0.005	15	0.197
13		7	2	0.5	2	8430	16860	33720	33720	0.1	208144	0.0241	0.005	15	0.197
14	Background control	1	2	0.5	1	65	130	260	260	0.1	208144	0.0000	0.005	15	0.000
15		2	2	0.5	2	56	112	224	224	0.1	208144	0.0000	0.005	15	0.000
16		3	2	0.5	2	58	116	232	232	0.1	208144	0.0000	0.005	15	0.000
17		4	2	0.5	2	58	116	232	232	0.1	208144	0.0000	0.005	15	0.000
18		5	2	0.5	2	58	116	232	232	0.1	208144	0.0000	0.005	15	0.000
19		6	2	0.5	2	58	116	232	232	0.1	208144	0.0000	0.005	15	0.000
20		7	2	0.5	2	58	116	232	232	0.1	208144	0.0000	0.005	15	0.000
21		8	2	0.5	2	58	116	232	232	0.1	208144	0.0000	0.005	15	0.000
22		9	2	0.5	2	58	116	232	232	0.1	208144	0.0000	0.005	15	0.000
23	100% ASIN	1-1	2	0.5	1	187	374	748	748	0.1	208144	0.0004	0.005	15	0.003
24		2-1	2	0.5	2	187	374	748	748	0.1	208144	0.0004	0.005	15	0.003
25		3-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
26		4-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
27		5-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
28		6-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
29		7-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
30		8-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
31		9-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
32		10-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
33		11-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
34		12-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
35		13-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
36		14-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
37		15-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
38		16-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
39		17-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
40		18-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
41		19-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
42		20-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
43		21-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
44		22-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
45		23-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
46		24-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
47		25-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
48		26-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
49		27-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
50		28-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
51		29-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
52		30-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
53		31-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
54		32-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
55		33-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
56		34-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
57		35-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
58		36-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
59		37-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
60		38-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
61		39-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
62		40-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
63		41-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
64		42-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
65		43-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
66		44-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
67		45-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
68		46-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
69		47-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003
70		48-1	2	0.5	2	155	310	620	620	0.1	208144	0.0004	0.005	15	0.003

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date: 3/13/2007															
Chemical															
IO CR11606 # Concentrations tested 12545-13															
Counter data															
Assay Ref 12535-08 13MR2049-P00															
Technician ID PRP															
Replicate # 2 Positive Control 4-OH Androst Pos Ctl Tubes/Conc 2															
Sample ID															
Sample type	Replicate #	Alq	Nominal total volume (mL)	Alq #	DP/Mtq	DP/MtL	Ave DP/MtL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed Total DPM corrected for background (Background Tubes)	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
7	CR11606	1-1	2	0.5	1	8398	16776	33448	0.1	208144	33215	0.0331	0.005	15	0.221
8					2	8398	16572		0.1	208144	31153	0.0311	0.005	15	0.207
9		1-2	2	0.5	1	7807	15514	15993		208144		0.0334	0.005	15	0.222
10					2	7808	15772		0.1	208144	33468	0.0368	0.005	15	0.246
11		1-3	2	0.5	1	8457	16914	16651		208144		0.0379	0.005	15	0.252
12					2	83943	16788	33702		208144	32419	0.0320	0.005	15	0.213
13		2-1	2	0.5	1	8283	16598	15999		208144		0.0356	0.005	15	0.238
14					2	8283	16598		0.1	208144	30945	0.0309	0.005	15	0.207
15		2-2	2	0.5	1	8457	16914	16651		208144		0.0349	0.005	15	0.244
16					2	8457	16598	15999		208144	30945	0.0309	0.005	15	0.207
17		2-3	2	0.5	1	80544	16598	32652		208144		0.0347	0.005	15	0.241
18					2	8272	16544	32652		208144	32419	0.0320	0.005	15	0.213
19		3-1	2	0.5	1	80445	16032	32340		208144		0.0354	0.005	15	0.238
20					2	8124	16248		0.1	208144	30945	0.0309	0.005	15	0.207
21		3-2	2	0.5	1	8029	16038	17857		208144		0.0356	0.005	15	0.244
22					2	8028	17856		0.1	208144	30945	0.0309	0.005	15	0.207
23		3-3	2	0.5	1	8235	16470	19462		208144		0.0349	0.005	15	0.244
24					2	8227	16454	38536		208144	31053	0.0310	0.005	15	0.207
25		4-1	2	0.5	1	8674	19468	19268		208144		0.0349	0.005	15	0.244
26					2	8674	19468		0.1	208144	30945	0.0309	0.005	15	0.207
27		4-2	2	0.5	1	7816	16884	16658		208144		0.0347	0.005	15	0.241
28					2	7816	16884		0.1	208144	30945	0.0309	0.005	15	0.207
29		4-3	2	0.5	1	8610	17820	17600		208144		0.0347	0.005	15	0.241
30					2	8610	17820		0.1	208144	30945	0.0309	0.005	15	0.207
31		5-1	2	0.5	1	8313	18626	18447		208144		0.0347	0.005	15	0.241
32					2	8313	18626		0.1	208144	30945	0.0309	0.005	15	0.207
33		5-2	2	0.5	1	9213	18426	18320		208144		0.0347	0.005	15	0.241
34					2	9107	18514	36840		208144	30945	0.0309	0.005	15	0.207
35		5-3	2	0.5	1	8897	19794	19640		208144		0.0347	0.005	15	0.241
36					2	8743	19680		0.1	208144	30945	0.0309	0.005	15	0.207
37		6-1	2	0.5	1	8855	17910	17805		208144		0.0347	0.005	15	0.241
38					2	8754	17598		0.1	208144	30945	0.0309	0.005	15	0.207
39		6-2	2	0.5	1	8701	17522	17515		208144		0.0347	0.005	15	0.241
40					2	8701	17522		0.1	208144	30945	0.0309	0.005	15	0.207
41		6-3	2	0.5	1	9104	18208	18254		208144		0.0347	0.005	15	0.241
42					2	9150	18300		0.1	208144	30945	0.0309	0.005	15	0.207
43		7-1	2	0.5	1	8940	17840	17822		208144		0.0347	0.005	15	0.241
44					2	8882	17764		0.1	208144	30945	0.0309	0.005	15	0.207
45		7-2	2	0.5	1	7710	15420	15161		208144		0.0347	0.005	15	0.241
46					2	8461	16802		0.1	208144	30945	0.0309	0.005	15	0.207
47		7-3	2	0.5	1	9358	19278	19085		208144		0.0347	0.005	15	0.241
48					2	9429	18558		0.1	208144	30945	0.0309	0.005	15	0.207
49		8-1	2	0.5	1	9439	18988	19102		208144		0.0347	0.005	15	0.241
50					2	9013	18005		0.1	208144	30945	0.0309	0.005	15	0.207
51		8-2	2	0.5	1	8558	17138	17354		208144		0.0347	0.005	15	0.241
52					2	8558	17138		0.1	208144	30945	0.0309	0.005	15	0.207
53		8-3	2	0.5	1	9042	18984	18148		208144		0.0347	0.005	15	0.241
54					2	9106	18212		0.1	208144	30945	0.0309	0.005	15	0.207

Aromatase Assay Spreadsheet

	R	S
1		
2		
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5		OC check - aliquot variance
6		
7		% RE
8		1.00
9		1.00
10		0.99
11		1.01
12		1.00
13		1.00
14		1.00
15		1.00
16		1.00
17		0.99
18		1.01
19		1.00
20		1.00
21		1.01
22		0.99
23		1.00
24		1.00
25		1.00
26		1.00
27		1.00
28		1.00
29		1.00
30		0.99
31		1.01
32		0.99
33		1.01
34		0.99
35		1.01
36		0.99
37		1.01
38		0.99
39		1.00
40		1.00
41		1.00
42		1.00
43		1.00
44		0.99
45		1.01
46		1.01
47		0.99
48		0.99
49		1.01
50		1.01
51		0.99
52		1.01
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A		B		C		D		E		F		G		H		I		J		K		L	
1	Assay Date	3/13/2007		Test Chemical ID	CR11606			Counter data filename	13MR2049.P00			# Concentrations	8	Microsome ID	12635-13								
2	NB Ref.	12535-68		Replicate #								2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc								
3	Technician ID	PRP																					
4	Control Type	Portion	Average	SD																			
5	Full activity	Overall	0.204	0.033																			
6	Background (uncorrected)	Overall	0.002	0.000																			
7																							
8																							
9																							
10	Positive Control	Level	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity																	
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003																	
12	4-OH ASDN	1	2	1.00E-06	-5.00	0.003																	
13																							
14	4-OH ASDN	2	1	1.00E-06	-6.00	0.023																	
15	4-OH ASDN	2	2	1.00E-06	-6.00	0.020																	
16																							
17	4-OH ASDN	3	1	5.00E-07	-6.30	0.041																	
18	4-OH ASDN	3	2	5.00E-07	-6.30	0.036																	
19																							
20	4-OH ASDN	4	1	1.00E-07	-7.00	0.118																	
21	4-OH ASDN	4	2	1.00E-07	-7.00	0.121																	
22																							
23	4-OH ASDN	5	1	5.00E-08	-7.30	0.163																	
24	4-OH ASDN	5	2	5.00E-08	-7.30	0.156																	
25																							
26	4-OH ASDN	6	1	1.00E-08	-8.00	0.213																	
27	4-OH ASDN	6	2	1.00E-08	-8.00	0.212																	
28																							
29	4-OH ASDN	7	1	1.00E-09	-9.00	0.218																	
30	4-OH ASDN	7	2	1.00E-09	-9.00	0.241																	
31																							
32	4-OH ASDN	8	1	1.00E-10	-10.00	0.246																	
33	4-OH ASDN	8	2	1.00E-10	-10.00	0.216																	
34																							
35																							
36																							
37	Test Chemical	Level	Replicate	Test chemical M	Log(test chemical)	Activity																	
38	CR11606	1	1	1.00E-03	-3.00	0.221																	
39	CR11606	1	2	1.00E-03	-3.00	0.207																	
40	CR11606	1	3	1.00E-03	-3.00	0.222																	
41	CR11606	2	1	1.00E-04	-4.00	0.246																	
42	CR11606	2	2	1.00E-04	-4.00	0.252																	
43	CR11606	2	3	1.00E-04	-4.00	0.215																	
44	CR11606	3	1	1.00E-05	-5.00	0.213																	
45	CR11606	3	2	1.00E-05	-5.00	0.236																	
46	CR11606	3	3	1.00E-05	-5.00	0.244																	
47	CR11606	4	1	1.00E-06	-6.00	0.207																	
48	CR11606	4	2	1.00E-06	-6.00	0.232																	
49	CR11606	4	3	1.00E-06	-6.00	0.242																	
50	CR11606	5	1	1.00E-07	-7.00	0.244																	
51	CR11606	5	2	1.00E-07	-7.00	0.242																	
52	CR11606	5	3	1.00E-07	-7.00	0.255																	
53	CR11606	6	1	1.00E-08	-8.00	0.235																	
54	CR11606	6	2	1.00E-08	-8.00	0.231																	
55	CR11606	6	3	1.00E-08	-8.00	0.241																	
56	CR11606	7	1	1.00E-09	-9.00	0.235																	
57	CR11606	7	2	1.00E-09	-9.00	0.213																	
58	CR11606	7	3	1.00E-09	-9.00	0.252																	
59	CR11606	8	1	1.00E-10	-10.00	0.262																	
60	CR11606	8	2	1.00E-10	-10.00	0.229																	
61	CR11606	8	3	1.00E-10	-10.00	0.240																	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/14/2007	Test Chemical ID	CR11606	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-71	Counter data filename	14MR2056.P00					
3	Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/14/2007	Test Chemical ID	CR11606	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-71	Counter data filename	14MR2056.P00				Pos Ctl Tubes/Conc	
3	Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN			2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0192		36850		1919271			
8	2	0.0191		36304		1900733			
9	3	0.0201		40808		2030249			
10	4	0.0194		40006		2062165			
11	5	0.0192		39910		2078646			
12						Average DPM/g soln		1998213	
13						SD		82644	
14						CV		4.14	
15						µCi/g soln		0.9001	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1183 g			
25	Mass of dilution B used in substrate prep					4.5548 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56158982 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.9001			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5616	+	0.011		
37				=	0.573	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.572	µCi/µg ASDN			
41					9.995.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/14/2007	Test Chemical ID	CR11606	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-71	Counter data filename	14MR2056.P00					
3			Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
5			Microsome Dilution Details								
6											
7			Dilution A								
8			0.12 mL microsome Stock used								
9			2.4 mL total volume								
10			20 dilution factor								
11			Dilution B								
12			1.5 mL microsome Dilution A used								
13			60 mL total volume								
14			40 dilution factor								
15			Dilution C (if applicable)								
16			mL microsome Dilution B used								
17			mL total volume								
18			dilution factor								
19			800 total dilution factor								
20											
21			Protein Concentration (stock microsomes, mg/mL):								
22			Protein Concentration (dilution added to assay, mg/mL):								
23											
24											
25											
26											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Test # Chemical Concentrations As: 3/14/2007 ID C tested 8. Microsome ID 12535-13									
2	Counter data NB 12535-71 filename 14MR2056.P00									
3	Te ch nic ian ID PRP Replicate # 3 Positive Control 4-OH ASD Tubes/Conc 2									
4										
5	Vial	Tin	DPM1	tSIE						Round DPM
6	2	5	36850.43	14501.02	0	12.75	485.96			36850
7	3	5	36304.23	14315.22	0	12.74	485.21			36304
8	4	5	40807.63	16025.62	0	12.77	484.37			40808
9	5	5	40005.89	15671.1	0	12.7	483.31			40006
10	6	5	39909.98	15647.22	0	12.74	482.49			39910
11	8	5	9350.14	4000.22	0	13.66	547.82			9350
12	9	5	9438.05	4042.9	0	13.73	549.08			9438
13	10	5	8242.11	3526.18	0	13.71	548.62			8242
14	11	5	8117.98	3490.82	0	13.59	549.32			8118
15	12	5	7240.75	3110.58	0	13.68	548.7			7241
16	13	5	7427.51	3177.98	0	13.75	547.4			7428
17	14	5	7895.38	3373.22	0	13.67	548.11			7895
18	15	5	7512.98	3220.26	0	13.75	549.09			7513
19	16	5	50.78	19.78	0	13.26	545.86			51
20	17	5	54.59	26.38	0	13.38	547.54			55
21	18	5	41.71	18.02	0	11.67	548			42
22	19	5	41.65	20.82	0	13.08	548.99			42
23	20	5	50.85	23.42	0	12.55	548.86			51
24	21	5	55.05	21.38	0	13.39	548.87			55
25	22	5	53.05	23.42	0	14.62	547.1			53
26	23	5	50.85	23.02	0	12.01	548.87			51
27	25	5	137.03	56.38	0	13.58	546.86			137
28	26	5	145.56	61.98	0	13.75	546.34			146
29	27	5	133.15	56.42	0	13.61	544.21			133
30	28	5	134.43	57.82	0	12.19	546.18			134
31	29	5	890.86	383.42	0	13.84	544.63			891
32	30	5	879.62	380.22	0	13.49	545.79			880
33	31	5	804.79	344.82	0	13.34	545.11			805
34	32	5	823.39	350.42	0	14.08	546.02			823
35	33	5	1425.65	616.78	0	13.42	544.1			1426
36	34	5	1432.14	610.48	0	13.84	544.08			1432
37	35	5	1328.51	568.62	0	13.83	542.69			1329
38	36	5	1302.96	548.82	0	13.67	542.42			1303
39	37	5	4389.31	1849.62	0	13.72	540.15			4389
40	38	5	4205.04	1770.78	0	13.62	542.51			4205
41	39	5	4371.69	1858.86	0	13.64	544.94			4372
42	40	5	4443.79	1897.62	0	13.57	545.68			4444
43	41	5	5363.94	2277.62	0	13.55	541.09			5364
44	42	5	5303.99	2260.38	0	13.52	543.97			5304
45	43	5	5608.29	2385.06	0	13.49	541.87			5608
46	44	5	4836.8	2075.7	0	13.65	545.74			4837
47	45	5	7713.69	3272.58	0	13.55	540.42			7714
48	46	5	7564.94	3191.78	0	13.84	541.03			7565
49	47	5	6396.19	2726.78	0	13.58	541.49			6396
50	48	5	6103.43	2607.02	0	13.56	542.87			6103
51	49	5	7942.01	3396.22	0	13.54	542.54			7942
52	50	5	7887.12	3345.26	0	13.6	541.9			7887
53	51	5	7322.94	3119.66	0	13.56	539.85			7323
54	52	5	7624.91	3219.78	0	13.56	537.87			7625
55	53	5	8278.46	3541.66	0	13.56	541.43			8276
56	54	5	8460.57	3577.58	0	13.53	538.01			8461
57	55	5	7609.71	3236.42	0	13.58	539.9			7610
58	56	5	7582.78	3203.42	0	13.68	539.4			7583
59	58	5	7530.88	3174.78	0	13.57	539.12			7531
60	59	5	7414.14	3187.06	0	13.51	543.19			7414
61	60	5	6202.91	2829.62	0	13.48	542.97			6203
62	61	5	6009.67	2551.98	0	13.63	542.7			6010
63	62	5	7225.89	3083.58	0	13.59	544.39			7226
64	63	5	7201.21	3056.42	0	13.65	543.94			7201
65	64	5	8842.71	3733.46	0	13.6	539.58			8843
66	65	5	8568.97	3652.5	0	13.66	546.23			8569
67	66	5	8850.04	3779.46	0	13.57	546.27			8850
68	67	5	9180.05	3908.1	0	13.65	546.08			9180
69	68	5	8262.31	3539.42	0	13.63	546.95			8262
70	69	5	8162.7	3485.18	0	13.68	546.1			8163

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	8789.17	3721.08	0	13.61	542.43				8789
72	71	5	8382.82	3603.18	0	13.55	547.13				8383
73	72	5	8126.85	3455.22	0	13.69	544.43				8127
74	73	5	8081.79	3466.38	0	13.58	548.76				8082
75	74	5	7901.96	3369.02	0	13.6	546.42				7902
76	75	5	7713.19	3326.98	0	13.57	548.6				7713
77	76	5	8914.72	3813.38	0	13.61	546.98				8915
78	77	5	8690.88	3734.98	0	13.55	547.48				8691
79	78	5	8618.38	3673.82	0	13.7	548.46				8618
80	79	5	7612.79	3272.58	0	13.69	549.37				7613
81	80	5	7832.56	3358.38	0	13.63	549.18				7833
82	81	5	7959.01	3411.62	0	13.73	548.87				7959
83	82	5	5578.74	2384.54	0	13.61	546.63				5579
84	83	5	5538.79	2364.78	0	13.82	550.01				5537
85	84	5	8717.95	3739.82	0	13.66	549.52				8718
86	85	5	8639.22	3681.22	0	13.68	546.69				8639
87	86	5	9171.11	3951.82	0	13.68	550.07				9171
88	87	5	8952.39	3842.02	0	13.75	549.86				8952
89	88	5	8479.09	3640.02	0	13.68	549.03				8479
90	89	5	8481.17	3629.22	0	13.69	551.02				8481
91	90	5	8464.36	2757.42	0	13.68	546.93				8464
92	91	5	8462.4	2772.42	0	13.65	550.1				8462
93	92	5	8491.88	3621.18	0	13.69	547.28				8492
94	93	5	8346.59	3563.62	0	13.73	546.2				8346
95	94	5	8709.21	3750.58	0	13.67	548.03				8709
96	95	5	8633.28	3723.38	0	13.66	551.78				8633
97	96	5	6820.04	2939.06	0	13.66	550.61				6820

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
98	97	5	6720.32	2893.82	0	13.71	550.65				6720
99	98	5	7842.48	3352.78	0	13.74	548.29				7842
100	99	5	7731.11	3290.22	0	13.79	549.21				7731
101	100	5	7134.68	3068.78	0	13.69	550.18				7135
102	101	5	7076.62	3008.98	0	13.77	547.39				7077
103	102	5	8737.85	3725.46	0	13.74	549.43				8738
104	103	5	8758.81	3770.62	0	13.73	551.92				8757
105	104	5	8185.01	3497.22	0	13.7	547.31				8185
106	105	5	8260.64	3532.22	0	13.72	549.11				8281
107	107	5	234486.34	108409.7	0	15.04	627.13				234486
109											0
109											0
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111											0
112											0
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
				Test	Chemical										
1				Assay Date	3/14/2017	CS11806	# Concentrations tested	8	Microsome ID	12535-13					
2				NB Ref	12535-71	14MP2018 P100	Counter dist	14MP2018 P100							
3				10	10		Replicate	3	Positive Control	4-ON-ASDN P100					
4				REP											
5	Sample ID	Replicates	Nonvol total vol (mL)	Ala Volume (mL)	CPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (cpm)	Calculate nmol ³ H ₂ O formed Total DPM corrected for background (background (Tubes)	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Incubation time (min)		
6	Full activity control	2	2	0.5	1	9250	18788	37576	0.1	199821	37776	0.0374	15	0.249	
7		2	2	0.5	2	8438	16876	33752	0.1	199821	33752	0.0374	15	0.217	
8		2	2	0.5	1	6242	12484	24968	0.1	199821	24968	0.0374	15	0.194	
9		2	2	0.5	2	8118	16236	32472	0.1	199821	32472	0.0374	15	0.204	
10		2	2	0.5	1	7241	14482	28964	0.1	199821	28964	0.0374	15	0.190	
11		2	2	0.5	2	7428	14856	29712	0.1	199821	29712	0.0374	15	0.200	
12		2	2	0.5	1	7895	15790	31580	0.1	199821	31580	0.0374	15	0.200	
13		2	2	0.5	2	7513	15026	30052	0.1	199821	30052	0.0374	15	0.200	
14	Background control	1	2	0.5	1	51	102	204	0.1	199821	204	0.0000	15	0.000	
15		2	2	0.5	2	55	110	220	0.1	199821	220	0.0000	15	0.000	
16		2	2	0.5	1	42	84	168	0.1	199821	168	0.0000	15	0.000	
17		2	2	0.5	2	44	88	176	0.1	199821	176	0.0000	15	0.000	
18		2	2	0.5	1	51	102	204	0.1	199821	204	0.0000	15	0.000	
19		2	2	0.5	2	55	110	220	0.1	199821	220	0.0000	15	0.000	
20		2	2	0.5	1	53	106	212	0.1	199821	212	0.0000	15	0.000	
21		2	2	0.5	2	59	118	236	0.1	199821	236	0.0000	15	0.000	
22	4-ON-ASDN	1-1	2	0.5	1	137	274	548	0.1	199821	548	0.0044	15	0.002	
23		2	2	0.5	2	146	292	584	0.1	199821	584	0.0044	15	0.002	
24		1-2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
25		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
26		1-3	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
27		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
28		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
29		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
30		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
31		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
32		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
33		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
34		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
35		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
36		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
37		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
38		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
39		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
40		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
41		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
42		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
43		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
44		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
45		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
46		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
47		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
48		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
49		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
50		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
51		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
52		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
53		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
54		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
55		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
56		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
57		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
58		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
59		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
60		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
61		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
62		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
63		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
64		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
65		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
66		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
67		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
68		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	
69		2	2	0.5	2	134	268	536	0.1	199821	536	0.0044	15	0.002	
70		2	2	0.5	1	133	266	532	0.1	199821	532	0.0044	15	0.002	

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		Assay Date	3/14/2007	Test Chemical	CR11605	# Concentrations tested	12535-13									
2		NB Ref	12535-71	Chemical ID	14M42056.P100											
3		Technician ID	ppp	Replicate #	3	Positive Control	4-OH ASDM Pro C1 Tunasi/Conc	2								
4																
5		Sample ID														
6		Replicate Level	Volume (mL)	Aliq. #	DP (mL)	Ave DPM/mL	Total DPM	Volume of substrate solution used (assay tube) (mL)	Total DPM in assay tube (initial)	Calculate nmoL ³ H ₂ O formed	Final [protein] in assay (mg/mL)	nmoL ³ H ₂ O formed	Incubation time (min)	Aromatase activity (nmoL estrogen forming/mg protein/min)		
7	CR11605	1-1	2	0.5	1	7531	15062	0.1	19821	29890	0.0297	0.005	15	0.188		
8		1-2	2	0.5	2	7414	14826	0.1	19821	24526	0.0542	0.005	15	0.162		
9		1-3	2	0.5	1	8203	12405	0.1	19821	28054	0.0787	0.005	15	0.191		
10		2-1	2	0.5	2	7201	14402	0.1	19821	34524	0.0945	0.005	15	0.231		
11		2-2	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
12		2-3	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
13		3-1	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
14		3-2	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
15		3-3	2	0.5	2	8092	16164	0.1	19821	34144	0.0942	0.005	15	0.228		
16		4-1	2	0.5	2	7713	15426	0.1	19821	31030	0.0910	0.005	15	0.207		
17		4-2	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		
18		4-3	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		
19		5-1	2	0.5	2	7531	15062	0.1	19821	29890	0.0297	0.005	15	0.188		
20		5-2	2	0.5	2	7414	14826	0.1	19821	24526	0.0542	0.005	15	0.162		
21		5-3	2	0.5	2	8203	12405	0.1	19821	28054	0.0787	0.005	15	0.191		
22		6-1	2	0.5	2	7201	14402	0.1	19821	34524	0.0945	0.005	15	0.231		
23		6-2	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
24		6-3	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
25		7-1	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
26		7-2	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
27		7-3	2	0.5	2	8092	16164	0.1	19821	34144	0.0942	0.005	15	0.228		
28		8-1	2	0.5	2	7713	15426	0.1	19821	31030	0.0910	0.005	15	0.207		
29		8-2	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		
30		8-3	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		
31		9-1	2	0.5	2	7531	15062	0.1	19821	29890	0.0297	0.005	15	0.188		
32		9-2	2	0.5	2	7414	14826	0.1	19821	24526	0.0542	0.005	15	0.162		
33		9-3	2	0.5	2	8203	12405	0.1	19821	28054	0.0787	0.005	15	0.191		
34		10-1	2	0.5	2	7201	14402	0.1	19821	34524	0.0945	0.005	15	0.231		
35		10-2	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
36		10-3	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
37		11-1	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
38		11-2	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
39		11-3	2	0.5	2	8092	16164	0.1	19821	34144	0.0942	0.005	15	0.228		
40		12-1	2	0.5	2	7713	15426	0.1	19821	31030	0.0910	0.005	15	0.207		
41		12-2	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		
42		12-3	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		
43		13-1	2	0.5	2	7531	15062	0.1	19821	29890	0.0297	0.005	15	0.188		
44		13-2	2	0.5	2	7414	14826	0.1	19821	24526	0.0542	0.005	15	0.162		
45		13-3	2	0.5	2	8203	12405	0.1	19821	28054	0.0787	0.005	15	0.191		
46		14-1	2	0.5	2	7201	14402	0.1	19821	34524	0.0945	0.005	15	0.231		
47		14-2	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
48		14-3	2	0.5	2	8493	16986	0.1	19821	35860	0.0959	0.005	15	0.239		
49		15-1	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
50		15-2	2	0.5	2	8153	16304	0.1	19821	34144	0.0942	0.005	15	0.228		
51		15-3	2	0.5	2	8092	16164	0.1	19821	34144	0.0942	0.005	15	0.228		
52		16-1	2	0.5	2	7713	15426	0.1	19821	31030	0.0910	0.005	15	0.207		
53		16-2	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		
54		16-3	2	0.5	2	8815	17630	0.1	19821	35012	0.0950	0.005	15	0.234		

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		
6		QC check - aliquot variance
7		% RE
8		1.01
9		0.99
10		1.02
11		0.98
12		1.00
13		1.00
14		1.00
15		0.98
16		1.02
17		1.01
18		0.99
19		1.02
20		0.98
21		1.00
22		1.00
23		1.01
24		0.98
25		1.01
26		0.99
27		1.00
28		0.98
29		1.01
30		0.99
31		1.00
32		1.00
33		1.00
34		1.01
35		0.99
36		1.00
37		1.00
38		1.00
39		1.00
40		1.00
41		1.00
42		0.98
43		1.00
44		1.01
45		0.99
46		1.01
47		0.99
48		1.00
49		1.00
50		1.00
51		1.00
52		1.00
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/14/2007	CR11606	Test Chemical ID	14MR2056.P00	# Concentrations	8	Microsome ID	12535-13		
2	NB Ref	12535-71	Counter data filename								
3	Technician ID	PRP	Replicate #			3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Cone	2	
4											
5	Control Type	Average	SD								
6	Full activity	0.216	0.024								
7	Background (uncorrected)	0.001	0.000								
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log[Positive Ctl]	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.002						
12	4-OH ASDN	2	1.00E-05	-5.00	0.002						
13	4-OH ASDN	1	1.00E-06	-6.00	0.022						
14	4-OH ASDN	2	1.00E-06	-6.00	0.020						
15	4-OH ASDN	1	5.00E-07	-6.30	0.037						
16	4-OH ASDN	2	5.00E-07	-6.30	0.034						
17	4-OH ASDN	1	1.00E-07	-7.00	0.113						
18	4-OH ASDN	2	1.00E-07	-7.00	0.116						
19	4-OH ASDN	1	5.00E-08	-7.30	0.141						
20	4-OH ASDN	2	5.00E-08	-7.30	0.138						
21	4-OH ASDN	1	1.00E-08	-8.00	0.202						
22	4-OH ASDN	2	1.00E-08	-8.00	0.165						
23	4-OH ASDN	1	1.00E-09	-9.00	0.210						
24	4-OH ASDN	2	1.00E-09	-9.00	0.198						
25	4-OH ASDN	1	1.00E-10	-10.00	0.222						
26	4-OH ASDN	2	1.00E-10	-10.00	0.201						
27	4-OH ASDN	1									
28	4-OH ASDN	2									
29	4-OH ASDN	1									
30	4-OH ASDN	2									
31	4-OH ASDN	1									
32	4-OH ASDN	2									
33	4-OH ASDN	1									
34	4-OH ASDN	2									
35	4-OH ASDN	1									
36	4-OH ASDN	2									
37	Test Chemical	Replicate	Test chemical [M]	Log[Test chemical]	Activity						
38	CR11606	1	1.00E-03	-3.00	0.198						
39	CR11606	2	1.00E-03	-3.00	0.162						
40	CR11606	3	1.00E-03	-3.00	0.151						
41	CR11606	1	1.00E-04	-4.00	0.231						
42	CR11606	2	1.00E-04	-4.00	0.239						
43	CR11606	3	1.00E-04	-4.00	0.218						
44	CR11606	1	1.00E-05	-5.00	0.228						
45	CR11606	2	1.00E-05	-5.00	0.216						
46	CR11606	3	1.00E-05	-5.00	0.207						
47	CR11606	1	1.00E-06	-6.00	0.234						
48	CR11606	2	1.00E-06	-6.00	0.215						
49	CR11606	3	1.00E-06	-6.00	0.209						
50	CR11606	1	1.00E-07	-7.00	0.147						
51	CR11606	2	1.00E-07	-7.00	0.230						
52	CR11606	3	1.00E-07	-7.00	0.240						
53	CR11606	1	1.00E-08	-8.00	0.226						
54	CR11606	2	1.00E-08	-8.00	0.171						
55	CR11606	3	1.00E-08	-8.00	0.223						
56	CR11606	1	1.00E-09	-9.00	0.230						
57	CR11606	2	1.00E-09	-9.00	0.179						
58	CR11606	3	1.00E-09	-9.00	0.206						
59	CR11606	1	1.00E-10	-10.00	0.188						
60	CR11606	2	1.00E-10	-10.00	0.232						
61	CR11606	3	1.00E-10	-10.00	0.218						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/1/2007	Test Chemical ID	CR11607	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-57	Counter data filename	01MR2342.P00					
3	Technician	ase	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/1/2007	Test Chemical ID	CR11607	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-57	Counter data filename	01MR2342.P00					
3	Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0198		40343		2037525			
8	2	0.0203		39848		1962956			
9	3	0.0198		42151		2128838			
10	4	0.0200		37976		1898800			
11	5	0.0199		40685		2044472			
12						Average DPM/g soln		2014518	
13						SD		87386	
14						CV		4.34	
15						µCi/g soln		0.9074	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.0	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1148 g			
25	Mass of dilution B used in substrate prep					4.5693 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56364535 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.9074			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36			=	0.5636	+	0.011			
37			=	0.575	µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39		= (µCi/g soln.)/(µg ASDN/g soln.)							
40		=		1.579 µCi/µg ASDN					
41				1.004.E+06 dpm/nmol					

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/1/2007	Test Chemical ID	CR11607	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-57	Counter data filename	01MR2342.P00					
3			Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctl	2
4											
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	As	3/1/2007	Test Chemical ID	# Concentrations tested	8	Microsome ID	12535-13			
2	Nr	12535-57	Counter data filename	01MR2342.P00						
3	T									
4	e									
5	c									
6	h									
7	n									
8	i									
9	a									
10	n									
11	i									
12	d									
13	a									
14	s									
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Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	12733.15	5738.24	0	12.9	533.27				12733
66	53	5	12709.39	5734.08	0	12.91	531.55				12709
67											
68											
69	54	5	13306.87	5941.92	0	12.94	527.94				13307
70	55	5	13720.55	6146.32	0	12.94	530.96				13721
71	56	5	12718.95	5750.64	0	12.91	532.8				12719
72	57	5	12943.53	5847.64	0	12.98	534.46				12944
73											
74											
75	59	5	279.52	124.44	0	13.53	531.85				280
76	60	5	288.88	133.28	0	12.85	532.34				289
77	61	5	308.86	136.72	0	12.44	531.48				309
78	62	5	287.56	131.28	0	12.98	532.79				288
79	63	5	330.52	148.48	0	12.95	535.73				331
80	64	5	315.4	146.64	0	12.71	533.6				315
81	65	5	3283.1	1476.04	0	12.92	528.97				3283
82	66	5	3333.71	1490.04	0	13.01	532.68				3334
83	67	5	2990.12	1342.08	0	12.91	533.68				2990
84	68	5	3079.2	1384.8	0	12.79	531.94				3079
85	69	5	3253.99	1456.12	0	13.19	535.64				3254
86	70	5	3324.96	1494.88	0	12.86	533.43				3325
87	71	5	10501.69	4720.92	0	12.91	532.32				10502
88	72	5	10730.71	4847.92	0	12.92	531.7				10731
89	73	5	10049.89	4563.88	0	12.98	536.25				10050
90	74	5	10062.13	4534.88	0	12.93	530.79				10062
91	75	5	9289.54	4189.28	0	12.94	532.21				9290
92	76	5	9329.71	4176.04	0	12.91	529.69				9330
93	77	5	14031.47	6291.64	0	13.03	532.76				14031
94	78	5	14264.69	6374.52	0	12.99	530.81				14265
95	79	5	12630.47	5689.44	0	13	533.12				12630
96	80	5	12805.31	5746.24	0	12.96	532.79				12805
97	81	5	11193.4	5076.28	0	12.92	536.21				11193
98	82	5	11215.18	5033.88	0	12.92	530.81				11215
99	84	5	12693.15	5735.88	0	12.96	532.88				12693
100	85	5	12785.73	5761.64	0	12.98	536.03				12786
101	86	5	12980.57	5856.64	0	12.97	535.52				12981
102	87	5	13178.55	5956.88	0	12.89	532.78				13179
103	88	5	12118.38	5428.64	0	12.94	530.29				12118
104	89	5	12399.61	5579.56	0	12.96	532.92				12400
105	90	5	13380.62	6019.88	0	12.96	533.87				13381
106	91	5	13735.77	6196.52	0	12.95	530.8				13736
107	92	5	12030.61	5396.88	0	12.93	531.52				12031
108	93	5	12199.69	5503.64	0	12.95	532.14				12200
109	94	5	12784.78	5767.12	0	12.92	532.75				12785
110	95	5	12880.41	5801.04	0	12.91	532.01				12880
111	96	5	13094.47	5891.96	0	12.91	531.63				13094
112	97	5	13031.99	5885.16	0	13.06	535.65				13032
113	98	5	12358.4	5592.68	0	12.87	532.6				12358
114	99	5	12747.53	5728.88	0	12.97	531.15				12748
115	100	5	12598.66	5658.28	0	12.95	530.16				12599
116	101	5	12637.33	5657.08	0	12.98	530.32				12637
117	102	5	13388.35	5997.64	0	12.97	530.39				13388
118	103	5	13660.25	6116.28	0	12.92	530.23				13660
119	104	5	12974.96	5829.68	0	12.96	531.69				12975
120	105	5	13186.42	5952.72	0	12.93	532.89				13186
121	106	5	12731.24	5761.88	0	13.02	535.6				12731
122	107	5	12651.07	5713.92	0	12.92	532.62				12651
123	109	5	229212.33	53493.88	0	9.13	251.48				229212

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
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Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								
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Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Concentrations tested	# Concentrations tested	Microsome ID	12535-13								</

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		QC check: standard variance			
5		QC value not within mean $\pm$ 10%			
6					
7		1.01			
8		0.99			
9		1.00			
10		1.00			
11		1.00			
12		1.00			
13		1.00			
14		1.00			
15		0.99			
16		1.07			
17		0.95			
18		1.03			
19					
20					
21		0.99			
22		1.05			
23		1.05			
24		0.95			
25		1.01			
26		0.99			
27					
28		1.00			
29		1.00			
30		0.99			
31		1.02			
32					
33					
34		0.99			
35		1.01			
36		1.00			
37		1.00			
38		1.00			
39					
40		0.99			
41		1.01			
42		1.00			
43		1.00			
44		1.00			
45					
46					
47		0.99			
48		1.01			
49		1.00			
50		1.00			
51					
52		0.99			
53		1.01			
54		0.99			
55		1.01			
56					
57					
58		1.00			
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63					
64		0.99			
65		1.02			
66		0.99			
67		1.01			
68					
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	3/12/2007	Test Chemical ID	CR11607	# Concentrations tested	4								
2		WB Ref.	12535-57	Comp. date	01MR2342P00										
3		Technician ID	1	Replicate #	1	Positive Control	4-QH-ASDN Pos. Ctrl. Tubes/Comp.								
4															
5		Sample ID													
6	Sample type	Replicate/Level	Nominal total volume (mL)	Aliq. #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed Total DPM corrected for background (background tubes)	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein)
7	CR11607	1-1	2	0.5	280	560	560	1136	0.1	201452	804	0.009	0.005	15	0.005
8		1-2	2	0.5	280	578	578	1194	0.1	201452	860	0.010	0.005	15	0.005
9		1-3	2	0.5	280	618	597	1194	0.1	201452	960	0.011	0.005	15	0.007
10		2-1	2	0.5	280	576	645	1292	0.1	201452	1098	0.0129	0.005	15	0.008
11		2-2	2	0.5	280	652	667	1324	0.1	201452	1300	0.0119	0.005	15	0.008
12		2-3	2	0.5	280	652	667	1324	0.1	201452	1300	0.0119	0.005	15	0.008
13		3-1	2	0.5	280	590	606	1216	0.1	201452	1184	0.0129	0.005	15	0.008
14		3-2	2	0.5	280	618	606	1216	0.1	201452	1224	0.0129	0.005	15	0.008
15		3-3	2	0.5	280	652	606	1216	0.1	201452	1224	0.0129	0.005	15	0.008
16		4-1	2	0.5	280	21004	21233	42465	0.1	201452	42232	0.0421	0.005	15	0.289
17		4-2	2	0.5	280	20100	20132	40264	0.1	201452	40230	0.0399	0.005	15	0.266
18		4-3	2	0.5	280	20164	18520	37240	0.1	201452	37005	0.0369	0.005	15	0.246
19		5-1	2	0.5	280	18500	20206	38706	0.1	201452	38553	0.0369	0.005	15	0.246
20		5-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
21		5-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
22		6-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
23		6-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
24		6-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
25		7-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
26		7-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
27		7-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
28		8-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
29		8-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
30		8-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
31		9-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
32		9-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
33		9-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
34		10-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
35		10-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
36		10-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
37		11-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
38		11-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
39		11-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
40		12-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
41		12-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
42		12-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
43		13-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
44		13-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
45		13-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
46		14-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
47		14-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
48		14-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
49		15-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
50		15-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
51		15-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
52		16-1	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
53		16-2	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246
54		16-3	2	0.5	280	20302	20206	40508	0.1	201452	40356	0.0369	0.005	15	0.246

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot volume
6		% RE
7		0.98
8		1.02
9		1.04
10		0.98
11		0.98
12		0.98
13		1.01
14		0.99
15		1.01
16		1.01
17		0.99
18		1.01
19		0.99
20		1.01
21		1.03
22		1.00
23		1.00
24		1.00
25		0.99
26		1.01
27		0.99
28		1.01
29		1.00
30		1.00
31		1.00
32		1.00
33		0.99
34		1.01
35		0.99
36		1.01
37		0.99
38		1.01
39		0.99
40		1.01
41		1.00
42		1.00
43		1.00
44		1.00
45		0.98
46		1.02
47		1.00
48		1.00
49		0.99
50		1.01
51		0.99
52		1.01
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/1/2007	Chemical ID	CR11607	# Concentrations tested			8	Microsoma ID	12535-13
2		NB Ref.	12535-57	Replicate #	01MR2342 P00	1	Positive Control	4.0H ASDN	Pos CH Tubes/Conc		2
3		Technician ID									
4		Control Type	SD								
5		Portion									
6		Full activity	0.343								
7		Background									
8		Uncorrected	0.002								
9											
10		Positive Control									
11		4.0H ASDN	1	1.00E-05	5.00	0.003					
12		4.0H ASDN	2	1.00E-05	5.00	0.003					
13		4.0H ASDN	1	1.00E-06	6.00	0.025					
14		4.0H ASDN	2	1.00E-06	5.00	0.024					
15		4.0H ASDN	1	1.00E-07	6.00	0.039					
16		4.0H ASDN	2	1.00E-07	6.00	0.046					
17		4.0H ASDN	1	1.00E-08	7.00	0.141					
18		4.0H ASDN	2	1.00E-08	7.00	0.141					
19		4.0H ASDN	1	1.00E-09	8.00	0.353					
20		4.0H ASDN	2	1.00E-09	8.00	0.283					
21		4.0H ASDN	1	1.00E-10	10.00	0.357					
22		4.0H ASDN	2	1.00E-10	10.00	0.338					
23		4.0H ASDN	1	1.00E-11	10.00	0.357					
24		4.0H ASDN	2	1.00E-11	10.00	0.338					
25		4.0H ASDN	1	1.00E-12	10.00	0.357					
26		4.0H ASDN	2	1.00E-12	10.00	0.338					
27		4.0H ASDN	1	1.00E-13	10.00	0.357					
28		4.0H ASDN	2	1.00E-13	10.00	0.338					
29		4.0H ASDN	1	1.00E-14	10.00	0.357					
30		4.0H ASDN	2	1.00E-14	10.00	0.338					
31		4.0H ASDN	1	1.00E-15	10.00	0.357					
32		4.0H ASDN	2	1.00E-15	10.00	0.338					
33		4.0H ASDN	1	1.00E-16	10.00	0.357					
34		4.0H ASDN	2	1.00E-16	10.00	0.338					
35		4.0H ASDN	1	1.00E-17	10.00	0.357					
36		4.0H ASDN	2	1.00E-17	10.00	0.338					
37		Test Chemical									
38		CR11607	1	1.00E-03	3.00	0.006					
39		CR11607	2	1.00E-03	3.00	0.006					
40		CR11607	1	1.00E-04	4.00	0.007					
41		CR11607	2	1.00E-04	4.00	0.006					
42		CR11607	1	1.00E-05	5.00	0.007					
43		CR11607	2	1.00E-05	5.00	0.006					
44		CR11607	1	1.00E-06	6.00	0.007					
45		CR11607	2	1.00E-06	6.00	0.006					
46		CR11607	1	1.00E-07	7.00	0.007					
47		CR11607	2	1.00E-07	7.00	0.006					
48		CR11607	1	1.00E-08	8.00	0.007					
49		CR11607	2	1.00E-08	8.00	0.006					
50		CR11607	1	1.00E-09	9.00	0.007					
51		CR11607	2	1.00E-09	9.00	0.006					
52		CR11607	1	1.00E-10	10.00	0.007					
53		CR11607	2	1.00E-10	10.00	0.006					
54		CR11607	1	1.00E-11	10.00	0.007					
55		CR11607	2	1.00E-11	10.00	0.006					
56		CR11607	1	1.00E-12	10.00	0.007					
57		CR11607	2	1.00E-12	10.00	0.006					
58		CR11607	1	1.00E-13	10.00	0.007					
59		CR11607	2	1.00E-13	10.00	0.006					
60		CR11607	1	1.00E-14	10.00	0.007					
61		CR11607	2	1.00E-14	10.00	0.006					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/6/2007	Test Chemical ID	CR11607	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-60	Counter data filename	06MR2221.P00					
3	Technician		Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/6/2007	Test Chemical ID	CR11807	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-60	Counter data filename	06MR2221.P00					
3	Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0192		38974		2029896			
8	2	0.0198		35872		1811717			
9	3	0.0201		41920		2085572			
10	4	0.0190		40442		2128526			
11	5	0.0206		41626		2020680			
12						Average DPM/g soln		2015278	
13						SD		121908	
14						CV		6.05	
15						µCi/g soln		0.9078	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.0	10		1000.00				
20	Dilution A			100	10.00				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0698 g			
25	Mass of dilution B used in substrate prep					4.5476 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56353317 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.9078			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5635	+	0.011		
37				=	0.575	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=		1.580	µCi/µg ASDN					
41			1.004.E+06	dpm/nmol					

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/6/2007	Test Chemical ID	CR11607	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-60	Counter data filename	06MR2221.P00					
3			Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4											
5	Microsome Dilution Details										
6											
7				0.12 mL microsome Stock used					Positive Control Concentrations		
8				2.4 mL total volume					Level	Final Concentration (M)	
9				20 dilution factor					1	1.00E-05	
10									2	1.00E-06	
11				1.5 mL microsome Dilution A used					3	5.00E-07	
12				60 mL total volume					4	1.00E-07	
13				40 dilution factor					5	5.00E-08	
14									6	1.00E-08	
15				mL microsome Dilution B used					7	1.00E-09	
16				mL total volume					8	1.00E-10	
17				dilution factor							
18		NA							Test Chemical Concentrations		
19				800 (total dilution factor)					Level	Final Concentration (M)	
20									1	2.50E-04	
21									2	1.00E-04	
22				Protein Concentration (stock microsomes, mg/mL):					3	5.00E-05	
23				Protein Concentration (dilution added to assay, mg/mL):					4	2.50E-05	
24									5	1.00E-05	
25									6	5.00E-06	
26									7	1.00E-06	
									8	1.00E-07	

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Test Chemical # Concentrations A: 3/6/2007 ID CI tested B: Microsome ID 12535-13									
2	Counter data NI 12535-60 filename 06MR2221.P00									
3	T e c h n i c i a n i D ase Replicate # 2 Positive Control 4-OH ASD Tubes/Conc 2									
4										
5	Vial #	Ti	DPM1	tSIE			Round DPM			
6	2	5	38974.33	18859.32	0	14.15	612.38	38974		
7	3	5	35871.52	17371.72	0	14.13	611.04	35872		
8	4	5	41920.11	20399.88	0	14.19	616.22	41920		
9	5	5	40441.68	19569	0	14.07	609.29	40442		
10	6	5	41625.8	20235.4	0	14.19	615.66	41626		
11	8	5	12610.73	5640.08	0	12.9	526.55	12611		
12	9	5	12421.66	5606	0	12.86	529.9	12422		
13	10	5	14683.02	8570.88	0	12.93	529.69	14683		
14	11	5	14940.06	6679.4	0	12.92	531.17	14940		
15	12	5	14583.77	8528.52	0	12.94	529.19	14584		
16	13	5	14553.7	8558.4	0	12.89	529.92	14554		
17	14	5	11686.56	5261.04	0	12.85	529.7	11687		
18	15	5	12066.34	5423.32	0	12.83	527.77	12066		
19	17	5	53.6	22.04	0	12.52	531.13	54		
20	18	5	49.94	23.8	0	12.64	527.2	50		
21	19	5	60.93	27.6	0	12.03	529.39	61		
22	20	5	66.16	30.28	0	15.85	529.59	66		
23	21	5	55.34	23.4	0	12.27	529.4	55		
24	22	5	75	34.04	0	13.82	529.47	75		
25	23	5	67.54	28.64	0	14.32	531.1	68		
26	24	5	59.88	26.64	0	11.71	529.77	60		
27	26	5	168.52	71.84	0	12.8	530.7	169		
28	27	5	150.07	69.6	0	11.95	530.47	150		
29	28	5	165.79	71.04	0	12.89	530.5	166		
30	29	5	154.33	67.52	0	13.25	532.59	154		
31										
32										
33	30	5	949.62	426.28	0	12.91	529.22	950		
34	31	5	996.91	440.04	0	12.98	530.23	997		
35	32	5	1001.27	446.8	0	12.98	530.23	1001		
36	33	5	959.45	427.6	0	12.98	528.9	959		
37										
38										
39	34	5	1664.24	746.28	0	13.06	530.14	1664		
40	35	5	1694.9	772.2	0	12.86	530.27	1695		
41	36	5	1668.53	746.6	0	13.21	531.69	1669		
42	37	5	1607	725.2	0	12.87	529	1607		
43										
44										
45	38	5	5708.99	2571.4	0	12.76	529.19	5709		
46	39	5	5796.62	2809.2	0	12.88	530.64	5797		
47	40	5	5458.54	2466.2	0	12.88	531.72	5459		
48	41	5	5638.34	2527.8	0	12.9	529.62	5638		
49										
50										
51	42	5	8392.65	3783.44	0	12.9	530.32	8393		
52	43	5	8602.33	3832.4	0	12.95	527.38	8602		
53	44	5	8027.88	3574.6	0	12.69	528.74	8028		
54	45	5	8051.19	3820.4	0	12.87	530.05	8051		
55										
56										
57	46	5	12750.02	5717	0	12.86	529.43	12750		
58	47	5	13080.49	5838.16	0	12.93	527.49	13080		
59	48	5	12528.92	5822.08	0	12.82	530.38	12529		
60	49	5	12534.58	5608.92	0	12.93	529.16	12535		
61										
62										
63	50	5	14059.08	6348.44	0	13.01	535.83	14059		
64	51	5	14365.75	6450.6	0	12.89	532.5	14366		

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	14238.05	6416	0	12.87	530.15				14238
66	53	5	14136.2	6371.64	0	12.94	532.27				14136
67											
68											
69	54	5	14169.4	6452.64	0	12.89	533.26				14169
70	55	5	14440.99	6482.4	0	12.92	531.09				14441
71	56	5	14872.72	6709.44	0	12.87	530.93				14873
72	57	5	14772.88	6679.84	0	12.9	532.79				14773
73											
74											
75	59	5	1617.18	729.2	0	12.85	531.52				1617
76	60	5	1587.99	707.88	0	13.05	531.71				1588
77	61	5	1550.25	708.28	0	12.82	531.43				1550
78	62	5	1558.4	695.64	0	13.09	530.83				1558
79	63	5	1527.67	703.8	0	12.43	530.4				1528
80	64	5	1548.93	698.8	0	12.99	527.8				1550
81	65	5	3388.28	1524.44	0	12.94	531				3388
82	66	5	3422.32	1546.8	0	12.83	530.06				3422
83	67	5	3518.18	1576.72	0	12.9	530.59				3518
84	68	5	3522.27	1578.88	0	12.92	530.45				3522
85	69	5	3362.12	1502.2	0	12.95	531.58				3362
86	70	5	3399.18	1534.2	0	12.8	530.56				3399
87	71	5	5763.66	2593.84	0	12.99	531.6				5764
88	72	5	5884.44	2640.84	0	12.85	531.3				5884
89	73	5	5600.39	2545.24	0	12.89	531.3				5600
90	74	5	5584.32	2512.48	0	12.88	528.88				5584
91	75	5	5484.22	2454.8	0	12.93	529.93				5484
92	76	5	5418.78	2442.64	0	12.82	530.76				5419
93	77	5	7855.25	3538.4	0	12.89	529.26				7855
94	78	5	8019.23	3614	0	12.96	533.92				8019
95	79	5	7960.52	3563	0	12.98	530.88				7961
96	80	5	7854.22	3546.24	0	12.81	530.09				7854
97	81	5	7562.21	3401.6	0	12.93	530.5				7562
98	82	5	7544.45	3389.2	0	12.93	529.83				7544
99	84	5	10470.04	4685.68	0	13	530.62				10470
100	85	5	10415.66	4667.88	0	12.94	530.54				10416
101	86	5	10254.92	4606.64	0	12.9	530.91				10255
102	87	5	10266.52	4633.52	0	12.86	531.86				10267
103	88	5	10385.73	4676.84	0	12.98	531.98				10386
104	89	5	10670.79	4774.04	0	12.87	527.63				10671
105	90	5	11758.08	5272.52	0	12.99	532.89				11758
106	91	5	12305.29	5497.6	0	12.88	528.84				12305
107	92	5	11465.65	5159.2	0	12.94	534.08				11466
108	93	5	11534.75	5175.72	0	12.93	532.25				11535
109	94	5	11710.19	5222.4	0	12.95	528.46				11710
110	95	5	11598.56	5186.6	0	12.97	528.52				11599
111	96	5	13281.03	5936.44	0	12.97	529.81				13281
112	97	5	13469.27	6060.64	0	12.91	529.74				13469
113	98	5	13164.81	5910.44	0	12.96	529.12				13165
114	99	5	12958.02	5839.6	0	12.95	532.99				12958
115	100	5	13053.3	5837.08	0	13	532.19				13053
116	101	5	13213.66	5917.8	0	12.97	530.82				13214
117	102	5	13671.76	6133.6	0	12.91	529.5				13672
118	103	5	13933.51	6259.64	0	12.95	530.84				13934
119	104	5	13992.73	6239.2	0	12.93	529.78				13993
120	105	5	14031.5	6316.48	0	12.93	531.03				14032
121	106	5	12871.68	5788.84	0	12.94	531.17				12872
122	107	5	13214.04	5920.44	0	12.93	529.53				13214
123	109	5	232917.3	53106.96	0	9.06	246.88				232917

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance			
6		Real T value not within mean $\pm$ 10%			
7		1.01			
8		0.99			
9		0.99			
10		1.01			
11		1.00			
12		1.00			
13		0.99			
14		1.02			
15		1.04			
16		0.96			
17		1.04			
18		1.04			
19		0.98			
20		1.06			
21		1.06			
22		0.94			
23		1.06			
24		0.94			
25		1.04			
26		0.96			
27		0.98			
28		0.98			
29		1.02			
30		1.02			
31		0.98			
32		0.98			
33		0.98			
34		0.99			
35		0.99			
36		1.01			
37		1.02			
38		0.98			
39		0.98			
40		0.98			
41		1.01			
42		0.98			
43		0.98			
44		1.02			
45		0.98			
46		0.98			
47		1.01			
48		1.00			
49		1.00			
50		1.00			
51		0.99			
52		1.01			
53		1.00			
54		1.00			
55		1.00			
56		0.99			
57		1.01			
58		1.00			
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63		0.99			
64		1.01			
65		1.01			
66		1.01			
67		1.00			
68		1.00			
69		1.00			
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Refill/Assay	Volume (mL)	Aliq #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPH	Volume of substrate solution used (mL)	Total DPH in assay tube (background)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estradiol formed/mg protein/min)
1	CR11607	36/2007	0.5	1	1817	3224	3205	6410	0.1	201528	5766	0.0061	0.005	15	0.041
2	CR11607	36/2007	0.5	2	1508	3176	3108	6216	0.1	201528	5972	0.0059	0.005	15	0.040
3	CR11607	36/2007	0.5	3	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
4	CR11607	36/2007	0.5	4	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
5	CR11607	36/2007	0.5	5	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
6	CR11607	36/2007	0.5	6	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
7	CR11607	36/2007	0.5	7	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
8	CR11607	36/2007	0.5	8	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
9	CR11607	36/2007	0.5	9	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
10	CR11607	36/2007	0.5	10	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
11	CR11607	36/2007	0.5	11	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
12	CR11607	36/2007	0.5	12	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
13	CR11607	36/2007	0.5	13	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
14	CR11607	36/2007	0.5	14	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
15	CR11607	36/2007	0.5	15	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
16	CR11607	36/2007	0.5	16	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
17	CR11607	36/2007	0.5	17	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
18	CR11607	36/2007	0.5	18	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
19	CR11607	36/2007	0.5	19	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
20	CR11607	36/2007	0.5	20	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
21	CR11607	36/2007	0.5	21	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
22	CR11607	36/2007	0.5	22	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
23	CR11607	36/2007	0.5	23	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
24	CR11607	36/2007	0.5	24	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
25	CR11607	36/2007	0.5	25	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
26	CR11607	36/2007	0.5	26	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
27	CR11607	36/2007	0.5	27	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
28	CR11607	36/2007	0.5	28	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
29	CR11607	36/2007	0.5	29	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
30	CR11607	36/2007	0.5	30	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
31	CR11607	36/2007	0.5	31	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
32	CR11607	36/2007	0.5	32	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
33	CR11607	36/2007	0.5	33	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
34	CR11607	36/2007	0.5	34	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
35	CR11607	36/2007	0.5	35	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
36	CR11607	36/2007	0.5	36	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
37	CR11607	36/2007	0.5	37	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
38	CR11607	36/2007	0.5	38	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
39	CR11607	36/2007	0.5	39	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
40	CR11607	36/2007	0.5	40	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
41	CR11607	36/2007	0.5	41	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
42	CR11607	36/2007	0.5	42	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
43	CR11607	36/2007	0.5	43	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
44	CR11607	36/2007	0.5	44	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
45	CR11607	36/2007	0.5	45	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
46	CR11607	36/2007	0.5	46	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
47	CR11607	36/2007	0.5	47	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
48	CR11607	36/2007	0.5	48	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
49	CR11607	36/2007	0.5	49	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
50	CR11607	36/2007	0.5	50	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
51	CR11607	36/2007	0.5	51	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
52	CR11607	36/2007	0.5	52	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
53	CR11607	36/2007	0.5	53	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039
54	CR11607	36/2007	0.5	54	1508	3108	3108	6216	0.1	201528	5912	0.0059	0.005	15	0.039

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot Variance
6		% RE
7		1.01
8		0.99
9		1.00
10		0.99
11		0.99
12		1.01
13		1.00
14		1.00
15		1.00
16		1.00
17		0.99
18		1.01
19		0.99
20		1.01
21		1.00
22		1.00
23		1.01
24		0.99
25		0.99
26		1.01
27		1.01
28		0.99
29		1.00
30		1.00
31		1.00
32		1.00
33		1.00
34		1.00
35		0.99
36		1.01
37		0.99
38		1.00
39		1.00
40		1.00
41		1.00
42		1.00
43		0.99
44		1.01
45		1.01
46		0.99
47		0.99
48		1.01
49		0.99
50		1.01
51		1.00
52		1.00
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/6/2007	Chemical ID	CR11607	# Concentrations tested			8	Microsoma ID	12535-13
2		MB Ref.	12535-60	Reagent	05MR2221.P00	2	Positive Control	4-OH ASDN			
3		Technician ID		Replicate #							2
4											
5	Control Type	Average	SD								
6	Full activity	0.355	0.039								
7	Background (uncorrected)	0.002	0.000								
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.003						
12	4-OH ASDN	1	2	-5.00	0.003						
13	4-OH ASDN	2	1	-6.00	0.024						
14	4-OH ASDN	2	2	-6.00	0.024						
15	4-OH ASDN	3	1	-6.30	0.043						
16	4-OH ASDN	3	2	-6.30	0.042						
17	4-OH ASDN	4	1	-7.00	0.151						
18	4-OH ASDN	4	2	-7.00	0.146						
19	4-OH ASDN	5	1	-7.30	0.224						
20	4-OH ASDN	5	2	-7.30	0.212						
21	4-OH ASDN	6	1	-8.00	0.341						
22	4-OH ASDN	6	2	-8.00	0.331						
23	4-OH ASDN	7	1	-9.00	0.376						
24	4-OH ASDN	7	2	-9.00	0.375						
25	4-OH ASDN	8	1	-10.00	0.378						
26	4-OH ASDN	8	2	-10.00	0.392						
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical) M	Activity						
38	CR11607	1	1	-3.60	0.041						
39	CR11607	1	2	-3.60	0.040						
40	CR11607	1	3	-3.60	0.039						
41	CR11607	2	1	-4.00	0.089						
42	CR11607	2	2	-4.00	0.092						
43	CR11607	2	3	-4.00	0.088						
44	CR11607	3	1	-4.30	0.153						
45	CR11607	3	2	-4.30	0.147						
46	CR11607	3	3	-4.30	0.143						
47	CR11607	4	1	-4.60	0.208						
48	CR11607	4	2	-4.60	0.206						
49	CR11607	4	3	-4.60	0.199						
50	CR11607	5	1	-5.00	0.276						
51	CR11607	5	2	-5.00	0.271						
52	CR11607	5	3	-5.00	0.278						
53	CR11607	6	1	-5.30	0.316						
54	CR11607	6	2	-5.30	0.304						
55	CR11607	6	3	-5.30	0.308						
56	CR11607	7	1	-6.00	0.353						
57	CR11607	7	2	-6.00	0.345						
58	CR11607	7	3	-6.00	0.347						
59	CR11607	8	1	-7.00	0.385						
60	CR11607	8	2	-7.00	0.370						
61	CR11607	8	3	-7.00	0.345						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/7/2007	Test Chemical ID	CR11607	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-63	Counter data filename	07MR2157.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/7/2007	Test Chemical ID	CR11607	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-63	Counter data filename	07MR2157.P00				Pos Ctl	
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN		Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0201		35163		1749403			
8	2	0.0201		39308		1955622			
9	3	0.0200		38868		1943400			
10	4	0.0199		39633		1991608			
11	5	0.0201		39012		1940898			
12						Average DPM/g soln		1916186	
13						SD		95409	
14						CV		4.98	
15						µCi/g soln		0.8631	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0357 g			
25	Mass of dilution B used in substrate prep					4.5348 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56376734 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8631			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5638	+	0.011		
37				=	0.574	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.503	µCi/µg ASDN			
41					9.556.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/7/2007	Test Chemical ID	CR11607	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-63	Counter data filename	07MR2157.P00				Pos Ctl	
3			Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN		Tubes/Conc	2
4											
5	Microsome Dilution Details							Positive Control Concentrations			
6								Level	Final Concentration (M)		
7	Dilution A			0.12 mL microsome Stock used				1	1.00E-05		
8				2.4 mL total volume				2	1.00E-06		
9				20 dilution factor				3	5.00E-07		
10								4	1.00E-07		
11	Dilution B			1.5 mL microsome Dilution A used				5	5.00E-08		
12				60 mL total volume				6	1.00E-08		
13				40 dilution factor				7	1.00E-09		
14								8	1.00E-10		
15	Dilution C (if applicable)			mL microsome Dilution B used							
16				mL total volume							
17		NA		dilution factor							
18											
19				800 total dilution factor							
20											
21	Protein Concentration (stock microsomes, mg/mL):				8.00			Test Chemical Concentrations			
22	Protein Concentration (dilution added to assay, mg/mL):				0.01			Level	Final Concentration (M)		
23								1	2.50E-04		
24								2	1.00E-04		
25								3	5.00E-05		
26								4	2.50E-05		
								5	1.00E-05		
								6	5.00E-06		
								7	1.00E-06		
								8	1.00E-07		

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Test Chemical # Concentrations Ac 3/7/2007 ID Ct tested 8 Microsome ID 12535-13									
2		Counter data filename 07MR2157.P00									
3		Pos Ctl Replicate # 3 Positive Control 4-OH ASDI Tubes/Conc 2									
4											
5		Vial # Ti DPM1 tSIE Round DPM									
6		2	5	35162.71	17135.3	0	14.18	618.63			35163
7		3	5	39308.43	19149.78	0	14.11	615.73			39308
8		4	5	38868.05	18852.78	0	14.13	612.38			38868
9		5	5	39632.77	19377.58	0	14.12	617.39			39633
10		6	5	39011.77	19047.3	0	14.16	618.25			39012
11		8	5	12572.43	5662.74	0	12.93	533.02			12572
12		9	5	12551.62	5681.3	0	13.01	536.48			12552
13		10	5	12677.42	5726.74	0	12.95	532.63			12677
14		11	5	12530.87	5652.18	0	12.88	536.09			12531
15		12	5	11510.78	5203.9	0	12.94	536.03			11511
16		13	5	11363.74	5127.78	0	12.91	534.53			11364
17		14	5	11434.2	5150.1	0	12.98	536.33			11434
18		15	5	11418.78	5183.74	0	12.96	537.97			11419
19		17	5	62.53	28.14	0	14.1	536.75			63
20		18	5	64	28.7	0	13.98	531.96			64
21		19	5	65.58	31.14	0	12.82	533.96			66
22		20	5	70.41	33.74	0	13.72	535.44			70
23		21	5	62.83	27.14	0	13.16	539.36			63
24		22	5	61.47	27.9	0	11.4	535.09			61
25		23	5	81.4	27.5	0	14.17	535.83			81
26		24	5	101.13	47.1	0	15.09	536.45			101
27		26	5	147.57	65.54	0	12.9	534.46			148
28		27	5	158	70.9	0	13.39	532.15			158
29		28	5	155.63	67.14	0	13.58	533.19			156
30		29	5	152.24	71.3	0	13.01	535.32			152
31											
32											
33		30	5	869.48	394.3	0	12.94	535.1			869
34		31	5	898.04	406.98	0	12.83	534.22			898
35		32	5	858.95	384.34	0	12.95	533.92			859
36		33	5	846.99	390.5	0	12.91	539.6			847
37											
38											
39		34	5	1471.14	653.1	0	12.92	534.99			1471
40		35	5	1537.64	699.5	0	12.93	536.85			1538
41		36	5	1445.14	658.94	0	12.85	536.27			1445
42		37	5	1472	660.7	0	13.05	537.06			1472
43											
44											
45		38	5	4110.8	1875.34	0	12.94	536			4111
46		39	5	4202.75	1896.1	0	13.01	538.53			4203
47		40	5	4457.88	1997.74	0	12.86	530.01			4458
48		41	5	4336.04	1970.34	0	12.94	534.98			4336
49											
50											
51		42	5	6605.76	3018.42	0	12.89	534.97			6606
52		43	5	6808.55	3068.7	0	13.02	537.15			6809
53		44	5	6564.53	2980.34	0	12.94	535.95			6565
54		45	5	6497.29	2964.58	0	12.91	536.94			6497
55											
56											
57		46	5	10624.24	4791.9	0	12.96	533.81			10624
58		47	5	10495.11	4743.54	0	12.91	532.58			10496
59		48	5	9731.05	4400.54	0	12.96	534.28			9731
60		49	5	9668.18	4487.7	0	12.86	535.24			9668
61											
62											
63		50	5	10484.59	4745.14	0	13.01	537.26			10485
64		51	5	10429.33	4725.54	0	12.91	537.3			10429

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	10769.7	4865.18	0	12.97	534.32				10770
66	53	5	10741.62	4818.48	0	12.97	533.48				10742
67											
68											
69	54	5	11368.07	5128.34	0	13	535.92				11368
70	55	5	11233.15	5090.14	0	12.94	536.46				11233
71	56	5	9751.88	4410.78	0	12.91	534.56				9752
72	57	5	9786.42	4410.98	0	12.89	532.72				9786
73											
74											
75	59	5	1370.36	617.98	0	12.88	534.82				1370
76	60	5	1399.66	629.26	0	13.09	537.78				1400
77	61	5	1321.22	609.3	0	12.71	535.72				1321
78	62	5	1367.52	614.1	0	12.98	536.06				1368
79	63	5	1360.35	625.14	0	12.87	534.26				1360
80	64	5	1399.71	630.3	0	12.82	534.98				1400
81	65	5	3094.29	1400.3	0	12.84	534.94				3094
82	66	5	3276.2	1468.14	0	13.01	532.73				3276
83	67	5	3124.66	1407.14	0	12.94	536.21				3125
84	68	5	3107.54	1402.1	0	12.83	534.72				3108
85	69	5	3386.15	1510.9	0	12.94	532.73				3386
86	70	5	3211.2	1466.34	0	12.91	535.29				3211
87	71	5	4901.53	2205.9	0	12.86	533.07				4902
88	72	5	5064.84	2283.9	0	12.96	536.05				5065
89	73	5	4941.02	2235.5	0	12.93	535.75				4941
90	74	5	4881.93	2188.78	0	13.03	535.03				4882
91	75	5	5009.29	2264.3	0	13.03	534.29				5009
92	76	5	4966.37	2251.7	0	12.98	535.4				4966
93	77	5	7014.45	3193.98	0	12.97	540.2				7014
94	78	5	6967.26	3164.14	0	12.88	534.09				6967
95	79	5	6755.87	3055.58	0	13.03	534.92				6756
96	80	5	6956.64	3145.54	0	12.89	535.39				6957
97	81	5	7034.91	3162.1	0	13.04	534.44				7035
98	82	5	7135.67	3216.34	0	13.03	536.22				7136
99	84	5	9203.85	4164.34	0	12.92	533.69				9204
100	85	5	9429.93	4255.54	0	12.96	534.36				9430
101	86	5	8901.53	4003.34	0	12.92	532.11				8902
102	87	5	9420.62	4208.1	0	13.03	531.95				9421
103	88	5	8837.19	3986.74	0	12.88	533.67				8837
104	89	5	9116.5	4100.58	0	12.93	535.32				9117
105	90	5	10624.31	4812.14	0	12.96	535.8				10624
106	91	5	10793.65	4869.58	0	12.99	535.84				10794
107	92	5	8669.94	3949.7	0	12.95	536.99				8670
108	93	5	8716.98	3935.94	0	13	534.93				8717
109	94	5	10161.26	4566.18	0	12.99	533.69				10161
110	95	5	10494.71	4675.38	0	13.08	532.81				10495
111	96	5	11144.03	5025.3	0	12.97	534.78				11144
112	97	5	11293.77	5086.34	0	12.91	532.05				11294
113	98	5	11602.71	5263.78	0	12.95	536.78				11603
114	99	5	12012.88	5418.38	0	12.93	534.7				12013
115	100	5	10968.57	4913.18	0	12.99	537.42				10969
116	101	5	10851.2	4961.3	0	12.92	534.42				10851
117	102	5	11802.2	5344.1	0	12.86	531.36				11802
118	103	5	12041.95	5496.74	0	12.92	536.86				12042
119	104	5	11629.27	5260.34	0	12.95	534.89				11629
120	105	5	11920.01	5390.74	0	12.87	532.19				11920
121	106	5	10809.75	4902.3	0	12.85	533.2				10810
122	107	5	11131.82	5072.1	0	12.91	537.58				11132
123	109	5	229515.95	53747.74	0	9.12	251.89				229516

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance			
6		Red B value not within 10% of 10%			
7		1.00			
8		1.00			
9		1.01			
10		0.99			
11		1.01			
12		0.99			
13		1.00			
14		1.00			
15		0.99			
16		1.01			
17		0.97			
18		1.03			
19		1.02			
20		0.98			
21		0.97			
22		0.97			
23		1.03			
24		1.01			
25		0.99			
26		0.99			
27		0.99			
28		0.98			
29		0.98			
30		1.02			
31		1.01			
32		0.99			
33		0.98			
34		0.98			
35		1.02			
36		0.99			
37		1.01			
38		1.01			
39		0.99			
40		0.99			
41		1.01			
42		1.01			
43		0.99			
44		0.99			
45		1.01			
46		0.98			
47		1.02			
48		1.01			
49		0.99			
50		0.99			
51		1.01			
52		1.01			
53		0.99			
54		0.99			
55		0.99			
56		1.01			
57		1.01			
58		1.00			
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63		1.01			
64		1.01			
65		0.99			
66		1.00			
67		1.00			
68		1.00			
69		1.00			
70		1.00			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1	Assay Date			3/7/2007	Chemical ID	CR11907	# Concentrations tested			Microsome ID	107935-13					
2	NR Rxn			12535-63	Assay data	07MR2157 PRO										
3	Technician ID				Replicate #	3	Positive Control			4-OH ASDM	Pos Ctrl Conc					
4																
5	Sample ID				Calculate DPM in aqueous portion after extraction											
6	Sample type	Replicator Level	Nominal total volume (mL)	Aliq volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (m/mL)	Total DPM corrected for background (background tubes)	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
7	CR11607	1-1	2	0.5	1	1370	2740	2770	5540	0.1	191619	5289	0.0055	0.005	15	0.037
8				0.5	2	1400	2800				191619	5104	0.0053	0.005	15	0.036
9		1-2	2	0.5	1	1321	2642	2689	5378	0.1	191619	5248	0.0056	0.005	15	0.037
10				0.5	2	1388	2736				191619	5248	0.0056	0.005	15	0.037
11		1-3	2	0.5	1	1360	2720	2760	5520	0.1	191619	5248	0.0056	0.005	15	0.037
12				0.5	2	1360	2720				191619	5248	0.0056	0.005	15	0.037
13		2-1	2	0.5	1	1370	2740	2760	5520	0.1	191619	5248	0.0056	0.005	15	0.037
14				0.5	2	1370	2740				191619	5248	0.0056	0.005	15	0.037
15		2-2	2	0.5	1	1370	2740	2760	5520	0.1	191619	5248	0.0056	0.005	15	0.037
16				0.5	2	1370	2740				191619	5248	0.0056	0.005	15	0.037
17		2-3	2	0.5	1	1386	2772	2807	5614	0.1	191619	5248	0.0056	0.005	15	0.037
18				0.5	2	1386	2772				191619	5248	0.0056	0.005	15	0.037
19		3-1	2	0.5	1	1402	2804	2837	5674	0.1	191619	5248	0.0056	0.005	15	0.037
20				0.5	2	1402	2804				191619	5248	0.0056	0.005	15	0.037
21		5-2	2	0.5	1	1411	2822	2853	5704	0.1	191619	5248	0.0056	0.005	15	0.037
22				0.5	2	1411	2822				191619	5248	0.0056	0.005	15	0.037
23		3-3	2	0.5	1	1429	2858	2889	5778	0.1	191619	5248	0.0056	0.005	15	0.037
24				0.5	2	1429	2858				191619	5248	0.0056	0.005	15	0.037
25		4-1	2	0.5	1	1447	2894	2925	5848	0.1	191619	5248	0.0056	0.005	15	0.037
26				0.5	2	1447	2894				191619	5248	0.0056	0.005	15	0.037
27		4-2	2	0.5	1	1465	2930	2961	5912	0.1	191619	5248	0.0056	0.005	15	0.037
28				0.5	2	1465	2930				191619	5248	0.0056	0.005	15	0.037
29		4-3	2	0.5	1	1483	2966	3000	5982	0.1	191619	5248	0.0056	0.005	15	0.037
30				0.5	2	1483	2966				191619	5248	0.0056	0.005	15	0.037
31		5-1	2	0.5	1	1501	3002	3033	6062	0.1	191619	5248	0.0056	0.005	15	0.037
32				0.5	2	1501	3002				191619	5248	0.0056	0.005	15	0.037
33		5-2	2	0.5	1	1519	3038	3069	6136	0.1	191619	5248	0.0056	0.005	15	0.037
34				0.5	2	1519	3038				191619	5248	0.0056	0.005	15	0.037
35		5-3	2	0.5	1	1537	3074	3105	6210	0.1	191619	5248	0.0056	0.005	15	0.037
36				0.5	2	1537	3074				191619	5248	0.0056	0.005	15	0.037
37		6-1	2	0.5	1	1555	3110	3141	6284	0.1	191619	5248	0.0056	0.005	15	0.037
38				0.5	2	1555	3110				191619	5248	0.0056	0.005	15	0.037
39		6-2	2	0.5	1	1573	3146	3177	6358	0.1	191619	5248	0.0056	0.005	15	0.037
40				0.5	2	1573	3146				191619	5248	0.0056	0.005	15	0.037
41		6-3	2	0.5	1	1591	3182	3213	6432	0.1	191619	5248	0.0056	0.005	15	0.037
42				0.5	2	1591	3182				191619	5248	0.0056	0.005	15	0.037
43		7-1	2	0.5	1	1609	3218	3249	6506	0.1	191619	5248	0.0056	0.005	15	0.037
44				0.5	2	1609	3218				191619	5248	0.0056	0.005	15	0.037
45		7-2	2	0.5	1	1627	3254	3285	6580	0.1	191619	5248	0.0056	0.005	15	0.037
46				0.5	2	1627	3254				191619	5248	0.0056	0.005	15	0.037
47		7-3	2	0.5	1	1645	3290	3321	6654	0.1	191619	5248	0.0056	0.005	15	0.037
48				0.5	2	1645	3290				191619	5248	0.0056	0.005	15	0.037
49		8-1	2	0.5	1	1663	3326	3357	6728	0.1	191619	5248	0.0056	0.005	15	0.037
50				0.5	2	1663	3326				191619	5248	0.0056	0.005	15	0.037
51		8-2	2	0.5	1	1681	3362	3393	6802	0.1	191619	5248	0.0056	0.005	15	0.037
52				0.5	2	1681	3362				191619	5248	0.0056	0.005	15	0.037
53		8-3	2	0.5	1	1699	3398	3429	6876	0.1	191619	5248	0.0056	0.005	15	0.037
54				0.5	2	1699	3398				191619	5248	0.0056	0.005	15	0.037

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC Check - aliquot variance
5		
6		% RE
7		0.99
8		1.01
9		0.98
10		1.02
11		0.99
12		1.01
13		0.97
14		1.03
15		1.00
16		1.00
17		1.03
18		0.97
19		0.98
20		1.02
21		1.01
22		0.99
23		1.00
24		1.00
25		1.00
26		0.99
27		1.01
28		0.98
29		1.01
30		1.01
31		0.99
32		1.01
33		0.97
34		1.03
35		0.98
36		1.02
37		0.99
38		1.01
39		1.00
40		1.00
41		0.99
42		1.02
43		0.98
44		1.01
45		0.98
46		1.02
47		1.00
48		1.00
49		0.98
50		1.01
51		0.99
52		1.01
53		0.98
54		1.01

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L
1			Assay Date	3/7/2007	Chemical ID	CR11607	# Concentrations tested			8	Microsome ID	12535-13
2			NB Ref. technician ID	12535-43	Counter data filename	07MR2157.P00	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
3					Replicate #							
4			Average	SD								
5	Control Type	Portion										
6	Full activity	Overall	0.333	0.019	Bkg % of Full							
7	Background	Overall	0.002	0.000			0.6 (Red If >1%)					
8	(uncorrected)											
9												
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity							
11	4-OH ASDN	1	1	-5.00	0.002							
12	4-OH ASDN	1	2	1.00E-05	0.002							
13												
14	4-OH ASDN	2	1	1.00E-06	0.023							
15	4-OH ASDN	2	2	1.00E-06	0.022							
16												
17	4-OH ASDN	3	1	5.00E-07	0.040							
18	4-OH ASDN	3	2	5.00E-07	0.039							
19												
20	4-OH ASDN	4	1	1.00E-07	0.114							
21	4-OH ASDN	4	2	1.00E-07	0.121							
22												
23	4-OH ASDN	5	1	5.00E-08	0.185							
24	4-OH ASDN	5	2	5.00E-08	0.180							
25												
26	4-OH ASDN	6	1	1.00E-08	0.253							
27	4-OH ASDN	6	2	1.00E-08	0.272							
28												
29	4-OH ASDN	7	1	1.00E-09	0.290							
30	4-OH ASDN	7	2	1.00E-09	0.288							
31												
32	4-OH ASDN	8	1	1.00E-10	0.313							
33	4-OH ASDN	8	2	1.00E-10	0.271							
34												
35												
36												
37	Test Chemical	Level	Replicate	Log(Positive Ctl)	Activity							
38	CR11607	1	1	2.50E-04	0.037							
39	CR11607	1	2	2.50E-04	0.036							
40	CR11607	1	3	2.50E-04	0.037							
41	CR11607	2	1	1.00E-04	0.087							
42	CR11607	2	2	1.00E-04	0.086							
43	CR11607	2	3	1.00E-04	0.090							
44	CR11607	3	1	5.00E-05	0.137							
45	CR11607	3	2	5.00E-05	0.135							
46	CR11607	3	3	5.00E-05	0.137							
47	CR11607	4	1	2.50E-05	0.193							
48	CR11607	4	2	2.50E-05	0.189							
49	CR11607	4	3	2.50E-05	0.186							
50	CR11607	5	1	1.00E-05	0.258							
51	CR11607	5	2	1.00E-05	0.254							
52	CR11607	5	3	1.00E-05	0.249							
53	CR11607	6	1	5.00E-06	0.297							
54	CR11607	6	2	5.00E-06	0.241							
55	CR11607	6	3	5.00E-06	0.286							
56	CR11607	7	1	1.00E-06	0.311							
57	CR11607	7	2	1.00E-06	0.328							
58	CR11607	7	3	1.00E-06	0.303							
59	CR11607	8	1	1.00E-07	0.331							
60	CR11607	8	2	1.00E-07	0.327							
61	CR11607	8	3	1.00E-07	0.304							
62												
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Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/16/2007	Test Chemical ID	CR11608	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-73	Counter data filename	16MR2113.P00					
3	Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/16/2007	Test Chemical ID	CR11608	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-73	Counter data filename	16MR2113.P00					
3	Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0212		40768		1923019			
8	2	0.0204		37998		1862696			
9	3	0.0200		38643		1932150			
10	4	0.0200		40055		2002750			
11	5	0.0200		40047		2002350			
12						Average DPM/g soln		1944593	
13						SD		59257	
14						CV		3.05	
15						µCi/g soln		0.8759	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0890 g			
25	Mass of dilution B used in substrate prep					4.5514 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56210268 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8759			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5621	+	0.011		
37				=	0.573	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=	1.529	µCi/µg ASDN						
41		9.723 E+05	dpm/nmol						

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/16/2007	Test Chemical ID	CR11608	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-73	Counter data filename	16MR2113.P00					
3			Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctrl Tubes/Conc	2
4											
5	Microsome Dilution Details							Positive Control Concentrations			
6								Level	Final Concentration (M)		
7	Dilution A			0.12 mL microsome Stock used				1	1.00E-05		
8				2.4 mL total volume				2	1.00E-06		
9				20 dilution factor				3	5.00E-07		
10								4	1.00E-07		
11	Dilution B			1.5 mL microsome Dilution A used				5	5.00E-08		
12				60 mL total volume				6	1.00E-08		
13				40 dilution factor				7	1.00E-09		
14								8	1.00E-10		
15	Dilution C (if applicable)			mL microsome Dilution B used							
16				mL total volume							
17		NA		dilution factor				Test Chemical Concentrations			
18								Level	Final Concentration (M)		
19				800 total dilution factor				1	1.00E-03		
20								2	1.00E-04		
21	Protein Concentration (stock microsomes, mg/mL):				8.00			3	1.00E-05		
22	Protein Concentration (dilution added to assay, mg/mL):				0.01			4	1.00E-06		
23								5	1.00E-07		
24								6	1.00E-08		
25								7	1.00E-09		
26								8	1.00E-10		

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	As: 3/16/2007	Chemical ID	Concentrations	Cl	tested	8	Microsome ID	12535-13		
2	NB 12535-73	Counter data filename	16MR2113.P00							
3	ch	Replicate	Positive		Pos Cl					
4	nic SLB	#	1	Control	4-OH ASDN	Tubes/Conc	2			
5	Vial	Tin	DPM1		ISIE					Round DPM
6	2	5	40768.47	18040.39	0	12.01	487.72			40768
7	3	5	37998.86	16570.9	0	11.89	478.96			37999
8	4	5	38642.57	18786.12	0	11.91	478.64			38643
9	5	5	40055.46	17513.86	0	11.91	479.16			40055
10	6	5	40047.2	17399.96	0	11.87	477.87			40047
11	8	5	16208.25	7517.12	0	12.78	533.37			16208
12	9	5	16260.28	7572.33	0	12.76	537.67			16260
13	10	5	15342.52	7156.58	0	12.71	537.81			15343
14	11	5	15362.89	7129.51	0	12.75	534.29			15363
15	12	5	15818.27	7379.18	0	12.72	534.72			15818
16	13	5	15647.02	7319.12	0	12.66	534.24			15647
17	14	5	15760.14	7326.67	0	12.74	533.77			15750
18	15	5	15768.31	7354.27	0	12.72	534.63			15768
19	16	5	62.69	28.83	0	14.34	535.09			63
20	17	5	57.98	26.03	0	12.24	534.37			58
21	18	5	67.02	31.73	0	13.43	535.71			67
22	19	5	57.65	30.41	0	12.49	532.38			58
23	20	5	75.11	33.97	0	12.11	535.33			75
24	21	5	62.77	26.11	0	14	535.7			63
25	22	5	54.95	28.88	0	9.97	536.04			55
26	23	5	63.73	33.75	0	12.32	536.58			64
27	25	5	185.77	94.04	0	12.15	533.93			196
28	26	5	184.11	89.87	0	13.2	536.21			184
29	27	5	179.56	85.52	0	11.68	535.49			180
30	28	5	180	84.37	0	12.38	536.02			180
31										
32										
33	29	5	996.47	470.56	0	12.69	536.38			996
34	30	5	982.88	455.96	0	12.75	535.37			983
35	31	5	1017.91	471.42	0	12.74	534.83			1018
36	32	5	993.08	467.16	0	12.66	533.51			993
37										
38										
39	33	5	1841.31	854.68	0	12.84	534.16			1841
40	34	5	1772.95	829	0	12.52	535.18			1773
41	35	5	1766.81	828.62	0	12.74	533.86			1767
42	36	5	1691.32	790.48	0	12.75	535.01			1691
43										
44										
45	37	5	5811.96	2706.22	0	12.76	535.43			5812
46	38	5	5971.21	2779.14	0	12.68	534.14			5971
47	39	5	6043.09	2815.71	0	12.74	537.55			6043
48	40	5	5970.45	2798.18	0	12.63	533.24			5970
49										
50										
51	41	5	8458.56	3938.37	0	12.72	535.18			8459
52	42	5	8280.38	3877.59	0	12.87	536.21			8280
53	43	5	8926.59	4179.86	0	12.76	538.24			8927
54	44	5	9118.82	4217.51	0	12.78	532.03			9117
55										
56										
57	45	5	14216.77	6560.35	0	12.78	533.11			14217
58	46	5	14178.53	6579.96	0	12.76	532.97			14179
59	47	5	14025.46	6622.76	0	12.71	531.93			14025
60	48	5	14060.69	6588.35	0	12.71	534.72			14061
61										
62										
63	49	5	15915.88	7480.74	0	12.73	538.55			15916
64	50	5	16179.69	7605.18	0	12.72	532.82			16180
65	51	5	15838.36	7232.46	0	12.83	533.92			15838
66	52	5	15738.77	7277.04	0	12.84	533.72			15739
67										
68										
69	53	5	16659.3	7724.55	0	12.78	535.76			16658
70	54	5	16389.68	7624.63	0	12.75	536.12			16390
71	55	5	15675.51	7292.43	0	12.81	535.57			15676
72	56	5	15943.13	7383.74	0	12.76	532.9			15943
73										
74										

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	92.1	39.34	0	13.67	535.59				92
76	59	5	97.01	46.12	0	13.39	535.78				97
77	60	5	94.61	42.17	0	14.19	537.42				95
78	61	5	86.94	40.14	0	12.98	536				87
79	62	5	77.77	37.12	0	11.95	534.51				78
80	63	5	75.63	35.42	0	11.9	534.59				76
81	64	5	388.84	184.47	0	12.77	534.59				389
82	65	5	378.77	179.45	0	12.41	535.68				377
83	66	5	391.63	182.44	0	12.98	536.16				392
84	67	5	399.05	177.62	0	13.16	536.69				399
85	68	5	386.41	179.47	0	12.78	533.29				386
86	69	5	402.88	190.57	0	12.48	532.59				403
87	70	5	2687.67	1238.66	0	12.81	533.67				2688
88	71	5	2596.42	1256.42	0	12.66	535.39				2696
89	72	5	2667.29	1251.35	0	12.6	536.33				2667
90	73	5	2627.34	1226.83	0	12.67	536.55				2627
91	74	5	2573.52	1201.13	0	12.81	533.67				2574
92	75	5	2541.68	1189.83	0	12.84	537.71				2542
93	76	5	10517.13	4895.76	0	12.71	534.58				10517
94	77	5	10239.28	4794.76	0	12.76	535.31				10238
95	78	5	10087.67	4882.6	0	12.73	535.2				10088
96	79	5	10010.14	4658.33	0	12.74	535.69				10010
97	80	5	10280.91	4767.71	0	12.75	534.59				10281
98	81	5	10253.18	4739.87	0	12.71	531.9				10253
99	82	5	15572.28	7250.9	0	12.8	534.01				15572
100	83	5	15403.17	7208.99	0	12.73	535.75				15403
101	85	5	14228.55	6630.8	0	12.75	536.18				14229
102	86	5	13901.21	6481.69	0	12.79	537.63				13901
103	87	5	15018.2	6864.71	0	12.76	533.3				15016
104	88	5	15150.67	7100.02	0	12.74	534.6				15151
105	89	5	16501.96	7706.44	0	12.89	536.37				16502
106	90	5	16574.71	7679	0	12.79	534.2				16575
107	91	5	16160.52	7479.58	0	12.75	532.62				16161
108	92	5	16195.11	7499.36	0	12.78	534.43				16195
109	93	5	15810.4	7327.12	0	12.69	532.95				15810
110	94	5	15995.81	7431.27	0	12.68	532.59				15996
111	95	5	15703.28	7297.72	0	12.75	532.8				15703
112	96	5	15950.6	7388.26	0	12.71	531.25				15951
113	97	5	16194.36	7532.49	0	12.7	534.09				16194
114	98	5	16262.71	7548.7	0	12.7	533.62				16263
115	99	5	15588.27	7273.28	0	12.72	537.23				15588
116	100	5	15912.8	7387.58	0	12.7	533.39				15913
117	101	5	15395.92	7184.9	0	12.66	533.87				15396
118	102	5	16039.43	7452.49	0	12.72	533.38				16039
119	103	5	15265.34	7121.47	0	12.79	537.03				15265
120	104	5	15360.95	7177.63	0	12.77	537.62				15361
121	105	5	16001.05	7476.01	0	12.74	536.78				16001
122	106	5	15785.37	7383.87	0	12.71	535.62				15785
123	107	5	231036.64	35997.84	0	7.78	171.32				231037
124	108	5	19.18	1.77	0	12.7	474.98				18

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance			
6		Had it value not within mean +/- 10%			
7		1.00			
8		1.00			
9		1.00			
10		1.00			
11		0.99			
12		0.99			
13		1.00			
14		1.00			
15		1.04			
16		0.98			
17		1.07			
18		0.93			
19		1.09			
20		0.91			
21		0.92			
22		1.08			
23		0.97			
24		1.00			
25		1.00			
26		1.00			
27		#DIV/0!			
28		#DIV/0!			
29		1.01			
30		0.99			
31		1.01			
32		0.99			
33		#DIV/0!			
34		#DIV/0!			
35		0.96			
36		0.96			
37		1.02			
38		0.98			
39		#DIV/0!			
40		#DIV/0!			
41		0.99			
42		1.01			
43		1.01			
44		0.99			
45		#DIV/0!			
46		#DIV/0!			
47		1.01			
48		0.96			
49		0.99			
50		1.01			
51		#DIV/0!			
52		#DIV/0!			
53		1.00			
54		1.00			
55		1.00			
56		#DIV/0!			
57		#DIV/0!			
58		0.99			
59		1.01			
60		1.00			
61		1.00			
62		#DIV/0!			
63		#DIV/0!			
64		1.01			
65		0.99			
66		0.99			
67		1.01			
68		#DIV/0!			
69		#DIV/0!			
70		#DIV/0!			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Sample ID	Sample Type	Replicate Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/protein/min)
1	CR11608	1-1	2	0.5	1	92	184	184	376	0.1	194459	127	0.0001	0.005	15	0.001
2	CR11608	1-2	2	0.5	1	95	190	182	364	0.1	194459	113	0.0001	0.005	15	0.001
3	CR11608	1-3	2	0.5	1	87	174	154	308	0.1	194459	57	0.0001	0.005	15	0.000
4	CR11608	1-4	2	0.5	1	76	152	152	304	0.1	194459	1281	0.0013	0.005	15	0.004
5	CR11608	2-1	2	0.5	1	303	758	768	1532	0.1	194459	1331	0.0014	0.005	15	0.009
6	CR11608	2-2	2	0.5	1	377	943	761	1522	0.1	194459	1327	0.0014	0.005	15	0.009
7	CR11608	2-3	2	0.5	1	383	958	769	1538	0.1	194459	10517	0.0108	0.005	15	0.072
8	CR11608	2-4	2	0.5	1	403	1008	5384	10768	0.1	194459	10337	0.0108	0.005	15	0.071
9	CR11608	3-1	2	0.5	1	2668	5375	5384	10768	0.1	194459	9961	0.0003	0.005	15	0.068
10	CR11608	3-2	2	0.5	1	2667	5334	5384	10768	0.1	194459	41259	0.0024	0.005	15	0.283
11	CR11608	3-3	2	0.5	1	2974	5948	5115	10232	0.1	194459	39945	0.0041	0.005	15	0.274
12	CR11608	4-1	2	0.5	1	10517	21034	20755	41510	0.1	194459	40817	0.0420	0.005	15	0.280
13	CR11608	4-2	2	0.5	1	10048	20096	20096	40192	0.1	194459	61889	0.0635	0.005	15	0.423
14	CR11608	4-3	2	0.5	1	10281	20562	20534	41068	0.1	194459	55019	0.0576	0.005	15	0.384
15	CR11608	5-1	2	0.5	1	15712	31424	30979	61958	0.1	194459	60853	0.0818	0.005	15	0.412
16	CR11608	5-2	2	0.5	1	14229	28458	28130	56260	0.1	194459	69503	0.0878	0.005	15	0.452
17	CR11608	5-3	2	0.5	1	13801	27602	30167	60334	0.1	194459	64451	0.0683	0.005	15	0.442
18	CR11608	6-1	2	0.5	1	15161	30302	33077	66154	0.1	194459	53351	0.0652	0.005	15	0.434
19	CR11608	6-2	2	0.5	1	16575	33150	32566	64712	0.1	194459	62057	0.0849	0.005	15	0.437
20	CR11608	6-3	2	0.5	1	16185	32370	31008	62016	0.1	194459	64653	0.0865	0.005	15	0.443
21	CR11608	7-1	2	0.5	1	15946	31892	31654	63308	0.1	194459	62751	0.0845	0.005	15	0.430
22	CR11608	7-2	2	0.5	1	15946	31892	32457	64914	0.1	194459	62819	0.0844	0.005	15	0.429
23	CR11608	7-3	2	0.5	1	15588	31176	31901	63802	0.1	194459	61001	0.0827	0.005	15	0.418
24	CR11608	8-1	2	0.5	1	15386	30792	31435	62870	0.1	194459	63321	0.0851	0.005	15	0.434
25	CR11608	8-2	2	0.5	1	16039	32078	30626	61252	0.1	194459					
26	CR11608	8-3	2	0.5	1	15385	30769	31786	63572	0.1	194459					
27	CR11608	9-1	2	0.5	1	16001	32002	31870	63740	0.1	194459					
28	CR11608	9-2	2	0.5	1	15735	31470			0.1	194459					

Aromatase Assay Spreadsheet

	N	S
1		
2		
3		
4		
5		QC Check: aliquot reference
6		% RE
7		0.97
8		1.03
9		1.04
10		0.95
11		1.01
12		0.99
13		1.02
14		0.98
15		0.99
16		1.01
17		0.99
18		1.01
19		1.00
20		1.00
21		1.01
22		0.98
23		1.01
24		0.96
25		1.01
26		0.99
27		1.00
28		1.00
29		1.00
30		1.00
31		1.00
32		0.99
33		1.01
34		0.98
35		1.00
36		1.00
37		1.00
38		1.00
39		1.00
40		1.00
41		0.99
42		1.01
43		0.99
44		1.01
45		1.00
46		1.00
47		0.98
48		1.01
49		0.98
50		1.02
51		1.00
52		1.00
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/16/2007	CR11608	Test Chemical ID	16MR2113.P00	1	Positive Control	4-OH ASDN	8	Microsome ID	12535-13
2	RB Ref	12535-73	SLB	Counter data filename	Replicate #					Pos Ctl Tubes/Conc	2
3	Technician ID	SLB									
4	Control Type	Average	SD								
5	Full activity	0.431	0.010		Bkg % of Full						
6	Background (unconnected)	0.002	0.000								
7											
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	2	1.00E-05	-5.00	0.003						
13	4-OH ASDN	1	1.00E-06	-6.00	0.026						
14	4-OH ASDN	2	1.00E-06	-6.00	0.026						
15	4-OH ASDN	1	5.00E-07	-8.30	0.048						
16	4-OH ASDN	2	5.00E-07	-8.30	0.048						
17	4-OH ASDN	1	1.00E-07	-7.00	0.160						
18	4-OH ASDN	2	1.00E-07	-7.00	0.160						
19	4-OH ASDN	1	5.00E-08	-7.30	0.228						
20	4-OH ASDN	2	5.00E-08	-7.30	0.246						
21	4-OH ASDN	1	1.00E-08	-8.00	0.388						
22	4-OH ASDN	2	1.00E-08	-8.00	0.383						
23	4-OH ASDN	1	1.00E-09	-9.00	0.438						
24	4-OH ASDN	2	1.00E-09	-9.00	0.429						
25	4-OH ASDN	1	1.00E-10	-10.00	0.451						
26	4-OH ASDN	2	1.00E-10	-10.00	0.432						
27	4-OH ASDN	1									
28	4-OH ASDN	2									
29	4-OH ASDN	1									
30	4-OH ASDN	2									
31	4-OH ASDN	1									
32	4-OH ASDN	2									
33	4-OH ASDN	1									
34	4-OH ASDN	2									
35	4-OH ASDN	1									
36	4-OH ASDN	2									
37	Test Chemical	Replicate	Test chemical M	Log(test chemical)	Activity						
38	CR11608	1	1.00E-03	-3.00	0.001						
39	CR11608	2	1.00E-03	-3.00	0.001						
40	CR11608	1	1.00E-03	-3.00	0.000						
41	CR11608	2	1.00E-04	-4.00	0.009						
42	CR11608	1	1.00E-04	-4.00	0.009						
43	CR11608	2	1.00E-04	-4.00	0.009						
44	CR11608	1	1.00E-05	-5.00	0.072						
45	CR11608	2	1.00E-05	-5.00	0.071						
46	CR11608	1	1.00E-05	-5.00	0.068						
47	CR11608	2	1.00E-06	-6.00	0.283						
48	CR11608	1	1.00E-06	-6.00	0.274						
49	CR11608	2	1.00E-06	-6.00	0.260						
50	CR11608	1	1.00E-07	-7.00	0.423						
51	CR11608	2	1.00E-07	-7.00	0.384						
52	CR11608	1	1.00E-07	-7.00	0.412						
53	CR11608	2	1.00E-08	-8.00	0.452						
54	CR11608	1	1.00E-08	-8.00	0.442						
55	CR11608	2	1.00E-08	-8.00	0.434						
56	CR11608	1	1.00E-09	-9.00	0.432						
57	CR11608	2	1.00E-09	-9.00	0.443						
58	CR11608	1	1.00E-09	-9.00	0.430						
59	CR11608	2	1.00E-10	-10.00	0.429						
60	CR11608	1	1.00E-10	-10.00	0.418						
61	CR11608	2	1.00E-10	-10.00	0.434						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11608	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-77	Counter data filename	20MR2111.P00					
3	Technician		Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11608	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-77	Counter data filename	20MR2111.P00					
3	Technician ID	SLB	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0213		40798		1915399			
8	2	0.0208		37441		1800048			
9	3	0.0206		39708		1927573			
10	4	0.0206		39985		1941019			
11	5	0.0202		43286		2142871			
12						Average DPM/g soln		1945382	
13						SD		123854	
14						CV		6.37	
15						µCi/g soln		0.8763	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.03	10		1003.00				
20	Dilution A			100	10.03				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1202 g			
25	Mass of dilution B used in substrate prep					4.5633 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56365482 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8763			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5637	+	0.011		
37				=	0.574 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.526 µCi/µg ASDN				
41					9.701.E+05 dpm/nmol				

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/20/2007	Test Chemical ID	CR11608	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-77	Counter data filename	20MR2111.P00					
3			Technician ID	SLB	Replicate #	2	Positive Control	14-OH ASDN	P6S CH	Tubes/Conc	2
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											
26											

Aromalase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Chemical Concentrations As: 3/20/2007 ID C tested 8 Microsome ID 12535-13									
2	Counter data NB 12535-77 filename 20MR2111.P00									
3	ch	Replicate	Positive	Pos Ctl						
4	nic SLB	# 2	Control	4-OH ASDN	Tubes/Conc	2				
5	Vial	Tin	DPM1			tSIE				
6	2	5	40798.44	18286.79	0	12.26	502.94			
7	3	5	37440.57	16343.06	0	11.97	482.09			
8	4	5	39707.72	17414.34	0	12.1	469.01			
9	5	5	39964.53	17498.82	0	11.93	480.41			
10	6	5	43285.9	18765.79	0	11.95	478.23			
11	8	5	15188.4	7054.53	0	12.73	533.42			
12	9	5	15519.69	7158.2	0	12.77	530.83			
13	10	5	3042.46	1483.48	0	13.32	575.98			
14	11	5	0	0	0	811.54	0			
15	12	5	14306.05	6653.26	0	12.68	531.54			
16	13	5	14471.88	6885.47	0	12.73	528.73			
17	14	5	13421.73	6219.05	0	12.66	531.04			
18	15	5	13590.84	6317.7	0	12.72	533.34			
19	16	5	52.43	24.91	0	11.35	530.78			
20	17	5	53.8	26.01	0	11.45	530.46			
21	18	5	47.51	23.93	0	11.27	532.99			
22	19	5	52.46	23.19	0	9.73	531.99			
23	20	5	55.96	26.9	0	14.56	535.47			
24	21	5	44.02	21.76	0	12.87	531.39			
25	22	5	55.5	29.5	0	13.56	530.06			
26	23	5	40.09	20.54	0	10.25	532.11			
27	25	5	150.27	75.75	0	12.48	533.47			
28	26	5	165.92	75.86	0	12.83	529.74			
29	27	5	153.86	72.87	0	12.98	531.12			
30	28	5	142.1	66.75	0	12.73	533.03			
31										
32										
33	29	5	920.45	421.71	0	12.89	534.71			
34	30	5	926.43	435.13	0	12.96	534.62			
35	31	5	891.44	406.04	0	13.15	530.09			
36	32	5	831.93	380.29	0	12.8	532.04			
37										
38										
39	33	5	1570.11	728.47	0	12.7	531.25			
40	34	5	1645.39	785.91	0	12.53	531.9			
41	35	5	1537.84	720.86	0	12.58	532.42			
42	36	5	1524.67	701.6	0	12.69	534.76			
43										
44										
45	37	5	5394.81	2496.01	0	12.74	530.45			
46	38	5	5273.57	2470.24	0	12.62	530.78			
47	39	5	5098.52	2376.98	0	12.87	531.28			
48	40	5	5319.57	2460.1	0	12.8	532.49			
49										
50										
51	41	5	7628.1	3564.3	0	12.65	532.19			
52	42	5	7834.97	3610.39	0	12.77	532.13			
53	43	5	7599.81	3502.58	0	12.77	531.82			
54	44	5	7541.08	3504.14	0	12.57	531.94			
55										
56										
57	45	5	12466.67	5783.71	0	12.72	531.37			
58	46	5	12494.6	5765.7	0	12.8	531.89			
59	47	5	12332.39	5693.84	0	12.71	527.28			
60	48	5	12173.94	5665.48	0	12.65	531.52			
61										
62										
63	49	5	14020.95	6500.72	0	12.81	534.15			
64	50	5	14073.67	6500.89	0	12.74	531.06			
65	51	5	14222.79	6576.38	0	12.7	530.1			
66	52	5	14475.01	6712.56	0	12.69	532.11			
67										
68										
69	53	5	13919.01	6455.14	0	12.68	530.95			
70	54	5	13984.44	6468.23	0	12.76	533.03			
71	55	5	14267.14	6823.51	0	12.72	531.96			
72	56	5	14240.69	6586.07	0	12.7	529.35			
73										
74										
75	58	5	289.97	139.64	0	12.53	536			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
76	59	5	320.45	151.82	0	13.11	530.97				320
77	60	5	344.16	153.36	0	12.93	534.41				344
78	61	5	303.05	150.56	0	11.87	530.53				303
79	62	5	311.87	142.29	0	12.7	530.53				312
80	63	5	330.36	152.64	0	12.88	530.12				330
81	64	5	608.77	282.17	0	12.67	530.22				609
82	65	5	625.64	289.37	0	12.56	532.23				626
83	66	5	581.04	274	0	12.79	530.46				581
84	67	5	598.97	277.68	0	12.91	532.36				599
85	68	5	599.2	275.95	0	13.01	531.49				599
86	69	5	572.65	267.46	0	12.7	532.46				573
87	70	5	2201.46	1026.8	0	12.71	532.8				2201
88	71	5	2407.73	1125.64	0	12.55	530.21				2408
89	72	5	2197.31	1031.91	0	12.77	534.41				2197
90	73	5	2225.07	1029.68	0	12.79	534.17				2225
91	74	5	2072.69	987.63	0	12.68	533.76				2073
92	75	5	2172.7	1005.08	0	12.64	530.65				2173
93	76	5	3765.78	1735.02	0	12.67	530.26				3766
94	77	5	3637.41	1688.84	0	12.79	531.71				3637
95	78	5	3716.87	1717.39	0	12.71	532.09				3717
96	79	5	3668.98	1714.69	0	12.73	533.92				3669
97	80	5	3617.77	1699.44	0	12.81	535.5				3618
98	81	5	3728.06	1743.91	0	12.63	531.91				3728
99	83	5	5843.94	2724.77	0	12.76	534.25				5844
100	84	5	6023.73	2778.83	0	12.82	534.07				6024
101	85	5	5904.96	2745.28	0	12.68	530.56				5905
102	86	5	5826.53	2696.48	0	12.69	530.3				5827
103	87	5	5485.63	2523.63	0	12.76	532.18				5486
104	88	5	5556.42	2580.46	0	12.73	532.48				5556
105	89	5	8787.83	4079.09	0	12.7	531.41				8788
106	90	5	8875.53	4103.68	0	12.75	532.23				8876
107	91	5	8496.09	3959.14	0	12.59	529.73				8496
108	92	5	8827.13	4019.1	0	12.64	532.82				8827
109	93	5	8562.96	3994.19	0	12.71	533.75				8563
110	94	5	8567.42	3985.89	0	12.72	530.84				8567
111	95	5	13138.18	6084.94	0	12.8	532.59				13138
112	96	5	13460.79	6222.6	0	12.71	533.43				13461
113	97	5	13206.86	6139.51	0	12.74	533.72				13207
114	98	5	13277.95	6209.81	0	12.65	530.86				13278
115	99	5	13243.11	6135.94	0	12.61	529.98				13243
116	100	5	13198.3	6115.47	0	12.81	533.42				13198
117	101	5	14163.69	6550.14	0	12.8	531.53				14164
118	102	5	14177.11	6571.28	0	12.75	533.2				14177
119	103	5	14171.32	6604.89	0	12.73	533.5				14171
120	104	5	13922.01	6494.67	0	12.7	533.03				13922
121	105	5	14077.19	6560.88	0	12.71	533.77				14077
122	106	5	14261.3	6639.12	0	12.72	531.73				14261
123	107	5	228838.23	36284.66	0	7.8	173.12				228838
124	108	5	0	0.3	0	0	475.31				0
125											0
126											0
127											0
128											0
129											0
130											0
131											0
132											0
133											0
134											0
135											0
136											0
137											0
138											0
139											0
140											0
141											0
142											0
143											0
144											0
145											0
146											0
147											0

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
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Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
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Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
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Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
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Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							
Assay Date	Chemical ID	CR11608	# Concentrations tested	12635-13	2	OH ASDN	Pos Ctl Tubes/Conc	2							

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance Red if value not within mean $\pm$ 10%			
6					
7		0.99			
8		1.01			
9		1.00			
10		0.99		Background activity uncorrected for background	
11		1.01			
12		0.99			
13		1.01			
14		0.98		nmol ³ H-O ₂ /nmol	B/A activity
15		1.01		2.19E-04	0.001
16		0.98			
17		1.02		2.09E-04	0.001
18		0.96			
19		1.04		2.06E-04	0.001
20				1.98E-04	0.001
21					
22		0.95			
23		1.05			
24		1.04			
25		0.96			
26					
27					
28					
29		1.00			
30		1.00			
31		1.03			
32		0.97			
33					
34		0.98			
35		1.02			
36		1.00			
37					
38		1.00			
39					
40					
41		1.01			
42		0.99			
43		0.98			
44		1.02			
45					
46					
47		0.99			
48		1.01			
49		1.00			
50		1.00			
51					
52					
53		1.00			
54		1.00			
55		1.00			
56		0.99			
57					
58					
59		1.00			
60		1.00			
61		0.99			
62		1.01			
63					
64					
65		1.00			
66		1.00			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate Level	Normalised Volume (mL)	Aliq Volume (mL)	Aliq #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPH	Volume of substrate solution used (mL)	Total DPM in assay tube (nM)	Calculate nMol ³ H ₂ O formed	nMol ³ H ₂ O formed	Free (protein) in assay (mg/mL)	Inhibition time (min)	Aromatase activity (nMol estrogen converted/mg protein/min)
1	1	2	0.5	1	280	580	810	1220	0.1	194538	1019	0.0011	0.005	15	0.007
2	2	2	0.5	1	280	580	810	1220	0.1	194538	1019	0.0011	0.005	15	0.007
3	3	2	0.5	1	344	680	847	1284	0.1	194538	1083	0.0011	0.005	15	0.007
4	4	2	0.5	1	303	606	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
5	5	2	0.5	1	312	624	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
6	6	2	0.5	1	330	660	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
7	7	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
8	8	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
9	9	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
10	10	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
11	11	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
12	12	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
13	13	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
14	14	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
15	15	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
16	16	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
17	17	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
18	18	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
19	19	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
20	20	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
21	21	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
22	22	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
23	23	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
24	24	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
25	25	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
26	26	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
27	27	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
28	28	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
29	29	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
30	30	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
31	31	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
32	32	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
33	33	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
34	34	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
35	35	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
36	36	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
37	37	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
38	38	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
39	39	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
40	40	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
41	41	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
42	42	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
43	43	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
44	44	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
45	45	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
46	46	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
47	47	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
48	48	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
49	49	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
50	50	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
51	51	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
52	52	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
53	53	2	0.5	1	309	618	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007
54	54	2	0.5	1	326	652	842	1284	0.1	194538	1083	0.0011	0.005	15	0.007

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC Check - allowed variance
6		% RE
7		0.95
8		1.05
9		1.05
10		0.94
11		0.97
12		1.03
13		0.99
14		1.01
15		0.98
16		1.02
17		0.96
18		0.98
19		1.04
20		0.99
21		1.01
22		0.98
23		1.02
24		1.02
25		1.02
26		0.98
27		1.01
28		0.99
29		1.01
30		0.98
31		1.02
32		1.01
33		0.99
34		1.01
35		0.99
36		1.01
37		1.00
38		1.00
39		0.99
40		1.01
41		1.00
42		0.98
43		1.01
44		1.00
45		1.00
46		1.00
47		1.00
48		1.00
49		1.00
50		1.00
51		1.01
52		0.99
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/20/2007	Test Chemical ID	CR1608	20MR2111.P00	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13	
2	Assay Date	3/20/2007	Test Chemical ID	CR1608	20MR2111.P00	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13	
3	Assay Date	3/20/2007	Test Chemical ID	CR1608	20MR2111.P00	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13	
4	Assay Date	3/20/2007	Test Chemical ID	CR1608	20MR2111.P00	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	12535-13	
5	Control Type	Portion	Average	SD							
6	Full activity	Overall	0.400	0.023							
7	Background (uncorrected)	Overall	0.001	0.000							
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.003						
12	4-OH ASDN	2	2	-5.00	0.003						
13	4-OH ASDN	3	3	-6.00	0.024						
14	4-OH ASDN	4	4	-6.00	0.022						
15	4-OH ASDN	5	5	-7.00	0.043						
16	4-OH ASDN	6	6	-8.00	0.041						
17	4-OH ASDN	7	7	-9.00	0.145						
18	4-OH ASDN	8	8	-10.00	0.142						
19	4-OH ASDN	9	9	-10.00	0.211						
20	4-OH ASDN	10	10	-10.00	0.207						
21	4-OH ASDN	11	11	-10.00	0.342						
22	4-OH ASDN	12	12	-10.00	0.335						
23	4-OH ASDN	13	13	-10.00	0.385						
24	4-OH ASDN	14	14	-10.00	0.393						
25	4-OH ASDN	15	15	-10.00	0.382						
26	4-OH ASDN	16	16	-10.00	0.390						
27	4-OH ASDN	17	17	-10.00							
28	4-OH ASDN	18	18	-10.00							
29	4-OH ASDN	19	19	-10.00							
30	4-OH ASDN	20	20	-10.00							
31	4-OH ASDN	21	21	-10.00							
32	4-OH ASDN	22	22	-10.00							
33	4-OH ASDN	23	23	-10.00							
34	4-OH ASDN	24	24	-10.00							
35	4-OH ASDN	25	25	-10.00							
36	4-OH ASDN	26	26	-10.00							
37	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
38	CR1608	1	1	-4.00	0.007						
39	CR1608	2	2	-4.00	0.008						
40	CR1608	3	3	-4.00	0.007						
41	CR1608	4	4	-4.30	0.016						
42	CR1608	5	5	-4.30	0.015						
43	CR1608	6	6	-4.30	0.015						
44	CR1608	7	7	-5.00	0.062						
45	CR1608	8	8	-5.00	0.059						
46	CR1608	9	9	-5.00	0.057						
47	CR1608	10	10	-5.30	0.100						
48	CR1608	11	11	-5.30	0.100						
49	CR1608	12	12	-5.30	0.100						
50	CR1608	13	13	-5.60	0.162						
51	CR1608	14	14	-5.60	0.160						
52	CR1608	15	15	-5.60	0.160						
53	CR1608	16	16	-6.00	0.241						
54	CR1608	17	17	-6.00	0.234						
55	CR1608	18	18	-6.00	0.234						
56	CR1608	19	19	-7.00	0.384						
57	CR1608	20	20	-7.00	0.363						
58	CR1608	21	21	-7.00	0.362						
59	CR1608	22	22	-8.00	0.382						
60	CR1608	23	23	-8.00	0.385						
61	CR1608	24	24	-8.00	0.388						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	3/21/2007	Test	Chemical ID	CR11608	# Concentrations	tested	8	Microsome ID	12535-13
2	NB Ref.	12535-81	Counter data	filename	21MR2113.P00					
	Technician		Pos Ctl							
3	ID	SLB	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc			2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/21/2007	Test Chemical ID	CR11608	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-81	Counter data filename	21MR2113.P00					
3	Technician ID	SLB	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0209		39278		1879330			
8	2	0.0195		36976		1896205			
9	3	0.0203		40278		1984138			
10	4	0.0202		39686		1964653			
11	5	0.0200		37777		1888850			
12						Average DPM/g soln	1922635		
13						SD	48123		
14						CV	2.50		
15						µCi/g soln	0.8661		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0872 g			
25	Mass of dilution B used in substrate prep					4.5471 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56169662 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8661			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5617	+	0.011		
37				=	0.572	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.513	µCi/µg ASDN			
41					9.622.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

[illegible]

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		As:	3/21/2007	Chemical ID	Concentrations	tested	8	Microsome ID	12535-13		
2		NB	12535-81	Counter date	filename	21MR2113.P00					
3		ch	SLB	Replicate	Positive	Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2	
4											
5		Vial	Tin	DPM1		ISIE					Round DPM
6	2	5	39277.86	17026.88	0	11.72	471.26				39278
7	3	5	36975.7	15954.05	0	11.75	469.56				36976
8	4	5	40277.94	17352.12	0	11.77	471.37				40278
9	5	5	39686.11	17093.79	0	11.78	470.42				39686
10	6	5	37776.86	16368.15	0	11.81	473.5				37777
11	8	5	14078.08	6596.88	0	12.61	533.31				14078
12	9	5	14413.28	6699.93	0	12.5	528.22				14413
13	10	5	14847.61	6866.41	0	12.65	529.77				14848
14	11	5	14765.72	6828.11	0	12.58	526.71				14766
15	12	5	14098.68	6516.81	0	12.57	526.93				14099
16	13	5	14610.55	6757.85	0	12.66	529.84				14611
17	14	5	13757.8	6392.45	0	12.59	528.81				13758
18	15	5	13568.76	6325.92	0	12.65	533.57				13569
19	16	5	78.41	36.14	0	12.74	527.74				78
20	17	5	78.51	37.6	0	14.1	530.24				79
21	18	5	57.05	28.01	0	13.77	533.36				57
22	19	5	80.44	36.57	0	12.11	530.54				80
23	20	5	75.59	33.01	0	15.24	527.14				76
24	21	5	70.07	33.01	0	12.52	529.28				70
25	22	5	71.04	29.3	0	15.93	532.93				71
26	23	5	69.7	31.43	0	15.44	533.22				70
27	25	5	145.06	70.51	0	12.61	526.86				145
28	26	5	158.7	75.45	0	12.58	528.38				159
29	27	5	148.62	69.48	0	12.61	529.68				149
30	28	5	149.99	68.73	0	12.8	530.96				150
31											
32											
33	29	5	840.59	393.98	0	12.61	528.57				841
34	30	5	867.73	404.5	0	12.87	531.11				868
35	31	5	850.38	397.76	0	12.68	528.82				850
36	32	5	828.72	388.63	0	12.54	530.56				829
37											
38											
39	33	5	1604.94	749.21	0	12.78	530.9				1605
40	34	5	1691.74	780.76	0	12.6	526.9				1692
41	35	5	1625.6	706.05	0	12.57	530.19				1526
42	36	5	1394.77	648.92	0	12.53	529.33				1395
43											
44											
45	37	5	5195.07	2402.98	0	12.67	527.16				5195
46	38	5	5011.27	2325.61	0	12.89	531.01				5011
47	39	5	5026.03	2342.7	0	12.64	530.12				5026
48	40	5	5319.64	2456.58	0	12.68	528.65				5320
49											
50											
51	41	5	7709.58	3569.84	0	12.55	527.87				7710
52	42	5	7791.96	3595.75	0	12.64	528.05				7792
53	43	5	8140.27	3758.21	0	12.64	527.63				8140
54	44	5	8078.49	3748.26	0	12.62	529.12				8078
55											
56											
57	45	5	12674.01	5825.87	0	12.63	526.6				12674
58	46	5	12508.95	5823.09	0	12.65	531.05				12509
59	47	5	12370.66	5751.63	0	12.54	526.7				12371
60	48	5	11770.02	5469.43	0	12.71	530.68				11770
61											
62											
63	49	5	14229.07	6613.45	0	12.61	531.09				14229
64	50	5	14099.94	6574.79	0	12.82	530.79				14100
65	51	5	13978.05	6464.86	0	12.62	527.31				13978
66	52	5	13184.85	6085.21	0	12.69	527.91				13185
67											
68											
69	53	5	14179.19	6606.54	0	12.63	531.67				14179
70	54	5	14385.55	6696.72	0	12.6	531.31				14386
71	55	5	13829.39	6424.05	0	12.64	529.98				13829
72	56	5	14267.51	6641.37	0	12.68	531.81				14268
73											
74											
75	57	5	381.15	171.97	0	13.04	530.37				381

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
76	58	5	374.91	165.78	0	13.26	530.38				375
77	60	5	365.03	168.13	0	12.93	528.25				365
78	61	5	368.86	173.73	0	12.82	529.96				369
79	62	5	397.7	179.77	0	13.08	531.55				398
80	63	5	367.41	167.65	0	13.14	530.27				367
81	64	5	654.8	304.54	0	12.92	528.42				655
82	65	5	666.09	310.4	0	12.47	528.6				666
83	66	5	651.48	300.28	0	12.25	529.56				651
84	67	5	635.72	304.18	0	12.72	530.99				636
85	68	5	704.48	321.22	0	12.91	525.42				704
86	69	5	688.7	320.2	0	12.49	529.04				689
87	70	5	2454.47	1140.43	0	12.75	532.93				2454
88	71	5	2505.54	1170.28	0	12.75	533.06				2506
89	72	5	2354.42	1093.66	0	12.72	530.26				2354
90	73	5	2472.54	1134.74	0	12.69	528.79				2473
91	74	5	2434.59	1121.08	0	12.83	533.23				2435
92	75	5	2483.37	1159.42	0	12.58	527.75				2483
93	76	5	4021.49	1887.14	0	12.46	531.69				4021
94	77	5	3960.16	1836.97	0	12.63	530.54				3960
95	78	5	3910.85	1821.25	0	12.76	531.34				3911
96	79	5	4055.44	1901.97	0	12.63	531.75				4055
97	80	5	4035.62	1870.9	0	12.85	532.6				4036
98	81	5	4017.07	1860.02	0	12.68	531.32				4017
99	83	5	6019.97	2806.54	0	12.68	532.75				6020
100	84	5	6100.04	2812.7	0	12.75	531.3				6100
101	85	5	6237.71	2858.41	0	12.75	529.86				6238
102	86	5	5932.24	2744.66	0	12.76	532.38				5932
103	87	5	6236.87	2901.82	0	12.64	530.1				6237
104	88	5	5986.4	2784.97	0	12.57	530.49				5986
105	89	5	9106.59	4227.74	0	12.68	531.76				9107
106	90	5	9104.31	4223.33	0	12.72	532.73				9104
107	91	5	9145.26	4267.94	0	12.55	532				9145
108	92	5	9239.65	4325.11	0	12.57	530.32				9240
109	93	5	9210.08	4251.14	0	12.75	528.71				9210
110	94	5	8986.31	4193.32	0	12.65	530.13				8986
111	95	5	13883.77	6466.41	0	12.71	532.46				13884
112	96	5	13880.18	6480.25	0	12.54	529.21				13880
113	97	5	13863.47	6429.88	0	12.68	529.88				13863
114	98	5	13767.27	6404.37	0	12.58	531.04				13767
115	99	5	13326.94	6185.78	0	12.64	529.31				13327
116	100	5	13247.59	6191.84	0	12.61	532.63				13248
117	101	5	14508.29	6749.39	0	12.67	532.36				14508
118	102	5	13817.69	6439.05	0	12.7	531.82				13818
119	103	5	14474.92	6782.84	0	12.62	533.52				14475
120	104	5	14629.89	6790.35	0	12.64	528.04				14630
121	105	5	14391.36	6693.44	0	12.63	529.83				14391
122	106	5	14410.29	6653.91	0	12.69	529.84				14410
123	107	5	228470.08	36592.69	0	7.79	173.61				228470
124	108	5	9.22	1.58	0	29.07	477.56				9
125											0
126											0

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Volume (mL)	Aliq (mL)	Assay Date	Chemical ID	Test Chemical	Concentrations tested	8 Microsome ID	4-OH ASDN	Pos Ctrl Tubes/Conc	2				
1				3/21/2007	GR11608	GR11608									
2						Control only									
3						21MR2113 P00									
4						Replicate #									
5						3	Positive Control								
6						3	Positive Control								
7						3	Positive Control								
8						3	Positive Control								
9						3	Positive Control								
10						3	Positive Control								
11						3	Positive Control								
12						3	Positive Control								
13						3	Positive Control								
14						3	Positive Control								
15						3	Positive Control								
16						3	Positive Control								
17						3	Positive Control								
18						3	Positive Control								
19						3	Positive Control								
20						3	Positive Control								
21						3	Positive Control								
22						3	Positive Control								
23						3	Positive Control								
24						3	Positive Control								
25						3	Positive Control								
26						3	Positive Control								
27						3	Positive Control								
28						3	Positive Control								
29						3	Positive Control								
30						3	Positive Control								
31						3	Positive Control								
32						3	Positive Control								
33						3	Positive Control								
34						3	Positive Control								
35						3	Positive Control								
36						3	Positive Control								
37						3	Positive Control								
38						3	Positive Control								
39						3	Positive Control								
40						3	Positive Control								
41						3	Positive Control								
42						3	Positive Control								
43						3	Positive Control								
44						3	Positive Control								
45						3	Positive Control								
46						3	Positive Control								
47						3	Positive Control								
48						3	Positive Control								
49						3	Positive Control								
50						3	Positive Control								
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52						3	Positive Control								
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58						3	Positive Control								
59						3	Positive Control								
60						3	Positive Control								
61						3	Positive Control								
62						3	Positive Control								
63						3	Positive Control								
64						3	Positive Control								
65						3	Positive Control								
66						3	Positive Control								
67						3	Positive Control								
68						3	Positive Control								
69						3	Positive Control								
70						3	Positive Control								

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance			
6		Had if value not within mean +/- 10%			
7		0.99			
8		1.01			
9		1.00			
10		0.99			
11		1.02			
12		1.01			
13		0.99			
14		1.01			
15		0.99			
16		1.01			
17					
18					
19		1.02			
20		0.99			
21		1.01			
22		0.99			
23		1.02			
24		1.00			
25		1.00			
26		1.00			
27		#DIV/0!			
28		#DIV/0!			
29		0.99			
30		1.02			
31		1.01			
32		0.99			
33		#DIV/0!			
34		#DIV/0!			
35		0.97			
36		1.03			
37		1.04			
38		0.95			
39		#DIV/0!			
40		#DIV/0!			
41		1.02			
42		0.98			
43		0.97			
44		1.03			
45		#DIV/0!			
46		#DIV/0!			
47		0.99			
48		1.01			
49		1.00			
50		1.00			
51		#DIV/0!			
52		#DIV/0!			
53		1.01			
54		0.99			
55		1.02			
56		0.99			
57		#DIV/0!			
58		#DIV/0!			
59		1.00			
60		1.00			
61		1.03			
62		0.97			
63		#DIV/0!			
64		#DIV/0!			
65		0.99			
66		0.99			
67		0.99			
68		1.02			
69		#DIV/0!			
70		#DIV/0!			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Chemical ID	Chemical	Test	Assay Date	Chemical ID	Chemical	Test	Assay Date	Chemical ID	Chemical	Test	Assay Date	Chemical ID	Chemical	Test
3/21/2007	CR11609	125-53-51	Counter data	3/21/2007	CR11609	125-53-51	Counter data	3/21/2007	CR11609	125-53-51	Counter data	3/21/2007	CR11609	125-53-51	Counter data
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34
35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37
38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39
40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40
41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44
45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46
47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47
48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48
49	49	49	49	49	49	49	49	49	49	49	49	49	49	49	49
50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
51	51	51	51	51	51	51	51	51	51	51	51	51	51	51	51
52	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52
53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		OK check - aliquot
5		variance
6		% RE
7		1.01
8		0.99
9		0.99
10		1.01
11		1.04
12		0.96
13		0.99
14		1.01
15		1.01
16		0.99
17		1.01
18		0.99
19		0.99
20		1.01
21		0.99
22		1.02
23		0.99
24		1.01
25		1.01
26		0.99
27		0.99
28		1.02
29		1.00
30		1.00
31		0.99
32		1.01
33		1.03
34		0.97
35		1.02
36		0.98
37		1.01
38		1.00
39		0.99
40		1.01
41		1.01
42		0.99
43		1.00
44		1.00
45		1.00
46		1.00
47		1.00
48		1.02
49		0.98
50		0.99
51		0.99
52		1.01
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/21/2007	Test Chemical ID	CR11608	21MP2113.P00	3	Positive Control	4-OH ASDN	For Cili Tubas/Conc	2	
2	ND Ref.	12535-81	Counter data file								
3	Technician ID	SLB	Replicate #								
4	Control Type	Average	SD								
5	Full activity	0.383	0.013								
6	Background	0.002	0.000								
7	(uncorrected)										
8											
9											
10	Positive Control	Replicate	[Positive Cili M]	Log(Positive Cili)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.002						
12	4-OH ASDN	2	1.00E-05	-5.00	0.002						
13	4-OH ASDN	1	1.00E-05	-6.00	0.022						
14	4-OH ASDN	2	1.00E-05	-6.00	0.021						
15	4-OH ASDN	1	1.00E-05	-6.00	0.044						
16	4-OH ASDN	2	1.00E-05	-6.00	0.039						
17	4-OH ASDN	1	5.00E-07	-7.00	0.139						
18	4-OH ASDN	2	5.00E-07	-7.00	0.141						
19	4-OH ASDN	1	1.00E-07	-7.00	0.213						
20	4-OH ASDN	2	1.00E-07	-7.00	0.223						
21	4-OH ASDN	1	5.00E-08	-8.00	0.347						
22	4-OH ASDN	2	5.00E-08	-8.00	0.332						
23	4-OH ASDN	1	1.00E-08	-9.00	0.391						
24	4-OH ASDN	2	1.00E-08	-9.00	0.374						
25	4-OH ASDN	1	1.00E-10	-10.00	0.394						
26	4-OH ASDN	2	1.00E-10	-10.00	0.367						
27	4-OH ASDN	1									
28	4-OH ASDN	2									
29	4-OH ASDN	1									
30	4-OH ASDN	2									
31	4-OH ASDN	1									
32	4-OH ASDN	2									
33	4-OH ASDN	1									
34	4-OH ASDN	2									
35	4-OH ASDN	1									
36	4-OH ASDN	2									
37	Test Chemical	Replicate	[Test chemical] M	Log(test chemical)	Activity						
38	CR11608	1	1.00E-04	-4.00	0.008						
39	CR11608	2	1.00E-04	-4.00	0.008						
40	CR11608	1	1.00E-04	-4.00	0.009						
41	CR11608	2	1.00E-04	-4.00	0.016						
42	CR11608	1	5.00E-05	-4.30	0.016						
43	CR11608	2	5.00E-05	-4.30	0.017						
44	CR11608	1	1.00E-05	-5.00	0.067						
45	CR11608	2	1.00E-05	-5.00	0.065						
46	CR11608	1	1.00E-05	-5.00	0.086						
47	CR11608	2	1.00E-05	-5.00	0.109						
48	CR11608	1	5.00E-06	-5.30	0.108						
49	CR11608	2	5.00E-06	-5.30	0.110						
50	CR11608	1	2.50E-06	-5.60	0.166						
51	CR11608	2	2.50E-06	-5.60	0.167						
52	CR11608	1	1.00E-06	-6.00	0.250						
53	CR11608	2	1.00E-06	-6.00	0.253						
54	CR11608	1	1.00E-06	-6.00	0.250						
55	CR11608	2	1.00E-06	-6.00	0.250						
56	CR11608	1	1.00E-07	-7.00	0.383						
57	CR11608	2	1.00E-07	-7.00	0.381						
58	CR11608	1	1.00E-07	-7.00	0.366						
59	CR11608	2	1.00E-07	-7.00	0.360						
60	CR11608	1	1.00E-08	-8.00	0.401						
61	CR11608	2	1.00E-08	-8.00	0.397						
62	CR11608	1	1.00E-08	-8.00	0.397						
63	CR11608	2	1.00E-08	-8.00	0.397						
64	CR11608	1	1.00E-08	-8.00	0.397						
65	CR11608	2	1.00E-08	-8.00	0.397						
66	CR11608	1	1.00E-08	-8.00	0.397						
67	CR11608	2	1.00E-08	-8.00	0.397						
68	CR11608	1	1.00E-08	-8.00	0.397						
69	CR11608	2	1.00E-08	-8.00	0.397						
70	CR11608	1	1.00E-08	-8.00	0.397						
71	CR11608	2	1.00E-08	-8.00	0.397						
72	CR11608	1	1.00E-08	-8.00	0.397						
73	CR11608	2	1.00E-08	-8.00	0.397						
74	CR11608	1	1.00E-08	-8.00	0.397						
75	CR11608	2	1.00E-08	-8.00	0.397						
76	CR11608	1	1.00E-08	-8.00	0.397						
77	CR11608	2	1.00E-08	-8.00	0.397						
78	CR11608	1	1.00E-08	-8.00	0.397						
79	CR11608	2	1.00E-08	-8.00	0.397						
80	CR11608	1	1.00E-08	-8.00	0.397						
81	CR11608	2	1.00E-08	-8.00	0.397						
82	CR11608	1	1.00E-08	-8.00	0.397						
83	CR11608	2	1.00E-08	-8.00	0.397						
84	CR11608	1	1.00E-08	-8.00	0.397						
85	CR11608	2	1.00E-08	-8.00	0.397						
86	CR11608	1	1.00E-08	-8.00	0.397						
87	CR11608	2	1.00E-08	-8.00	0.397						
88	CR11608	1	1.00E-08	-8.00	0.397						
89	CR11608	2	1.00E-08	-8.00	0.397						
90	CR11608	1	1.00E-08	-8.00	0.397						
91	CR11608	2	1.00E-08	-8.00	0.397						
92	CR11608	1	1.00E-08	-8.00	0.397						
93	CR11608	2	1.00E-08	-8.00	0.397						
94	CR11608	1	1.00E-08	-8.00	0.397						
95	CR11608	2	1.00E-08	-8.00	0.397						
96	CR11608	1	1.00E-08	-8.00	0.397						
97	CR11608	2	1.00E-08	-8.00	0.397						
98	CR11608	1	1.00E-08	-8.00	0.397						
99	CR11608	2	1.00E-08	-8.00	0.397						
100	CR11608	1	1.00E-08	-8.00	0.397						
101	CR11608	2	1.00E-08	-8.00	0.397						
102	CR11608	1	1.00E-08	-8.00	0.397						
103	CR11608	2	1.00E-08	-8.00	0.397						
104	CR11608	1	1.00E-08	-8.00	0.397						
105	CR11608	2	1.00E-08	-8.00	0.397						
106	CR11608	1	1.00E-08	-8.00	0.397						
107	CR11608	2	1.00E-08	-8.00	0.397						
108	CR11608	1	1.00E-08	-8.00	0.397						
109	CR11608	2	1.00E-08	-8.00	0.397						
110	CR11608	1	1.00E-08	-8.00	0.397						
111	CR11608	2	1.00E-08	-8.00	0.397						
112	CR11608	1	1.00E-08	-8.00	0.397						
113	CR11608	2	1.00E-08	-8.00	0.397						
114	CR11608	1	1.00E-08	-8.00	0.397						
115	CR11608	2	1.00E-08	-8.00	0.397						
116	CR11608	1	1.00E-08	-8.00	0.397						
117	CR11608	2	1.00E-08	-8.00	0.397						
118	CR11608	1	1.00E-08	-8.00	0.397						
119	CR11608	2	1.00E-08	-8.00	0.397						
120	CR11608	1	1.00E-08	-8.00	0.397						
121	CR11608	2	1.00E-08	-8.00	0.397						
122	CR11608	1	1.00E-08	-8.00	0.397						
123	CR11608	2	1.00E-08	-8.00	0.397						
124	CR11608	1	1.00E-08	-8.00	0.397						
125	CR11608	2	1.00E-08	-8.00	0.397						
126	CR11608	1	1.00E-08	-8.00	0.397						
127	CR11608	2	1.00E-08	-8.00	0.397						
128	CR11608	1	1.00E-08	-8.00	0.397						
129	CR11608	2	1.00E-08	-8.00	0.397						
130	CR11608	1	1.00E-08	-8.00	0.397						
131	CR11608	2	1.00E-08	-8.00	0.397						
132	CR11608	1	1.00E-08	-8.00	0.397						
133	CR11608	2	1.00E-08	-8.00	0.397						
134	CR11608	1	1.00E-08	-8.00	0.397						
135	CR11608	2	1.00E-08	-8.00	0.397						
136	CR11608	1	1.00E-08	-8.00	0.397						
137	CR11608	2	1.00E-08	-8.00	0.397						
138	CR11608	1	1.00E-08	-8.00	0.397						
139	CR11608	2	1.00E-08	-8.00	0.397						
140	CR11608	1	1.00E-08	-8.00	0.397						
141	CR11608	2	1.00E-08	-8.00	0.397						
142	CR11608	1	1.00E-08	-8.00	0.397						
143	CR11608	2	1.00E-08	-8.00	0.397						
144	CR11608	1	1.00E-08	-8.00	0.397						
145	CR11608	2	1.00E-08	-8.00	0.397						
146	CR11608	1	1.00E-08	-8.00	0.397						
147	CR11608	2	1								

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	3/22/2007	Test	Chemical ID	CR11609	# Concentrations	tested	8	Microsome ID	12535-13
2	NB Ref.	12535-85	Counter data	filename	22MR2054.P00					
3	Technician		Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc			
	ID	SLB								2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/22/2007	Test Chemical ID	CR11609	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-85	Counter data filename	22MR2054.P00					
3	Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Alq.		DPM/g soln.			
7	1	0.0213		39574		1857934			
8	2	0.0212		38331		1808066			
9	3	0.0211		39546		1874218			
10	4	0.0210		40276		1917905			
11	5	0.0211		41045		1945261			
12						Average DPM/g soln	1880677		
13						SD	53358		
14						CV	2.84		
15						µCi/g soln	0.8472		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1438 g			
25	Mass of dilution B used in substrate prep					4.5808 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56361423 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.010 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8472			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5636	+	0.010		
37				=	0.574	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.476 µCi/µg ASDN								
41	9.385 E+05 dpm/nmol								

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/22/2007	Test Chemical ID	CR11609	# Concentrations tested		8	Microsome ID	12535-13
2		NB Ref	12535-85	Counter data filename	22MR2054.P00					
3		Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	4-OH ASDN	Pos Ctrl	2
4										
5	Microsome Dilution Details									
6										
7	Dilution A		0.12 mL microsome Stock used				Positive Control	Final Concentration (M)		
8			2.4 mL total volume				Level	1	1.00E-05	
9			20 dilution factor					2	1.00E-06	
10								3	5.00E-07	
11	Dilution B		1.5 mL microsome Dilution A used					4	1.00E-07	
12			60 mL total volume					5	5.00E-08	
13			40 dilution factor					6	1.00E-08	
14								7	1.00E-09	
15	Dilution C (if applicable)		mL microsome Dilution B used					8	1.00E-10	
16			mL total volume							
17			dilution factor							
18	NA									
19			800 total dilution factor							
20										
21	Protein Concentration (stock microsomes, mg/mL):			8.00						
22	Protein Concentration (dilution added to assay, mg/mL):			0.01						
23										
24										
25										
26										

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Assay Date	3/22/2007	Chemical ID	CR11609	# Concentrations tested	8	Microsome ID	12535-13		
2	NB Ref.	12535-85	Counter data filename	22MR2054.P00						
3	Technician ID	SLB	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2		
4										
5	Vial	Time (min)	DPM1		ISIE					Round DPM
6	2	5	39574.11	17142.25	0	11.86	475.04			39574
7	3	5	38331.31	16567.88	0	11.8	474.26			38331
8	4	5	39545.94	17170.06	0	11.78	474.65			39546
9	5	5	40275.64	17603.16	0	11.79	477			40276
10	6	5	41045	18009.95	0	11.77	476.89			41045
11	8	5	14606.77	6792.38	0	12.72	533.92			14607
12	9	5	14302.47	6650.28	0	12.71	533.62			14302
13	10	5	14820.42	6919.04	0	12.66	535.25			14820
14	11	5	15071.33	7047.41	0	12.68	534.6			15071
15	12	5	13938	6477.63	0	12.65	533.58			13938
16	13	5	14124.33	6581.93	0	12.65	532.86			14124
17	14	5	13651.17	6404.14	0	12.72	536.58			13651
18	15	5	13778.65	6428.23	0	12.68	534.44			13779
19	16	5	58.85	28.21	0	11.52	533.21			59
20	17	5	53.17	28.62	0	12.66	534.97			53
21	18	5	35.24	17.07	0	13.04	531.71			35
22	19	5	44.19	20.24	0	13.13	533.28			44
23	20	5	49.36	25.58	0	12.79	535.08			49
24	21	5	52.4	25.9	0	12.76	536.59			52
25	22	5	80.3	26.66	0	15.41	533.9			80
26	23	5	54.22	27.8	0	11.21	535.45			54
27	25	5	159.65	76.24	0	12.3	531.41			160
28	26	5	153.58	70.91	0	12.47	532.44			154
29	27	5	161.6	77.58	0	13.42	531.61			162
30	28	5	162.83	74.96	0	13.45	536.86			163
31										0
32										0
33	29	5	919.26	430.41	0	12.71	534.12			919
34	30	5	832.05	396.94	0	12.29	531.93			832
35	31	5	854.34	397.54	0	12.81	534.75			854
36	32	5	842.91	394.7	0	12.62	533.39			843
37										0
38										0
39	33	5	1516.98	718.15	0	12.67	533.98			1517
40	34	5	1537.01	728.45	0	12.64	535.5			1537
41	35	5	1669.85	779.74	0	12.59	534.34			1670
42	36	5	1610.82	750.6	0	12.79	533.06			1611
43										0
44										0
45	37	5	5194.37	2426.26	0	12.59	534.41			5194
46	38	5	5067.73	2374	0	12.55	535.21			5068
47	39	5	5053.34	2368.56	0	12.69	534.19			5053
48	40	5	5162.55	2407.31	0	12.68	532.03			5163
49										0
50										0
51	41	5	7745.61	3638.33	0	12.61	536.69			7746
52	42	5	7664.3	3587.59	0	12.66	536.27			7664
53	43	5	7856.54	3661.87	0	12.54	531.25			7859
54	44	5	7948.34	3679.55	0	12.71	532.58			7948
55										0
56										0
57	45	5	12525	5855.3	0	12.88	536.32			12525
58	46	5	11964.01	5584.32	0	12.65	533.51			11964
59	47	5	12135.31	5669.24	0	12.83	534.23			12135
60	48	5	12403.13	5744.87	0	12.61	529.55			12403
61										0
62										0
63	49	5	13560.71	6339.03	0	12.61	534.15			13561
64	50	5	13602.08	6339.37	0	12.71	536.14			13602
65	51	5	13927.07	6497	0	12.67	533.01			13927
66	52	5	14235.68	6655.2	0	12.7	536.27			14236
67										0
68										0
69	53	5	14191.46	6597.2	0	12.69	531.62			14191
70	54	5	13926.56	6507.75	0	12.6	533.71			13927
71	55	5	13882.63	6487.07	0	12.75	536.92			13883
72	56	5	13965.83	6519.27	0	12.67	535.8			13966
73										0
74										0
75	58	5	69.4	34.26	0	13.78	540.1			69

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
76	59	5	68.37	32.68		0	12.66	538.15			68
77	60	5	69.47	33.49		0	13.61	537.46			69
78	61	5	64.03	32.76		0	13.74	538.4			64
79	62	5	86.3	39.32		0	14.41	534.79			86
80	63	5	73.18	37.25		0	12.14	541.09			73
81	64	5	63.66	29.76		0	11.96	538.53			64
82	65	5	60.37	28.25		0	12.04	534.78			60
83	66	5	66.14	30.81		0	13.89	538.36			66
84	67	5	72.6	34.09		0	12.39	534.77			73
85	68	5	42.62	25.09		0	10.62	534.8			43
86	69	5	56.56	30.8		0	12.94	530.33			59
87	70	5	70.54	35.38		0	12.54	534.79			71
88	71	5	81.94	42.13		0	12.44	535.1			82
89	72	5	67.67	33.95		0	11.91	534.19			68
90	73	5	68.74	34.11		0	10.71	534.91			69
91	74	5	57.83	34.2		0	11.26	535.51			58
92	75	5	78.94	36.1		0	13.67	532.77			79
93	76	5	215.97	101.99		0	13.04	535.55			216
94	77	5	204.35	92.88		0	13.13	532.85			204
95	78	5	184.25	85		0	12.97	538.52			184
96	79	5	207.65	99.69		0	13.22	535.29			208
97	80	5	224.85	105.06		0	12.49	537.03			225
98	81	5	222.84	98.62		0	13.31	531.59			223
99	83	5	1326.98	622.41		0	12.37	535.45			1327
100	84	5	1329.42	617.77		0	12.85	532.89			1329
101	85	5	1313.72	610.94		0	12.63	532.29			1314
102	86	5	1333.21	613.99		0	12.79	534.38			1333
103	87	5	1342.45	621.54		0	12.78	533.26			1342
104	88	5	1305.85	619.54		0	12.5	536.03			1306
105	89	5	8553.2	3077.16		0	12.71	535.24			8553
106	90	5	6722.98	3131.5		0	12.71	533.03			6723
107	91	5	6729.84	3111.94		0	12.7	532.47			6730
108	92	6	6573.96	3118.72		0	12.62	536.83			6574
109	93	5	6667.6	3125.47		0	12.59	533.82			6668
110	94	5	6597.84	3106.76		0	12.64	534.14			6598
111	95	5	12893.11	6013.3		0	12.65	534.1			12893
112	96	5	12990.11	6047.07		0	12.75	535.13			12990
113	97	5	13231.77	6157.49		0	12.67	533.24			13232
114	98	5	13055.52	6083.23		0	12.64	530.69			13056
115	99	5	12885.04	5981.89		0	12.63	531.25			12885
116	100	5	13109.18	6083.13		0	12.73	533.58			13109
117	101	5	14173.11	6605.03		0	12.66	533.78			14173
118	102	5	14210.47	6607.31		0	12.7	533.95			14210
119	103	5	0	0		0	0	607.73			0
120	104	5	11.01	5.83		0	27.51	607.76			11
121	105	5	14140.29	6591.13		0	12.67	535.83			14140
122	106	5	13839.8	6468.26		0	12.7	535.33			13840
123	107	5	226808.73	36325.65		0	7.78	173.58			226809
124	108	5	1.23	0		0	5.93	481.84			1

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Volume (mL)	Alt.	Alq. #	DPH/min	DPH/mL	Ave DPH/mL	Total DPM	Volume of substrate solution (mL)	Total DPM in assay tube (cpm)	Calculate and ³ H ₂ O formed (nmol ³ H ₂ O formed)	Test DPM corrected for background (background tubes)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (fmol estrogen formed/mg protein-min)
1	Full activity control	2	0.5	1	14697	29394	29394	57818	0.1	18265	57615	0.0614	0.005	15	0.423
2	Full activity control	2	0.5	2	14332	28664	28664	57472	0.1	18068	5978	0.0615	0.005	15	0.317
3	Full activity control	2	0.5	3	14220	28440	28440	56880	0.1	18068	5921	0.0615	0.005	15	0.398
4	Full activity control	2	0.5	4	13828	27656	27656	55312	0.1	18068	5487	0.0615	0.005	15	0.000
5	Full activity control	2	0.5	5	14124	28248	28248	56496	0.1	18068	5487	0.0615	0.005	15	0.000
6	Full activity control	2	0.5	6	13651	27302	27302	54604	0.1	18068	5487	0.0615	0.005	15	0.000
7	Full activity control	2	0.5	7	13779	27558	27558	55116	0.1	18068	5487	0.0615	0.005	15	0.000
8	Full activity control	2	0.5	8	59	118	118	224	0.1	18068	5487	0.0615	0.005	15	0.000
9	Full activity control	2	0.5	9	35	70	70	154	0.1	18068	5487	0.0615	0.005	15	0.000
10	Full activity control	2	0.5	10	44	88	88	202	0.1	18068	5487	0.0615	0.005	15	0.000
11	Full activity control	2	0.5	11	49	98	98	228	0.1	18068	5487	0.0615	0.005	15	0.000
12	Full activity control	2	0.5	12	52	104	104	278	0.1	18068	5487	0.0615	0.005	15	0.000
13	Full activity control	2	0.5	13	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
14	Full activity control	2	0.5	14	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
15	Full activity control	2	0.5	15	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
16	Full activity control	2	0.5	16	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
17	Full activity control	2	0.5	17	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
18	Full activity control	2	0.5	18	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
19	Full activity control	2	0.5	19	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
20	Full activity control	2	0.5	20	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
21	Full activity control	2	0.5	21	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
22	Full activity control	2	0.5	22	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
23	Full activity control	2	0.5	23	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
24	Full activity control	2	0.5	24	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
25	Full activity control	2	0.5	25	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
26	Full activity control	2	0.5	26	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
27	Full activity control	2	0.5	27	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
28	Full activity control	2	0.5	28	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
29	Full activity control	2	0.5	29	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
30	Full activity control	2	0.5	30	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
31	Full activity control	2	0.5	31	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
32	Full activity control	2	0.5	32	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
33	Full activity control	2	0.5	33	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
34	Full activity control	2	0.5	34	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
35	Full activity control	2	0.5	35	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
36	Full activity control	2	0.5	36	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
37	Full activity control	2	0.5	37	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
38	Full activity control	2	0.5	38	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
39	Full activity control	2	0.5	39	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
40	Full activity control	2	0.5	40	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
41	Full activity control	2	0.5	41	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
42	Full activity control	2	0.5	42	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
43	Full activity control	2	0.5	43	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
44	Full activity control	2	0.5	44	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
45	Full activity control	2	0.5	45	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
46	Full activity control	2	0.5	46	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
47	Full activity control	2	0.5	47	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
48	Full activity control	2	0.5	48	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
49	Full activity control	2	0.5	49	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
50	Full activity control	2	0.5	50	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
51	Full activity control	2	0.5	51	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
52	Full activity control	2	0.5	52	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
53	Full activity control	2	0.5	53	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
54	Full activity control	2	0.5	54	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
55	Full activity control	2	0.5	55	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
56	Full activity control	2	0.5	56	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
57	Full activity control	2	0.5	57	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
58	Full activity control	2	0.5	58	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
59	Full activity control	2	0.5	59	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
60	Full activity control	2	0.5	60	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
61	Full activity control	2	0.5	61	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
62	Full activity control	2	0.5	62	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
63	Full activity control	2	0.5	63	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
64	Full activity control	2	0.5	64	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
65	Full activity control	2	0.5	65	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
66	Full activity control	2	0.5	66	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
67	Full activity control	2	0.5	67	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
68	Full activity control	2	0.5	68	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
69	Full activity control	2	0.5	69	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000
70	Full activity control	2	0.5	70	54	108	108	288	0.1	18068	5487	0.0615	0.005	15	0.000

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check: aliquot variance			
6		Root of value not within mean $\pm$ 10%			
7					
8		1.01			
9		0.99			
10		1.01			
11		0.99			
12		1.01			
13		1.00			
14		1.00			
15		1.03			
16		0.95			
17					
18					
19		0.97			
20		1.03			
21		0.95			
22		1.02			
23		0.98			
24		1.00			
25		1.00			
26		EDNVP			
27		EDNVP			
28		EDNVP			
29		1.05			
30		0.95			
31		1.01			
32		0.99			
33		EDNVP			
34		EDNVP			
35		0.99			
36		1.01			
37		1.02			
38		0.98			
39		EDNVP			
40		EDNVP			
41		1.01			
42		0.99			
43		0.99			
44		1.01			
45		EDNVP			
46		EDNVP			
47		1.01			
48		0.99			
49		0.99			
50		1.01			
51		EDNVP			
52		EDNVP			
53		1.02			
54		0.98			
55		0.99			
56		1.01			
57		EDNVP			
58		EDNVP			
59		1.00			
60		1.00			
61		0.99			
62		1.01			
63		EDNVP			
64		EDNVP			
65		1.01			
66		0.99			
67		1.00			
68		1.00			
69		EDNVP			
70		EDNVP			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicates	Normal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay time (mL)	total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed (initial)	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen/nmol protein/min)
1	3/22/2007	2	0.5	1	69	138	137	274	0.1	185058	Total DPM corrected for background (Background Tubes)	71	0.005	15	0.001
2	3/22/2007	2	0.5	2	69	138	137	274	0.1	185058		63	0.005	15	0.001
3	3/22/2007	2	0.5	3	69	138	137	274	0.1	185058		115	0.005	15	0.001
4	3/22/2007	2	0.5	4	69	138	137	274	0.1	185058		45	0.005	15	0.001
5	3/22/2007	2	0.5	5	69	138	137	274	0.1	185058		79	0.005	15	0.001
6	3/22/2007	2	0.5	6	69	138	137	274	0.1	185058		1	0.005	15	0.001
7	3/22/2007	2	0.5	7	69	138	137	274	0.1	185058		103	0.005	15	0.001
8	3/22/2007	2	0.5	8	69	138	137	274	0.1	185058		71	0.005	15	0.001
9	3/22/2007	2	0.5	9	69	138	137	274	0.1	185058		63	0.005	15	0.001
10	3/22/2007	2	0.5	10	69	138	137	274	0.1	185058		115	0.005	15	0.001
11	3/22/2007	2	0.5	11	69	138	137	274	0.1	185058		45	0.005	15	0.001
12	3/22/2007	2	0.5	12	69	138	137	274	0.1	185058		79	0.005	15	0.001
13	3/22/2007	2	0.5	13	69	138	137	274	0.1	185058		1	0.005	15	0.001
14	3/22/2007	2	0.5	14	69	138	137	274	0.1	185058		103	0.005	15	0.001
15	3/22/2007	2	0.5	15	69	138	137	274	0.1	185058		71	0.005	15	0.001
16	3/22/2007	2	0.5	16	69	138	137	274	0.1	185058		63	0.005	15	0.001
17	3/22/2007	2	0.5	17	69	138	137	274	0.1	185058		115	0.005	15	0.001
18	3/22/2007	2	0.5	18	69	138	137	274	0.1	185058		45	0.005	15	0.001
19	3/22/2007	2	0.5	19	69	138	137	274	0.1	185058		79	0.005	15	0.001
20	3/22/2007	2	0.5	20	69	138	137	274	0.1	185058		1	0.005	15	0.001
21	3/22/2007	2	0.5	21	69	138	137	274	0.1	185058		103	0.005	15	0.001
22	3/22/2007	2	0.5	22	69	138	137	274	0.1	185058		71	0.005	15	0.001
23	3/22/2007	2	0.5	23	69	138	137	274	0.1	185058		63	0.005	15	0.001
24	3/22/2007	2	0.5	24	69	138	137	274	0.1	185058		115	0.005	15	0.001
25	3/22/2007	2	0.5	25	69	138	137	274	0.1	185058		45	0.005	15	0.001
26	3/22/2007	2	0.5	26	69	138	137	274	0.1	185058		79	0.005	15	0.001
27	3/22/2007	2	0.5	27	69	138	137	274	0.1	185058		1	0.005	15	0.001
28	3/22/2007	2	0.5	28	69	138	137	274	0.1	185058		103	0.005	15	0.001
29	3/22/2007	2	0.5	29	69	138	137	274	0.1	185058		71	0.005	15	0.001
30	3/22/2007	2	0.5	30	69	138	137	274	0.1	185058		63	0.005	15	0.001
31	3/22/2007	2	0.5	31	69	138	137	274	0.1	185058		115	0.005	15	0.001
32	3/22/2007	2	0.5	32	69	138	137	274	0.1	185058		45	0.005	15	0.001
33	3/22/2007	2	0.5	33	69	138	137	274	0.1	185058		79	0.005	15	0.001
34	3/22/2007	2	0.5	34	69	138	137	274	0.1	185058		1	0.005	15	0.001
35	3/22/2007	2	0.5	35	69	138	137	274	0.1	185058		103	0.005	15	0.001
36	3/22/2007	2	0.5	36	69	138	137	274	0.1	185058		71	0.005	15	0.001
37	3/22/2007	2	0.5	37	69	138	137	274	0.1	185058		63	0.005	15	0.001
38	3/22/2007	2	0.5	38	69	138	137	274	0.1	185058		115	0.005	15	0.001
39	3/22/2007	2	0.5	39	69	138	137	274	0.1	185058		45	0.005	15	0.001
40	3/22/2007	2	0.5	40	69	138	137	274	0.1	185058		79	0.005	15	0.001
41	3/22/2007	2	0.5	41	69	138	137	274	0.1	185058		1	0.005	15	0.001
42	3/22/2007	2	0.5	42	69	138	137	274	0.1	185058		103	0.005	15	0.001
43	3/22/2007	2	0.5	43	69	138	137	274	0.1	185058		71	0.005	15	0.001
44	3/22/2007	2	0.5	44	69	138	137	274	0.1	185058		63	0.005	15	0.001
45	3/22/2007	2	0.5	45	69	138	137	274	0.1	185058		115	0.005	15	0.001
46	3/22/2007	2	0.5	46	69	138	137	274	0.1	185058		45	0.005	15	0.001
47	3/22/2007	2	0.5	47	69	138	137	274	0.1	185058		79	0.005	15	0.001
48	3/22/2007	2	0.5	48	69	138	137	274	0.1	185058		1	0.005	15	0.001
49	3/22/2007	2	0.5	49	69	138	137	274	0.1	185058		103	0.005	15	0.001
50	3/22/2007	2	0.5	50	69	138	137	274	0.1	185058		71	0.005	15	0.001
51	3/22/2007	2	0.5	51	69	138	137	274	0.1	185058		63	0.005	15	0.001
52	3/22/2007	2	0.5	52	69	138	137	274	0.1	185058		115	0.005	15	0.001
53	3/22/2007	2	0.5	53	69	138	137	274	0.1	185058		45	0.005	15	0.001
54	3/22/2007	2	0.5	54	69	138	137	274	0.1	185058		79	0.005	15	0.001

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		OP check - aliquot
5		variance
6		% RE
7		1.01
8		0.99
9		1.04
10		0.96
11		1.05
12		0.92
13		1.03
14		0.97
15		0.96
16		1.04
17		
18		
19		0.93
20		1.07
21		0.99
22		1.01
23		
24		
25		1.03
26		0.97
27		0.94
28		1.06
29		1.00
30		1.00
31		1.00
32		1.00
33		0.99
34		1.01
35		1.01
36		0.99
37		0.99
38		1.01
39		1.01
40		0.99
41		1.01
42		0.99
43		1.00
44		1.00
45		#VALUE!
46		#VALUE!
47		0.99
48		1.01
49		1.00
50		1.00
51		#VALUE!
52		#VALUE!
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/22/2007	Test Chemical ID	CR11609	12535-85	22MR2054.P00	1	Positive Control	4-OH ASDN	8 Microsome ID	12535-13
2	RB Ref.	SLB	Conc. dose Name	Replicate #						Pos Cl Tubes/Cubic	2
3											
4											
5	Control Type	Average	SD								
6	Full activity	0.405	0.015								
7	Background (uncorrected)	0.001	0.000								
8											
9											
10	Positive Control	Replicate	Positive Cl M	Log(Positive Cl)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	2	1.00E-05	-5.00	0.003						
13	4-OH ASDN	1	1.00E-06	-6.00	0.023						
14	4-OH ASDN	2	1.00E-06	-6.00	0.023						
15	4-OH ASDN	1	5.00E-07	-6.30	0.042						
16	4-OH ASDN	2	5.00E-07	-6.30	0.045						
17	4-OH ASDN	1	1.00E-07	-7.00	0.144						
18	4-OH ASDN	2	1.00E-07	-7.00	0.144						
19	4-OH ASDN	1	5.00E-08	-7.30	0.217						
20	4-OH ASDN	2	5.00E-08	-7.30	0.223						
21	4-OH ASDN	1	1.00E-08	-8.00	0.346						
22	4-OH ASDN	2	1.00E-08	-8.00	0.347						
23	4-OH ASDN	1	1.00E-09	-9.00	0.364						
24	4-OH ASDN	2	1.00E-09	-9.00	0.399						
25	4-OH ASDN	1	1.00E-10	-10.00	0.358						
26	4-OH ASDN	2	1.00E-10	-10.00	0.395						
27	4-OH ASDN	1									
28	4-OH ASDN	2									
29	4-OH ASDN	1									
30	4-OH ASDN	2									
31	4-OH ASDN	1									
32	4-OH ASDN	2									
33	4-OH ASDN	1									
34	4-OH ASDN	2									
35	4-OH ASDN	1									
36	4-OH ASDN	2									
37	Test Chemical	Replicate	Test chemical M	Log(test chemical)	Activity						
38	CR11609	1	1.00E-03	-3.00	0.001						
39	CR11609	2	1.00E-03	-3.00	0.000						
40	CR11609	1	1.00E-03	-3.00	0.001						
41	CR11609	2	1.00E-04	-4.00	0.000						
42	CR11609	1	1.00E-04	-4.00	0.001						
43	CR11609	2	1.00E-04	-4.00	0.000						
44	CR11609	1	1.00E-05	-5.00	0.001						
45	CR11609	2	1.00E-05	-5.00	0.001						
46	CR11609	1	1.00E-06	-6.00	0.001						
47	CR11609	2	1.00E-06	-6.00	0.005						
48	CR11609	1	1.00E-06	-6.00	0.004						
49	CR11609	2	1.00E-06	-6.00	0.005						
50	CR11609	1	1.00E-07	-7.00	0.036						
51	CR11609	2	1.00E-07	-7.00	0.036						
52	CR11609	1	1.00E-07	-7.00	0.033						
53	CR11609	2	1.00E-08	-8.00	0.187						
54	CR11609	1	1.00E-08	-8.00	0.188						
55	CR11609	2	1.00E-08	-8.00	0.187						
56	CR11609	1	1.00E-09	-9.00	0.365						
57	CR11609	2	1.00E-09	-9.00	#VALUE!						
58	CR11609	1	1.00E-09	-9.00	0.368						
59	CR11609	2	1.00E-09	-9.00	0.402						
60	CR11609	1	1.00E-10	-10.00	#VALUE!						
61	CR11609	2	1.00E-10	-10.00	0.396						
62	CR11609	1	1.00E-10	-10.00	0.396						
63	CR11609	2	1.00E-10	-10.00	0.396						
64	CR11609	1	1.00E-10	-10.00	0.396						
65	CR11609	2	1.00E-10	-10.00	0.396						
66	CR11609	1	1.00E-10	-10.00	0.396						
67	CR11609	2	1.00E-10	-10.00	0.396						
68	CR11609	1	1.00E-10	-10.00	0.396						
69	CR11609	2	1.00E-10	-10.00	0.396						
70	CR11609	1	1.00E-10	-10.00	0.396						
71	CR11609	2	1.00E-10	-10.00	0.396						
72	CR11609	1	1.00E-10	-10.00	0.396						
73	CR11609	2	1.00E-10	-10.00	0.396						
74	CR11609	1	1.00E-10	-10.00	0.396						
75	CR11609	2	1.00E-10	-10.00	0.396						
76	CR11609	1	1.00E-10	-10.00	0.396						
77	CR11609	2	1.00E-10	-10.00	0.396						
78	CR11609	1	1.00E-10	-10.00	0.396						
79	CR11609	2	1.00E-10	-10.00	0.396						
80	CR11609	1	1.00E-10	-10.00	0.396						
81	CR11609	2	1.00E-10	-10.00	0.396						
82	CR11609	1	1.00E-10	-10.00	0.396						
83	CR11609	2	1.00E-10	-10.00	0.396						
84	CR11609	1	1.00E-10	-10.00	0.396						
85	CR11609	2	1.00E-10	-10.00	0.396						
86	CR11609	1	1.00E-10	-10.00	0.396						
87	CR11609	2	1.00E-10	-10.00	0.396						
88	CR11609	1	1.00E-10	-10.00	0.396						
89	CR11609	2	1.00E-10	-10.00	0.396						
90	CR11609	1	1.00E-10	-10.00	0.396						
91	CR11609	2	1.00E-10	-10.00	0.396						
92	CR11609	1	1.00E-10	-10.00	0.396						
93	CR11609	2	1.00E-10	-10.00	0.396						
94	CR11609	1	1.00E-10	-10.00	0.396						
95	CR11609	2	1.00E-10	-10.00	0.396						
96	CR11609	1	1.00E-10	-10.00	0.396						
97	CR11609	2	1.00E-10	-10.00	0.396						
98	CR11609	1	1.00E-10	-10.00	0.396						
99	CR11609	2	1.00E-10	-10.00	0.396						
100	CR11609	1	1.00E-10	-10.00	0.396						
101	CR11609	2	1.00E-10	-10.00	0.396						
102	CR11609	1	1.00E-10	-10.00	0.396						
103	CR11609	2	1.00E-10	-10.00	0.396						
104	CR11609	1	1.00E-10	-10.00	0.396						
105	CR11609	2	1.00E-10	-10.00	0.396						
106	CR11609	1	1.00E-10	-10.00	0.396						
107	CR11609	2	1.00E-10	-10.00	0.396						
108	CR11609	1	1.00E-10	-10.00	0.396						
109	CR11609	2	1.00E-10	-10.00	0.396						
110	CR11609	1	1.00E-10	-10.00	0.396						
111	CR11609	2	1.00E-10	-10.00	0.396						
112	CR11609	1	1.00E-10	-10.00	0.396						
113	CR11609	2	1.00E-10	-10.00	0.396						
114	CR11609	1	1.00E-10	-10.00	0.396						
115	CR11609	2	1.00E-10	-10.00	0.396						
116	CR11609	1	1.00E-10	-10.00	0.396						
117	CR11609	2	1.00E-10	-10.00	0.396						
118	CR11609	1	1.00E-10	-10.00	0.396						
119	CR11609	2	1.00E-10	-10.00	0.396						
120	CR11609	1	1.00E-10	-10.00	0.396						
121	CR11609	2	1.00E-10	-10.00	0.396						
122	CR11609	1	1.00E-10	-10.00	0.396						
123	CR11609	2	1.00E-10	-10.00	0.396						
124	CR11609	1	1.00E-10	-10.00	0.396						
125	CR11609	2	1.00E-10	-10.00	0.396						
126	CR11609	1	1.00E-10	-10.00	0.396						
127	CR11609	2	1.00E-10	-10.00	0.396						
128	CR11609	1	1.00E-10	-10.00	0.396						
129	CR11609	2	1.00E-10	-10.00	0.396						
130	CR11609	1	1.00E-10	-10.00	0.396						
131	CR11609	2	1.00E-10	-10.00	0.396						
132	CR11609	1	1.00E-10	-10.00	0.396						
133	CR11609	2	1.00E-10	-10.00	0.396						
134	CR11609	1	1.00E-10	-10.00	0.396						
135	CR11609	2	1.00E-10	-10.00	0.396						
136	CR11609	1	1.00E-10	-10.00	0.396						
137	CR11609	2	1.00E-10	-10.00	0.396						
138	CR11609	1	1.00E-10	-10.00	0.396						
139	CR11609	2	1.00E-10	-10.00	0.396						
140	CR11609	1	1.00E-10	-10.00	0.396						
141	CR11609	2	1.00E-10	-10.00	0.396						
142	CR11609	1	1.00E-10	-10.00	0.396						
143	CR11609	2	1.00E-10	-10.00	0.396						
144	CR11609	1	1.00E-10	-10.00	0.396						
145	CR11609	2	1.00E-10	-10.00	0.396						
146	CR11609	1	1.00								

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
	Test # Concentrations								
1	Assay Date	3/27/2007	Chemical ID	CR11609	tested		8	Microsome ID	12535-13
2	NB Ref.	12535-88	Counter data filename	27MR2035.P00					
	Technician		Pos Ctl						
3	ID	SLB	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/27/2007	Test Chemical ID	CR11609	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-88	Counter data filename	27MR2035.P00					
3	Technician ID	SLB	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0214		37359		1745748			
8	2	0.0206		38527		1870243			
9	3	0.0205		38766		1891024			
10	4	0.0208		39314		1890096			
11	5	0.0206		40272		1954951			
12						Average DPM/g soln	1870412		
13						SD	76649		
14						CV	4.10		
15						µCi/g soln	0.8425		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1379 g			
25	Mass of dilution B used in substrate prep					4.5639 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.561942 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.010	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8425			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5619	+	0.010		
37				=	0.572	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.472	µCi/µg ASDN			
41					9.362.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/27/2007	Test Chemical ID	CR11609	# Concentrations tested	8	Microsome ID	12535-13		
2		NB Ref.	12535-88	Counter data filename	27MR2035.P00						
3		Technician		Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2		
4		ID	SLB								
5		Vial	Time (min)	DPM1			ISIE				Round DPM
6	2	5	37359.44	16709.23	0	12.14	498.82				37359
7	3	5	38528.95	16738.41	0	11.9	477.11				38527
8	4	5	38765.67	16858.48	0	11.82	475.34				38766
9	5	5	39314.34	17132.03	0	11.85	477.53				39314
10	6	5	40272.31	17482.81	0	11.83	474.97				40272
11	8	5	14125.47	6590.76	0	12.84	537.96				14125
12	9	5	14178.19	6600.23	0	12.69	532.96				14179
13	10	5	13400.57	6259.83	0	12.71	536.39				13401
14	11	5	13426.25	6216.13	0	12.69	530.6				13426
15	12	5	13369.45	6227.29	0	12.68	532.39				13369
16	13	5	13337.62	6213.25	0	12.71	531.34				13338
17	14	5	12717.37	5919.4	0	12.69	533.93				12717
18	15	5	13196.99	6144.14	0	12.78	536.4				13197
19	16	5	82.13	27.74	0	15.62	533.63				82
20	17	5	84.33	28.77	0	12.56	533.89				84
21	18	5	71.82	31.92	0	16.41	530.92				72
22	19	5	64.59	31.31	0	14.27	533.2				65
23	20	5	74.29	39.03	0	13.18	536.02				74
24	21	5	58.59	30.34	0	13.93	535.56				60
25	22	5	62.27	30.49	0	14.81	534.25				62
26	23	5	60.51	29.5	0	13.34	532.97				61
27	25	5	202.19	94.11	0	12.72	533.2				202
28	26	5	142.04	70.04	0	11.86	532.91				142
29	27	5	162.12	74.43	0	12.92	535.79				162
30	28	5	160.67	75.24	0	12.99	532.58				161
31											
32											
33	29	5	847.78	392.52	0	12.76	527.94				848
34	30	5	868.43	400.56	0	12.52	527.55				868
35	31	5	776.61	355.3	0	12.67	527.89				777
36	32	5	800.25	380.1	0	12.46	530.94				800
37											
38											
39	33	5	1499.56	699.56	0	12.57	532.88				1500
40	34	5	1465.97	675.79	0	12.55	532.53				1466
41	35	5	1293.29	595.24	0	12.75	530.95				1293
42	36	5	1458.68	667.75	0	12.92	529.96				1459
43											
44											
45	37	5	4708.65	2189.2	0	12.77	534.81				4709
46	38	5	4771.98	2219.02	0	12.74	535.1				4772
47	39	5	4409.94	2045.67	0	12.69	533.62				4410
48	40	5	4577.62	2114.25	0	12.83	532.02				4578
49											
50											
51	41	5	7367.35	3403.7	0	12.63	530.47				7367
52	42	5	7149.83	3329.86	0	12.69	533.41				7150
53	43	5	6689.45	3123.11	0	12.67	533.74				6689
54	44	5	6847.22	3208.55	0	12.68	535.83				6847
55											
56											
57	45	5	11535.42	5367.62	0	12.74	534.76				11535
58	46	5	11664.53	5414.04	0	12.73	532.97				11665
59	47	5	10739.35	5009.22	0	12.66	532.55				10739
60	48	5	10895.85	5051.31	0	12.75	533				10896
61											
62											
63	49	5	13084.65	6067.39	0	12.67	530.61				13085
64	50	5	13058.88	6058.51	0	12.64	529.71				13059
65	51	5	13110.91	6067.03	0	12.61	527.28				13111
66	52	5	13118.53	6098.21	0	12.67	532.32				13119
67											
68											
69	53	5	13377.27	6229.94	0	12.7	533.16				13377
70	54	5	13593.65	6273.22	0	12.76	529.73				13594
71	55	5	12458.29	5782.22	0	12.71	531.71				12458
72	56	5	12917.89	5988.3	0	12.7	530.91				12918
73											
74											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	100.79	48.07		0	12.73	533.95			101
76	59	5	103.49	48.77		0	12.5	532.4			103
77	60	5	97.5	48.19		0	11.46	531.53			98
78	61	5	100.33	47.49		0	12.81	534.46			100
79	62	5	96.63	45.58		0	12.01	534.29			97
80	63	5	99.39	45.42		0	15.4	533.06			99
81	64	5	329.79	142.29		0	13.24	531.34			330
82	65	5	296.71	144		0	12.77	533.78			297
83	66	5	291.37	139.7		0	12.6	533.58			291
84	67	5	326.84	153.76		0	13.02	535.11			327
85	68	5	279.48	129.79		0	13.5	531.52			279
86	69	5	299.84	144.21		0	12.95	531.3			300
87	70	5	2102.38	962.18		0	12.76	524.9			2102
88	71	5	2063.77	961.19		0	12.71	529.77			2064
89	72	5	2096.89	953.4		0	12.83	529.49			2097
90	73	5	2110.38	986.16		0	12.5	529.97			2110
91	74	5	2006.78	929.54		0	12.77	530.8			2007
92	75	5	2013.83	937.74		0	12.65	531.52			2014
93	76	5	5537.8	2576.96		0	12.75	534.46			5538
94	77	5	5539.16	2580.66		0	12.67	531.27			5539
95	78	5	5556.77	2665.85		0	12.77	531.52			5557
96	79	5	5433.01	2519.29		0	12.67	533.94			5433
97	80	5	5435.03	2522.67		0	12.72	530.26			5435
98	81	5	5260.93	2452.31		0	12.72	532.39			5261
99	83	5	8359.53	3864.9		0	12.83	534.16			8360
100	84	5	8457.83	3915.15		0	12.69	531.62			8458
101	85	5	8423.21	3934.43		0	12.69	531.93			8423
102	86	5	8326.96	3855.86		0	12.77	534.35			8327
103	87	5	8256.19	3821.34		0	12.8	532.52			8256
104	88	5	8215.45	3847.99		0	12.7	535.03			8215
105	89	5	11537.46	5332.14		0	12.7	530.44			11537
106	90	5	11532.98	5369.33		0	12.82	533.82			11533
107	91	5	11762.96	5396.41		0	12.69	526.74			11763
108	92	5	11700.22	5433.38		0	12.61	530.13			11700
109	93	5	11515.59	5308.03		0	12.67	525.83			11516
110	94	5	11384.05	5318.86		0	12.56	530.86			11384
111	95	5	12495.71	5815.03		0	12.68	531.82			12496
112	96	5	12721.07	5888.68		0	12.75	529.92			12721
113	97	5	12877.37	5986.91		0	12.74	532.61			12877
114	98	5	12811.65	5936.22		0	12.74	531.3			12812
115	99	5	12271.67	5642.37		0	12.73	526.67			12272
116	100	5	12249	5691.28		0	12.67	529.63			12249
117	101	5	13309.33	6189.04		0	12.69	533.96			13309
118	102	5	13263.23	6114.5		0	12.66	528.42			13263
119	103	5	13203.64	6142.17		0	12.71	533.03			13204
120	104	5	13726.2	6299.7		0	12.72	528.47			13726
121	105	5	13264.58	6118.99		0	12.66	526.99			13265
122	106	5	13111.25	6130.09		0	12.72	534.78			13111
123	107	5	229718.63	35813.49		0	7.78	171.2			229719
124	108	5	23.04	7.74		0	22.56	462.39			23

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Nominal total volume (mL)	Aliq Volume (mL)	Aliq #	DPH/dilq	DPH/mL	Ave DPH/mL	Total DPH	Volume of substrate solution used assay tube (mL)	total DPM in assay tube (initial)	Calculate net H_2O formed	net H_2O formed	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrone formed/mg protein/min)
1	Full activity control	2	0.5	1	14175	28350	28350	56700	0.1	187041		56700	0.005	15	
2		2	0.5	2	14175	28350	28350	56700	0.1	187041		56700	0.005	15	
3		2	0.5	1	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
4		2	0.5	2	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
5		2	0.5	1	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
6		2	0.5	2	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
7		2	0.5	1	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
8		2	0.5	2	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
9		2	0.5	1	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
10		2	0.5	2	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
11		2	0.5	1	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
12		2	0.5	2	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
13		2	0.5	1	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
14		2	0.5	2	13401	26802	26802	53604	0.1	187041		53604	0.005	15	
15	Background control	2	0.5	1	13197	26394	26394	52788	0.1	187041		52788	0.005	15	
16		2	0.5	2	13197	26394	26394	52788	0.1	187041		52788	0.005	15	
17		2	0.5	1	64	128	128	256	0.1	187041		256	0.0001	15	
18		2	0.5	2	64	128	128	256	0.1	187041		256	0.0001	15	
19		2	0.5	1	72	144	144	288	0.1	187041		288	0.0001	15	
20		2	0.5	2	72	144	144	288	0.1	187041		288	0.0001	15	
21		2	0.5	1	60	120	120	240	0.1	187041		240	0.0001	15	
22		2	0.5	2	60	120	120	240	0.1	187041		240	0.0001	15	
23		2	0.5	1	61	122	122	244	0.1	187041		244	0.0001	15	
24		2	0.5	2	61	122	122	244	0.1	187041		244	0.0001	15	
25	4-OH ASDN	2	0.5	1	102	204	204	408	0.1	187041		408	0.0001	15	
26		2	0.5	2	102	204	204	408	0.1	187041		408	0.0001	15	
27		2	0.5	1	102	204	204	408	0.1	187041		408	0.0001	15	
28		2	0.5	2	102	204	204	408	0.1	187041		408	0.0001	15	
29		2	0.5	1	0	0	0	0	0.1	187041		0	0.0001	15	
30		2	0.5	2	0	0	0	0	0.1	187041		0	0.0001	15	
31		2	0.5	1	868	1736	1736	3472	0.1	187041		3472	0.005	15	
32		2	0.5	2	868	1736	1736	3472	0.1	187041		3472	0.005	15	
33		2	0.5	1	868	1736	1736	3472	0.1	187041		3472	0.005	15	
34		2	0.5	2	868	1736	1736	3472	0.1	187041		3472	0.005	15	
35		2	0.5	1	1668	3336	3336	6672	0.1	187041		6672	0.005	15	
36		2	0.5	2	1668	3336	3336	6672	0.1	187041		6672	0.005	15	
37		2	0.5	1	1293	2586	2586	5172	0.1	187041		5172	0.005	15	
38		2	0.5	2	1293	2586	2586	5172	0.1	187041		5172	0.005	15	
39		2	0.5	1	1458	2916	2916	5832	0.1	187041		5832	0.005	15	
40		2	0.5	2	1458	2916	2916	5832	0.1	187041		5832	0.005	15	
41		2	0.5	1	4799	9598	9598	19196	0.1	187041		19196	0.005	15	
42		2	0.5	2	4799	9598	9598	19196	0.1	187041		19196	0.005	15	
43		2	0.5	1	4799	9598	9598	19196	0.1	187041		19196	0.005	15	
44		2	0.5	2	4799	9598	9598	19196	0.1	187041		19196	0.005	15	
45		2	0.5	1	4578	9156	9156	18312	0.1	187041		18312	0.005	15	
46		2	0.5	2	4578	9156	9156	18312	0.1	187041		18312	0.005	15	
47		2	0.5	1	756	1512	1512	3024	0.1	187041		3024	0.005	15	
48		2	0.5	2	756	1512	1512	3024	0.1	187041		3024	0.005	15	
49		2	0.5	1	7159	14318	14318	28636	0.1	187041		28636	0.005	15	
50		2	0.5	2	7159	14318	14318	28636	0.1	187041		28636	0.005	15	
51		2	0.5	1	6869	13738	13738	27476	0.1	187041		27476	0.005	15	
52		2	0.5	2	6869	13738	13738	27476	0.1	187041		27476	0.005	15	
53		2	0.5	1	6847	13694	13694	27388	0.1	187041		27388	0.005	15	
54		2	0.5	2	6847	13694	13694	27388	0.1	187041		27388	0.005	15	
55		2	0.5	1	11535	23070	23070	46140	0.1	187041		46140	0.005	15	
56		2	0.5	2	11535	23070	23070	46140	0.1	187041		46140	0.005	15	
57		2	0.5	1	10959	21918	21918	43836	0.1	187041		43836	0.005	15	
58		2	0.5	2	10959	21918	21918	43836	0.1	187041		43836	0.005	15	
59		2	0.5	1	10485	20970	20970	41940	0.1	187041		41940	0.005	15	
60		2	0.5	2	10485	20970	20970	41940	0.1	187041		41940	0.005	15	
61		2	0.5	1	13085	26170	26170	52340	0.1	187041		52340	0.005	15	
62		2	0.5	2	13085	26170	26170	52340	0.1	187041		52340	0.005	15	
63		2	0.5	1	13111	26222	26222	52444	0.1	187041		52444	0.005	15	
64		2	0.5	2	13111	26222	26222	52444	0.1	187041		52444	0.005	15	
65		2	0.5	1	13119	26238	26238	52476	0.1	187041		52476	0.005	15	
66		2	0.5	2	13119	26238	26238	52476	0.1	187041		52476	0.005	15	
67		2	0.5	1	13377	26754	26754	53508	0.1	187041		53508	0.005	15	
68		2	0.5	2	13377	26754	26754	53508	0.1	187041		53508	0.005	15	
69		2	0.5	1	13584	27168	27168	54336	0.1	187041		54336	0.005	15	
70		2	0.5	2	13584	27168	27168	54336	0.1	187041		54336	0.005	15	
71		2	0.5	1	13116	26232	26232	52464	0.1	187041		52464	0.005	15	
72		2	0.5	2	13116	26232	26232	52464	0.1	187041		52464	0.005	15	
73		2	0.5	1	0	0	0	0	0.1	187041		0	0.005	15	
74		2	0.5	2	0	0	0	0	0.1	187041		0	0.005	15	

Aromatase Assay Spreadsheet

	R	S	T	U	V
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5		QC check - aliquot variance			
6		Red if value not within mean +/- 10%			
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
	Assay Date	3/27/2007	Chemical ID	CR11609	# Concentrations tested	12/35-13											
1	MB Ref	12635-98	27MR0205-P00	2	Positive Control	4-OH-ASED	Pos Ct Tubes/Conc	2									
2	Technician ID	SLR	Replicate #														
3																	
4																	
5	Sample ID	Replicate	Volume (mL)	APR #	DPM/mL	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used in assay tube (mL)	Total DPM in assay tube (final)	Calculate nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estradiol formed/mg protein/min)			
6	2-1	2	0.5	1	101	202	204	408	0.1	187041	148	0.0002	15	0.001			
7	2-2	2	0.5	2	103	206	196	396	0.1	187041	136	0.0001	15	0.001			
8	2-3	2	0.5	3	98	196	196	392	0.1	187041	132	0.0001	15	0.001			
9	3-1	2	0.5	1	97	194	196	392	0.1	187041	984	0.0011	15	0.007			
10	3-2	2	0.5	2	339	650	627	1254	0.1	187041	976	0.0010	15	0.007			
11	3-3	2	0.5	3	297	594	579	1158	0.1	187041	888	0.0010	15	0.006			
12	4-1	2	0.5	1	300	600	4166	8332	0.1	187041	8072	0.0086	15	0.057			
13	4-2	2	0.5	2	2102	4204	4207	8414	0.1	187041	8154	0.0087	15	0.058			
14	4-3	2	0.5	3	2097	4194	4207	8414	0.1	187041	7782	0.0083	15	0.055			
15	5-1	2	0.5	1	2014	4028	4021	8042	0.1	187041	21894	0.0234	15	0.156			
16	5-2	2	0.5	2	5539	11078	11077	22154	0.1	187041	21720	0.0232	15	0.155			
17	5-3	2	0.5	3	5557	11114	10880	21760	0.1	187041	21132	0.0226	15	0.150			
18	6-1	2	0.5	1	5435	10870	10606	21212	0.1	187041	33276	0.0757	15	0.238			
19	6-2	2	0.5	2	8360	16720	16812	33624	0.1	187041	33240	0.0755	15	0.237			
20	6-3	2	0.5	3	8438	16876	16740	33480	0.1	187041	32582	0.0749	15	0.233			
21	7-1	2	0.5	1	8715	17430	17471	34942	0.1	187041	43880	0.0480	15	0.327			
22	7-2	2	0.5	2	11537	23074	23070	46140	0.1	187041	46056	0.0486	15	0.332			
23	7-3	2	0.5	3	11703	23406	23483	46966	0.1	187041	46540	0.0486	15	0.324			
24	8-1	2	0.5	1	12496	24992	25017	50034	0.1	187041	50174	0.0536	15	0.367			
25	8-2	2	0.5	2	12771	25542	25599	51198	0.1	187041	51118	0.0545	15	0.364			
26	8-3	2	0.5	3	12812	25624	24521	49042	0.1	187041	48782	0.0521	15	0.347			
27	9-1	2	0.5	1	13089	26178	26572	53144	0.1	187041	52884	0.0565	15	0.377			
28	9-2	2	0.5	2	13263	26526	26930	53860	0.1	187041	53600	0.0573	15	0.382			
29	9-3	2	0.5	3	13776	27552	26376	52752	0.1	187041	52482	0.0551	15	0.374			
30	10-1	2	0.5	1	13953	27906	28300	55806	0.1	187041	55806	0.0589	15	0.389			
31	10-2	2	0.5	2	14129	28258	28652	57316	0.1	187041	57316	0.0599	15	0.394			
32	10-3	2	0.5	3	14305	28610	29004	58019	0.1	187041	58019	0.0609	15	0.399			
33	11-1	2	0.5	1	14481	28962	29356	58918	0.1	187041	58918	0.0619	15	0.404			
34	11-2	2	0.5	2	14657	29314	29708	59422	0.1	187041	59422	0.0629	15	0.409			
35	11-3	2	0.5	3	14833	29666	30060	60330	0.1	187041	60330	0.0639	15	0.414			
36	12-1	2	0.5	1	15009	30018	30412	60826	0.1	187041	60826	0.0649	15	0.419			
37	12-2	2	0.5	2	15185	30370	30764	61730	0.1	187041	61730	0.0659	15	0.424			
38	12-3	2	0.5	3	15361	30722	31116	62634	0.1	187041	62634	0.0669	15	0.429			
39	13-1	2	0.5	1	15537	31074	31468	63142	0.1	187041	63142	0.0679	15	0.434			
40	13-2	2	0.5	2	15713	31426	31820	64046	0.1	187041	64046	0.0689	15	0.439			
41	13-3	2	0.5	3	15889	31778	32172	64950	0.1	187041	64950	0.0699	15	0.444			
42	14-1	2	0.5	1	16065	32130	32524	65854	0.1	187041	65854	0.0709	15	0.449			
43	14-2	2	0.5	2	16241	32482	32876	66758	0.1	187041	66758	0.0719	15	0.454			
44	14-3	2	0.5	3	16417	32834	33228	67662	0.1	187041	67662	0.0729	15	0.459			
45	15-1	2	0.5	1	16593	33186	33580	68566	0.1	187041	68566	0.0739	15	0.464			
46	15-2	2	0.5	2	16769	33538	33932	69470	0.1	187041	69470	0.0749	15	0.469			
47	15-3	2	0.5	3	16945	33890	34284	70374	0.1	187041	70374	0.0759	15	0.474			
48	16-1	2	0.5	1	17121	34242	34636	71278	0.1	187041	71278	0.0769	15	0.479			
49	16-2	2	0.5	2	17297	34594	34988	72182	0.1	187041	72182	0.0779	15	0.484			
50	16-3	2	0.5	3	17473	34946	35340	73086	0.1	187041	73086	0.0789	15	0.489			
51	17-1	2	0.5	1	17649	35298	35692	73990	0.1	187041	73990	0.0799	15	0.494			
52	17-2	2	0.5	2	17825	35650	36044	74894	0.1	187041	74894	0.0809	15	0.499			
53	17-3	2	0.5	3	18001	36002	36396	75798	0.1	187041	75798	0.0819	15	0.504			
54	18-1	2	0.5	1	18177	36354	36748	76702	0.1	187041	76702	0.0829	15	0.509			
55	18-2	2	0.5	2	18353	36706	37100	77606	0.1	187041	77606	0.0839	15	0.514			
56	18-3	2	0.5	3	18529	37058	37452	78510	0.1	187041	78510	0.0849	15	0.519			
57	19-1	2	0.5	1	18705	37410	37804	79414	0.1	187041	79414	0.0859	15	0.524			
58	19-2	2	0.5	2	18881	37762	38156	80318	0.1	187041	80318	0.0869	15	0.529			
59	19-3	2	0.5	3	19057	38114	38508	81222	0.1	187041	81222	0.0879	15	0.534			
60	20-1	2	0.5	1	19233	38466	38860	82126	0.1	187041	82126	0.0889	15	0.539			
61	20-2	2	0.5	2	19409	38818	39212	83030	0.1	187041	83030	0.0899	15	0.544			
62	20-3	2	0.5	3	19585	39170	39564	83934	0.1	187041	83934	0.0909	15	0.549			
63	21-1	2	0.5	1	19761	39522	39916	84838	0.1	187041	84838	0.0919	15	0.554			
64	21-2	2	0.5	2	19937	39874	40268	85742	0.1	187041	85742	0.0929	15	0.559			
65	21-3	2	0.5	3	20113	40226	40620	86646	0.1	187041	86646	0.0939	15	0.564			
66	22-1	2	0.5	1	20289	40578	40972	87550	0.1	187041	87550	0.0949	15	0.569			
67	22-2	2	0.5	2	20465	40930	41324	88454	0.1	187041	88454	0.0959	15	0.574			
68	22-3	2	0.5	3	20641	41282	41676	89358	0.1	187041	89358	0.0969	15	0.579			
69	23-1	2	0.5	1	20817	41634	42028	90262	0.1	187041	90262	0.0979	15	0.584			
70	23-2	2	0.5	2	20993	41986	42380	91166	0.1	187041	91166	0.0989	15	0.589			
71	23-3	2	0.5	3	21169	42338	42732	92070	0.1	187041	92070	0.0999	15	0.594			
72	24-1	2	0.5	1	21345	42690	43084	92974	0.1	187041	92974	0.1009	15	0.599			
73	24-2	2	0.5	2	21521	43042	43436	93878	0.1	187041	93878	0.1019	15	0.604			
74	24-3	2	0.5	3	21697	43394	43788	94782	0.1	187041	94782	0.1029	15	0.609			
75	25-1	2	0.5	1	21873	43746	44140	95686	0.1	187041	95686	0.1039	15	0.614			
76	25-2	2	0.5	2	22049	44098	44492	96590	0.1	187041	96590	0.1049	15	0.619			
77	25-3	2	0.5	3	22225	44450	44844	97494	0.1	187041	97494	0.1059	15	0.624			
78	26-1	2	0.5	1	22401	44802	45196	98398	0.1	187041	98398	0.1069	15	0.629			
79	26-2	2	0.5	2	22577	45154	45548	99302	0.1	187041	99302	0.1079	15	0.634			
80	26-3	2	0.5	3	22753	45506	45900	100206	0.1	187041	100206	0.1089	15	0.639			
81	27-1	2	0.5	1	22929	45858	46252	101110	0.1	187041	101110	0.1099	15	0.644			
82	27-2	2	0.5	2	23105	46210	46604	102014	0.1	187041	102014	0.1109	15	0.649			
83	27-3	2	0.5	3	23281	46562	46956	102918	0.1	187041	102918	0.1119	15	0.654			
84	28-1	2	0.5	1	23457	46914	47308	103822	0.1	187041	103822	0.1129	15	0.659			
85	28-2	2	0.5	2	23633	47266	47660	104726	0.1	187041	104726	0.1139	15	0.664			
86	28-3	2	0.5	3	23809	47618	48012	105630	0.1	187041	105630	0.1149	15	0.669			
87	29-1	2	0.5	1	23985	47970	48364	106534	0.1	187041	106534	0.1159	15	0.674			
88	29-2	2	0.5	2	24161	48322	48716	107438	0.1	187041	107438	0.1169	15	0.679			
89	29-3	2	0.5	3	24337	48674	49068	108342	0.1	187041	108342	0.1179	15	0.684			
90	30-1	2	0.5	1	24513	49026	49420	109246	0.1	187041	109246	0.1189	15	0.689			
91	30-2	2	0.5	2	24689	49378	49772	110150	0.1								

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		0.98
8		1.01
9		0.99
10		1.01
11		0.99
12		1.01
13		1.05
14		0.95
15		0.94
16		1.04
17		0.96
18		1.04
19		0.99
20		0.99
21		1.00
22		1.00
23		1.05
24		1.00
25		1.00
26		1.00
27		1.01
28		0.99
29		1.02
30		0.95
31		0.95
32		1.01
33		1.01
34		0.99
35		1.00
36		1.00
37		1.00
38		1.00
39		1.00
40		1.00
41		1.01
42		0.99
43		0.99
44		1.01
45		1.00
46		1.00
47		1.00
48		1.00
49		1.00
50		1.00
51		0.99
52		1.02
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Test Chemical	Assay Date	3/27/2007	Test Chemical ID	CR11609	# Concentrations			8	Microsome ID	12535-13
2	4-OH ASDN	NU Ref	12535-88	Counter data file name	27MR2035.P00	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
3	4-OH ASDN	Technician ID	SLB	Replicate #							
4	Control Type	Portion	SD								
5	Full activity	Average	0.382		Bkg % of Full						
6	Background (uncorrected)	Overall	0.002			0.5 (Red if >1%)					
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.003						
12	4-OH ASDN	1	2	-5.00	0.003						
13	4-OH ASDN	2	1	-6.00	0.023						
14	4-OH ASDN	2	2	-6.00	0.021						
15	4-OH ASDN	3	1	-8.30	0.040						
16	4-OH ASDN	3	2	-8.30	0.037						
17	4-OH ASDN	4	1	-7.00	0.133						
18	4-OH ASDN	4	2	-7.00	0.126						
19	4-OH ASDN	5	1	-7.30	0.205						
20	4-OH ASDN	5	2	-7.30	0.191						
21	4-OH ASDN	6	1	-8.00	0.329						
22	4-OH ASDN	6	2	-8.00	0.306						
23	4-OH ASDN	7	1	-9.00	0.371						
24	4-OH ASDN	7	2	-9.00	0.372						
25	4-OH ASDN	8	1	-10.00	0.382						
26	4-OH ASDN	8	2	-10.00	0.360						
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical) M	Activity						
38	CR11609	1	1	-5.00	0.001						
39	CR11609	1	2	-5.00	0.001						
40	CR11609	2	1	-6.00	0.007						
41	CR11609	2	2	-6.00	0.007						
42	CR11609	3	1	-7.00	0.057						
43	CR11609	3	2	-7.00	0.058						
44	CR11609	4	1	-7.00	0.055						
45	CR11609	4	2	-7.60	0.156						
46	CR11609	5	1	-7.60	0.155						
47	CR11609	5	2	-8.00	0.238						
48	CR11609	6	1	-8.00	0.237						
49	CR11609	6	2	-8.00	0.233						
50	CR11609	7	1	-8.60	0.327						
51	CR11609	7	2	-8.60	0.332						
52	CR11609	8	1	-9.00	0.324						
53	CR11609	8	2	-9.00	0.357						
54	CR11609	9	1	-9.00	0.364						
55	CR11609	9	2	-9.00	0.347						
56	CR11609	10	1	-10.00	0.377						
57	CR11609	10	2	-10.00	0.382						
58	CR11609	11	1	-10.00	0.374						
59	CR11609	11	2	-10.00	0.382						
60	CR11609	12	1	-10.00	0.374						
61	CR11609	12	2	-10.00	0.382						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/28/2007	Test Chemical ID	CR11609	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-91	Counter data filename	28MR2055.P00					
3	Technician ID	SLB	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/28/2007	Test Chemical ID	CR11609	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-91	Counter data filename	28MR2055.P00					
3	Technician ID	SLB	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0211		39579		1875782			
8	2	0.0210		38879		1851381			
9	3	0.0212		41730		1968396			
10	4	0.0210		40290		1918571			
11	5	0.0212		43450		2049528			
12						Average DPM/g soln		1932732	
13						SD		79018	
14						CV		4.09	
15						µCi/g soln		0.8706	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0891 g			
25	Mass of dilution B used in substrate prep					4.5537 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56406861 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln				0.8706				
31	b. Specific activity of [³ H]ASDN (µCi/nmol)				23500000				
32	c. Molecular wt of ASDN (mg/nmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36			=	0.5641	+	0.011			
37			=	0.575	µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.515 µCi/µg ASDN								
41	9.632.E+05 dpm/nmol								

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Test				# Concentrations					
2		Assay Date	3/28/2007	Chemical ID	CR11609	tested	8	Microsome ID	12535-13		
3		NB Ref.	12535-91	Counter data filename	28MR2055.P00	Pos Ctl					
4		Technician	SLB	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc	2		
5		Vial	Time (min)	DPM1			ISIE			Round DPM	
6	2	5	39579.39	17508.95	0	12.07	491.2			39579	
7	3	5	38879.1	17042.46	0	11.92	482.56			38879	
8	4	5	41729.81	18443.97	0	12.02	489.34			41730	
9	5	5	40289.7	17570.13	0	11.89	477.81			40290	
10	6	5	43450.32	18769.89	0	11.92	476.63			43450	
11	8	5	14699.55	6850.44	0	12.69	532.8			14700	
12	9	5	14744.78	6836.39	0	12.63	529.77			14745	
13	10	5	14902.39	6904.23	0	12.6	527.75			14902	
14	11	5	14957.17	6934.92	0	12.68	531.72			14957	
15	12	5	14166.59	6661.45	0	12.64	536.31			14187	
16	13	5	14078.12	6549.78	0	12.64	532.69			14078	
17	14	5	13889.53	6430.86	0	12.67	530.33			13890	
18	15	5	13951.54	6473.5	0	12.66	530.66			13952	
19	16	5	60.31	31.29	0	9.85	533.26			60	
20	17	5	53.57	26.57	0	10.2	532.87			54	
21	18	5	65.24	33.87	0	11.54	529.11			65	
22	19	5	54.64	25.57	0	11.82	533.93			55	
23	20	5	121.84	55.73	0	13.04	532.57			122	
24	21	5	88.1	38.89	0	12.84	529.95			88	
25	22	5	105.77	47.37	0	13.31	538.6			106	
26	23	5	129.56	58.87	0	12.98	535.4			130	
27	25	5	146.69	66.27	0	12.36	527.38			147	
28	26	5	174.01	80.67	0	13.11	529.5			174	
29	27	5	131.93	61.95	0	13.65	541.02			132	
30	28	5	117.19	55.88	0	12.16	541.66			117	
31											
32											
33	29	5	856.99	407.52	0	12.37	529.35			857	
34	30	5	924.71	424.87	0	12.72	531.21			925	
35	31	5	877.51	412.96	0	12.82	533.82			878	
36	32	5	970.01	443.98	0	12.68	533.56			970	
37											
38											
39	33	5	1517.81	711.61	0	12.56	531.75			1518	
40	34	5	1658.58	757.11	0	12.81	531.51			1659	
41	35	5	1618.78	752.4	0	12.65	532.72			1619	
42	36	5	1824.35	756.08	0	12.59	530.16			1624	
43											
44											
45	37	5	5066.59	2385.5	0	12.63	532.31			5067	
46	38	5	5190.21	2421.23	0	12.63	532.68			5190	
47	39	5	4362.34	2035.08	0	12.69	536.86			4362	
48	40	5	4403.64	2080.92	0	12.69	534.16			4404	
49											
50											
51	41	5	7942.8	3680.11	0	12.66	530.58			7943	
52	42	5	7720.44	3600.28	0	12.56	532.79			7720	
53	43	5	7742.01	3625.79	0	12.6	530.78			7742	
54	44	5	7736.88	3614.69	0	12.61	533.46			7737	
55											
56											
57	45	5	12523.03	5829.15	0	12.64	530.19			12523	
58	46	5	12580.72	5830.37	0	12.58	527.3			12581	
59	47	5	12545.18	5828.22	0	12.63	526.83			12545	
60	48	5	12633.17	5836.17	0	12.62	530.12			12633	
61											
62											
63	49	5	13936.57	6448.89	0	12.6	530.13			13937	
64	50	5	13356.89	6191.74	0	12.61	529			13357	
65	51	5	14140.14	6576.6	0	12.66	532.4			14140	
66	52	5	14201.68	6577.28	0	12.65	530.59			14202	
67											
68											
69	53	5	13772.04	6412.92	0	12.65	531.93			13772	
70	54	5	13965.25	6448.63	0	12.64	529.61			13965	
71	55	5	14115.27	6541.3	0	12.69	530.51			14115	
72	56	5	13900.08	6444.71	0	12.69	529.78			13900	
73											
74											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	58	5	106.56	45.98		0 14.14	530.82				107
76	59	5	83.39	38.17		0 13.31	533.34				83
77	60	5	74.67	35.5		0 13.11	533.93				75
78	61	5	63.66	31.19		0 12.79	532.22				64
79	62	5	79.51	34.48		0 15.88	533.52				80
80	63	5	92.27	38.1		0 12.63	533.77				92
81	64	5	300.5	141.58		0 13.08	533.39				301
82	65	5	294.58	132.15		0 13.05	534.37				295
83	66	5	220.68	102.61		0 12.79	531.23				221
84	67	5	237.89	105.2		0 13.05	533.27				238
85	68	5	217.61	102.78		0 11.95	532.85				218
86	69	5	215.78	98.97		0 13.22	530.26				216
87	70	5	2182.12	1002.16		0 12.78	529.08				2182
88	71	5	2245.13	1028.54		0 12.67	525.84				2245
89	72	5	1473.45	683.32		0 12.53	531.23				1473
90	73	5	1504.57	689.51		0 12.64	531.18				1505
91	74	5	1531.71	711.74		0 12.89	532.77				1532
92	75	5	1498.94	706.93		0 12.66	533.09				1499
93	76	5	5148.13	2406.09		0 12.66	535.42				5148
94	77	5	5326.25	2479.94		0 12.74	534.64				5326
95	78	5	3830.32	1786.52		0 12.67	534.08				3830
96	79	5	3701.25	1717.77		0 12.73	532.74				3701
97	80	5	4287.8	1996.44		0 12.62	533.2				4288
98	81	5	4266.06	1975.06		0 12.71	529.21				4266
99	83	5	9074.74	4227.34		0 12.64	529.97				9075
100	84	5	9074.27	4220.78		0 12.82	529.24				9074
101	85	5	6591.71	3068.29		0 12.67	533.79				6592
102	86	5	6978.6	3218.95		0 12.63	530.51				6979
103	87	5	6842.72	3151.02		0 12.72	529.04				6843
104	88	5	6687.64	3132.52		0 12.62	532.01				6688
105	89	5	12616.87	5835.82		0 12.64	530.01				12617
106	90	5	12626.48	5862.69		0 12.69	530.6				12626
107	91	5	10420.28	4836.67		0 12.63	530.83				10420
108	92	5	10575.49	4870.68		0 12.58	528.1				10575
109	93	5	10523.54	4859.46		0 12.61	528.44				10524
110	94	5	10477.24	4868.27		0 12.59	529.4				10477
111	95	5	13753.33	6395.36		0 12.61	530.62				13753
112	96	5	13714.98	6340.03		0 12.57	528.11				13715
113	97	5	11651.54	5416.56		0 12.67	529.73				11652
114	98	5	11465.79	5356.23		0 12.47	529.49				11466
115	99	5	11243.07	5212.86		0 12.67	530.32				11243
116	100	5	11198.33	5222.03		0 12.57	528.57				11198
117	101	5	14369.27	6649.12		0 12.63	529.96				14369
118	102	5	14330.09	6651.48		0 12.64	528.6				14330
119	103	5	12331.74	5721.69		0 12.68	533.83				12332
120	104	5	12593.4	5839.12		0 12.63	528.48				12593
121	105	5	12312.33	5742.4		0 12.61	528.84				12312
122	106	5	12398.77	5758.11		0 12.54	527.28				12389
123	107	5	226239.63	35696.74		0 7.75	171.85				226240
124	108	5	8.22	0		0 36.1	480.59				8

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Assay Date	3/26/2007	Chemical ID	CR11099	# Concentrations tested	4	Microplate ID	42935-13									
NB Ref. Technician ID	12535-91	28MR2055 P100														
SLB	3	Positive Control														
Sample ID	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Sample type	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control	Full activity control
Replicate	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Nominal total volume (mL)	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Aliq Volume (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
DPMS#	14700	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400
Aliq #	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Ave DPMS#	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400	29400
Total OPM	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560
Volume of substrate solution (mL)	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
total OPM in assay tube (initial)	193273	193273	193273	193273	193273	193273	193273	193273	193273	193273	193273	193273	193273	193273	193273	193273
Calculate initial ³ H ₂ O formed (total DPMS# background) (Tubes)	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560	58560
nmol ³ H ₂ O formed	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009	0.0009
Final protein (in assay) (mg/mL)	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005
Incubation time (min)	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
Aromatase activity (nmol ³ H ₂ O formed/mg protein/min)	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405	0.405

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
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7					
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
					Test											
					Chemical ID	CR11609	# Concentrations tested									
1	Assay Date	3/28/2007								8 Microsome ID	12535-13					
2	NB Ref.	12535-91				28NR2055 P00										
3																
4	Technician ID	SLB			Replicate #	31	Positive Control			4-OH ASDN Pos. Ctrl Tubes/Conc						

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC Check - aliquot variance
5		
6		% RE
7		
8		1.08
9		0.92
10		0.93
11		1.07
12		
13		1.01
14		0.94
15		0.96
16		1.04
17		1.00
18		1.00
19		0.95
20		0.99
21		1.01
22		1.01
23		0.94
24		0.96
25		1.02
26		1.02
27		0.99
28		1.00
29		1.00
30		1.00
31		1.00
32		0.97
33		1.03
34		1.01
35		0.99
36		1.00
37		1.00
38		1.00
39		0.95
40		1.01
41		1.00
42		1.00
43		1.00
44		1.00
45		1.01
46		0.99
47		1.00
48		1.00
49		1.00
50		0.96
51		1.01
52		1.00
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/28/2007	Test Chemical ID	CR11609	# Concentrations					
2		NB Ref	12535-01	Counter data filename	28MR2055.P00						
3		Technician ID	SLB	Replicate #		3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4	Control Type	Average	SD								
5	Full activity	0.397	0.013								
6	Background (unconnected)	0.002	0.001								
7											
8											
9											
10	Positive Control	Replicate	[Positive Ctl] M	Log[Positive Ctl]	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.002						
12	4-OH ASDN	2	1.00E-06	-6.00	0.001						
13	4-OH ASDN	1	1.00E-06	-6.00	0.022						
14	4-OH ASDN	2	1.00E-06	-6.00	0.023						
15	4-OH ASDN	1	5.00E-07	-6.30	0.042						
16	4-OH ASDN	2	5.00E-07	-6.30	0.043						
17	4-OH ASDN	1	1.00E-07	-7.00	0.140						
18	4-OH ASDN	2	1.00E-07	-7.00	0.119						
19	4-OH ASDN	1	5.00E-08	-7.30	0.214						
20	4-OH ASDN	2	5.00E-08	-7.30	0.212						
21	4-OH ASDN	1	1.00E-08	-8.00	0.346						
22	4-OH ASDN	2	1.00E-08	-8.00	0.346						
23	4-OH ASDN	1	1.00E-08	-8.00	0.375						
24	4-OH ASDN	2	1.00E-08	-8.00	0.390						
25	4-OH ASDN	1	1.00E-10	-10.00	0.382						
26	4-OH ASDN	2	1.00E-10	-10.00	0.386						
27	4-OH ASDN	1									
28	4-OH ASDN	2									
29	4-OH ASDN	1									
30	4-OH ASDN	2									
31	4-OH ASDN	1									
32	4-OH ASDN	2									
33	4-OH ASDN	1									
34	4-OH ASDN	2									
35	4-OH ASDN	1									
36	4-OH ASDN	2									
37	Test Chemical	Replicate	Test chemical [M]	Log[Test chemical]	Activity						
38	CR11609	1	1.00E-05	-5.00	0.000						
39	CR11609	2	1.00E-05	-5.00	0.000						
40	CR11609	1	1.00E-06	-6.00	0.000						
41	CR11609	2	1.00E-06	-6.00	0.006						
42	CR11609	1	1.00E-06	-6.00	0.004						
43	CR11609	2	1.00E-06	-6.00	0.004						
44	CR11609	1	1.00E-07	-7.00	0.039						
45	CR11609	2	1.00E-07	-7.00	0.059						
46	CR11609	1	1.00E-07	-7.00	0.040						
47	CR11609	2	1.00E-07	-7.00	0.143						
48	CR11609	1	2.50E-08	-7.60	0.102						
49	CR11609	2	2.50E-08	-7.60	0.116						
50	CR11609	1	1.00E-08	-8.00	0.249						
51	CR11609	2	1.00E-08	-8.00	0.186						
52	CR11609	1	1.00E-08	-8.00	0.185						
53	CR11609	2	2.50E-09	-8.60	0.347						
54	CR11609	1	2.50E-09	-8.60	0.288						
55	CR11609	2	2.50E-09	-8.60	0.289						
56	CR11609	1	1.00E-09	-9.00	0.378						
57	CR11609	2	1.00E-09	-9.00	0.318						
58	CR11609	1	1.00E-09	-9.00	0.308						
59	CR11609	2	1.00E-10	-10.00	0.395						
60	CR11609	1	1.00E-10	-10.00	0.343						
61	CR11609	2	1.00E-10	-10.00	0.340						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/22/2007	Test Chemical ID	CR11610	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-86	Counter data filename	22MR2028.P00					
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/22/2007	Test Chemical ID	CR11610	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-86	Counter data filename	22MR2028.P00					
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Allquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0194		35545		1832216			
8	2	0.0193		35067		1816943			
9	3	0.0194		41666		2147732			
10	4	0.0198		38382		1938485			
11	5	0.0190		40635		2138684			
12						Average DPM/g soln		1974812	
13						SD		160726	
14						CV		8.14	
15						µCi/g soln		0.8896	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1269 g			
25	Mass of dilution B used in substrate prep					4.5639 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.5627026 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8896			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5627	+	0.011		
37				=	0.574	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.551 µCi/µg ASDN				
41					9.861 E+05 dpm/nmol				

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/22/2007	Test Chemical ID	CR11610	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-86	Counter data filename	22MR2028.P00					
3			Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Test #								
		Chemical Concentrations								
2		Asst 3/22/2007	ID C	tested		8	Microsome ID	12535-13		
3		Counter data								
4		NB 12535-38	filename	22MR2028.P00						
5		Te								
6		ch								
7		nic								
8		lan								
9		ID PRP	Replicate	Positive	Pos CU					
10			# 1	Control	4-OH ASD Tubes/Conc				2	
11										
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
60	59	5	1019.27	438.52	0	13.64	545.88				1019
61	60	5	926.96	394.72	0	13.83	546.82				927
62	61	5	899.97	378.88	0	13.7	549.85				900
63	62	5	936.37	396.32	0	13.71	547.7				936
64	63	5	881.17	373.36	0	13.61	547.38				881
65	64	5	2207.41	945.72	0	13.63	549.5				2207
66	65	5	2157.52	917.52	0	13.79	548.65				2158
67	66	5	2130.61	928.24	0	13.6	548.56				2140
68	67	5	2108.19	902.44	0	13.82	549.81				2106
69	68	5	2061.43	886.92	0	13.6	546.21				2061
70	69	5	2062.07	880.76	0	13.63	551.58				2062
71	70	5	6380.41	2710.96	0	13.7	545.74				6380
72	71	5	6427.66	2729.92	0	13.78	548.53				6428
73	72	5	6474.19	2748.56	0	13.71	548.27				6474
74	73	5	6298.41	2679.76	0	13.79	547.5				6298
75	74	5	6268.98	2690.12	0	13.72	548.54				6297
76	75	5	6326.14	2688.6	0	13.67	546.32				6326
77	76	5	11599.75	4960.36	0	13.7	549.25				11600
78	77	5	11755.9	5001.96	0	13.72	544.96				11756
79	78	5	11745.44	5020.92	0	13.79	550.51				11745
80	79	5	11476.2	4883.12	0	13.69	545.83				11476
81	80	5	11464.98	4891.08	0	13.68	548.8				11465
82	81	5	11261.79	4626.96	0	13.67	549.93				11262
83	82	5	12892.25	5518.4	0	13.77	550.54				12892
84	83	5	12550.88	5332.92	0	13.76	545.62				12551
85	84	5	12930.84	5507.76	0	13.89	546.3				12931
86	85	5	12656.94	5401.96	0	13.68	547.88				12657
87	86	5	12588.21	5362.52	0	13.73	547.34				12588
88	87	5	12496.59	5358.76	0	13.79	550.02				12499
89	88	5	12567.17	5398.2	0	13.67	550.51				12567
90	89	5	12471.47	5320.16	0	13.72	549.18				12471
91	90	5	13212.82	5598.12	0	13.76	545.1				13213
92	91	5	12810.41	5382.36	0	13.8	548.66				12810
93	92	5	12730.55	5432.52	0	13.71	547.85				12731
94	93	5	12625.57	5368.16	0	13.72	545.23				12627
95	94	5	12718.29	5445.72	0	13.65	548.18				12718
96	95	5	12688.4	5407.96	0	13.76	547.59				12688
97	96	5	12598.61	5357.16	0	13.75	545.1				12599
98	97	5	12439.35	5320.96	0	13.7	548.72				12439
99	98	5	11952.88	5067.76	0	13.72	547.21				11953
100	99	5	12014.37	5110.72	0	13.81	547.17				12014
101	100	5	12710.18	5394.16	0	13.73	547.98				12710
102	101	5	12675.48	5421.8	0	13.72	549.6				12675
103	102	5	12778.07	5448.8	0	13.73	548.18				12778
104	103	5	12640	5377.32	0	13.8	548.02				12640
105	104	5	12076.87	5185.18	0	13.74	550.6				12077
106	105	5	11970.2	5123.56	0	13.81	550.46				11970
107	106	5	235686.39	108105.2	0	15.08	621.96				235686

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		QC check - aliquot			
5		variance			
6		Rad if value not			
7		within mean +/-			
8		10%			
9		1.01			
10		0.99			
11		0.99			
12		1.01			
13		1.00			
14		1.00			
15		1.00			
16		0.94			
17		0.95			
18		1.05			
19		0.99			
20		1.01			
21		0.99			
22		1.00			
23		1.00			
24		0.98			
25		0.98			
26		1.02			
27					
28		1.01			
29		0.99			
30		1.02			
31		0.99			
32					
33		1.00			
34		1.00			
35		1.00			
36		1.00			
37		1.00			
38		1.00			
39					
40					
41		0.99			
42		1.01			
43		1.00			
44		1.00			
45					
46		0.99			
47		1.01			
48		0.99			
49		1.01			
50					
51					
52		1.00			
53		1.00			
54		1.00			
55		0.99			
56		1.01			
57					
58		1.00			
59		1.00			
60		1.00			
61		1.00			
62					
63					
64		1.01			
65		0.99			
66		1.00			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Sample Type	Replicates	Assay Date	Test Chemical ID	# Concentrations tested	# Microsome ID	125-15-13	4-OH ASEDN	Post Cell Tubes/Conc	2	Calculate nmol ³ H ₂ O formed	Total DPM corrected for background (Background Tubes)	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
1	CR11510	1-1	2	0.5	1057	2114	2076	4152	197481	0.1	197481	0.0019	0.005	15	0.026
2	CR11510	1-2	2	0.5	1019	2038	1877	3854	197481	0.1	197481	0.0034	0.005	15	0.023
3	CR11510	1-3	2	0.5	927	1854	1877	3854	197481	0.1	197481	0.0034	0.005	15	0.023
4	CR11510	2-1	2	0.5	900	1800	1877	3854	197481	0.1	197481	0.0034	0.005	15	0.023
5	CR11510	2-2	2	0.5	881	1762	1877	3854	197481	0.1	197481	0.0034	0.005	15	0.023
6	CR11510	2-3	2	0.5	2207	4414	4385	8730	197481	0.1	197481	0.0086	0.005	15	0.067
7	CR11510	3-1	2	0.5	2138	4276	4246	8492	197481	0.1	197481	0.0083	0.005	15	0.066
8	CR11510	3-2	2	0.5	2140	4280	4246	8492	197481	0.1	197481	0.0083	0.005	15	0.066
9	CR11510	3-3	2	0.5	2103	4206	4123	8246	197481	0.1	197481	0.0081	0.005	15	0.064
10	CR11510	4-1	2	0.5	2083	4166	4123	8246	197481	0.1	197481	0.0081	0.005	15	0.064
11	CR11510	5-1	2	0.5	6380	12760	12888	25616	197481	0.1	197481	0.0257	0.005	15	0.171
12	CR11510	5-2	2	0.5	6429	12853	12772	25544	197481	0.1	197481	0.0256	0.005	15	0.171
13	CR11510	5-3	2	0.5	6474	12948	12772	25544	197481	0.1	197481	0.0253	0.005	15	0.169
14	CR11510	6-1	2	0.5	8297	16594	16523	32946	197481	0.1	197481	0.0471	0.005	15	0.314
15	CR11510	6-2	2	0.5	8320	16640	16523	32946	197481	0.1	197481	0.0469	0.005	15	0.312
16	CR11510	6-3	2	0.5	11796	23592	23356	46712	197481	0.1	197481	0.0458	0.005	15	0.306
17	CR11510	7-1	2	0.5	11745	23490	23221	46442	197481	0.1	197481	0.0458	0.005	15	0.306
18	CR11510	7-2	2	0.5	11465	22930	22727	45454	197481	0.1	197481	0.0513	0.005	15	0.342
19	CR11510	7-3	2	0.5	11262	22524	22443	45086	197481	0.1	197481	0.0516	0.005	15	0.344
20	CR11510	8-1	2	0.5	12581	25192	25443	50886	197481	0.1	197481	0.0512	0.005	15	0.341
21	CR11510	8-2	2	0.5	12582	25193	25443	50886	197481	0.1	197481	0.0512	0.005	15	0.341
22	CR11510	8-3	2	0.5	12581	25192	25443	50886	197481	0.1	197481	0.0512	0.005	15	0.341
23	CR11510	9-1	2	0.5	12459	24908	25087	50174	197481	0.1	197481	0.0505	0.005	15	0.337
24	CR11510	9-2	2	0.5	12457	24906	25087	50174	197481	0.1	197481	0.0505	0.005	15	0.337
25	CR11510	9-3	2	0.5	12471	24942	25087	50174	197481	0.1	197481	0.0505	0.005	15	0.337
26	CR11510	10-1	2	0.5	13213	26426	25923	51846	197481	0.1	197481	0.0521	0.005	15	0.347
27	CR11510	10-2	2	0.5	12817	25634	25558	50716	197481	0.1	197481	0.0512	0.005	15	0.341
28	CR11510	10-3	2	0.5	12817	25634	25558	50716	197481	0.1	197481	0.0512	0.005	15	0.341
29	CR11510	11-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
30	CR11510	11-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
31	CR11510	11-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
32	CR11510	12-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
33	CR11510	12-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
34	CR11510	12-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
35	CR11510	13-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
36	CR11510	13-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
37	CR11510	13-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
38	CR11510	14-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
39	CR11510	14-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
40	CR11510	14-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
41	CR11510	15-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
42	CR11510	15-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
43	CR11510	15-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
44	CR11510	16-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
45	CR11510	16-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
46	CR11510	16-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
47	CR11510	17-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
48	CR11510	17-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
49	CR11510	17-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
50	CR11510	18-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
51	CR11510	18-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
52	CR11510	18-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
53	CR11510	19-1	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
54	CR11510	19-2	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337
55	CR11510	19-3	2	0.5	12637	25274	25366	50732	197481	0.1	197481	0.0508	0.005	15	0.337

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC check - aliquot variance
5		
6		% RE
7		1.02
8		0.98
9		1.01
10		0.99
11		1.03
12		0.97
13		1.01
14		0.99
15		1.01
16		0.98
17		1.00
18		0.99
19		1.00
20		1.00
21		1.01
22		0.99
23		1.00
24		1.00
25		0.99
26		1.01
27		1.01
28		0.99
29		1.01
30		0.99
31		1.01
32		0.99
33		1.01
34		0.99
35		1.00
36		1.00
37		1.00
38		1.00
39		1.00
40		1.02
41		0.99
42		1.00
43		1.00
44		1.01
45		0.99
46		0.99
47		1.00
48		1.00
49		1.00
50		1.00
51		1.01
52		0.99
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/22/2007	Test Chemical ID	GR11610						
2		NB Ref	12535-86	Counterplate filename	22MR0208.P00						
3		Technician ID	PRP	Replicate #		1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4											
5	Control Type	Average	SD								
6	Full activity	0.334	0.014		Bkg % of Full						
7	Background (unconnected)	0.002	0.000								
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1.00E-05	-3.00	0.002						
12	4-OH ASDN	2	1.00E-05	-5.00	0.002						
13	4-OH ASDN	1	1.00E-06	-6.00	0.020						
14	4-OH ASDN	2	1.00E-06	-6.00	0.020						
15	4-OH ASDN	1	1.00E-07	-7.00	0.040						
16	4-OH ASDN	2	1.00E-07	-7.00	0.040						
17	4-OH ASDN	1	1.00E-08	-8.00	0.301						
18	4-OH ASDN	2	1.00E-08	-8.00	0.301						
19	4-OH ASDN	1	1.00E-09	-9.00	0.349						
20	4-OH ASDN	2	1.00E-09	-9.00	0.349						
21	4-OH ASDN	1	1.00E-10	-10.00	0.361						
22	4-OH ASDN	2	1.00E-10	-10.00	0.361						
23	4-OH ASDN	1	1.00E-11	-11.00	0.348						
24	4-OH ASDN	2	1.00E-11	-11.00	0.348						
25	4-OH ASDN	1	1.00E-12	-12.00	0.323						
26	4-OH ASDN	2	1.00E-12	-12.00	0.323						
27	4-OH ASDN	1	1.00E-13	-13.00	0.294						
28	4-OH ASDN	2	1.00E-13	-13.00	0.294						
29	4-OH ASDN	1	1.00E-14	-14.00	0.346						
30	4-OH ASDN	2	1.00E-14	-14.00	0.346						
31	4-OH ASDN	1	1.00E-15	-15.00	0.381						
32	4-OH ASDN	2	1.00E-15	-15.00	0.381						
33	4-OH ASDN	1	1.00E-16	-16.00	0.348						
34	4-OH ASDN	2	1.00E-16	-16.00	0.348						
35											
36											
37	Test Chemical	Replicate	Test chemical M	Log(test chemical)	Activity						
38	CR11610	1	1.00E-03	-3.00	0.026						
39	CR11610	2	1.00E-03	-3.00	0.023						
40	CR11610	1	1.00E-03	-3.00	0.023						
41	CR11610	2	1.00E-04	-4.00	0.057						
42	CR11610	1	1.00E-04	-4.00	0.054						
43	CR11610	2	1.00E-04	-4.00	0.054						
44	CR11610	1	1.00E-05	-5.00	0.171						
45	CR11610	2	1.00E-05	-5.00	0.171						
46	CR11610	1	1.00E-05	-5.00	0.169						
47	CR11610	2	1.00E-06	-6.00	0.314						
48	CR11610	1	1.00E-06	-6.00	0.312						
49	CR11610	2	1.00E-06	-6.00	0.306						
50	CR11610	1	1.00E-07	-7.00	0.342						
51	CR11610	2	1.00E-07	-7.00	0.344						
52	CR11610	1	1.00E-07	-7.00	0.337						
53	CR11610	2	1.00E-08	-8.00	0.337						
54	CR11610	1	1.00E-08	-8.00	0.347						
55	CR11610	2	1.00E-08	-8.00	0.341						
56	CR11610	1	1.00E-09	-9.00	0.341						
57	CR11610	2	1.00E-09	-9.00	0.337						
58	CR11610	1	1.00E-09	-9.00	0.322						
59	CR11610	2	1.00E-10	-10.00	0.341						
60	CR11610	1	1.00E-10	-10.00	0.342						
61	CR11610	2	1.00E-10	-10.00	0.323						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
			Test	# Concentrations					
1	Assay Date	3/27/2007	Chemical ID	CR11610		tested	8		Microsome ID 12535-13
2	NB Ref.	12535-89	Counter data filename	27MR2052.P00 & 28MR2055.P00					
	Technician						Pos Ctl		
3	ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/27/2007	Test Chemical ID	CR11610	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-89	Counter data filename	27MR2052.P00 & 28MR2055.P00					
3	Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0195		35544		1822769			
8	2	0.0198		36834		1860303			
9	3	0.0194		40486		2088907			
10	4	0.0200		42385		2119250			
11	5	0.0196		40069		2044337			
12						Average DPM/g soln		1986713	
13						SD		135815	
14						CV		6.84	
15						µCi/g soln		0.8949	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0054 g			
25	Mass of dilution B used in substrate prep					4.4884 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56179289 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8949			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5618	+	0.011		
37				=	0.573	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.563	µCi/µg ASDN			
41					9.935.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/27/2007	Test Chemical ID	CR11610	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-89	Counter data filename		27MR2052.P00 & 28MR2055.P00				
3			Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
5	Microsome Dilution Details								Positive Control Concentrations		
6								Level	Final Concentration (M)		
7	Dilution A			0.12 mL microsome Stock used				1	1.00E-05		
8				2.4 mL total volume				2	1.00E-06		
9				20 dilution factor				3	5.00E-07		
10								4	1.00E-07		
11	Dilution B			1.5 mL microsome Dilution A used				5	5.00E-08		
12				60 mL total volume				6	1.00E-08		
13				40 dilution factor				7	1.00E-09		
14								8	1.00E-10		
15	Dilution C (if applicable)			mL microsome Dilution B used							
16				mL total volume							
17		NA		dilution factor							
18									Test Chemical Concentrations		
19				800 total dilution factor				Level	Final Concentration (M)		
20								1	5.00E-05		
21	Protein Concentration (stock microsomes, mg/mL):				8.00			2	2.50E-05		
22	Protein Concentration (dilution added to assay, mg/mL):				0.01			3	1.00E-05		
23								4	5.00E-06		
24								5	2.50E-06		
25								6	1.00E-06		
26								7	1.00E-07		
								8	1.00E-08		

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Test # Chemical Concentrations Assay 3/27/2007 ID CR11610 tested 8 Microsome ID 12535-13 Counter data NB 12535-89 filename 27MR2052.P00 & 28MR2055.P00 Technician Replicate # 2 Positive Control 4-OH ASD Tubes/Conc 2									
2											
3											
4											
5	Vial	Tin	DPM1								Round DPM
6	2	5	35544.04	13785.5	0	12.64	475.71				35544
7	3	5	36833.55	14631.1	0	12.84	491.75				36834
8	4	5	40486.02	15853.1	0	12.72	482.8				40486
9	5	5	42384.64	16672	0	12.72	485.39				42385
10	6	5	40068.87	15570.8	0	12.66	477.8				40069
11	8	5	13989.39	5952.94	0	13.7	547.58				13989
12	9	5	13804.88	5871.38	0	13.65	543.47				13805
13	10	5	14685.78	6261.94	0	13.61	545.78				14686
14	11	5	14487.82	6170.74	0	13.7	546.48				14488
15	12	5	12555.85	5307.1	0	13.76	545.65				12556
16	13	5	12364.23	5281.38	0	13.72	545.08				12364
17	14	5	13283.93	5639.82	0	13.59	542.69				12979
18	15	5	12979.18	5539.78	0	13.68	545.19				59
19	16	5	59.41	23.34	0	15.77	543.04				66
20	17	5	66.25	24.98	0	16.51	549.11				58
21	18	5	58.19	21.98	0	13.84	545.08				71
22	19	5	71.38	24.94	0	14.99	545.05				57
23	20	5	56.5	20.58	0	14.63	546.94				52
24	21	5	52.27	18.14	0	14.91	544.97				63
25	22	5	63.18	24.34	0	15.45	545.91				56
26	23	5	56.31	21.34	0	14.04	545.4				182
27	25	5	182.41	74.74	0	14.58	548.4				144
28	26	5	144.41	56.14	0	13.33	547.03				147
29	27	5	146.76	60.66	0	15.85	545.74				152
30	28	5	151.8	60.94	0	13.71	547.74				922
31	29	5	921.84	393.18	0	13.86	547.41				885
32	30	5	884.57	380.78	0	13.99	546.66				866
33	31	5	885.88	383.98	0	13.6	548.48				833
34	32	5	832.89	353.34	0	13.9	546.84				1612
35	33	5	1612.42	688.62	0	13.58	546.5				1626
36	34	5	1525.6	655.34	0	13.88	547.76				1475
37	35	5	1474.69	622.94	0	13.73	547.02				1459
38	36	5	1459.06	614.38	0	13.71	545.05				5428
39	37	5	5427.61	2309.94	0	13.63	546.83				5377
40	38	5	5376.95	2294.82	0	13.62	542.97				5118
41	39	5	5117.79	2168.38	0	13.76	548.04				5115
42	40	5	5114.96	2178.58	0	13.75	547.81				7204
43	41	5	7204.07	3067.94	0	13.7	546.52				7382
44	42	5	7381.54	3144.42	0	13.8	548.03				7606
45	43	5	7606.91	3245.78	0	13.66	547.25				7556
46	44	5	7556.02	3219.54	0	13.66	549.04				12025
47	45	5	12024.56	5153.98	0	13.68	546.24				12264
48	46	5	12264.27	5223.74	0	13.75	548.79				11338
49	47	5	11337.69	4862.74	0	13.78	549.67				11294
50	48	5	11293.61	4829.42	0	13.7	546.49				13544
51	49	5	13543.58	5769.54	0	13.65	547.45				13480
52	50	5	13479.8	5753.38	0	13.68	546.42				13216
53	51	5	13216.4	5631.62	0	13.71	545.45				13363
54	52	5	13362.58	5666.54	0	13.87	547.73				12630
55	53	5	12629.74	5399.62	0	13.72	545.74				12866
56	54	5	12866.02	5482.54	0	13.76	550.35				13621
57	55	5	13621.04	5848.94	0	13.75	547.15				13651
58	56	5	13651.11	5801.94	0	13.65	543.14				2562
59	58	5	2562.45	1087.18	0	13.68	544.86				2528
60	59	5	2528.14	1069.94	0	13.94	548.18				2800
61	60	5	2799.71	1186.18	0	13.87	546.98				2762
62	61	5	2761.61	1173.78	0	13.86	551.44				2421
63	62	5	2421.33	1029.94	0	13.82	549.64				2371
64	63	5	2370.93	1000.14	0	13.62	550.17				4446
65	64	5	4446.48	1903.78	0	13.85	546.03				4497
66	65	5	4496.54	1897.18	0						

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
67	66	5	4349.93	1846.58		0	13.7	547.96			4350
68	67	5	4308.9	1839.34		0	13.79	550.09			4309
69	68	5	4217.39	1806.42		0	13.74	548.19			4217
70	69	5	4262.09	1805.78		0	13.71	548.89			4262
71	70	5	7074.61	3028.98		0	13.76	548.27			7075
72	71	5	7111.84	3050.94		0	13.77	550.86			7112
73	72	5	7090.09	3032.14		0	13.69	546.73			7090
74	73	5	7107.89	3027.58		0	13.81	547.61			7108
75	74	5	7277.03	3085.02		0	13.74	547.32			7277
76	75	5	7043.09	2997.38		0	13.72	545.6			7043
77	76	5	9754.95	4191.3		0	14.09	556.64			9755
78	77	5	9788.26	4179.86		0	13.98	554.14			9788
79	78	5	9236.77	3943.14		0	13.81	549.13			9237
80	79	5	9045.73	3853.58		0	13.62	545.79			9046
81	80	5	9370	4047.74		0	13.65	550.3			9370
82	81	5	9319.07	3998.14		0	13.65	548.23			9319
83	82	5	10966.28	4664.74		0	13.71	545.63			10966
84	83	5	11077.37	4693.66		0	13.72	546.02			11077
85	84	5	11131.31	4723.38		0	13.76	545.73			11131
86	85	5	11323.53	4793.22		0	13.76	548.18			11324

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
87	86	5	10757.73	4571.18		0	13.71	546.66			10758
88	87	5	10473.94	4496.22		0	13.65	546.64			10474
89	88	5	12674.73	5413.38		0	13.74	547.28			12675
90	89	5	12552.09	5330.18		0	13.77	545.54			12552
91	90	5	12304.1	5240.54		0	13.67	546.65			12304
92	91	5	12186.76	5171.78		0	13.71	543.96			12189
93	92	5	12640.1	5384.02		0	13.74	546.9			12640
94	93	5	12524.77	5359.18		0	13.77	549.81			12525
95	94	5	13172.85	5619.62		0	13.65	547.96			13173
96	95	5	13220.05	5617.38		0	13.76	549.09			13220
97	96	5	13279.74	5668.34		0	13.73	547.26			13280
98	97	5	13128.96	5578.94		0	13.74	546.97			13129
99	98	5	13249.92	5643.46		0	13.71	547.75			13250
100	99	5	13085.48	5574.5		0	13.7	549.58			13085
101	100	5	14116.32	6011.22		0	13.7	547.86			14116
102	101	5	14082.1	5990.42		0	13.74	546.27			14082
103	102	5	13665.04	5831.58		0	13.71	549.33			13665
104	103	5	13508.81	5737.14		0	13.76	547.74			13509
105	104	5	13256.59	5673.98		0	13.6	548.79			13257
106	105	5	13068.2	5567.78		0	13.7	546.4			13068
107	106	5	234734.23	107879		0	15.02	621.98			234734

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Assay ID	Test Chemical	# Concentrations tested	Microsome ID	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc	2						
Assay Date	Assay ID	Test Chemical	# Concentrations tested	Microsome ID	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc	2						
Assay Date	Assay ID	Test Chemical	# Concentrations tested	Microsome ID	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc	2						
Assay Date	Assay ID	Test Chemical	# Concentrations tested	Microsome ID	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc	2						
Assay Date	Assay ID	Test Chemical	# Concentrations tested	Microsome ID	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc	2						
Assay Date	Assay ID	Test Chemical	# Concentrations tested	Microsome ID	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc	2						
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Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - signal variance			
6		Red T value not within mean +/- 10%			
7		1.01			
8		0.99			
9		1.01			
10		0.99			
11		1.01			
12		0.99			
13		1.01			
14		0.99			
15		0.99			
16		1.00			
17					
18					
19		1.00			
20		0.99			
21		1.00			
22		0.99			
23					
24					
25		0.99			
26		1.02			
27					
28					
29		1.02			
30		0.99			
31		1.02			
32		0.99			
33					
34					
35		1.03			
36		0.97			
37		1.01			
38		0.99			
39					
40					
41		1.00			
42		1.00			
43		1.00			
44		1.00			
45					
46					
47		0.99			
48		1.01			
49		1.00			
50		1.00			
51					
52					
53		0.99			
54		1.01			
55		1.00			
56		1.00			
57					
58					
59		1.00			
60		0.99			
61		1.01			
62					
63					
64					
65		0.99			
66		1.01			
67		1.00			
68					
69		1.00			
70					

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		OC check - aliquot variance
5		
6		% RE
7		1.01
8		0.99
9		1.01
10		0.99
11		1.01
12		0.99
13		0.99
14		1.01
15		1.00
16		1.00
17		0.99
18		1.01
19		1.01
20		1.00
21		1.00
22		1.00
23		1.02
24		0.98
25		1.00
26		1.00
27		1.01
28		0.99
29		1.00
30		1.00
31		0.99
32		1.01
33		0.99
34		1.01
35		1.01
36		0.99
37		1.00
38		1.00
39		1.00
40		1.00
41		1.00
42		1.00
43		1.00
44		1.00
45		1.01
46		0.99
47		1.00
48		0.99
49		1.00
50		1.00
51		1.01
52		0.99
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/27/2007	CR11610	CR11610	27MR2052 P00 & 28MR2055 P00	2	Positive Control	4 OH ASDN	Pos Ctl Tubes/Conc	12535-13	
2	NE Ref	12535-09	12535-09	12535-09	12535-09	2	Positive Control	4 OH ASDN	Pos Ctl Tubes/Conc	12535-13	
3	Technician ID	PRP	PRP	PRP	PRP	2	Positive Control	4 OH ASDN	Pos Ctl Tubes/Conc	12535-13	
4	Control Type	Portion	SD								
5	Full activity	Overall	0.025								
6	Background (unconnected)	Overall	0.000								
7											
8											
9											
10	Positive Control	Replicate	[Positive Ctl M]	Log[Positive Ctl]	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	2	1.00E-05	-5.00	0.002						
13	4-OH ASDN	1	1.00E-06	-6.00	0.023						
14	4-OH ASDN	2	1.00E-06	-6.00	0.021						
15	4-OH ASDN	3	1.00E-07	-6.30	0.040						
16	4-OH ASDN	4	1.00E-07	-6.30	0.038						
17	4-OH ASDN	5	1.00E-07	-6.30	0.143						
18	4-OH ASDN	6	1.00E-07	-6.30	0.136						
19	4-OH ASDN	7	1.00E-08	-7.30	0.194						
20	4-OH ASDN	8	1.00E-08	-7.30	0.202						
21	4-OH ASDN	9	1.00E-08	-8.00	0.324						
22	4-OH ASDN	10	1.00E-08	-8.00	0.302						
23	4-OH ASDN	11	1.00E-09	-9.00	0.361						
24	4-OH ASDN	12	1.00E-09	-9.00	0.355						
25	4-OH ASDN	13	1.00E-10	-10.00	0.341						
26	4-OH ASDN	14	1.00E-10	-10.00	0.364						
27	4-OH ASDN	15	1.00E-10	-10.00	0.364						
28	4-OH ASDN	16	1.00E-10	-10.00	0.364						
29	4-OH ASDN	17	1.00E-10	-10.00	0.364						
30	4-OH ASDN	18	1.00E-10	-10.00	0.364						
31	4-OH ASDN	19	1.00E-10	-10.00	0.364						
32	4-OH ASDN	20	1.00E-10	-10.00	0.364						
33	4-OH ASDN	21	1.00E-10	-10.00	0.364						
34	4-OH ASDN	22	1.00E-10	-10.00	0.364						
35	4-OH ASDN	23	1.00E-10	-10.00	0.364						
36	4-OH ASDN	24	1.00E-10	-10.00	0.364						
37	Test Chemical	Replicate	[Test chemical M]	Log[Test chemical]	Activity						
38	CR11610	1	5.00E-06	-4.30	0.087						
39	CR11610	2	5.00E-06	-4.30	0.073						
40	CR11610	3	5.00E-06	-4.30	0.063						
41	CR11610	4	5.00E-06	-4.30	0.118						
42	CR11610	5	5.00E-06	-4.30	0.115						
43	CR11610	6	5.00E-06	-4.30	0.112						
44	CR11610	7	5.00E-06	-4.30	0.189						
45	CR11610	8	5.00E-06	-4.30	0.189						
46	CR11610	9	5.00E-06	-4.30	0.191						
47	CR11610	10	5.00E-06	-4.30	0.261						
48	CR11610	11	5.00E-06	-4.30	0.244						
49	CR11610	12	5.00E-06	-4.30	0.249						
50	CR11610	13	5.00E-06	-4.30	0.284						
51	CR11610	14	5.00E-06	-4.30	0.300						
52	CR11610	15	5.00E-06	-4.30	0.283						
53	CR11610	16	5.00E-06	-4.30	0.337						
54	CR11610	17	5.00E-06	-4.30	0.327						
55	CR11610	18	5.00E-06	-4.30	0.336						
56	CR11610	19	5.00E-06	-4.30	0.353						
57	CR11610	20	5.00E-06	-4.30	0.353						
58	CR11610	21	5.00E-06	-4.30	0.352						
59	CR11610	22	5.00E-06	-4.30	0.377						
60	CR11610	23	5.00E-06	-4.30	0.363						
61	CR11610	24	5.00E-06	-4.30	0.362						
62	CR11610	25	5.00E-06	-4.30	0.362						
63	CR11610	26	5.00E-06	-4.30	0.362						
64	CR11610	27	5.00E-06	-4.30	0.362						
65	CR11610	28	5.00E-06	-4.30	0.362						
66	CR11610	29	5.00E-06	-4.30	0.362						
67	CR11610	30	5.00E-06	-4.30	0.362						
68	CR11610	31	5.00E-06	-4.30	0.362						
69	CR11610	32	5.00E-06	-4.30	0.362						
70	CR11610	33	5.00E-06	-4.30	0.362						
71	CR11610	34	5.00E-06	-4.30	0.362						
72	CR11610	35	5.00E-06	-4.30	0.362						
73	CR11610	36	5.00E-06	-4.30	0.362						
74	CR11610	37	5.00E-06	-4.30	0.362						
75	CR11610	38	5.00E-06	-4.30	0.362						
76	CR11610	39	5.00E-06	-4.30	0.362						
77	CR11610	40	5.00E-06	-4.30	0.362						
78	CR11610	41	5.00E-06	-4.30	0.362						
79	CR11610	42	5.00E-06	-4.30	0.362						
80	CR11610	43	5.00E-06	-4.30	0.362						
81	CR11610	44	5.00E-06	-4.30	0.362						
82	CR11610	45	5.00E-06	-4.30	0.362						
83	CR11610	46	5.00E-06	-4.30	0.362						
84	CR11610	47	5.00E-06	-4.30	0.362						
85	CR11610	48	5.00E-06	-4.30	0.362						
86	CR11610	49	5.00E-06	-4.30	0.362						
87	CR11610	50	5.00E-06	-4.30	0.362						
88	CR11610	51	5.00E-06	-4.30	0.362						
89	CR11610	52	5.00E-06	-4.30	0.362						
90	CR11610	53	5.00E-06	-4.30	0.362						
91	CR11610	54	5.00E-06	-4.30	0.362						
92	CR11610	55	5.00E-06	-4.30	0.362						
93	CR11610	56	5.00E-06	-4.30	0.362						
94	CR11610	57	5.00E-06	-4.30	0.362						
95	CR11610	58	5.00E-06	-4.30	0.362						
96	CR11610	59	5.00E-06	-4.30	0.362						
97	CR11610	60	5.00E-06	-4.30	0.362						
98	CR11610	61	5.00E-06	-4.30	0.362						
99	CR11610	62	5.00E-06	-4.30	0.362						
100	CR11610	63	5.00E-06	-4.30	0.362						
101	CR11610	64	5.00E-06	-4.30	0.362						
102	CR11610	65	5.00E-06	-4.30	0.362						
103	CR11610	66	5.00E-06	-4.30	0.362						
104	CR11610	67	5.00E-06	-4.30	0.362						
105	CR11610	68	5.00E-06	-4.30	0.362						
106	CR11610	69	5.00E-06	-4.30	0.362						
107	CR11610	70	5.00E-06	-4.30	0.362						
108	CR11610	71	5.00E-06	-4.30	0.362						
109	CR11610	72	5.00E-06	-4.30	0.362						
110	CR11610	73	5.00E-06	-4.30	0.362						
111	CR11610	74	5.00E-06	-4.30	0.362						
112	CR11610	75	5.00E-06	-4.30	0.362						
113	CR11610	76	5.00E-06	-4.30	0.362						
114	CR11610	77	5.00E-06	-4.30	0.362						
115	CR11610	78	5.00E-06	-4.30	0.362						
116	CR11610	79	5.00E-06	-4.30	0.362						
117	CR11610	80	5.00E-06	-4.30	0.362						
118	CR11610	81	5.00E-06	-4.30	0.362						
119	CR11610	82	5.00E-06	-4.30	0.362						
120	CR11610	83	5.00E-06	-4.30	0.362						
121	CR11610	84	5.00E-06	-4.30	0.362						
122	CR11610	85	5.00E-06	-4.30	0.362						
123	CR11610	86	5.00E-06	-4.30	0.362						
124	CR11610	87	5.00E-06	-4.30	0.362						
125	CR11610	88	5.00E-06	-4.30	0.362						
126	CR11610	89	5.00E-06	-4.30	0.362						
127	CR11610	90	5.00E-06	-4.30	0.362						
128	CR11610	91	5.00E-06	-4.30	0.362						
129	CR11610	92	5.00E-06	-4.30	0.362						
130	CR11610	93	5.00E-06	-4.30	0.362						
131	CR11610	94	5.00E-06	-4.30	0.362						
132	CR11610	95	5.00E-06	-4.30	0.362						
133	CR11610	96	5.00E-06	-4.30	0.362						
134	CR11610	97	5.00E-06	-4.30	0.362						
135	CR11610	98	5.00E-06	-4.30	0.362						
136	CR11610	99	5.00E-06	-4.30	0.362						
137	CR11610	100	5.00E-06	-4.30	0.362						
138	CR11610	101	5.00E-06	-4.30	0.362						
139	CR11610	102	5.00E-06	-4.30	0.362						
140	CR11610	103	5.00E-06	-4.30	0.362						
141	CR11610	104	5.00E-06	-4.30	0.362						
142	CR11610	105	5.00E-06	-4							

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
			Test		# Concentrations				
1	Assay Date	3/28/2007	Chemical ID	CR11610	tested			8 Microsome ID	12535-13
2	NB Ref.	12535-92	Counter data filename	28MR2027.P00				Pos Ctl	
3	Technician	PRP	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/28/2007	Test Chemical ID	CR11610	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-92	Counter data filename	28MR2027.P00					
3	Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Alq.		DPM/g soln.			
7	1	0.0196		37028		1889184			
8	2	0.0194		40328		2078763			
9	3	0.0196		39388		2009592			
10	4	0.0199		39731		1996533			
11	5	0.0195		37534		1924821			
12						Average DPM/g soln		1979778	
13						SD		74490	
14						CV		3.76	
15						µCi/g soln		0.8918	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0744 g			
25	Mass of dilution B used in substrate prep					4.5160 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56041712 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8918			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5604	+	0.011		
37				=	0.571	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.561	µCi/µg ASDN			
41					9.925.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/28/2007	Test Chemical ID	CR11610	# Concentrations tested	8	Microsome ID	12535-13	
2			NB Ref	12535-92	Counter data filename	28MR2027.P00					
3			Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl	2	
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	As:	3/28/2007	Test Chemical ID CR11610		# Concentrations tested		8	Microsome ID	12535-13	
2	NB	12535-92	Counter data filename 28MR2027.P00							
3	Te				Positive Control		Pos Ctl			
4	ch						4-OH ASDI Tubes/Conc		2	
5	nic									
6	ian									
7	ID PRP		Replicate #		3					
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	3080.5	1318.7		0	13.69	546.64			3081
72	71	5	2998.54	1278.3		0	13.66	547.62			2999
73	72	5	3201.73	1362.46		0	13.83	546.19			3202
74	73	5	3336.01	1413.98		0	13.82	543.92			3336
75	74	5	3298.78	1393.86		0	13.81	544.9			3299
76	75	5	3332.02	1408.66		0	13.9	546.85			3332
77	76	5	4570.02	1980.14		0	13.77	547.25			4570
78	77	5	4618.12	1961.3		0	13.67	545.36			4618
79	78	5	4422.73	1881.1		0	13.95	546.94			4423
80	79	5	4372.73	1852.46		0	13.89	545.76			4373
81	80	5	4572.85	1933.86		0	13.8	546.52			4573
82	81	5	4531.32	1914.9		0	13.87	545.41			4531

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
83	82	5	5486.85	2328.5		0	13.75	548.87			5496
84	83	5	5367.83	2265.5		0	13.8	544.75			5368
85	84	5	5546.51	2356.14		0	13.85	548.24			5547
86	85	5	5481.4	2326.54		0	13.89	547.12			5481
87	86	5	5489.37	2328.74		0	13.77	545.17			5489
88	87	5	5504.35	2329.3		0	13.82	548.35			5504
89	88	5	6444.82	2769.86		0	13.62	548.47			6445
90	89	5	6535.2	2763.38		0	13.81	543.85			6535
91	90	5	7085.73	2987.74		0	13.85	545.26			7086
92	91	5	6962.44	2972.5		0	13.76	547.96			6962
93	92	5	6941.79	2965.06		0	13.81	548.35			6942
94	93	5	6785.48	2891.74		0	13.94	547.99			6785
95	94	5	7427.32	3144.54		0	13.82	546.64			7427
96	95	5	7466.59	3171.86		0	13.83	547.45			7467
97	96	5	7851.19	3338.46		0	13.82	546.47			7851
98	97	5	7800.02	3288.34		0	13.81	543.84			7800
99	98	5	7974.88	3408.58		0	13.8	546.18			7975
100	99	5	8087.18	3447.58		0	13.74	547.68			8087
101	100	5	7385.68	3131.5		0	13.8	544.51			7386
102	101	5	7471.72	3156.46		0	13.79	544.31			7472
103	102	5	7658.86	3250.14		0	13.87	545.42			7659
104	103	5	7550.79	3204.46		0	13.83	548.51			7551
105	104	5	7333.3	3110.7		0	13.89	549.04			7333
106	105	5	7791.83	3313.5		0	13.75	543.84			7792
107	106	5	234186.17	107618.02		0	15.17	625.98			234186
108											0
109											0
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1		Assay Date	3/26/2007	Test Chemical ID	CR11610	# Concentrations tested				8 Micrograms ID	12535-13						
2		NB Ref. Technician ID	12535-92	Course date	26MAR2027	P00											
3		Replicate #	3	Positive Controls						14-CH ASDM (For Oil Tubes/Conv							
4																	
5		Sample ID															
6		Sample type	Replicate	Nominal conc. (nM)	Aliq. #	DPM/aliq	DPM/mL	Ave. DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate (nmol ³ H ₂ O formed) (Total DPM corrected for background) (Background tubes)	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Inactivation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	
7		Full activity control	1	2	0.5	2512	1256	1256	3372	0.1	197978	3372	0.0337	0.005	15	0.225	
8			2	0.5	2	6349	1899	1899	3624	0.1	197978	3624	0.0363	0.005	15	0.242	
9			3	0.5	2	9064	18128	18128	30936	0.1	197978	30936	0.0309	0.005	15	0.206	
10			4	0.5	2	7761	15542	15542	23946	0.1	197978	23946	0.0239	0.005	15	0.200	
11			1	0.5	2	7446	14890	14890	236	0.1	197978	236	0.0023	0.005	15	0.000	
12			2	0.5	2	7549	15098	15098	222	0.1	197978	222	0.0022	0.005	15	0.000	
13			3	0.5	2	57	114	114	276	0.1	197978	276	0.0027	0.005	15	0.000	
14			4	0.5	2	54	108	108	236	0.1	197978	236	0.0023	0.005	15	0.000	
15			1	0.5	2	61	122	122	554	0.1	197978	554	0.0055	0.005	15	0.002	
16			2	0.5	2	138	276	276	454	0.1	197978	454	0.0045	0.005	15	0.002	
17			3	0.5	2	108	216	216	227	0.1	197978	227	0.0022	0.005	15	0.002	
18			4	0.5	2	119	238	238	218	0.1	197978	218	0.0021	0.005	15	0.002	
19			1	0.5	2	681	1362	1359	2718	0.1	197978	2718	0.0027	0.005	15	0.002	
20			2	0.5	2	678	1356	1297	2534	0.1	197978	2534	0.0025	0.005	15	0.002	
21			3	0.5	2	656	1312	1262	394	0.1	197978	394	0.0039	0.005	15	0.002	
22			4	0.5	2	631	1262	1262	4524	0.1	197978	4524	0.0045	0.005	15	0.002	
23			1	0.5	2	1153	2306	2306	4524	0.1	197978	4524	0.0045	0.005	15	0.002	
24			2	0.5	2	1108	2218	6226	12452	0.1	197978	12452	0.0124	0.005	15	0.002	
25			3	0.5	2	1013	2026	1968	394	0.1	197978	394	0.0039	0.005	15	0.002	
26			4	0.5	2	984	1968	1968	6944	0.1	197978	6944	0.0069	0.005	15	0.002	
27			1	0.5	2	3532	7064	6944	13548	0.1	197978	13548	0.0134	0.005	15	0.002	
28			2	0.5	2	3412	6824	6226	12452	0.1	197978	12452	0.0124	0.005	15	0.002	
29			3	0.5	2	3172	6344	6108	12452	0.1	197978	12452	0.0124	0.005	15	0.002	
30			4	0.5	2	3054	6108	6108	8459	0.1	197978	8459	0.0084	0.005	15	0.002	
31			1	0.5	2	4571	9142	9031	15652	0.1	197978	15652	0.0156	0.005	15	0.002	
32			2	0.5	2	4467	8934	8459	15276	0.1	197978	15276	0.0152	0.005	15	0.002	
33			3	0.5	2	4267	8534	8459	15276	0.1	197978	15276	0.0152	0.005	15	0.002	
34			4	0.5	2	4162	8324	8459	15276	0.1	197978	15276	0.0152	0.005	15	0.002	
35			1	0.5	2	5458	10912	10914	21628	0.1	197978	21628	0.0216	0.005	15	0.002	
36			2	0.5	2	5458	10912	10914	21628	0.1	197978	21628	0.0216	0.005	15	0.002	
37			3	0.5	2	6344	12688	12705	25410	0.1	197978	25410	0.0254	0.005	15	0.002	
38			4	0.5	2	6311	12622	12705	25410	0.1	197978	25410	0.0254	0.005	15	0.002	
39			1	0.5	2	7826	15652	15276	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
40			2	0.5	2	7826	15652	15276	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
41			3	0.5	2	7647	15294	14366	28368	0.1	197978	28368	0.0284	0.005	15	0.002	
42			4	0.5	2	7453	14906	14284	28368	0.1	197978	28368	0.0284	0.005	15	0.002	
43			1	0.5	2	7101	14202	14284	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
44			2	0.5	2	7206	14412	14508	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
45			3	0.5	2	7300	14600	14508	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
46			4	0.5	2	7511	15022	14807	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
47			1	0.5	2	7596	15192	14807	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
48			2	0.5	2	7596	15192	14807	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
49			3	0.5	2	7596	15192	14807	30592	0.1	197978	30592	0.0306	0.005	15	0.002	
50			4	0.5	2	7596	15192	14807	30592	0.1	197978	30592	0.0306	0.005	15	0.002	

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC Check: aliquot variance
5		
6		% RE
7		0.99
8		1.02
9		0.98
10		1.02
11		1.00
12		1.00
13		1.02
14		0.98
15		1.00
16		1.00
17		1.02
18		0.98
19		1.01
20		0.98
21		0.98
22		1.02
23		1.00
24		1.00
25		0.99
26		1.01
27		1.01
28		0.99
29		1.00
30		1.00
31		1.01
32		0.99
33		1.01
34		0.99
35		1.00
36		0.99
37		1.01
38		1.01
39		0.99
40		1.01
41		1.01
42		0.99
43		1.00
44		1.00
45		1.00
46		1.00
47		0.99
48		1.01
49		0.99
50		1.01
51		1.01
52		0.98
53		0.97
54		1.03

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/28/2007	Test Chemical ID	CR11610	# Concentrations			8 Microsome ID	12535-13	
2		NE Ref.	12535-92	Counter data filename	28M1207.P00				Pos Ctl Tubes/Conc		
3		Technician ID	PRP	Replicate #		3	Positive Control	4-OH ASDN			2
4											
5	Control Type	Average	SD								
6	Full activity	0.218	0.019		Blg % of Full						
7	Background (uncorrected)	0.002	0.000			0.7 (Red # >1%)					
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.002						
12	4-OH ASDN	2	1.00E-05	-5.00	0.002						
13	4-OH ASDN	1	1.00E-06	-8.00	0.017						
14	4-OH ASDN	2	1.00E-06	-8.00	0.015						
15	4-OH ASDN	1	1.00E-06	-8.00	0.015						
16	4-OH ASDN	2	1.00E-06	-8.00	0.015						
17	4-OH ASDN	1	5.00E-07	-6.30	0.029						
18	4-OH ASDN	2	5.00E-07	-6.30	0.025						
19	4-OH ASDN	1	1.00E-07	-7.00	0.052						
20	4-OH ASDN	2	1.00E-07	-7.00	0.062						
21	4-OH ASDN	1	5.00E-08	-7.30	0.120						
22	4-OH ASDN	2	5.00E-08	-7.30	0.112						
23	4-OH ASDN	1	1.00E-08	-8.00	0.145						
24	4-OH ASDN	2	1.00E-08	-8.00	0.169						
25	4-OH ASDN	1	1.00E-09	-9.00	0.204						
26	4-OH ASDN	2	1.00E-09	-9.00	0.190						
27	4-OH ASDN	1	1.00E-10	-10.00	0.193						
28	4-OH ASDN	2	1.00E-10	-10.00	0.197						
29	4-OH ASDN	1	1.00E-10	-10.00	0.193						
30	4-OH ASDN	2	1.00E-10	-10.00	0.197						
31	4-OH ASDN	1	1.00E-10	-10.00	0.193						
32	4-OH ASDN	2	1.00E-10	-10.00	0.197						
33	4-OH ASDN	1	1.00E-10	-10.00	0.193						
34	4-OH ASDN	2	1.00E-10	-10.00	0.197						
35	4-OH ASDN	1	1.00E-10	-10.00	0.193						
36	4-OH ASDN	2	1.00E-10	-10.00	0.197						
37	Test Chemical	Replicate	Test Chemical M	Log(test chemical)	Activity						
38	CR11610	1	5.00E-05	-4.30	0.027						
39	CR11610	2	5.00E-05	-4.30	0.028						
40	CR11610	1	5.00E-05	-4.30	0.027						
41	CR11610	2	5.00E-05	-4.30	0.027						
42	CR11610	1	2.50E-05	-4.60	0.049						
43	CR11610	2	2.50E-05	-4.60	0.049						
44	CR11610	1	2.50E-05	-4.60	0.044						
45	CR11610	2	2.50E-05	-4.60	0.044						
46	CR11610	1	1.00E-05	-5.00	0.080						
47	CR11610	2	1.00E-05	-5.00	0.080						
48	CR11610	1	1.00E-05	-5.00	0.088						
49	CR11610	2	1.00E-05	-5.00	0.088						
50	CR11610	1	5.00E-06	-5.30	0.122						
51	CR11610	2	5.00E-06	-5.30	0.117						
52	CR11610	1	5.00E-06	-5.30	0.121						
53	CR11610	2	5.00E-06	-5.30	0.121						
54	CR11610	1	2.50E-06	-5.60	0.144						
55	CR11610	2	2.50E-06	-5.60	0.147						
56	CR11610	1	2.50E-06	-5.60	0.148						
57	CR11610	2	2.50E-06	-5.60	0.148						
58	CR11610	1	1.00E-06	-6.00	0.173						
59	CR11610	2	1.00E-06	-6.00	0.187						
60	CR11610	1	1.00E-06	-6.00	0.183						
61	CR11610	2	1.00E-06	-6.00	0.183						
62	CR11610	1	1.00E-07	-7.00	0.199						
63	CR11610	2	1.00E-07	-7.00	0.209						
64	CR11610	1	1.00E-07	-7.00	0.214						
65	CR11610	2	1.00E-07	-7.00	0.214						
66	CR11610	1	1.00E-08	-8.00	0.198						
67	CR11610	2	1.00E-08	-8.00	0.203						
68	CR11610	1	1.00E-08	-8.00	0.203						
69	CR11610	2	1.00E-08	-8.00	0.203						
70	CR11610	1	1.00E-08	-8.00	0.202						
71	CR11610	2	1.00E-08	-8.00	0.202						
72	CR11610	1	1.00E-08	-8.00	0.202						
73	CR11610	2	1.00E-08	-8.00	0.202						
74	CR11610	1	1.00E-08	-8.00	0.202						
75	CR11610	2	1.00E-08	-8.00	0.202						
76	CR11610	1	1.00E-08	-8.00	0.202						
77	CR11610	2	1.00E-08	-8.00	0.202						
78	CR11610	1	1.00E-08	-8.00	0.202						
79	CR11610	2	1.00E-08	-8.00	0.202						
80	CR11610	1	1.00E-08	-8.00	0.202						
81	CR11610	2	1.00E-08	-8.00	0.202						
82	CR11610	1	1.00E-08	-8.00	0.202						
83	CR11610	2	1.00E-08	-8.00	0.202						
84	CR11610	1	1.00E-08	-8.00	0.202						
85	CR11610	2	1.00E-08	-8.00	0.202						
86	CR11610	1	1.00E-08	-8.00	0.202						
87	CR11610	2	1.00E-08	-8.00	0.202						
88	CR11610	1	1.00E-08	-8.00	0.202						
89	CR11610	2	1.00E-08	-8.00	0.202						
90	CR11610	1	1.00E-08	-8.00	0.202						
91	CR11610	2	1.00E-08	-8.00	0.202						
92	CR11610	1	1.00E-08	-8.00	0.202						
93	CR11610	2	1.00E-08	-8.00	0.202						
94	CR11610	1	1.00E-08	-8.00	0.202						
95	CR11610	2	1.00E-08	-8.00	0.202						
96	CR11610	1	1.00E-08	-8.00	0.202						
97	CR11610	2	1.00E-08	-8.00	0.202						
98	CR11610	1	1.00E-08	-8.00	0.202						
99	CR11610	2	1.00E-08	-8.00	0.202						
100	CR11610	1	1.00E-08	-8.00	0.202						
101	CR11610	2	1.00E-08	-8.00	0.202						
102	CR11610	1	1.00E-08	-8.00	0.202						
103	CR11610	2	1.00E-08	-8.00	0.202						
104	CR11610	1	1.00E-08	-8.00	0.202						
105	CR11610	2	1.00E-08	-8.00	0.202						
106	CR11610	1	1.00E-08	-8.00	0.202						
107	CR11610	2	1.00E-08	-8.00	0.202						
108	CR11610	1	1.00E-08	-8.00	0.202						
109	CR11610	2	1.00E-08	-8.00	0.202						
110	CR11610	1	1.00E-08	-8.00	0.202						
111	CR11610	2	1.00E-08	-8.00	0.202						
112	CR11610	1	1.00E-08	-8.00	0.202						
113	CR11610	2	1.00E-08	-8.00	0.202						
114	CR11610	1	1.00E-08	-8.00	0.202						
115	CR11610	2	1.00E-08	-8.00	0.202						
116	CR11610	1	1.00E-08	-8.00	0.202						
117	CR11610	2	1.00E-08	-8.00	0.202						
118	CR11610	1	1.00E-08	-8.00	0.202						
119	CR11610	2	1.00E-08	-8.00	0.202						
120	CR11610	1	1.00E-08	-8.00	0.202						
121	CR11610	2	1.00E-08	-8.00	0.202						
122	CR11610	1	1.00E-08	-8.00	0.202						
123	CR11610	2	1.00E-08	-8.00	0.202						
124	CR11610	1	1.00E-08	-8.00	0.202						
125	CR11610	2	1.00E-08	-8.00	0.202						
126	CR11610	1	1.00E-08	-8.00	0.202						
127	CR11610	2	1.00E-08	-8.00	0.202						
128	CR11610	1	1.00E-08	-8.00	0.202						
129	CR11610	2	1.00E-08	-8.00	0.202						
130	CR11610	1	1.00E-08	-8.00	0.202						
131	CR11610	2	1.00E-08	-8.00	0.202						
132	CR11610	1	1.00E-08	-8.00	0.202						
133	CR11610	2	1.00E-08	-8.00	0.202						
134	CR11610	1	1.00E-08	-8.00	0.202						
135	CR11610	2	1.00E-08	-8.00	0.202						
136	CR11610	1	1.00E-08	-8.00	0.202						
137	CR11610	2	1.00E-08	-8.00	0.202						
138	CR11610	1	1.00E-08	-8.00	0.202						
139	CR11610	2	1.00E-08	-8.00	0.202						
140	CR11610	1	1.00E-08	-8.00	0.202						
141	CR11610	2	1.00E-08	-8.00	0.202						
142	CR11610	1	1.00E-08	-8.00	0.202						
143	CR11610	2	1.00E-08	-8.00	0.202						
144	CR11610	1	1.00E-08	-8.00							

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	3/16/2007	Test	Chemical ID	CR11611	# Concentrations	tested	8	Microsome ID	12535-13
2	NB Ref.	12535-74	Counter data	filename	17MR0034.P00					
	Technician									Pos Ctrl
3	ID	CKN	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2		

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/16/2007	Test Chemical ID	CR11611	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-74	Counter data filename	17MR0034.P00					
3	Technician ID	CKN	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0204		42226		2069902			
8	2	0.0205		43666		2130049			
9	3	0.0204		40520		1986275			
10	4	0.0209		42854		2050431			
11	5	0.0199		42189		2120050			
12						Average DPM/g soln		2071341	
13						SD		58084	
14						CV		2.80	
15						µCi/g soln		0.9330	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution						8.0215 g		
25	Mass of dilution B used in substrate prep						4.5556 g		
26	Concentration of nonradiolabeled ASDN in substrate soln.						0.56735578 µg/g		
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.9330				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					= 0.5674 + 0.011				
37					= 0.579 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.612 µCi/µg ASDN								
41	1.025.E+06 dpm/nmol								

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/16/2007	Test Chemical ID	CR11611	# Concentrations tested	8	Microsome ID	12535-13	
2		NB Ref	12535-74	Counter data filename	17MR0034.P00					
3		Technician ID	CKN	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctrl		2
4										
5	Microsome Dilution Details									
6										
7	Dilution A		0.12 mL microsome Stock used							
8			2.4 mL total volume							
9			20 dilution factor							
10										
11	Dilution B		1.5 mL microsome Dilution A used							
12			60 mL total volume							
13			40 dilution factor							
14										
15	Dilution C (if applicable)		mL microsome Dilution B used							
16			mL total volume							
17			dilution factor							
18		NA								
19			800 total dilution factor							
20										
21	Protein Concentration (stock microsomes, mg/mL):			8.00						
22	Protein Concentration (dilution added to assay, mg/mL):			0.01						
23										
24										
25										
26										

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/16/2007	Test Chemical ID	CR11611	# Concentrations tested	8	Microsome ID	12535-13		
2		NB Ref.	12535-74	Counter data filename	17MR0034.P00						
3		Technician ID	CKN	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2		
4											
5		Vial	Time (min)	DPM1		ISIE				Round DPM	
6											
7	2	5	42226.33	21222.75	0	13.62	593.01			42226	
8	3	5	43685.65	22177.46	0	13.57	600.23			43666	
9	4	5	40519.71	20495.75	0	13.53	595.79			40520	
10	5	5	42854.48	21761.53	0	13.6	601.68			42854	
11	6	5	42189.3	21330.95	0	13.52	595.83			42189	
12											
13	8	5	13879.72	6541.59	0	12.64	525.76			13880	
14	9	5	13436.94	6389.11	0	12.55	526.44			13437	
15	10	5	12918.42	6127.98	0	12.54	525.15			12918	
16	11	5	12841.81	6077.86	0	12.58	528.58			12842	
17	12	5	13484.5	6379.69	0	12.57	526.4			13485	
18	13	5	13449.71	6356.06	0	12.6	527.27			13450	
19	14	5	13850.83	6548.01	0	12.58	527.68			13851	
20	15	5	13819.33	6557.19	0	12.61	527.41			13819	
21	16	5	205.59	98	0	12.44	525.41			206	
22	17	5	189.47	90.45	0	12.08	526.22			189	
23	18	5	266.89	127.15	0	12.42	528.14			267	
24	19	5	243.99	114.05	0	11.75	526.77			244	
25	20	5	141.13	67.76	0	12.26	526.03			141	
26	21	5	159.1	75.74	0	13.13	524.89			159	
27	22	5	172.35	78.71	0	13.03	526.01			172	
28	23	5	155.82	71.53	0	12.31	526.39			156	
29											
30	25	5	402.05	192.69	0	12.61	528.28			402	
31	26	5	394.49	187.95	0	12.41	526.83			394	
32	27	5	238.7	115.9	0	12.33	525.55			239	
33	28	5	217.97	106.41	0	12.16	522.76			218	
34											
35											
36	29	5	1041.63	497.29	0	12.74	527.61			1042	
37	30	5	1015.08	481.05	0	12.42	525.34			1015	
38	31	5	874.2	420.57	0	12.29	526.91			874	
39	32	5	884.72	423.56	0	12.57	527.54			885	
40											
41											
42	33	5	1521.6	722.22	0	12.43	524.97			1522	
43	34	5	1506.22	708.66	0	12.39	525.64			1506	
44	35	5	1527.57	722.36	0	12.66	526.53			1528	
45	36	5	1502.97	713.81	0	12.72	527.44			1503	
46											
47											
48	37	5	6162.73	2942.67	0	12.62	528.27			6163	
49	38	5	6072.52	2867.26	0	12.57	526.49			6073	
50	39	5	5063.23	2391.76	0	12.48	524.12			5063	
51	40	5	4917.21	2319.72	0	12.68	527.18			4917	
52											
53											
54	41	5	7763.99	3684.38	0	12.62	527.36			7764	
55	42	5	7858.65	3715.74	0	12.64	528.71			7859	
56	43	5	7471.67	3539.47	0	12.57	528.25			7472	
57	44	5	7421.33	3494.06	0	12.65	527.09			7421	
58											
59											
60	45	5	11837.74	5588.18	0	12.63	526.21			11838	
61	46	5	11734.37	5531.57	0	12.62	527.16			11734	
62	47	5	12186.62	5746.4	0	12.59	526.75			12187	
63	48	5	12090.95	5727.88	0	12.57	526.91			12091	
64											
65											
66	49	5	12451.78	5904.69	0	12.62	529.22			12452	
67	50	5	12607.53	5923.99	0	12.6	524.55			12608	
68	51	5	14627.26	6944.35	0	12.57	527.94			14627	
69	52	5	15216.84	7141.84	0	12.52	523.85			15217	
70											
71											
72	53	5	12697.57	6052.8	0	12.47	525.1			12698	

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
73	54	5	12756.83	5999.1		0 12.54	522.83				12757
74	55	5	14685.39	6934.79		0 12.65	527.35				14685
75	56	5	14739.62	7006.64		0 12.57	527.54				14740
76											
77	58	5	14998.63	7103.08		0 12.56	526.12				14999
78	59	5	15005.75	7085.13		0 12.61	525.91				15006
79	60	5	14251.98	6722.89		0 12.62	526.38				14252
80	61	5	14170.21	6746.37		0 12.57	528.99				14170
81	62	5	14759.47	7000.81		0 12.57	525.04				14759
82	63	5	14688.49	6916.55		0 12.52	521.23				14688
83	64	5	15521.83	7307.85		0 12.57	524.02				15522
84	65	5	15234.98	7201.47		0 12.6	526.5				15235
85	66	5	15331.13	7284.39		0 12.53	526.74				15331
86	67	5	15411.48	7284.91		0 12.59	525.93				15411
87	68	5	14859.72	7049.21		0 12.53	523.9				14860
88	69	5	14890.4	7032.51		0 12.61	525.42				14890
89	70	5	15637.2	7411.4		0 12.61	527.77				15637
90	71	5	15631.23	7390.48		0 12.53	525.27				15631
91	72	5	15411.84	7273.84		0 12.63	526.36				15412
92	73	5	15338.42	7251.35		0 12.55	527.29				15338
93	74	5	15064.35	7107.77		0 12.59	525.98				15064
94	75	5	15175.43	7141.55		0 12.61	522.53				15175
95	76	5	15224.46	7228.54		0 12.59	528.38				15224
96	77	5	15122.79	7167.96		0 12.58	526.08				15123
97	78	5	14992.15	7071.41		0 12.59	524.61				14992
98	79	5	14983.91	7062.23		0 12.53	526.07				14984
99	80	5	14852.75	7062.08		0 12.55	526.65				14853
100	81	5	14861.18	7024.93		0 12.61	526.33				14861
101	82	5	15395.11	7287.81		0 12.62	528.58				15395
102	83	5	15045.86	7125.47		0 12.57	524.94				15046
103	84	5	14784.84	6981.02		0 12.61	525.57				14785
104	85	5	14721.5	7002.47		0 12.47	523.55				14722
105	86	5	13617.88	6428.52		0 12.56	526.59				13618
106	87	5	13370.64	6276.37		0 12.57	524.58				13371
107	88	5	15213.58	7174.6		0 12.57	523.5				15214
108	89	5	14963.1	7093.64		0 12.57	526.48				14963
109	90	5	15326.78	7251.65		0 12.6	526.6				15327
110	91	5	15240.81	7190.9		0 12.56	524.93				15241
111	92	5	14868.75	7077.3		0 12.58	527.63				14869
112	93	5	14675.63	6949.5		0 12.61	526.27				14676
113	94	5	14903.14	7042.61		0 12.68	527.4				14903
114	95	5	14984.45	7100.9		0 12.6	526.96				14984
115	96	5	14791.11	6954.24		0 12.54	522.95				14791
116	97	5	14767.41	7011.32		0 12.6	525.83				14767
117	98	5	13807.33	6570.03		0 12.56	527.95				13807
118	99	5	13785.67	6559.24		0 12.52	525.82				13786
119	100	5	15010.38	7098.35		0 12.64	526.92				15010
120	101	5	15023.86	7111.67		0 12.59	524.98				15024
121	102	5	15489.55	7333.54		0 12.58	524.99				15490
122	103	5	15311.25	7253.31		0 12.62	526.3				15311
123	104	5	14378.09	6824.45		0 12.54	525.8				14378
124	105	5	14665.04	6900.86		0 12.66	530.16				14665
125	106	5	236718.58	57184.46		0 8.75	238.87				236717

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P																																																																
1	Assay Date	3/16/2007	Chemical ID	ORI1611	# Concentrations tested	7	Microsome ID	71NR0034.P00	1	Positive Control	14-OH ASDN Pos Ctl Tubes/Conc	2	Calculate DPM in aqueous portion after extraction	Aliq #	Aliq Volume (mL)	Nominal total volume (mL)	DPM/aliq	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed (Background Tubes)	nmol ³ H ₂ O formed (0.0578)	Final [Product] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)																																																					
																											1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
																											1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
																											1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53
2	Assay Date	1/23/35-74	Chemical ID	ORI1611	# Concentrations tested	7	Microsome ID	71NR0034.P00	1	Positive Control	14-OH ASDN Pos Ctl Tubes/Conc	2	Calculate DPM in aqueous portion after extraction	Aliq #	Aliq Volume (mL)	Nominal total volume (mL)	DPM/aliq	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed (Background Tubes)	nmol ³ H ₂ O formed (0.0578)	Final [Product] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)																																																					
3	Assay Date		Chemical ID		# Concentrations tested		Microsome ID						Calculate DPM in aqueous portion after extraction	Aliq #	Aliq Volume (mL)	Nominal total volume (mL)	DPM/aliq	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed (Background Tubes)	nmol ³ H ₂ O formed (0.0578)	Final [Product] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)																																																					
4	Assay Date		Chemical ID		# Concentrations tested		Microsome ID						Calculate DPM in aqueous portion after extraction	Aliq #	Aliq Volume (mL)	Nominal total volume (mL)	DPM/aliq	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed (Background Tubes)	nmol ³ H ₂ O formed (0.0578)	Final [Product] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)																																																					

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
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5		
6		QC Check - aliquot variance
7		% RE
8		1.00
9		1.00
10		1.00
11		1.00
12		1.01
13		0.99
14		1.00
15		1.00
16		1.00
17		1.00
18		1.00
19		1.00
20		1.00
21		1.00
22		1.00
23		1.00
24		1.00
25		1.00
26		1.00
27		1.00
28		1.00
29		1.00
30		1.00
31		1.01
32		0.99
33		1.00
34		1.00
35		1.01
36		0.99
37		1.01
38		0.99
39		1.00
40		1.00
41		1.01
42		0.99
43		1.00
44		1.00
45		1.00
46		1.00
47		1.00
48		1.00
49		1.00
50		1.00
51		1.00
52		0.99
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/16/2007	Test Chemical ID	CR11611	17MR0034.F00	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
2	NE Ref	12535-74	Counter plate	17MR0034.F00	1	Replicate #					
3	Technician ID	CKN									
4	Control Type	Portion	SD								
5	Full activity	Overall	0.345								
6	Background	Overall	0.001								
7	(uncorrected)										
8											
9											
10	Positive Control	Level	Replicate	[Positive Ctl] M	Log[Positive Ctl]	Activity					
11	4-OH ASDN	1	1	1.00E-09	-9.00	0.005					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.001					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.022					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.018					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.034					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.034					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.164					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.125					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.198					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.189					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.302					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.311					
23	4-OH ASDN	7	1	1.00E-08	-9.00	0.321					
24	4-OH ASDN	7	2	1.00E-08	-9.00	0.363					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.325					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.378					
27	4-OH ASDN										
28	4-OH ASDN										
29	4-OH ASDN										
30	4-OH ASDN										
31	4-OH ASDN										
32	4-OH ASDN										
33	4-OH ASDN										
34	4-OH ASDN										
35	4-OH ASDN										
36	4-OH ASDN										
37	Test Chemical	Level	Replicate	[Test chemical] M	Log[Test chemical]	Activity					
38	CR11611	1	1	1.00E-03	-3.00	0.385					
39	CR11611	1	2	1.00E-03	-3.00	0.365					
40	CR11611	2	1	1.00E-03	-3.00	0.378					
41	CR11611	2	2	1.00E-04	-4.00	0.395					
42	CR11611	2	3	1.00E-04	-4.00	0.395					
43	CR11611	3	1	1.00E-05	-5.00	0.402					
44	CR11611	3	2	1.00E-05	-5.00	0.395					
45	CR11611	3	3	1.00E-05	-5.00	0.388					
46	CR11611	4	1	1.00E-06	-6.00	0.380					
47	CR11611	4	2	1.00E-06	-6.00	0.384					
48	CR11611	4	3	1.00E-06	-6.00	0.382					
49	CR11611	5	1	1.00E-07	-7.00	0.391					
50	CR11611	5	2	1.00E-07	-7.00	0.378					
51	CR11611	5	3	1.00E-07	-7.00	0.365					
52	CR11611	6	1	1.00E-08	-8.00	0.388					
53	CR11611	6	2	1.00E-08	-8.00	0.393					
54	CR11611	6	3	1.00E-08	-8.00	0.379					
55	CR11611	7	1	1.00E-09	-9.00	0.384					
56	CR11611	7	2	1.00E-09	-9.00	0.379					
57	CR11611	7	3	1.00E-09	-9.00	0.354					
58	CR11611	8	1	1.00E-10	-10.00	0.385					
59	CR11611	8	2	1.00E-10	-10.00	0.396					
60	CR11611	8	3	1.00E-10	-10.00	0.371					
61	CR11611										

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11611	# Concentrations tested	8 Microsome ID 12535-13			
2	NB Ref.	12535-78	Counter data filename	21MR0024.P00		Pos Ctl			
3	Technician ID	CKN	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID CR11611		# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-78	Counter data filename	21MR0024.P00				Pos Ctl Tubes/Conc	
3	Technician ID	CKN	Replicate #	2	Positive Control	4-OH ASDN			2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0203		39458		1943744			
8	2	0.0197		37910		1924365			
9	3	0.0200		38992		1949600			
10	4	0.0197		33902		1720914			
11	5	0.0199		40080		2014070			
12						Average DPM/g soln		1910539	
13						SD		111238	
14						CV		5.82	
15						µCi/g soln		0.8606	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor		[ASDN] in solution (µg/mL)			
19	Stock	10.03	10			1003.00			
20	Dilution A			100		10.03			
21	Dilution B			10		1.00			
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					7.9360 g			
25	Mass of dilution B used in substrate prep					4.4824 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.566513 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.010	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.8606				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					0.5665	+	0.010		
37					0.577	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40					1.492	µCi/µg ASDN			
41					9.483.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/20/2007	Test Chemical ID	CR11611	# Concentrations tested		8	Microsome ID	12535-13
2		NB Ref	12535-78	Counter data filename	21MR0024.P00					
3		Technician ID	CKN	Replicate #	2	Positive Control	4-OH ASDN	Pos Cl		2
4										
5	Microsome Dilution Details									
6										
7	Dilution A									
8		0.12 mL microsome Stock used								
9		2.4 mL total volume								
10		20 dilution factor								
11	Dilution B									
12		1.5 mL microsome Dilution A used								
13		60 mL total volume								
14		40 dilution factor								
15	Dilution C (if applicable)									
16		mL microsome Dilution B used								
17		mL total volume								
18		dilution factor								
19		800 total dilution factor								
20										
21	Protein Concentration (stock microsomes, mg/mL):			8.00						
22	Protein Concentration (dilution added to assay, mg/mL):			0.01						
23										
24										
25										
26										

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	Test Chemical ID CR11611 # Concentrations tested 8 Microsome ID 12535-13 Assay Date 3/20/2007									
2	Counter data NB Ref. 12535-78 filename 21MR0024.P00									
3	Technician Replicate Pos Ctl ID CKN # 2 Positive Control 4-OH ASDN Tubes/Conc 2									
4										
5	Vial	Time (min)	DPM1	ISIE				Round DPM		
6										
7	2	5	39458.11	19908.28	0	13.87	604.25			39458
8	3	5	37909.73	19132.07	0	13.79	601.62			37910
9	4	5	38992.08	19605.79	0	13.74	597.36			38992
10	5	5	33901.85	17058.09	0	13.79	601.56			33902
11	6	5	40079.81	20162.25	0	13.87	602.01			40080
12										
13	8	5	15072.29	7081.38	0	12.8	527.83			15072
14	9	5	15266.77	7145.77	0	12.75	525.5			15267
15	10	5	13770.82	6523.07	0	12.79	530.1			13771
16	11	5	14260.41	6710.73	0	12.68	526.44			14260
17	12	5	14483.81	6807.9	0	12.71	527.13			14484
18	13	5	14903.87	6975.41	0	12.77	525.94			14904
19	14	5	14522.77	6874.98	0	12.66	527.84			14523
20	15	5	14587.41	6873.41	0	12.72	527.69			14587
21	16	5	66.99	31.24	0	13.9	524.34			67
22	17	5	64.47	26.26	0	14.22	522.99			64
23	18	5	70.08	31.81	0	13.22	524.54			70
24	19	5	64.39	31.86	0	11.63	523.01			64
25	20	5	68.54	30.55	0	15.24	525.34			69
26	21	5	50.02	24.82	0	12.05	523.26			50
27	22	5	61.25	28.31	0	13.48	524.11			61
28	23	5	64.52	28.49	0	15.33	527.34			65
29										
30	25	5	160.73	77.65	0	12.02	527.29			161
31	26	5	162.02	79.77	0	12.71	522.92			162
32	27	5	163.52	79.6	0	12.81	526			164
33	28	5	149.64	72.77	0	12.78	525.65			150
34										
35										
36	29	5	773.11	366.89	0	12.61	524.35			773
37	30	5	767.56	362.02	0	12.75	525.12			768
38	31	5	923.22	427.95	0	12.8	524.98			923
39	32	5	899.68	435.8	0	12.38	525.32			900
40										
41										
42	33	5	1688.89	791.44	0	12.79	524.9			1689
43	34	5	1656.69	759.82	0	12.79	523.62			1657
44	35	5	1618.85	754.16	0	12.74	524.14			1617
45	36	5	1604.98	753.73	0	12.79	524.04			1605
46										
47										
48	37	5	5523.41	2598.4	0	12.79	525.64			5523
49	38	5	5432.28	2572.63	0	12.62	528.15			5432
50	39	5	5665.62	2654.27	0	12.69	525.68			5666
51	40	5	5661.86	2669.2	0	12.75	526.92			5692
52										
53										
54	41	5	9024	4228.98	0	12.76	526.15			9024
55	42	5	9081.04	4273.67	0	12.74	526.03			9081
56	43	5	8310.46	3908.48	0	12.76	525.62			8310
57	44	5	8326.24	3914.76	0	12.71	525.78			8326
58										
59										
60	45	5	13765.68	6445.08	0	12.8	524.9			13766
61	46	5	13762.5	6462.05	0	12.7	525.64			13763
62	47	5	13123.92	6166.14	0	12.67	526.96			13124
63	48	5	13138.75	6182.52	0	12.71	525.33			13139
64										
65										
66	49	5	15660.48	7319.51	0	12.75	524.69			15660
67	50	5	15482.86	7254.56	0	12.62	524.99			15483
68	51	5	15011.08	7041.97	0	12.71	527.11			15011
69	52	5	15158.44	7082.17	0	12.76	525.24			15158
70										
71										
72	53	5	15991.14	7529.12	0	12.71	524.88			15991

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
73	54	5	16081.83	7527.88		0	12.73	524.87			16082
74	55	5	15200.29	7140.24		0	12.68	525.62			15200
75	56	5	15177.79	7139.54		0	12.63	525.52			15178
76											
77	58	5	15521.56	7294.52		0	12.69	524.41			15522
78	59	5	15401.6	7237.79		0	12.7	526.37			15402
79	60	5	15006.34	7032.93		0	12.71	526.13			15006
80	61	5	14889.34	7043.42		0	12.72	527.85			14889
81	62	5	14283.79	6730.74		0	12.71	525.8			14284
82	63	5	14460.55	6809.67		0	12.73	527.57			14461
83	64	5	15451.39	7275.82		0	12.75	527.49			15451
84	65	5	15324.24	7167.64		0	12.68	524.44			15324
85	66	5	14960.16	7049.69		0	12.7	527.95			14960
86	67	5	14719.33	6878.35		0	12.77	524.39			14719
87	68	5	14385.88	6759.08		0	12.71	524.9			14386
88	69	5	14155.84	6647.2		0	12.78	527.52			14156
89	70	5	15878.42	7434.35		0	12.68	524.08			15878
90	71	5	15329.8	7208.15		0	12.73	525.73			15330
91	72	5	15159.57	7112.39		0	12.8	525.05			15160
92	73	5	15074.05	7057.31		0	12.69	525.79			15074
93	74	5	13822.61	6514.22		0	12.86	527.11			13823
94	75	5	13659.65	6429.78		0	12.73	528.07			13660
95	76	5	15223.67	7153.6		0	12.62	522.7			15224
96	77	5	15159.76	7155.13		0	12.7	527.34			15160
97	78	5	14388.61	6780.26		0	12.7	528.26			14389
98	79	5	14451.64	6752.09		0	12.73	523.82			14452
99	80	5	15716.03	7348.06		0	12.81	526.13			15716
100	81	5	15423.04	7260.97		0	12.84	526.41			15423
101	82	5	14777.71	6997.9		0	12.67	528.11			14778
102	83	5	14897.2	6995.21		0	12.69	526.1			14897
103	84	5	13644.58	6404.22		0	12.78	527.48			13645
104	85	5	13500.18	6333.87		0	12.71	527.82			13500
105	86	5	14174.16	6620.01		0	12.74	524.13			14174
106	87	5	14123.15	6701.6		0	12.61	526.34			14123
107	88	5	14449.35	6798.52		0	12.68	526.42			14449
108	89	5	14753.96	6881.01		0	12.76	524.82			14754
109	90	5	13855.81	6512.54		0	12.72	527.31			13856
110	91	5	13924.87	6548.38		0	12.71	525.67			13925
111	92	5	13842.89	6528.04		0	12.72	527.79			13843
112	93	5	13670.9	6406.51		0	12.82	528.17			13671
113	94	5	15109.84	7113.05		0	12.73	525.84			15110
114	95	5	15016.07	7099.74		0	12.7	526.37			15016
115	96	5	14973.34	7018.46		0	12.72	526.92			14973
116	97	5	15394.16	7199.42		0	12.71	522.37			15394
117	98	5	14338.42	6740.23		0	12.71	527.12			14338
118	99	5	14625.12	6663.97		0	12.71	524.72			14625
119	100	5	15084.71	7071.25		0	12.73	525.55			15085
120	101	5	15001.32	6999.39		0	12.75	523.25			15001
121	102	5	14708.51	6901.85		0	12.72	524.85			14709
122	103	5	14531.3	6856.15		0	12.67	523.27			14531
123	104	5	13594.4	6462.98		0	12.78	528.2			13594
124	105	5	13929.73	6501.87		0	12.8	525.42			13930
125	106	5	239483.94	57603.94		0	8.87	241.25			239484

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
	Assay Date	3/20/2007	CR11611	# Concentrations tested	12x35-13	8	Microtiter ID								
1	NB Ref. ID	12535-78	21MR0024 P10	Replicate #	2	Positive Control	4-OH-ASON Pos C8 Tubes/Conc	2							
2	CKN														
3															
4															
5	Sample ID														
6	Sample type														
7	Full activity control	1	2	0.5	15072	30144	30336	0.0776	0.1	191054	60423	0.0637	0.005	15	0.425
8		2	2	0.5	15072	30144	30336	0.0776	0.1	191054	59807	0.0584	0.005	15	0.392
9		3	2	0.5	13771	27542	28031	0.0776	0.1	191054	54681	0.0517	0.005	15	0.411
10		4	2	0.5	14464	28928	29568	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
11		5	2	0.5	14904	29808	30448	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
12		6	2	0.5	14904	29808	30448	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
13		7	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
14	Background control	1	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
15		2	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
16		3	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
17		4	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
18		5	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
19		6	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
20		7	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
21		8	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
22	4-OH-ASON	1	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
23		2	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
24		3	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
25		4	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
26		5	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
27		6	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
28		7	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
29		8	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
30		9	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
31		10	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
32		11	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
33		12	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
34		13	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
35		14	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
36		15	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
37		16	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
38		17	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
39		18	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
40		19	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
41		20	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
42		21	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
43		22	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
44		23	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
45		24	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
46		25	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
47		26	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
48		27	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
49		28	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
50		29	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
51		30	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
52		31	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
53		32	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
54		33	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
55		34	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
56		35	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
57		36	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
58		37	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
59		38	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
60		39	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
61		40	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
62		41	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
63		42	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
64		43	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
65		44	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
66		45	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
67		46	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
68		47	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
69		48	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407
70		49	2	0.5	14972	29944	30584	0.0776	0.1	191054	57056	0.0611	0.005	15	0.407

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

Assay Date 3/20/2007		Chemical ID CR11611	# Concentrations tested		B Microsome ID 12335-L3													
NB Ref. 12535-781		21MR0024.P00		2		Positive Control		4-OH ASDN		Pos Ctrl Tubes/Conc		2						
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q		
Sample ID	Replicate	Nominal total volume (mL)	Aliq volume (mL)	Aliq #	DPH/aliq	DPH/mL	Ave DPH/mL	Total DPH	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed (Background Tubes)	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/protein/min)			
7	CR11611	1-1	2	0.5	15522	30644	30924	61848	0.1	191054	51393	0.0650	0.005	15	0.433			
8		1-2	2	0.5	15402	30604	28950	57900	0.1	191054	59535	0.0628	0.005	15	0.419			
9		1-3	2	0.5	15006	30012	28695	57390	0.1	191054	57235	0.0604	0.005	15	0.402			
10		2-1	2	0.5	14888	29778	28745	57490	0.1	191054	51295	0.0646	0.005	15	0.431			
11		2-2	2	0.5	14481	28972	30775	61540	0.1	191054	59103	0.0623	0.005	15	0.415			
12		2-3	2	0.5	15074	30148	29879	59758	0.1	191054	58829	0.0659	0.005	15	0.400			
13		3-1	2	0.5	14719	29438	28542	57094	0.1	191054	62161	0.0654	0.005	15	0.437			
14		3-2	2	0.5	15078	31756	31208	62416	0.1	191054	60213	0.0635	0.005	15	0.423			
15		3-3	2	0.5	15160	30320	30234	60468	0.1	191054	54711	0.0577	0.005	15	0.385			
16		4-1	2	0.5	15074	30148	27493	54906	0.1	191054	60513	0.0638	0.005	15	0.425			
17		4-2	2	0.5	13600	27320	30384	60768	0.1	191054	57427	0.0606	0.005	15	0.404			
18		4-3	2	0.5	15160	30320	28841	57682	0.1	191054	62023	0.0654	0.005	15	0.436			
19		5-1	2	0.5	15423	30846	31139	62278	0.1	191054	58095	0.0623	0.005	15	0.415			
20		5-2	2	0.5	14719	29438	29675	59350	0.1	191054	54035	0.0570	0.005	15	0.380			
21		5-3	2	0.5	14887	29794	27145	54260	0.1	191054	60479	0.0613	0.005	15	0.409			
22		6-1	2	0.5	13600	27320	27009	54006	0.1	191054	59561	0.0584	0.005	15	0.386			
23		6-2	2	0.5	14174	28348	28297	56594	0.1	191054	55007	0.0583	0.005	15	0.388			
24		6-3	2	0.5	14123	28246	27203	54406	0.1	191054	60479	0.0613	0.005	15	0.409			
25		7-1	2	0.5	14449	28898	27811	55662	0.1	191054	59977	0.0578	0.005	15	0.385			
26		7-2	2	0.5	14754	29508	27514	55028	0.1	191054	60479	0.0635	0.005	15	0.422			
27		7-3	2	0.5	13823	27646	30276	60552	0.1	191054	60479	0.0638	0.005	15	0.425			
28		8-1	2	0.5	15110	30220	30126	60352	0.1	191054	57671	0.0608	0.005	15	0.405			
29		8-2	2	0.5	14973	29946	30367	60724	0.1	191054	59917	0.0632	0.005	15	0.421			
30		8-3	2	0.5	14338	28676	29893	59796	0.1	191054	58425	0.0616	0.005	15	0.411			
31		9-1	2	0.5	14625	29250	30085	60172	0.1	191054	54993	0.0590	0.005	15	0.387			
32		9-2	2	0.5	15001	30002	29340	58680	0.1	191054								
33		9-3	2	0.5	14709	29418	27624	55248	0.1	191054								

Aromatase Assay Spreadsheet

	R	S
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		QC check - aliquot variance
6		% RE
7		1.00
8		1.00
9		1.00
10		1.00
11		0.99
12		1.01
13		1.00
14		1.00
15		1.01
16		0.99
17		1.01
18		0.99
19		0.99
20		1.00
21		1.00
22		1.00
23		1.01
24		0.99
25		1.00
26		1.00
27		1.00
28		1.00
29		1.01
30		0.99
31		1.00
32		1.00
33		1.00
34		0.99
35		1.00
36		1.00
37		0.99
38		1.01
39		1.00
40		1.00
41		1.01
42		0.99
43		1.00
44		1.00
45		0.99
46		1.01
47		0.99
48		1.01
49		1.00
50		1.00
51		1.00
52		1.00
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/20/2007	Test Chemical ID	CR11611	8 Microsome ID	12535-13					
2	NG Ref.	12535-78	Counter data filename	21MR0024.P00	Pos OH Tubes/Conc	2					
3	Technician ID	CKN	Replicate #								
4											
5	Control Type	Average	SD								
6	Full activity	0.409	0.013								
7	Background (unconnected)	0.002	0.000								
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	2	1.00E-05	-5.00	0.003						
13	4-OH ASDN	1	1.00E-06	-6.00							
14	4-OH ASDN	2	1.00E-06	-6.00	0.024						
15	4-OH ASDN	1	1.00E-07	-7.00							
16	4-OH ASDN	2	1.00E-07	-7.00	0.045						
17	4-OH ASDN	1	5.00E-07	-6.30	0.044						
18	4-OH ASDN	2	5.00E-07	-6.30	0.152						
19	4-OH ASDN	1	1.00E-07	-7.00	0.158						
20	4-OH ASDN	2	1.00E-07	-7.00	0.233						
21	4-OH ASDN	1	5.00E-06	-7.30	0.232						
22	4-OH ASDN	2	5.00E-06	-7.30	0.385						
23	4-OH ASDN	1	1.00E-08	-8.00	0.387						
24	4-OH ASDN	2	1.00E-08	-8.00	0.436						
25	4-OH ASDN	1	1.00E-09	-9.00	0.422						
26	4-OH ASDN	2	1.00E-09	-9.00	0.449						
27	4-OH ASDN	1	1.00E-10	-10.00	0.425						
28	4-OH ASDN	2	1.00E-10	-10.00							
29	4-OH ASDN	1	1.00E-09	-9.00							
30	4-OH ASDN	2	1.00E-09	-9.00							
31	4-OH ASDN	1	1.00E-10	-10.00							
32	4-OH ASDN	2	1.00E-10	-10.00							
33	4-OH ASDN	1	1.00E-10	-10.00							
34	4-OH ASDN	2	1.00E-10	-10.00							
35	4-OH ASDN	1	1.00E-10	-10.00							
36	4-OH ASDN	2	1.00E-10	-10.00							
37	Test Chemical	Replicate	Test chemical M	Log(test chemical)	Activity						
38	CR11611	1	1.00E-05	-5.00	0.433						
39	CR11611	2	1.00E-05	-5.00	0.419						
40	CR11611	1	1.00E-06	-6.00	0.402						
41	CR11611	2	1.00E-06	-6.00	0.431						
42	CR11611	1	1.00E-06	-6.00	0.415						
43	CR11611	2	1.00E-06	-6.00	0.400						
44	CR11611	1	1.00E-07	-7.00	0.437						
45	CR11611	2	1.00E-07	-7.00	0.423						
46	CR11611	1	1.00E-07	-7.00	0.389						
47	CR11611	2	1.00E-07	-7.00	0.425						
48	CR11611	1	5.00E-08	-7.30	0.404						
49	CR11611	2	5.00E-08	-7.30	0.436						
50	CR11611	1	1.00E-08	-8.00	0.415						
51	CR11611	2	1.00E-08	-8.00	0.380						
52	CR11611	1	1.00E-08	-8.00	0.395						
53	CR11611	2	1.00E-08	-8.00	0.409						
54	CR11611	1	5.00E-09	-8.30	0.389						
55	CR11611	2	5.00E-09	-8.30	0.385						
56	CR11611	1	1.00E-09	-9.00	0.422						
57	CR11611	2	1.00E-09	-9.00	0.425						
58	CR11611	1	1.00E-09	-9.00	0.405						
59	CR11611	2	1.00E-09	-9.00	0.421						
60	CR11611	1	1.00E-10	-10.00	0.411						
61	CR11611	2	1.00E-10	-10.00	0.411						
62	CR11611	1	1.00E-10	-10.00	0.387						
63	CR11611	2	1.00E-10	-10.00							
64	CR11611	1	1.00E-10	-10.00							
65	CR11611	2	1.00E-10	-10.00							
66	CR11611	1	1.00E-10	-10.00							
67	CR11611	2	1.00E-10	-10.00							
68	CR11611	1	1.00E-10	-10.00							
69	CR11611	2	1.00E-10	-10.00							
70	CR11611	1	1.00E-10	-10.00							
71	CR11611	2	1.00E-10	-10.00							
72	CR11611	1	1.00E-10	-10.00							
73	CR11611	2	1.00E-10	-10.00							
74	CR11611	1	1.00E-10	-10.00							
75	CR11611	2	1.00E-10	-10.00							
76	CR11611	1	1.00E-10	-10.00							
77	CR11611	2	1.00E-10	-10.00							
78	CR11611	1	1.00E-10	-10.00							
79	CR11611	2	1.00E-10	-10.00							
80	CR11611	1	1.00E-10	-10.00							
81	CR11611	2	1.00E-10	-10.00							
82	CR11611	1	1.00E-10	-10.00							
83	CR11611	2	1.00E-10	-10.00							
84	CR11611	1	1.00E-10	-10.00							
85	CR11611	2	1.00E-10	-10.00							
86	CR11611	1	1.00E-10	-10.00							
87	CR11611	2	1.00E-10	-10.00							
88	CR11611	1	1.00E-10	-10.00							
89	CR11611	2	1.00E-10	-10.00							
90	CR11611	1	1.00E-10	-10.00							
91	CR11611	2	1.00E-10	-10.00							
92	CR11611	1	1.00E-10	-10.00							
93	CR11611	2	1.00E-10	-10.00							
94	CR11611	1	1.00E-10	-10.00							
95	CR11611	2	1.00E-10	-10.00							
96	CR11611	1	1.00E-10	-10.00							
97	CR11611	2	1.00E-10	-10.00							
98	CR11611	1	1.00E-10	-10.00							
99	CR11611	2	1.00E-10	-10.00							
100	CR11611	1	1.00E-10	-10.00							
101	CR11611	2	1.00E-10	-10.00							
102	CR11611	1	1.00E-10	-10.00							
103	CR11611	2	1.00E-10	-10.00							
104	CR11611	1	1.00E-10	-10.00							
105	CR11611	2	1.00E-10	-10.00							
106	CR11611	1	1.00E-10	-10.00							
107	CR11611	2	1.00E-10	-10.00							
108	CR11611	1	1.00E-10	-10.00							
109	CR11611	2	1.00E-10	-10.00							
110	CR11611	1	1.00E-10	-10.00							
111	CR11611	2	1.00E-10	-10.00							
112	CR11611	1	1.00E-10	-10.00							
113	CR11611	2	1.00E-10	-10.00							
114	CR11611	1	1.00E-10	-10.00							
115	CR11611	2	1.00E-10	-10.00							
116	CR11611	1	1.00E-10	-10.00							
117	CR11611	2	1.00E-10	-10.00							
118	CR11611	1	1.00E-10	-10.00							
119	CR11611	2	1.00E-10	-10.00							
120	CR11611	1	1.00E-10	-10.00							
121	CR11611	2	1.00E-10	-10.00							
122	CR11611	1	1.00E-10	-10.00							
123	CR11611	2	1.00E-10	-10.00							
124	CR11611	1	1.00E-10	-10.00							
125	CR11611	2	1.00E-10	-10.00							
126	CR11611	1	1.00E-10	-10.00							
127	CR11611	2	1.00E-10	-10.00							
128	CR11611	1	1.00E-10	-10.00							
129	CR11611	2	1.00E-10	-10.00							
130	CR11611	1	1.00E-10	-10.00							
131	CR11611	2	1.00E-10	-10.00							
132	CR11611	1	1.00E-10	-10.00							
133	CR11611	2	1.00E-10	-10.00							
134	CR11611	1	1.00E-10	-10.00							
135	CR11611	2	1.00E-10	-10.00							
136	CR11611	1	1.00E-10	-10.00							
137	CR11611	2	1.00E-10	-10.00							
138	CR11611	1	1.00E-10	-10.00							
139	CR11611	2	1.00E-10	-10.00							
140	CR11611	1	1.00E-10	-10.00							
141	CR11611	2	1.00E-10	-10.00							
142	CR11611	1	1.00E-10	-10.00							
143	CR11611	2	1.00E-10	-10.00							
144	CR11611	1	1.00E-10	-10.00							
145	CR11611	2	1.00E-10	-10.00							
146	CR11611	1	1.00E-10	-10.00							
147	CR11611	2	1.00E-10	-10.00							
148	CR11611	1	1.00E-10	-10.00							
149	CR11611	2	1.00E-10	-10.00							
150	CR11611	1	1.00E-10	-10.00							

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
			Test	# Concentrations					
1	Assay Date	3/21/2007	Chemical ID	CR11611		tested	8 Microsome ID		12535-13
2	NB Ref.	12535-82	Counter data filename	21MR2327.P00					
	Technician						Pos Ctrl		
3	ID	CKN	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/21/2007	Test Chemical ID	CR11611	# Concentrations tested		8 Microsome ID	12535-13	
2	NB Ref.	12535-82	Counter data filename	21MR2327.P00					
3	Technician ID	CKN	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0202		39896		1975050			
8	2	0.0195		35873		1839641			
9	3	0.0196		39434		2011939			
10	4	0.0204		42363		2076618			
11	5	0.0198		40111		2025808			
12						Average DPM/g soln		1985811	
13						SD		89456	
14						CV		4.50	
15						µCi/g soln		0.8945	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution				8.0216 g				
25	Mass of dilution B used in substrate prep				4.5896 g				
26	Concentration of nonradiolabeled ASDN in substrate soln.				0.57158303 µg/g				
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.8945				
31	b. Specific activity of [³ H]ASDN (µCi/mmol)				23500000				
32	c. Molecular wt of ASDN (mg/mmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36	= 0.5716 + 0.011								
37	= 0.582 µg ASDN/g soln.								
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.) / (µg ASDN/g soln.)								
40	= 1.536 µCi/µg ASDN								
41	9.764.E+05 dpm/nmol								

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/21/2007	Test Chemical ID	CR11611	# Concentrations tested	8	Microsome ID	12535-13	
2		NB Ref	12535-82	Counter data filename	21MR2327.P00					
3		Technician ID	CKN	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4										
5	Microsome Dilution Details									
6										
7	Dilution A									
8		0.12 mL microsome Stock used								
9		2.4 mL total volume								
10		20 dilution factor								
11	Dilution B									
12		1.5 mL microsome Dilution A used								
13		60 mL total volume								
14		40 dilution factor								
15	Dilution C (if applicable)									
16		mL microsome Dilution B used								
17		mL total volume								
18		dilution factor								
19		800 total dilution factor								
20										
21	Protein Concentration (stock microsomes, mg/mL):			8.00						
22	Protein Concentration (dilution added to assay, mg/mL):			0.01						
23										
24										
25										
26										

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		Test Chemical # Concentrations Assay Date 3/21/2007 ID CR11611 tested 8 Microsome ID 12535-13									
2		NB Ref. 12535-82 Counter data filename 21MRZ327.P00									
3		Technician Replicate Pos Ctl ID CKN # 3 Positive Control 4-OH ASDN Tubes/Conc 2									
4											
5		Vial Time (min)	DPM1	ISIE		Round DPM					
6											
7	2	5	39895.63	19933.64	0	13.59	589.53				39896
8	3	5	35873.01	17994.92	0	13.5	589.64				35873
9	4	5	39434.18	19739.31	0	13.58	589.63				39434
10	5	5	42362.7	21152.03	0	13.53	588.25				42363
11	6	5	40110.68	19959.08	0	13.61	587.52				40111
12											
13	8	5	13769.8	6465.1	0	12.69	523.8				13770
14	9	5	13689.88	6428.11	0	12.71	523.94				13690
15	10	5	13725.78	6445.82	0	12.68	525.59				13726
16	11	5	13988.46	6530.24	0	12.8	524.44				13988
17	12	5	13169.69	6152.67	0	12.69	523.85				13170
18	13	5	13010.07	6070.66	0	12.77	523.44				13010
19	14	5	13199.23	6197.51	0	12.7	526.96				13199
20	15	5	13305.38	6240.59	0	12.7	523.57				13305
21	16	5	62.7	30.75	0	12.64	522.92				63
22	17	5	68.94	30.9	0	15.43	526.09				69
23	18	5	63.43	35.91	0	12.5	525.49				63
24	19	5	51.72	25.43	0	13.11	526.98				52
25	20	5	35.2	19.17	0	13.49	524.95				35
26	21	5	84.86	28.23	0	13.74	527.02				65
27	22	5	56.99	29.58	0	11.89	522.81				57
28	23	5	43.89	20.83	0	10.4	523.72				44
29											
30	25	5	160.09	71.49	0	13.06	524.85				160
31	26	5	178.28	78.61	0	13.82	521.37				178
32	27	5	174.29	77.56	0	12.71	521.12				174
33	28	5	165.83	78.87	0	13.07	525.04				166
34											
35											
36	29	5	995.06	463.77	0	12.79	528.5				985
37	30	5	991.91	460.6	0	12.65	524.5				992
38	31	5	950.41	452	0	12.64	523.22				950
39	32	5	939.1	443.27	0	12.41	524.56				939
40											
41											
42	33	5	1760.93	831.91	0	12.79	524.43				1761
43	34	5	1795.98	838.82	0	12.68	522.94				1796
44	35	5	1575.05	744.8	0	12.65	522.92				1575
45	36	5	1581.17	732.91	0	13	523.49				1581
46											
47											
48	37	5	5427.62	2539.36	0	12.67	524.05				5428
49	38	5	5541.48	2589.43	0	12.75	523.69				5541
50	39	5	5519.95	2582.06	0	12.74	521.53				5520
51	40	5	5684.04	2648.76	0	12.85	523.72				5684
52											
53											
54	41	5	8456.42	3959.12	0	12.77	525.17				8456
55	42	5	8365.06	3924.5	0	12.68	522.05				8365
56	43	5	8000.88	3743.87	0	12.83	525.53				8001
57	44	5	7829.14	3682.6	0	12.71	523.69				7829
58											
59											
60	45	5	11640.93	5448.4	0	12.69	523.72				11641
61	46	5	11700.32	5476.46	0	12.73	525.09				11700
62	47	5	12308.4	5791.7	0	12.7	523.86				12308
63	48	5	12613.64	5893.25	0	12.7	523.75				12614
64											
65											
66	49	5	13689.51	6423.75	0	12.67	525.75				13690
67	50	5	13645.84	6397.5	0	12.71	524.25				13646
68	51	5	12781.74	6005	0	12.67	525.06				12782
69	52	5	12863.53	6018.59	0	12.74	524.94				12864
70											
71											
72	53	5	14881.18	6932.36	0	12.75	520.28				14881

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
73	54	5	14685.93	6857.36		0 12.73	523.99				14686
74	55	5	12754.58	5996.15		0 12.64	523.86				12755
75	56	5	13058.26	6113.19		0 12.74	525.36				13058
76											
77	58	5	13543.12	6363.19		0 12.7	524.39				13543
78	59	5	13511.52	6353.83		0 12.66	525.52				13512
79	60	5	13558.8	6351.46		0 12.73	525.57				13559
80	61	5	13582.03	6361.13		0 12.68	523.94				13582
81	62	5	12545.33	5912.69		0 12.77	526.47				12546
82	63	5	12761.09	5961.27		0 12.68	522.7				12761
83	64	5	14035.44	6582.07		0 12.74	523.85				14035
84	65	5	14127.25	6573.48		0 12.75	522.7				14127
85	66	5	13359.19	6285.24		0 12.71	526.42				13359
86	67	5	13532.28	6340.24		0 12.7	526.77				13532
87	68	5	13026.22	6102.61		0 12.73	522.45				13026
88	69	5	12912.52	6049.5		0 12.69	523.82				12913
89	70	5	14286.33	6687.29		0 12.72	525.77				14288
90	71	5	14089.27	6815.65		0 12.8	527.03				14089
91	72	5	12979.76	6121.45		0 12.72	525.79				12980
92	73	5	13414.56	6292.23		0 12.88	528.77				13415
93	74	5	13420.91	6297.09		0 12.77	524.86				13421
94	75	5	13444.92	6281.81		0 12.74	525.33				13445
95	76	5	13600.09	6366.85		0 12.74	524.65				13600
96	77	5	13579.6	6377.16		0 12.8	526.26				13580
97	78	5	6089.11	2877.86		0 12.68	527.56				6089
98	79	5	6257.27	2908.66		0 12.78	520.32				6257
99	80	5	13267.01	6201.09		0 12.74	524.58				13267
100	81	5	13309.25	6249.07		0 12.73	523.9				13309
101	82	5	13581.99	6362.82		0 12.73	524.09				13582
102	83	5	13382.18	6262.06		0 12.64	522.87				13382
103	84	5	13100.85	6123.15		0 12.74	524.45				13101
104	85	5	13291.34	6160.29		0 12.84	523.34				13291
105	86	5	12718.51	5977.96		0 12.73	524.36				12719
106	87	5	12797.52	6037.87		0 12.62	524.29				12798
107	88	5	10101.59	4717.28		0 12.68	525.05				10102
108	89	5	10088.16	4700.87		0 12.77	524.09				10088
109	90	5	13590.74	6382.07		0 12.83	528.17				13591
110	91	5	13432.12	6303.04		0 12.77	527.03				13432
111	92	5	13545.62	6335.82		0 12.85	528.81				13546
112	93	5	13383.91	6268.99		0 12.72	526.2				13384
113	94	5	13545.88	6309.41		0 12.73	521.55				13546
114	95	5	13212.19	6201.18		0 12.7	526.3				13212
115	96	5	13806.19	6434.51		0 12.78	524.29				13808
116	97	5	13584.84	6363.63		0 12.77	524.28				13585
117	98	5	12778.18	5991.09		0 12.74	525.08				12778
118	99	5	12807.11	5974.45		0 12.68	523.65				12807
119	100	5	13987.02	6558.18		0 12.7	523.54				13987
120	101	5	14013.23	6591.59		0 12.71	526.43				14013
121	102	5	14352.35	6722.38		0 12.75	523.88				14352
122	103	5	14160.2	6661.93		0 12.77	525.41				14160
123	104	5	13388.64	6243.54		0 12.76	525.72				13368
124	105	5	13076.99	6127.37		0 12.8	526.44				13077
125	106	5	238340.89	57496.26		0 8.86	240.99				238341
126											
127											
128											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
				Test Chemical ID	CR16111	a	Concentrations tested	8	Microsome ID	V235-13					
1			Assay Date	3/21/2007											
2			NS Ref Technician	12535-32	21MR237: P00										
3			CKN	4	3	Positive Control			4.0H ASDN Pos Ctl Tubes+Cont						
4															
5	Sample ID	Replicate	Aliq	DPH/mL	DPH/mL	DPH/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (uL)	Total DPM in assay tube (net)	Calculate net ³ H ₂ O formed Total DPM corrected for background (Background Tubes)	nmol ³ H ₂ O formed (mg/mL)	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg/min)
6	Sample type	1	2	0.5	1	2	27480	54920	0.1	189581	52004	0.0565	0.005	15	0.377
7	Full activity control	2	0.5	1	2	2	27390	54528	0.1	189581	52004	0.0565	0.005	15	0.377
8		3	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
9		4	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
10		5	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
11		6	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
12		7	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
13		8	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
14		9	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
15	Background control	1	2	0.5	1	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
16		2	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
17		3	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
18		4	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
19		5	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
20		6	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
21		7	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
22		8	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
23		9	0.5	1	2	2	27452	54928	0.1	189581	52004	0.0565	0.005	15	0.377
24	4.0H ASDN	1-1	2	0.5	1	2	338	676	0.1	196581	452	0.0095	0.005	15	0.003
25		2-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
26		3-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
27		4-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
28		5-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
29		6-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
30		7-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
31		8-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
32		9-1	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
33		1-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
34		2-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
35		3-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
36		4-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
37		5-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
38		6-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
39		7-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
40		8-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
41		9-2	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
42		1-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
43		2-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
44		3-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
45		4-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
46		5-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
47		6-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
48		7-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
49		8-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
50		9-3	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
51		1-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
52		2-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
53		3-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
54		4-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
55		5-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
56		6-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
57		7-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
58		8-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
59		9-4	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
60		1-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
61		2-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
62		3-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
63		4-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
64		5-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
65		6-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
66		7-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
67		8-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
68		9-5	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
69		1-6	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003
70		2-6	2	0.5	1	2	346	692	0.1	196581	452	0.0095	0.005	15	0.003

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

Assay Date		3/21/2007		Test Chemical		CR11811		# Concentrations tested		8 Mircosome ID		1233543					
NB Ref.		1233542		1233542		1233542		1233542		1233542		1233542		1233542		1233542	
Technician ID		CKN		Replicate #		3		Positive Control		4-OH Andro		Post Oil Tubes/Cont		2			
Sample ID		Replicate		Volume		Aliq		DPM/aliq		DPM/mL		Ave DPM/mL		Total DPM		Volume of substrates	
Sample		Level		mL		mL		mL		mL		mL		mL		mL	
1		1-1		0.5		0.5		13543		27086		27055		54110		0.1	
2		1-2		0.5		0.5		13559		27119		27121		54242		0.1	
3		1-3		0.5		0.5		13562		27124		27066		50672		0.1	
4		2-1		0.5		0.5		12701		25402		26162		52324		0.1	
5		2-2		0.5		0.5		14127		28254		26891		53782		0.1	
6		2-3		0.5		0.5		13532		27064		27939		51678		0.1	
7		3-1		0.5		0.5		12013		24026		28375		56750		0.1	
8		3-2		0.5		0.5		14098		28178		26395		52790		0.1	
9		3-3		0.5		0.5		13415		26830		26868		53732		0.1	
10		4-1		0.5		0.5		13445		26880		27160		54360		0.1	
11		4-2		0.5		0.5		13590		27180		12346		24892		0.1	
12		4-3		0.5		0.5		6267		12514		26576		53152		0.1	
13		5-1		0.5		0.5		13300		26618		26864		53623		0.1	
14		5-2		0.5		0.5		13392		26764		26302		51764		0.1	
15		5-3		0.5		0.5		13291		26582		25517		51034		0.1	
16		6-1		0.5		0.5		12798		25438		20170		40340		0.1	
17		6-2		0.5		0.5		10102		20204		27023		54046		0.1	
18		6-3		0.5		0.5		13501		27002		26930		53660		0.1	
19		7-1		0.5		0.5		13432		26864		26758		53516		0.1	
20		7-2		0.5		0.5		13394		26768		27391		54782		0.1	
21		7-3		0.5		0.5		13276		26552		25095		51170		0.1	
22		8-1		0.5		0.5		12807		25614		28000		56000		0.1	
23		8-2		0.5		0.5		14019		28038		29372		57024		0.1	
24		8-3		0.5		0.5		14352		28704		26446		52092		0.1	
25				0.5		0.5		13089		26738				52668			

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	3/21/2007	Test Chemical ID	CR11611	21MR2327 P00	3	Positive Control	4-OH ASDN	Pos Oil Tubes/Conc	12535-13	
2	NB Ref.	12535-02	Concentration	Replicate #							
3	Technician ID	CKN									
4											
5	Control Type	Portion	SD								
6	Full Activity	Overall	0.367								
7	Background (unconnected)	Overall	0.002								
8											
9											
10	Positive Control	Level	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity					
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.003					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.025					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.024					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.047					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.042					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.148					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.151					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.228					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.215					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.317					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.339					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.372					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.349					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.402					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.351					
27	4-OH ASDN										
28	4-OH ASDN										
29	4-OH ASDN										
30	4-OH ASDN										
31	4-OH ASDN										
32	4-OH ASDN										
33	4-OH ASDN										
34	4-OH ASDN										
35	4-OH ASDN										
36	4-OH ASDN										
37	Test Chemical	Level	Replicate	Test chemical M	Log(test chemical)	Activity					
38	CR11611	1	1	1.00E-05	-5.00	0.369					
39	CR11611	1	2	1.00E-05	-5.00	0.365					
40	CR11611	2	1	1.00E-06	-6.00	0.344					
41	CR11611	2	2	1.00E-06	-6.00	0.383					
42	CR11611	3	1	1.00E-07	-7.00	0.365					
43	CR11611	3	2	1.00E-07	-7.00	0.353					
44	CR11611	4	1	1.00E-08	-8.00	0.386					
45	CR11611	4	2	1.00E-08	-8.00	0.353					
46	CR11611	5	1	5.00E-08	-7.30	0.365					
47	CR11611	5	2	5.00E-08	-7.30	0.370					
48	CR11611	6	1	1.00E-08	-8.00	0.367					
49	CR11611	6	2	1.00E-08	-8.00	0.381					
50	CR11611	7	1	1.00E-09	-9.00	0.359					
51	CR11611	7	2	1.00E-09	-9.00	0.347					
52	CR11611	8	1	1.00E-10	-10.00	0.274					
53	CR11611	8	2	1.00E-10	-10.00	0.367					
54	CR11611					0.266					
55	CR11611					0.364					
56	CR11611					0.373					
57	CR11611					0.348					
58	CR11611					0.361					
59	CR11611					0.388					
60	CR11611					0.360					
61	CR11611					0.360					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/22/2007	Test Chemical ID	CR11612	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-87	Counter data filename	22MR2100.P00					
3	Technician		Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/22/2007	Test Chemical ID	CR11612	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-87	Counter data filename	22MR2100.P00					
3	Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0190		33743		1775947			
8	2	0.0194		31711		1634588			
9	3	0.0194		38483		1983660			
10	4	0.0197		41064		2084467			
11	5	0.0198		39760		2008081			
12						Average DPM/g soln		1897349	
13						SD		186082	
14						CV		9.81	
15						µCi/g soln		0.8547	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0004 g			
25	Mass of dilution B used in substrate prep					4.4832 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56149273 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.010	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8547			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5615	+	0.010		
37				=	0.572	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.494	µCi/µg ASDN			
41					9.502.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/22/2007	Test Chemical ID	CR11612	# Concentrations tested		8	Microsome ID	12535-13
2		NB Ref	12535-87	Counter data filename	22MR2100.P00				Pos Ctl	
3		Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN		Tubes/Conc	2
4										
5	Microsome Dilution Details									
6										
7	Dilution A		0.12 mL microsome Stock used							
8			2.4 mL total volume							
9			20 dilution factor							
10										
11	Dilution B		1.5 mL microsome Dilution A used							
12			60 mL total volume							
13			40 dilution factor							
14										
15	Dilution C (if applicable)		mL microsome Dilution B used							
16			mL total volume							
17			dilution factor							
18										
19			800 total dilution factor							
20										
21	Protein Concentration (stock microsomes, mg/mL):									
22	Protein Concentration (dilution added to assay, mg/mL):									
23										
24										
25										
26										

Data entry checked by _____ Date _____
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12535-87_CR11612_ASE_print.xls
Microsome & Chemical Dilutions

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
	Test # Chemical Concentrations 3/22/2007 ID Cl tested 8 Microsome ID 12535-13										
	Counter data 12535-87 filename 22MR2100.P00										
	T e c h n i c i a n i d										
	Replicate Positive Pos Ctl base # 1 Control 4-OH ASDI Tubes/Conc 2										
5	Vial Ti DPM1 ISIE Round DPM										
6	2	5	33743.36	16180.3	0	13.04	539.1				33743
7	3	5	31710.71	14191.86	0	13.14	540.28				31711
8	4	5	38482.59	17396.3	0	13.1	544.29				38483
9	5	5	41063.58	18584.14	0	13.32	550.86				41064
10	6	5	39760.04	18028.5	0	13.27	551.67				39760
11	8	5	17879.43	7919.86	0	12.91	526.31				17879
12	9	5	17540.56	7760.1	0	12.91	526.15				17541
13	10	5	16455.61	7301.48	0	13	528.53				16456
14	11	5	16541.05	7344.14	0	12.96	528.18				16541
15	12	5	14793.42	6593.3	0	13.01	533.47				14793
16	13	5	14483.4	6453.74	0	12.86	529.67				14483
17	14	5	14685.29	6511.5	0	13	528.01				14685
18	15	5	14630.3	6510.86	0	12.91	530.73				14630
19	17	5	60.14	27.14	0	13.95	529.12				60
20	18	5	61.3	30.14	0	12.2	528.82				61
21	19	5	55.11	24.94	0	13.15	528.68				55
22	20	5	67.59	31.46	0	11.17	531.12				68
23	21	5	68.41	29.06	0	14.29	528.76				68
24	22	5	75.76	35.1	0	13.67	529.47				76
25	23	5	65.72	30.7	0	13.47	528.57				66
26	24	5	66.92	27.94	0	13.37	528.97				67
27	26	5	183.72	79.86	0	12.93	529.69				184
28	27	5	196.82	82.9	0	12.89	529.21				196
29	28	5	172.88	76.9	0	12.78	528.67				173
30	29	5	184.14	80.94	0	13.6	529.1				184
31											
32											
33	30	5	1038.71	464.66	0	12.93	529.22				1039
34	31	5	1009.87	450.9	0	12.82	529.66				1010
35	32	5	1010.71	447.26	0	12.95	528.49				1011
36	33	5	1005.43	452.3	0	12.66	528.67				1005
37											
38											
39	34	5	1877.66	832.54	0	12.82	527.91				1878
40	35	5	1945.27	856.74	0	13	527.2				1945
41	36	5	1677.18	758.28	0	12.65	528.46				1677
42	37	5	1675.82	746.66	0	12.96	529.68				1676
43											
44											
45	38	5	6274.15	2822.68	0	12.85	529.99				6274
46	39	5	6231.81	2769.74	0	12.86	525.8				6232
47	40	5	5970.99	2655.66	0	12.96	530.54				5971
48	41	5	5850.12	2615.3	0	12.86	528.35				5850
49											
50											
51	42	5	9224.17	4072.28	0	12.96	527.03				9224
52	43	5	9315.32	4104.86	0	12.94	527.67				9315
53	44	5	8826.35	3945.68	0	12.96	529.33				8826
54	45	5	8985.17	3981.66	0	12.99	531.59				8988
55											
56											
57	46	5	13804.52	6132.1	0	12.92	528.65				13805
58	47	5	13882.3	6168.86	0	12.89	527.37				13882
59	48	5	12423.54	5495.22	0	12.93	525.79				12424
60	49	5	12689.46	5629.7	0	12.89	527.42				12689
61											
62											
63	50	5	13493.5	5961.22	0	12.89	526.7				13494
64	51	5	13444.81	5930.26	0	12.93	526.12				13445

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	14033.15	6278.3	0	12.88	531.12				14033
66	53	5	14477.48	6402.66	0	12.95	528.12				14477
67											
68											
69	54	5	14344.43	6334.66	0	13.02	528.31				14344
70	55	5	14877.81	6675.94	0	12.92	526.51				14878
71	56	5	14398.61	6395.14	0	12.95	527.92				14399
72	57	5	14559.52	6455.5	0	12.88	528.53				14560
73											
74											
75	59	5	9418.56	4200.66	0	12.93	529.12				9419
76	60	5	9597.75	4245.1	0	12.93	527.74				9598
77	61	5	9329.26	4155.46	0	12.87	528.36				9329
78	62	5	9378.91	4141.7	0	12.95	527.52				9379
79	63	5	9507.51	4222.66	0	12.91	527.56				9508
80	64	5	9525.15	4210.74	0	12.87	526.17				9525
81	65	5	13393.49	5892.66	0	13	527				13393
82	66	5	13367.24	5902.3	0	12.92	528.53				13367
83	67	5	14088.9	6181.5	0	12.91	526.11				14089
84	68	5	13854.96	6141.46	0	12.91	526.37				13855
85	69	5	14021.96	6239.1	0	12.92	528.79				14022
86	70	5	14188.53	6321.1	0	12.89	528.95				14189
87	71	5	14587.95	6460.94	0	12.93	528.15				14587
88	72	5	14500.79	6489.94	0	12.89	530.83				14501
89	73	5	14914.7	6625.1	0	12.9	528.3				14915
90	74	5	15009.92	6663.98	0	12.96	530.18				15010
91	75	5	15261.19	6778.1	0	12.89	528.89				15261
92	76	5	15323.51	6784.5	0	12.91	526.48				15324
93	77	5	15534.39	6892.7	0	12.9	527.27				15534
94	78	5	16097.02	7151.22	0	12.93	529.73				16097
95	79	5	14702.12	6530.3	0	12.91	528.9				14702
96	80	5	14828.74	6590.9	0	12.87	532.05				14829
97	81	5	15439.05	6845.26	0	12.93	528.05				15439
98	82	5	15547.88	6902.9	0	12.89	528.16				15548
99	84	5	15665.58	6939.54	0	12.92	528.85				15666
100	85	5	15685.68	6903.7	0	12.93	524.86				15686
101	86	5	15100.72	6709.54	0	12.92	529.26				15101
102	87	5	15238.01	6770.54	0	12.92	530.24				15238
103	88	5	15542.3	6891.9	0	12.96	527.73				15542
104	89	5	15628.99	6949.9	0	12.93	528.88				15629
105	90	5	15644.07	6941.74	0	12.89	527.94				15644
106	91	5	16164.7	7169.38	0	12.94	527.81				16165
107	92	5	15345.27	6839.94	0	12.84	527.05				15345
108	93	5	15181.49	6762.7	0	12.93	529.99				15181
109	94	5	15799.89	6996.94	0	12.97	528.38				15800
110	95	5	16153.87	7190.66	0	12.91	528.9				16154
111	96	5	16120.82	7155.94	0	12.86	527.09				16121
112	97	5	16361.07	7214.34	0	12.91	525.8				16361
113	98	5	14599.8	8475.5	0	12.95	526.74				14600
114	99	5	14793.82	8650.86	0	12.84	525.87				14794
115	100	5	15225.31	6777.1	0	12.88	527.82				15225
116	101	5	15574.08	6921.9	0	12.87	529.05				15574
117	102	5	15426.17	6872.1	0	12.97	530.34				15426
118	103	5	15769.6	6970.3	0	12.94	526.68				15770
119	104	5	14465.7	6491.66	0	12.84	532.39				14466
120	105	5	14995.39	6626.74	0	12.92	528.35				14995
121	106	5	15738.52	6986.74	0	12.82	527.34				15737
122	107	5	16329.92	7242.06	0	12.87	528.23				16330
123	109	5	227128.91	33600.94	0	7.98	173.88				227129
124											0
125											#REF!
126											#REF!
127											#REF!
128											#REF!
129											#REF!
130											#REF!
131											#REF!
132											#REF!
133											227129

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Assay ID	Assay Date	Assay ID	Text	Chemical	# Concentrations tested	Microsome ID	12535-13							
3/22/2007	CR111612	3/22/2007	CR111612	Chemical	22MR2100.P00	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							
12535-87	12535-87	12535-87	12535-87	Replicate	1	Positive Control	4-OH ASDN	2							

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		QC check - aliquot			
5		Red if value not within mean $\pm$ 10%			
6					
7		1.00			
8		0.99			
9		1.00			
10		1.01			
11		0.99			
12		1.00			
13		1.00			
14		0.99			
15		1.01			
16		1.01			
17					
18					
19		0.94			
20		1.05			
21		1.01			
22		0.97			
23		1.03			
24		0.97			
25		1.03			
26		1.03			
27					
28					
29		1.01			
30		0.99			
31		1.00			
32		1.00			
33					
34		0.98			
35		1.02			
36		1.00			
37					
38		1.00			
39					
40					
41		1.00			
42		1.00			
43		0.99			
44		0.99			
45					
46					
47		1.00			
48		1.00			
49		0.99			
50		1.01			
51					
52					
53		1.00			
54		0.99			
55		1.01			
56					
57					
58		1.00			
59		1.00			
60		0.98			
61		1.02			
62					
63					
64		0.98			
65		0.98			
66		0.97			
67		0.98			
68		1.01			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Sample ID	Sample type	Replicate Level	Assay Date	Test Chemical ID	CR1612	# Concentrations tested	B Microsome ID	12535-13	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (nmol)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	
1	CR1612	1-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	37774	0.0385	0.005	15	0.265	
2	CR1612	1-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	37150	0.0391	0.005	15	0.261	
3	CR1612	1-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	37806	0.0398	0.005	15	0.265	
4	CR1612	2-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	53060	0.0461	0.005	15	0.374	
5	CR1612	2-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	53588	0.0385	0.005	15	0.360	
6	CR1612	2-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	56162	0.0391	0.005	15	0.384	
7	CR1612	3-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	57916	0.0310	0.005	15	0.406	
8	CR1612	3-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	55930	0.0327	0.005	15	0.418	
9	CR1612	3-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	60910	0.0341	0.005	15	0.427	
10	CR1612	4-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	63002	0.0353	0.005	15	0.442	
11	CR1612	4-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	63802	0.0319	0.005	15	0.413	
12	CR1612	4-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	61114	0.0330	0.005	15	0.433	
13	CR1612	5-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	62444	0.0367	0.005	15	0.438	
14	CR1612	5-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	60416	0.0336	0.005	15	0.424	
15	CR1612	5-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	62082	0.0353	0.005	15	0.436	
16	CR1612	6-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	63358	0.0367	0.005	15	0.445	
17	CR1612	6-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	60792	0.0340	0.005	15	0.427	
18	CR1612	6-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	63646	0.0370	0.005	15	0.447	
19	CR1612	7-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	61104	0.0381	0.005	15	0.454	
20	CR1612	7-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	58528	0.0316	0.005	15	0.411	
21	CR1612	7-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	61338	0.0345	0.005	15	0.430	
22	CR1612	8-1	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	62132	0.0354	0.005	15	0.436	
23	CR1612	8-2	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	58682	0.0317	0.005	15	0.412	
24	CR1612	8-3	3/22/2007	CR1612	CR1612	1	12535-13	12535-13	0.1	189735	63974	0.0372	0.005	15	0.448	

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC check - aliquot variance
5		
6		% RE
7		0.98
8		1.01
9		1.00
10		1.00
11		1.00
12		1.00
13		1.00
14		1.00
15		1.01
16		0.99
17		0.99
18		1.01
19		1.00
20		1.00
21		1.00
22		1.00
23		1.00
24		1.00
25		0.98
26		1.02
27		1.00
28		1.00
29		1.00
30		1.00
31		1.00
32		1.00
33		1.00
34		1.00
35		1.00
36		1.00
37		0.98
38		1.02
39		1.01
40		0.98
41		1.01
42		0.98
43		1.01
44		0.98
45		1.01
46		1.01
47		0.99
48		1.01
49		0.99
50		1.01
51		0.98
52		1.02
53		0.98
54		1.02

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	3/27/2007	Test	Chemical ID	CR11612	# Concentrations	tested	8	Microsome ID	12535-13
2	NB Ref.	12535-90	Counter data	filename	27MR2107.P00					
3	Technician	ase	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc			2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/27/2007	Test Chemical ID	CR11612	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-90	Counter data filename	27MR2107.P00					
3	Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0193		37745		1956699			
8	2	0.0189		34713		1836667			
9	3	0.0196		42562		2171531			
10	4	0.0191		39035		2043717			
11	5	0.0199		35276		1772663			
12						Average DPM/g soln		1956055	
13						SD		159688	
14						CV		8.16	
15						µCi/g soln		0.8811	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1840 g			
25	Mass of dilution B used in substrate prep					4.5805 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56080902 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8811			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5608	+	0.011		
37				=	0.572	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40				=	1.542	µCi/µg ASDN			
41					9.802.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/27/2007	Test Chemical ID	CR11612	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-90	Counter data filename	27MR2107.P00					
3			Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc		2
4											
5	Microsome Dilution Details										
6											
7				0.12 mL microsome Stock used					Positive Control Concentrations		
8				2.4 mL total volume					Level	Final Concentration (M)	
9				20 dilution factor					1	1.00E-05	
10									2	1.00E-06	
11				1.5 mL microsome Dilution A used					3	5.00E-07	
12				60 mL total volume					4	1.00E-07	
13				40 dilution factor					5	5.00E-08	
14									6	1.00E-08	
15				mL microsome Dilution B used					7	1.00E-09	
16				mL total volume					8	1.00E-10	
17				dilution factor							
18											
19				800 total dilution factor					Test Chemical Concentrations		
20									Level	Final Concentration (M)	
21				Protein Concentration (stock microsomes, mg/mL):					1	5.00E-04	
22				Protein Concentration (dilution added to assay, mg/mL):					2	2.50E-04	
23									3	1.00E-04	
24									4	1.00E-05	
25									5	1.00E-06	
26									6	1.00E-07	
									7	1.00E-08	
									8	1.00E-09	

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
			Test		#					
1			Chemical	Concentrations						
	A:	3/27/2007	ID	Cl	tested	8	Microsome ID	12535-13		
2			Counter data							
	NI	12535-90	filename	27MR2107.P00						
3			Replicate		Pos Ctl					
	D ase		#	2	Positive Control 4-OH ASDI Tubes/Conc				2	
4										
5	Vial	TI	DPM1		ISIE					Round DPM
6	2	5	37744.82	16971.3	0	12.96	537.73			37745
7	3	5	34712.66	15854.46	0	13.13	551.95			34713
8	4	5	42562.08	19125.3	0	13.02	539.67			42562
9	5	5	39035.2	17748.22	0	13.1	549.14			39035
10	6	5	35275.88	16300.1	0	13.46	566.95			35276
11	8	5	16168.11	7191.54	0	12.88	529.56			16166
12	9	5	16151.57	7170.94	0	12.97	531.89			16152
13	10	5	15649.81	6960.7	0	13	533.41			15650
14	11	5	15386.79	6802.26	0	12.9	528.76			15387
15	12	5	12811.48	5697.66	0	12.89	530.44			12811
16	13	5	12695.49	5670.5	0	12.84	530.24			12695
17	14	5	14394.54	6480.86	0	12.9	534.27			14395
18	15	5	14618.73	6500.3	0	12.95	529.55			14619
19	17	5	70.33	32.86	0	12.58	531.83			70
20	18	5	73.8	32.1	0	13.4	531.86			74
21	19	5	61.18	29.26	0	10.78	533.73			61
22	20	5	46.72	21.26	0	11.6	532.31			47
23	21	5	56.8	26.06	0	14.78	532.48			57
24	22	5	55.94	26.14	0	11.05	532.06			56
25	23	5	80.86	28.5	0	14	532.29			61
26	24	5	71.19	31.94	0	14.04	531.08			71
27	26	5	174.71	78.46	0	13.43	534.08			176
28	27	5	173.5	80.66	0	12.65	530.02			174
29	28	5	156.54	74.1	0	12.98	534.31			159
30	29	5	175.33	81.7	0	11.95	532.08			175
31										
32										
33	30	5	973.52	433.06	0	13.02	531.75			974
34	31	5	969.9	442.06	0	12.67	534.69			970
35	32	5	958.92	428.14	0	12.78	533.51			959
36	33	5	921.16	411.7	0	12.79	532.36			921
37										
38										
39	34	5	1777.06	796.06	0	13.06	534.66			1777
40	35	5	1720.37	774.06	0	12.75	533.64			1720
41	36	5	1618.13	727.5	0	12.87	529.61			1618
42	37	5	1695.53	768.9	0	12.96	532.64			1696
43										
44										
45	38	5	5789.46	2596.3	0	12.86	529.95			5789
46	39	5	5946.65	2650.46	0	12.95	531.98			5947
47	40	5	5718.44	2544.74	0	12.65	530.51			5718
48	41	5	5678.04	2548.86	0	12.86	530.21			5678
49										
50										
51	42	5	8541.43	3832.74	0	12.87	531.77			8541
52	43	5	8694.15	3877.7	0	12.89	532.77			8694
53	44	5	8126.07	3633.26	0	12.94	533.3			8126
54	45	5	8130.3	3658.5	0	12.89	533.14			8130
55										
56										
57	46	5	13556.79	6067.1	0	12.94	533.62			13557
58	47	5	13573.69	6102.66	0	12.82	530.92			13574
59	48	5	13217.66	5862.3	0	12.89	527.94			13218
60	49	5	13218.44	5830.46	0	12.98	529			13218
61										
62										
63	50	5	14510.23	6455.18	0	12.86	530.39			14510
64	51	5	14300.64	6406.06	0	12.92	530.1			14301

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	14238.66	6356.74	0	12.95	532.77				14239
66	53	5	14474.85	6470.94	0	12.92	531.36				14475
67											
68											
68	54	5	14229.51	6359.98	0	12.92	533.16				14230
70	55	5	14492.35	6432.22	0	12.86	529.15				14492
71	56	5	14064.67	6291.85	0	12.89	533.23				14065
72	57	5	14177.58	6300.86	0	12.95	529.74				14178
73											
74											
76	59	5	11016.98	4932.34	0	12.86	530.79				11017
76	60	5	11219.49	5036.5	0	12.83	529.43				11219
77	61	5	10954.31	4906.1	0	12.96	531.64				10954
78	62	5	11014.3	4922.74	0	12.85	531.16				11014
79	63	5	9921.6	4412.06	0	12.95	531.03				9922
80	64	5	10048.11	4493.14	0	12.83	530.79				10046
81	65	5	12118.8	5421.46	0	12.89	533.14				12119
82	66	5	12337.31	5521.06	0	12.91	532.79				12337
83	67	5	11886.72	5304.5	0	12.9	531.17				11889
84	68	5	12005.32	5379.54	0	12.87	530.24				12005
85	69	5	12515.16	5597.66	0	12.88	532.17				12515
86	70	5	12491.23	5548.1	0	12.93	530.67				12491
87	71	5	13540.46	6066.66	0	12.83	532.41				13540
88	72	5	13479.61	6007.26	0	12.93	530.41				13480
89	73	5	13182.69	5910.1	0	12.84	532.07				13183
90	74	5	13291.86	5962.66	0	12.85	532.94				13292
91	75	5	13789.24	6158.46	0	12.91	530.95				13789
92	76	5	13918.44	6206.9	0	12.79	529.81				13918
93	77	5	14868.65	6674.74	0	12.88	531.89				14869
94	78	5	15085.59	6720.7	0	12.9	530.56				15088
95	79	5	14418.92	6458.66	0	12.83	529.65				14419
96	80	5	14513.96	6453.9	0	12.82	531.81				14514
97	81	5	14900.29	6673.06	0	12.9	533.08				14900
98	82	5	15088.26	6718.94	0	12.86	530.54				15088
99	84	5	14333.58	6405.46	0	12.93	533.15				14334
100	85	5	14710.23	6561.66	0	12.93	530.44				14710
101	86	5	13876.67	6200.46	0	12.91	530.19				13877
102	87	5	14072.87	6287.7	0	12.8	529.96				14073
103	88	5	14644.68	6505.46	0	12.97	531.49				14645
104	89	5	14866.92	6626.1	0	12.88	532.09				14866
105	90	5	14831.45	6637.08	0	12.91	531.97				14831
106	91	5	14871.79	6644.46	0	12.94	534.78				14872
107	92	5	14271.81	6363.46	0	12.94	532.3				14272
108	93	5	14253.45	6377.1	0	12.88	532.05				14253
109	94	5	14293.96	6417.46	0	12.92	532.61				14294
110	95	5	14714.18	6542.26	0	12.94	529.25				14714
111	96	5	14383.87	6393.86	0	12.9	529.35				14384
112	97	5	14408.49	6412.3	0	12.9	530.03				14408
113	98	5	13846.12	6168.46	0	12.85	530.5				13846
114	99	5	13770.91	6151.3	0	12.79	529.03				13771
115	100	5	14398.28	6418.54	0	12.83	529.8				14398
116	101	5	14525.08	6527.1	0	12.82	532.03				14525
117	102	5	14213.66	6357.9	0	12.9	531.88				14214
118	103	5	14397.83	6427.54	0	12.91	533.27				14398
119	104	5	14726.1	6544.1	0	12.83	530.54				14726
120	105	5	14935.18	6648.38	0	12.88	529.3				14935
121	106	5	15104.2	6744.5	0	12.86	530.53				15104
122	107	5	15089.47	6783.3	0	12.84	531.65				15089
123	109	5	225688.19	33177.14	0	7.92	172.51				225688
124											#REF!
125											#REF!
126											#REF!

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Sample type	Nonfinal total volume (mL)	Aliq Volumes (mL)	Calculate DPM in aqueous portion after extraction			Total DPM	Volume of substrate solution added (mL)		total DPM in assay tube (initial)	Calculate nmol H ₂ O formed (Background subtracted) (Tubes)	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol H ₂ O formed / (mg protein * min))
					DPH/mole	DPH/mole	Ave DPM/mL		0	0					
1	1	Full activity control	2	0.5	15166	32332	32316	84636	0.1	155606	84388	0.0057	0.005	15	0.436
2	2	Full activity control	2	0.5	18192	31300	31037	62074	0.1	195606	81826	0.0051	0.005	15	0.421
3	3	Full activity control	2	0.5	14600	31300	31037	62074	0.1	195606	81826	0.0051	0.005	15	0.421
4	4	Full activity control	2	0.5	15387	30774	30774	61548	0.1	195606	81826	0.0051	0.005	15	0.421
5	5	Full activity control	2	0.5	12811	25622	25605	51012	0.1	195606	51012	0.00518	0.005	15	0.345
6	6	Full activity control	2	0.5	12895	25390	25390	50780	0.1	195606	50780	0.00518	0.005	15	0.345
7	7	Full activity control	2	0.5	14395	28190	28190	56380	0.1	195606	56380	0.00518	0.005	15	0.345
8	8	Full activity control	2	0.5	14819	28248	28248	56496	0.1	195606	56496	0.00518	0.005	15	0.345
9	9	Full activity control	2	0.5	74	143	143	286	0.1	195606	286	0.0006	0.005	15	0.000
10	10	Full activity control	2	0.5	47	91	91	182	0.1	195606	182	0.0006	0.005	15	0.000
11	11	Full activity control	2	0.5	57	112	112	224	0.1	195606	224	0.0006	0.005	15	0.000
12	12	Full activity control	2	0.5	61	122	122	244	0.1	195606	244	0.0006	0.005	15	0.000
13	13	Full activity control	2	0.5	71	142	142	284	0.1	195606	284	0.0006	0.005	15	0.000
14	14	Full activity control	2	0.5	175	350	349	698	0.1	195606	698	0.0006	0.005	15	0.000
15	15	Full activity control	2	0.5	174	348	348	696	0.1	195606	696	0.0006	0.005	15	0.000
16	16	Full activity control	2	0.5	159	318	314	628	0.1	195606	628	0.0006	0.005	15	0.000
17	17	Full activity control	2	0.5	175	350	350	700	0.1	195606	700	0.0006	0.005	15	0.000
18	18	Full activity control	2	0.5	974	1948	1944	3888	0.1	195606	3888	0.0037	0.005	15	0.025
19	19	Full activity control	2	0.5	970	1940	1940	3880	0.1	195606	3880	0.0037	0.005	15	0.024
20	20	Full activity control	2	0.5	958	1918	1880	3760	0.1	195606	3760	0.0036	0.005	15	0.024
21	21	Full activity control	2	0.5	921	1842	1842	3684	0.1	195606	3684	0.0036	0.005	15	0.024
22	22	Full activity control	2	0.5	1777	3554	3497	6994	0.1	195606	6994	0.0069	0.005	15	0.046
23	23	Full activity control	2	0.5	1720	3440	3414	6828	0.1	195606	6828	0.0068	0.005	15	0.043
24	24	Full activity control	2	0.5	1619	3236	3314	6628	0.1	195606	6628	0.0066	0.005	15	0.043
25	25	Full activity control	2	0.5	1686	3372	3392	6784	0.1	195606	6784	0.0068	0.005	15	0.046
26	26	Full activity control	2	0.5	5789	11578	11736	23472	0.1	195606	23472	0.0237	0.005	15	0.158
27	27	Full activity control	2	0.5	5847	11694	11894	23788	0.1	195606	23788	0.0240	0.005	15	0.159
28	28	Full activity control	2	0.5	5718	11436	11388	22776	0.1	195606	22776	0.0230	0.005	15	0.153
29	29	Full activity control	2	0.5	5678	11356	11356	22712	0.1	195606	22712	0.0229	0.005	15	0.153
30	30	Full activity control	2	0.5	8541	17082	17235	34470	0.1	195606	34470	0.0349	0.005	15	0.230
31	31	Full activity control	2	0.5	8594	17188	17235	34470	0.1	195606	34470	0.0349	0.005	15	0.230
32	32	Full activity control	2	0.5	8190	16380	16256	32512	0.1	195606	32512	0.0329	0.005	15	0.219
33	33	Full activity control	2	0.5	13957	27914	27131	54262	0.1	195606	54262	0.0551	0.005	15	0.387
34	34	Full activity control	2	0.5	13674	27348	26436	52672	0.1	195606	52672	0.0537	0.005	15	0.358
35	35	Full activity control	2	0.5	13218	26436	26436	52872	0.1	195606	52872	0.0537	0.005	15	0.358
36	36	Full activity control	2	0.5	13218	26436	26436	52872	0.1	195606	52872	0.0537	0.005	15	0.358
37	37	Full activity control	2	0.5	14510	29020	28811	57622	0.1	195606	57622	0.0585	0.005	15	0.390
38	38	Full activity control	2	0.5	14301	28602	28602	57204	0.1	195606	57204	0.0585	0.005	15	0.389
39	39	Full activity control	2	0.5	14338	28676	28714	57428	0.1	195606	57428	0.0585	0.005	15	0.389
40	40	Full activity control	2	0.5	14476	28950	28950	57900	0.1	195606	57900	0.0585	0.005	15	0.389
41	41	Full activity control	2	0.5	14230	28460	28722	57444	0.1	195606	57444	0.0585	0.005	15	0.389
42	42	Full activity control	2	0.5	14092	28184	28184	56368	0.1	195606	56368	0.0585	0.005	15	0.389
43	43	Full activity control	2	0.5	14085	28160	28160	56320	0.1	195606	56320	0.0585	0.005	15	0.389
44	44	Full activity control	2	0.5	14178	28356	28356	56712	0.1	195606	56712	0.0585	0.005	15	0.389

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - adjust variance			
6		Red if value not within mean $\pm$ 10%			
7		1.00			
8		1.01			
9		1.01			
10		0.99			
11		1.00			
12		1.00			
13		0.99			
14		1.01			
15		0.97			
16		1.03			
17					
18					
19		1.01			
20		0.99			
21		0.92			
22		1.00			
23		1.00			
24		0.95			
25		1.05			
26					
27					
28					
29		1.00			
30		1.00			
31		1.02			
32		0.98			
33					
34		1.02			
35		0.98			
36		0.98			
37		1.02			
38					
39					
40					
41		0.99			
42		1.01			
43		1.00			
44		1.00			
45		1.00			
46					
47		0.99			
48		1.01			
49		1.00			
50		1.00			
51					
52					
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57		1.01			
58		0.99			
59		1.01			
60		0.99			
61		0.99			
62		1.01			
63					
64					
65		0.99			
66		1.01			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate/Level	Normal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volumes of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Fetal protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
1	6	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
2	7	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
3	8	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
4	9	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
5	10	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
6	11	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
7	12	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
8	13	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
9	14	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
10	15	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
11	16	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
12	17	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
13	18	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
14	19	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
15	20	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
16	21	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
17	22	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
18	23	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
19	24	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
20	25	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
21	26	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
22	27	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
23	28	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
24	29	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
25	30	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
26	31	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
27	32	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
28	33	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
29	34	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
30	35	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
31	36	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
32	37	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
33	38	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
34	39	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
35	40	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
36	41	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
37	42	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
38	43	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
39	44	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
40	45	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
41	46	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
42	47	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
43	48	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
44	49	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
45	50	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
46	51	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
47	52	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
48	53	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
49	54	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
50	55	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
51	56	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
52	57	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
53	58	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301
54	59	2	0.5	1	11017	22034	22236	44472	0.1	185606	46224	0.0461	0.005	15	0.301

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/27/2007	Chemical ID	CR11612	# Concentrations tested					
2		NB Ref.	12535-90	Counter data	27MR2107 P00				8	Microsome ID	12535-13
3		Technician ID	ase	Replicate #		2	Positive Control	4 OH ASDN	Pos Ctl Tubes/Conc		2
4		Control Type	SD								
5		Overall	0.399		Bkg % of Full						
6		Overall	0.002								
7											
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Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/28/2007	Test Chemical ID	CR11612	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-93	Counter data filename	28MR2046.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/26/2007	Test Chemical ID	CR11612	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-93	Counter data filename	28MR2046.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0194		33057		1703969			
8	2	0.0196		36935		1884439			
9	3	0.0192		38440		2002083			
10	4	0.0195		40496		2076718			
11	5	0.0197		40747		2068376			
12						Average DPM/g soln		1947117	
13						SD		156182	
14						CV		8.02	
15						µCi/g soln		0.8771	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.02	10		1002.00				
20	Dilution A			100	10.02				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1782 g			
25	Mass of dilution B used in substrate prep					4.5765 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.							0.56071666 µg/g	
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8771			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					=	0.5607	+	0.011	
37					=	0.571	µg ASDN/g soln.		
38	3) Calculate Solution Specific Activity								
39					= (µCi/g soln.)/(µg ASDN/g soln.)				
40					=	1.535	µCi/µg ASDN		
41						9.759.E+05	dpm/nmol		

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/28/2007	Test Chemical ID	CR11612	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-93	Counter data filename	28MR2046.P00					
3			Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
5	Microsome Dilution Details										
6											
7				0.12 mL microsome Stock used					Positive Control Concentrations		
8				2.4 mL total volume					Level	Final Concentration (M)	
9				20 dilution factor					1	1.00E-05	
10									2	1.00E-06	
11				1.5 mL microsome Dilution A used					3	5.00E-07	
12				60 mL total volume					4	1.00E-07	
13				40 dilution factor					5	5.00E-08	
14									6	1.00E-08	
15				mL microsome Dilution B used					7	1.00E-09	
16				mL total volume					8	1.00E-10	
17				dilution factor							
18		NA							Test Chemical Concentrations		
19				800 total dilution factor					Level	Final Concentration (M)	
20									1	5.00E-04	
21									2	2.50E-04	
22	Protein Concentration (stock microsomes, mg/mL):				8				3	1.00E-04	
23	Protein Concentration (dilution added to assay, mg/mL):				0.01				4	1.00E-05	
24									5	1.00E-06	
25									6	1.00E-07	
26									7	1.00E-08	
									8	1.00E-09	

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
	Test # Chemical Concentrations A: 3/28/2007 ID Cl tested 8 Microsome ID 12535-13										
	Counter data N 12535-93 filename: 28MR2046.P00										
	Replicate Positive Pos Cl D ase # 3 Control 4-OH ASD Tubes/Conc 2										
5	Vial	Ti	DPM1								Round DPM
6	2	5	33066.54	14745.8	0	13.03	534.58				33067
7	3	5	36934.86	16107.44	0	12.73	516.01				36935
8	4	5	38439.64	16622.8	0	12.57	505.8				38440
9	5	5	40495.9	17960.56	0	12.92	528.44				40496
10	6	5	40747.34	17840.44	0	12.87	519.44				40747
11	8	5	14445.47	6361.04	0	12.92	526.2				14445
12	9	5	14222.8	6303.44	0	12.88	527.18				14223
13	10	5	13781.67	6094.44	0	12.86	523.64				13782
14	11	5	13571.13	5991.84	0	12.87	525.32				13571
15	12	5	12398.13	5497.16	0	12.95	528.53				12398
16	13	5	12235.86	5410.04	0	12.89	524.5				12236
17	14	5	12268.85	5460.04	0	12.9	528.82				12269
18	15	5	12283.14	5412.36	0	12.92	526.62				12283
19	17	5	60.94	29.2	0	12.76	528.51				61
20	18	5	62.24	28	0	13.65	526.93				62
21	19	5	67.21	29.8	0	13.07	523.21				67
22	20	5	76.2	35.24	0	12.88	529.1				76
23	21	5	67.5	31.4	0	12.57	525.55				68
24	22	5	66.55	29.44	0	11.82	528.62				66
25	23	5	55.39	28.36	0	12.74	526.42				55
26	24	5	60.91	25.76	0	14.72	527.72				61
27	26	5	169.37	73.4	0	13.45	530.13				169
28	27	5	168.47	77.8	0	13.09	529.29				168
29	28	5	179.9	78.2	0	13.14	527.02				180
30	29	5	140.03	62.16	0	12.59	527.64				140
31											
32											
33	30	5	826.01	368.24	0	13.05	528.98				826
34	31	5	878.17	383	0	13.33	526.91				878
35	32	5	829.27	371.04	0	12.74	525.24				829
36	33	5	809.13	360.56	0	12.99	525.28				809
37											
38											
39	34	5	1494.01	660.04	0	12.74	528.99				1494
40	35	5	1479.66	657.24	0	13.05	526.31				1480
41	36	5	1428.39	630	0	12.82	528.38				1428
42	37	5	1401.4	632.36	0	12.94	529.19				1401
43											
44											
45	38	5	5101.76	2263.36	0	13.05	532.59				5102
46	39	5	4984.72	2216.16	0	12.99	528.58				4985
47	40	5	4766.58	2106.8	0	12.95	527.77				4767
48	41	5	4694.74	2077.64	0	12.97	526.11				4695
49											
50											
51	42	5	6188.62	2741.8	0	12.92	529.31				6189
52	43	5	6332.28	2780.36	0	12.88	524.06				6332
53	44	5	6577.43	2891	0	12.91	525.41				6577
54	45	5	6669.36	2946.24	0	12.84	526.45				6669
55											
56											
57	46	5	11164.94	4963.8	0	12.96	529.5				11165
58	47	5	11639.89	5114	0	13.04	528.13				11640
59	48	5	10728.54	4743.56	0	12.86	529.16				10729
60	49	5	10667.52	4727.16	0	12.82	524.25				10668
61											
62											
63	50	5	11750.23	5249.16	0	12.87	529.93				11750
64	51	5	11655.81	5163.96	0	12.85	525.84				11656

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	11751.8	5186.16	0	12.94	527.69				11752
66	53	5	11532.03	5096.4	0	12.89	526.86				11532
67											
68											
69	54	5	11887.24	5244.4	0	12.97	526.58				11887
70	55	5	11932.82	5320.8	0	12.98	530.06				11933
71	56	5	11956.72	5318.04	0	12.87	528.61				11957
72	57	5	12012.11	5324.8	0	13.01	530.44				12012
73											
74											
75	59	5	9860.27	4358.2	0	12.93	528.42				9860
76	60	5	9819.54	4322.96	0	12.96	528.9				9820
77	61	5	9702.2	4290.56	0	12.85	525.48				9702
78	62	5	9901.81	4341.4	0	12.95	525.59				9902
79	63	5	9534	4218.56	0	12.95	529.18				9534
80	64	5	9753.02	4332	0	12.95	529.13				9753
81	65	5	11064.66	4917.64	0	12.92	526.64				11065
82	66	5	11092.43	4897.48	0	12.9	529.81				11092
83	67	5	10598.48	4682.58	0	12.92	526.02				10598
84	68	5	10665.56	4741.04	0	12.89	527.68				10666
85	69	5	11342.74	5047.8	0	12.9	527.8				11343
86	70	5	11750.02	5215.08	0	13.03	529				11750
87	71	5	10778.96	4807.56	0	12.86	527.35				10779
88	72	5	11058.18	4863.64	0	13.04	525.43				11058
89	73	5	11605.55	5128	0	12.98	526.13				11606
90	74	5	11608.19	5135.96	0	12.9	527.63				11608
91	75	5	11311.54	4988.76	0	13.02	525.26				11312
92	76	5	11071.79	4890.6	0	12.96	525.59				11072
93	77	5	10883.8	4829.04	0	12.86	527.99				10884
94	78	5	10930.17	4854.4	0	12.93	527.4				10930
95	79	5	10912.81	4827.8	0	12.95	527.42				10913
96	80	5	10583.82	4712.4	0	12.83	525.42				10584
97	81	5	10975.19	4882.2	0	12.95	527.99				10975
98	82	5	11184.79	4948.96	0	12.97	528.55				11185
99	84	5	11306.69	4986.4	0	12.98	527.54				11307
100	85	5	11378.71	5035.8	0	12.96	528.6				11377
101	86	5	11157.88	4967.6	0	13.03	531.15				11158
102	87	5	10994.27	4887.6	0	13.01	530.64				10994
103	88	5	11057.22	4908.56	0	12.95	527.2				11057
104	89	5	11069.34	4877.76	0	12.97	525.94				11069
105	90	5	11426.56	5077.6	0	12.94	527.9				11427
106	91	5	11517.51	5056.76	0	13.02	527.87				11518
107	92	5	11575.87	5091.6	0	12.89	522.91				11576
108	93	5	11238.7	5023.2	0	12.93	530.99				11239
109	94	5	12260.13	5454.84	0	12.93	527.83				12260
110	95	5	12462.43	5520.28	0	12.93	526.03				12462
111	96	5	11351.04	5044.4	0	12.83	525.3				11351
112	97	5	11381.59	5006.2	0	12.9	522.41				11382
113	98	5	11138.47	4927.96	0	13.07	527.87				11138
114	99	5	11180.1	4937.72	0	12.93	527.33				11180
115	100	5	12234.28	5379.16	0	12.92	525.16				12234
116	101	5	12411.02	5487.8	0	12.91	524.16				12411
117	102	5	12824.56	5674.4	0	12.99	530.6				12825
118	103	5	12915.17	5741.2	0	12.94	529.09				12915
119	104	5	12487.26	5512.36	0	12.97	527.72				12487
120	105	5	12448.81	5472.84	0	12.85	523.02				12449
121	106	5	12688.02	5600.48	0	12.95	528.9				12688
122	107	5	12616.49	5668.2	0	12.97	529.84				12616
123	109	5	230680.02	33305.64	0	7.99	172.13				230680
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date	Assay Date
3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007	3/28/2007
CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612	CK11612
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200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000
200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000
200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000
200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000
200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000
200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000
200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000
200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200000000	200			

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance Read if value not within mean $\pm$ 10%			
6					
7		1.01			
8		0.99			
9		1.01			
10		1.01			
11		0.99			
12		1.00			
13		1.00			
14		0.99			
15		1.01			
16		1.01			
17		0.94			
18		1.06			
19		1.01			
20		0.99			
21		0.95			
22		1.05			
23		1.01			
24		1.03			
25					
26					
27					
28					
29		0.97			
30		1.03			
31		1.01			
32		0.99			
33					
34		1.01			
35		1.01			
36		1.01			
37		1.01			
38		0.99			
39					
40					
41		1.01			
42		0.99			
43		1.01			
44		0.95			
45					
46		0.99			
47		1.01			
48		0.99			
49		1.01			
50					
51					
52					
53		0.95			
54		1.02			
55		1.00			
56		1.00			
57					
58					
59		1.00			
60		1.00			
61		1.01			
62		0.99			
63					
64					
65		1.01			
66		1.00			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	
1		Assay Date	3/28/2007	Test Chemical	CR11512	# Concentrations tested	8	Microsome ID	12535-13							
2		NIS Ref.	1.2555-93	Control Reagent	20MR204E-P100											
3		Technician ID	ase	Replicate #	3	Predictive Control	4-OH ASDN	Pos. Ctrl Tubes/Conc								
4																
5	Sample ID	Calculate DPM in aqueous portion after extraction														
6	Sample Type	Replicated Level	Normal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/Walq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (fmol)	Total DPM corrected for background (Background tubes)	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
7	CR11512	1-1	2	0.5	1	9860	19720	19680	39360	0.1	19472	39102	0.0401	0.005	15	0.267
8		1-2	2	0.5	2	9620	19240	19504	39208	0.1	19472	38950	0.0399	0.005	15	0.266
9		1-3	2	0.5	1	9702	19404			0.1	19472	38916	0.0393	0.005	15	0.262
10		2-1	2	0.5	2	9802	19604	19287	38574	0.1	19472	38574	0.0393	0.005	15	0.262
11		2-2	2	0.5	1	9534	19068			0.1	19472	38516	0.0393	0.005	15	0.262
12		2-3	2	0.5	2	11065	22130	22167	44314	0.1	19472	44056	0.0451	0.005	15	0.301
13		3-1	2	0.5	1	10920	21840			0.1	19472	43770	0.0444	0.005	15	0.296
14		3-2	2	0.5	2	10588	21168	21264	42528	0.1	19472	42270	0.0433	0.005	15	0.289
15		3-3	2	0.5	2	10686	21332			0.1	19472	42528	0.0433	0.005	15	0.289
16		4-1	2	0.5	1	11343	22686	23003	46186	0.1	19472	45828	0.0471	0.005	15	0.314
17		4-2	2	0.5	2	11750	23500			0.1	19472	43416	0.0445	0.005	15	0.297
18		4-3	2	0.5	1	10739	21558	21837	43674	0.1	19472	43416	0.0445	0.005	15	0.297
19		5-1	2	0.5	2	11658	23316	23214	46428	0.1	19472	46170	0.0473	0.005	15	0.315
20		5-2	2	0.5	1	11606	23216			0.1	19472	46170	0.0473	0.005	15	0.315
21		5-3	2	0.5	2	11312	22624	22804	44768	0.1	19472	44510	0.0458	0.005	15	0.304
22		6-1	2	0.5	1	10772	21544			0.1	19472	44510	0.0458	0.005	15	0.304
23		6-2	2	0.5	2	10590	21180	21814	43678	0.1	19472	43370	0.0444	0.005	15	0.296
24		6-3	2	0.5	1	10913	21826			0.1	19472	43370	0.0444	0.005	15	0.296
25		7-1	2	0.5	2	10884	21168	21437	42894	0.1	19472	42736	0.0438	0.005	15	0.292
26		7-2	2	0.5	1	10873	21840			0.1	19472	42736	0.0438	0.005	15	0.292
27		7-3	2	0.5	2	11658	23316	22160	44320	0.1	19472	44062	0.0451	0.005	15	0.301
28		8-1	2	0.5	1	13077	26154	27684	45368	0.1	19472	45110	0.0462	0.005	15	0.308
29		8-2	2	0.5	2	11755	23516	22152	44304	0.1	19472	45110	0.0462	0.005	15	0.308
30		8-3	2	0.5	1	11558	23116			0.1	19472	45110	0.0462	0.005	15	0.308
31		9-1	2	0.5	2	11658	23316	22764	45368	0.1	19472	45110	0.0462	0.005	15	0.308
32		9-2	2	0.5	1	11755	23516			0.1	19472	45110	0.0462	0.005	15	0.308
33		9-3	2	0.5	2	10894	21888	22108	44252	0.1	19472	45110	0.0462	0.005	15	0.308
34		10-1	2	0.5	1	11067	22134			0.1	19472	45110	0.0462	0.005	15	0.308
35		10-2	2	0.5	2	11067	22134	22108	44252	0.1	19472	45110	0.0462	0.005	15	0.308
36		10-3	2	0.5	1	11067	22134			0.1	19472	45110	0.0462	0.005	15	0.308
37		11-1	2	0.5	2	11427	22854	22945	45890	0.1	19472	45832	0.0468	0.005	15	0.312
38		11-2	2	0.5	1	11576	23152			0.1	19472	45832	0.0468	0.005	15	0.312
39		11-3	2	0.5	2	12339	24678	24678	49356	0.1	19472	45832	0.0468	0.005	15	0.312
40		12-1	2	0.5	1	12339	24678			0.1	19472	45832	0.0468	0.005	15	0.312
41		12-2	2	0.5	2	12339	24678	24722	49444	0.1	19472	45832	0.0468	0.005	15	0.312
42		13-1	2	0.5	1	11351	22702			0.1	19472	45832	0.0468	0.005	15	0.312
43		13-2	2	0.5	2	11351	22702	22733	45466	0.1	19472	45832	0.0468	0.005	15	0.312
44		14-1	2	0.5	1	11382	22764			0.1	19472	45832	0.0468	0.005	15	0.312
45		14-2	2	0.5	2	11382	22764	22718	44696	0.1	19472	45832	0.0468	0.005	15	0.312
46		15-1	2	0.5	1	11800	23600			0.1	19472	45832	0.0468	0.005	15	0.312
47		15-2	2	0.5	2	11800	23600	24645	49280	0.1	19472	45832	0.0468	0.005	15	0.312
48		16-1	2	0.5	1	12411	24822			0.1	19472	45832	0.0468	0.005	15	0.312
49		16-2	2	0.5	2	12411	24822	25740	51480	0.1	19472	45832	0.0468	0.005	15	0.312
50		17-1	2	0.5	1	12915	25830			0.1	19472	45832	0.0468	0.005	15	0.312
51		17-2	2	0.5	2	12915	25830	24936	49872	0.1	19472	45832	0.0468	0.005	15	0.312
52		18-1	2	0.5	1	12449	24898			0.1	19472	45832	0.0468	0.005	15	0.312
53		18-2	2	0.5	2	12449	24898	24936	49872	0.1	19472	45832	0.0468	0.005	15	0.312
54		19-1	2	0.5	1	12449	24898			0.1	19472	45832	0.0468	0.005	15	0.312
55		19-2	2	0.5	2	12449	24898	24936	49872	0.1	19472	45832	0.0468	0.005	15	0.312

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC Check - aliquot
5		Variance
6		% RE
7		1.00
8		1.00
9		0.99
10		1.01
11		0.99
12		1.01
13		1.00
14		1.00
15		1.00
16		1.00
17		0.99
18		1.01
19		0.99
20		1.01
21		1.00
22		1.00
23		1.01
24		0.99
25		1.00
26		1.00
27		1.02
28		0.99
29		0.99
30		1.01
31		1.00
32		1.00
33		1.01
34		0.99
35		1.00
36		1.00
37		1.00
38		1.00
39		1.01
40		0.99
41		0.99
42		1.01
43		1.00
44		1.00
45		1.00
46		0.99
47		1.01
48		1.00
49		1.00
50		1.00
51		1.00
52		1.00
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Data	3/28/2007	Test Chemical ID	CR11612	# Concentrations tested					
2		NS Ref	12535-93	Chemical ID	28MR2046 P00				8	Microsome ID	12535-13
3		Technician ID	area	Replicate #		3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4	Control Type	Average	SD								
5	Full activity	0.358	0.028								
6	Background (uncorrected)	0.002	0.000								
7											
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	2	1.00E-05	-5.00	0.003						
13	4-OH ASDN	1	1.00E-06	-6.00	0.022						
14	4-OH ASDN	2	1.00E-06	-6.00	0.021						
15	4-OH ASDN	1	5.00E-07	-7.00	0.039						
16	4-OH ASDN	2	5.00E-07	-7.00	0.037						
17	4-OH ASDN	1	1.00E-07	-8.00	0.136						
18	4-OH ASDN	2	1.00E-07	-8.00	0.128						
19	4-OH ASDN	1	1.00E-08	-9.00	0.310						
20	4-OH ASDN	2	1.00E-08	-9.00	0.291						
21	4-OH ASDN	1	1.00E-09	-10.00	0.318						
22	4-OH ASDN	2	1.00E-09	-10.00	0.316						
23	4-OH ASDN	1	1.00E-10	-11.00	0.324						
24	4-OH ASDN	2	1.00E-10	-11.00	0.326						
25	4-OH ASDN	1									
26	4-OH ASDN	2									
27	4-OH ASDN	1									
28	4-OH ASDN	2									
29	4-OH ASDN	1									
30	4-OH ASDN	2									
31	4-OH ASDN	1									
32	4-OH ASDN	2									
33	4-OH ASDN	1									
34	4-OH ASDN	2									
35											
36											
37	Test Chemical	Replicate	Test Chemical M	Log(test chemical)	Activity						
38	CR11612	1	5.00E-04	-3.30	0.267						
39	CR11612	2	5.00E-04	-3.30	0.256						
40	CR11612	3	5.00E-04	-3.30	0.262						
41	CR11612	1	2.50E-04	-3.60	0.301						
42	CR11612	2	2.50E-04	-3.60	0.289						
43	CR11612	3	2.50E-04	-3.60	0.314						
44	CR11612	1	1.00E-04	-4.00	0.297						
45	CR11612	2	1.00E-04	-4.00	0.315						
46	CR11612	3	1.00E-04	-4.00	0.304						
47	CR11612	1	1.00E-05	-5.00	0.286						
48	CR11612	2	1.00E-05	-5.00	0.292						
49	CR11612	3	1.00E-05	-5.00	0.301						
50	CR11612	1	1.00E-06	-6.00	0.308						
51	CR11612	2	1.00E-06	-6.00	0.301						
52	CR11612	3	1.00E-06	-6.00	0.301						
53	CR11612	1	1.00E-07	-7.00	0.312						
54	CR11612	2	1.00E-07	-7.00	0.310						
55	CR11612	3	1.00E-07	-7.00	0.306						
56	CR11612	1	1.00E-08	-8.00	0.309						
57	CR11612	2	1.00E-08	-8.00	0.303						
58	CR11612	3	1.00E-08	-8.00	0.335						
59	CR11612	1	1.00E-09	-9.00	0.350						
60	CR11612	2	1.00E-09	-9.00	0.339						
61	CR11612	3	1.00E-09	-9.00	0.347						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	2/22/2007	Test	Chemical ID	CR11613	# Concentrations	tested	8	Microsome ID	12535-13
2	NB Ref.	12535-48	Counter data	filename	23FE0007.P00					
3	Technician		Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc			
	ID	ase								2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/22/2007	Test Chemical ID	CR11613	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-48	Counter data filename	23FE0007.P00				Pos Ctl	
3	Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN		Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0207		39131		1890386			
8	2	0.0197		36759		1865839			
9	3	0.0199		37936		1906332			
10	4	0.0202		38705		1916089			
11	5	0.0192		36654		1909063			
12						Average DPM/g soln		1897562	
13						SD		20024	
14						CV		1.06	
15						µCi/g soln		0.8548	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.04	10		1004.00				
20	Dilution A			100	10.04				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0915 g			
25	Mass of dilution B used in substrate prep					4.5643 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56634211 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.010 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8548			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					= 0.5663 + 0.010				
37					= 0.577 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=	1.482	µCi/µg ASDN						
41		9.423.E+05	dpm/nmol						

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	2/22/2007	Test Chemical ID	CR11613	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-48	Counter data filename	23FE0007.P00					
3			Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc		2
4											
5			Microsome Dilution Details								
6											
7			Dilution A								
8											
9											
10											
11			Dilution B								
12											
13											
14											
15			Dilution C (if applicable)								
16											
17											
18											
19											
20											
21			Protein Concentration (stock microsomes, mg/mL):								
22			Protein Concentration (dilution added to assay, mg/mL):								
23											
24											
25											
26											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	As	2/22/2007	Test Chemical	ID	CI	# Concentrations tested	8	Microsome ID	12535-13		
2	NI	12535-48	Counter data filename	23FE0007.P00							
3	D	859	Replicate # 1	Positive Control	4-OH ASD	Tubes/Conc	2				
4											
5	Vial #	Th	DPM1			ISIE					Round DPM
6	2	5	39131.4	18998.6	0	14.16	614.17				39131
7	3	5	36759.2	17902.32	0	14.21	616.96				36759
8	4	5	37935.83	18468	0	14.17	615.1				37936
9	5	5	38705.16	18796.2	0	14.06	610.63				38705
10	6	5	36653.64	17778.48	0	14.16	614.62				36654
11	8	5	10593.04	4762.16	0	12.99	533.69				10593
12	9	5	10791.29	4885.96	0	12.97	536.01				10791
13	10	5	9839.77	4395.08	0	13.07	534.49				9840
14	11	5	9716.71	4389.64	0	12.95	534.07				9717
15	12	5	9652.8	4354.28	0	12.94	534.76				9653
16	13	5	9604.59	4340.72	0	12.95	533.98				9605
17	14	5	11094.65	4998.92	0	12.97	533.82				11095
18	15	5	10889.3	4915.72	0	12.98	535.17				10889
19	17	5	45.53	23.88	0	12.44	532.6				46
20	18	5	66.07	30.12	0	14.35	534.01				66
21	19	5	55.89	31.88	0	11.95	535				56
22	20	5	60.4	27.28	0	13.94	534.14				60
23	21	5	69.98	33.88	0	13.4	532.73				70
24	22	5	56.98	30.72	0	12.13	532.94				69
25	23	5	56.24	24.68	0	13.58	537.38				56
26	24	5	84.61	28.88	0	12.71	536.42				65
27	26	5	124.22	55.12	0	12.23	534.51				124
28	27	5	156.83	68.72	0	12.7	534.91				157
29	28	5	136.91	62.12	0	13.13	534.77				137
30	29	5	128.76	61.56	0	12.4	534.56				129
31											
32											
33	30	5	729.79	321.32	0	13.08	530.45				730
34	31	5	740.17	335.48	0	12.59	534.8				740
35	32	5	682.21	307.16	0	13.38	532.7				682
36	33	5	708.68	323.32	0	12.94	537.36				709
37											
38											
39	34	5	1258.22	558.88	0	12.94	532.4				1256
40	35	5	1231.15	549.36	0	13.06	534.29				1231
41	36	5	1144.82	519.08	0	13.03	537.51				1145
42	37	5	1117.1	502.92	0	12.8	533.8				1117
43											
44											
45	38	5	4018.63	1806.96	0	13.05	532.94				4019
46	39	5	4101.18	1830.88	0	13.05	534				4101
47	40	5	3817.08	1707.6	0	13.09	536.04				3817
48	41	5	3729.52	1677.28	0	13.05	531.75				3730
49											
50											
51	42	5	5716.8	2562.08	0	12.99	530.89				5717
52	43	5	5764.15	2611.76	0	13.01	534.6				5764
53	44	5	5605.43	2504.76	0	13.01	532.64				5605
54	45	5	5717.86	2598.08	0	13.05	535.61				5718
55											
56											
57	46	5	8631.66	3892.88	0	13.02	533.88				8632
58	47	5	8411.77	3802.28	0	13	535.43				8412
59	48	5	8421.82	3796.12	0	12.89	531.07				8422
60	49	5	8496.68	3821.28	0	13.04	533.39				8497
61											
62											
63	50	5	9119.4	4109.52	0	13	534.67				9119
64	51	5	9174.15	4136.96	0	12.98	535.32				9174

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	9639.72	4355.48	0	12.94	533.44				9640
66	53	5	9719.29	4378.16	0	13.03	535.88				9719
67											
68											
69	54	5	8966.04	4027.52	0	12.99	534.34				8966
70	55	5	8943.13	4011.08	0	13.01	534.35				8943
71	56	5	9224.53	4158.48	0	12.92	533.43				9225
72	57	5	9255.92	4176.88	0	13	534.23				9256
73											
74											
75	59	5	7176.96	3243.72	0	13.04	536.88				7177
76	60	5	7279.92	3287.12	0	12.91	534.2				7280
77	61	5	6509.4	2930.52	0	12.93	535.21				6509
78	62	5	6634.78	2978.28	0	13.03	534.1				6635
79	63	5	6143.73	2780.36	0	13.05	535.78				6144
80	64	5	6388.73	2864.28	0	13.04	533.83				6387
81	65	5	8176.81	3674.68	0	12.93	531.81				8177
82	66	5	8110.9	3657.36	0	13.02	535.43				8111
83	67	5	9206.56	4115.28	0	13.04	531.09				9207
84	68	5	9155.18	4104.28	0	13.05	532.45				9155
85	69	5	7930.88	3579.12	0	12.89	531.63				7931
86	70	5	7890.13	3578.72	0	12.97	534.96				7890

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
87	71	5	8512.11	3838.28	0	12.99	532.6				8512
88	72	5	8485.23	3862.68	0	12.93	534.56				8486
89	73	5	8386.18	3827.4	0	12.89	535.25				8386
90	74	5	8473.94	3788.48	0	13.02	529.3				8474
91	75	5	8276.64	3742.4	0	13.04	535.64				8277
92	76	5	8320.12	3745.08	0	12.96	532.91				8320
93	77	5	9566.73	4300.69	0	12.86	530.25				9566
94	78	5	9588.92	4308.52	0	13.01	533.02				9589
95	79	5	9351.94	4217.08	0	13.02	534.6				9352
96	80	5	9582.32	4306.48	0	13.05	533.75				9582
97	81	5	8957.4	4013.52	0	13.06	532.73				8957
98	82	5	8992.13	4038.28	0	13.05	534.61				8992
99	83	5	10028.71	4548.12	0	13	538.3				10029
100	84	5	10180.92	4590.52	0	12.92	533.42				10181
101	85	5	10161.39	4578.16	0	12.96	535.24				10161
102	86	5	9992.84	4492.12	0	12.96	532.01				9993
103	87	5	10236.59	4642.16	0	12.94	535.8				10237
104	88	5	10311.08	4670.68	0	13	535				10311
105	89	5	10287.97	4618.36	0	13.02	532.5				10288
106	90	5	10114.04	4569.2	0	12.97	532.5				10114
107	91	5	9966.99	4491.72	0	12.99	534.51				9967
108	92	5	9926.35	4455.48	0	12.95	531.56				9926
109	93	5	10545.25	4703.76	0	13.02	530.53				10545
110	94	5	10548.32	4741.8	0	13	531.58				10548
111	95	5	9959.96	4457.76	0	13.02	532.49				9960
112	96	5	10114.48	4534.48	0	12.99	531.48				10114
113	97	5	9951.96	4478.6	0	12.95	533.56				9952
114	98	5	10197.85	4591.92	0	12.99	531.65				10198
115	99	5	10371.01	4681.28	0	12.98	535.55				10371
116	100	5	10605.71	4767.88	0	12.93	530.6				10606
117	101	5	9953.12	4471.48	0	13	535.15				9953
118	102	5	9643.73	4350.52	0	12.97	533.66				9644
119	103	5	9641.92	4445.72	0	12.97	533.55				9642
120	104	5	9771.66	4427.88	0	13	534.28				9772
121	105	5	10738.11	4814.56	0	12.97	531.98				10738
122	106	5	10440.43	4692.36	0	12.93	530.86				10440
123	108	5	234303.52	53790.88	0	9.09	248.25				234304
124											0
125											0
126											0
127											0
128											0
129											0
130											0
131											0
132											0
133											0

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Assay Date	Assay Date	Assay Date	Test Chemical ID	Chemical ID	# Concentrations tested	8 Microsomes ID	72355-13							
2/27/2007	2/27/2007	2/27/2007	2/27/2007	CR116113	CR116113	2	23FE0007 P00	4-OH ASDM	Free Cell Tubes/Cont	2					
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
6	6	6	6	6	6	6	6	6	6	6	6	6	6	6	6
7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8
9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9
10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
11	11	11	11	11	11	11	11	11	11	11	11	11	11	11	11
12	12	12	12	12	12	12	12	12	12	12	12	12	12	12	12
13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13
14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14
15	15	15	15	15	15	15	15	15	15	15	15	15	15	15	15
16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16
17	17	17	17	17	17	17	17	17	17	17	17	17	17	17	17
18	18	18	18	18	18	18	18	18	18	18	18	18	18	18	18
19	19	19	19	19	19	19	19	19	19	19	19	19	19	19	19
20	20	20	20	20	20	20	20	20	20	20	20	20	20	20	20
21	21	21	21	21	21	21	21	21	21	21	21	21	21	21	21
22	22	22	22	22	22	22	22	22	22	22	22	22	22	22	22
23	23	23	23	23	23	23	23	23	23	23	23	23	23	23	23
24	24	24	24	24	24	24	24	24	24	24	24	24	24	24	24
25	25	25	25	25	25	25	25	25	25	25	25	25	25	25	25
26	26	26	26	26	26	26	26	26	26	26	26	26	26	26	26
27	27	27	27	27	27	27	27	27	27	27	27	27	27	27	27
28	28	28	28	28	28	28	28	28	28	28	28	28	28	28	28
29	29	29	29	29	29	29	29	29	29	29	29	29	29	29	29
30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
31	31	31	31	31	31	31	31	31	31	31	31	31	31	31	31
32	32	32	32	32	32	32	32	32	32	32	32	32	32	32	32
33	33	33	33	33	33	33	33	33	33	33	33	33	33	33	33
34	34	34	34	34	34	34	34	34	34	34	34	34	34	34	34
35	35	35	35	35	35	35	35	35	35	35	35	35	35	35	35
36	36	36	36	36	36	36	36	36	36	36	36	36	36	36	36
37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37
38	38	38	38	38	38	38	38	38	38	38	38	38	38	38	38
39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39
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41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
42	42	42	42	42	42	42	42	42	42	42	42	42	42	42	42
43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44
45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45
46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46
47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47
48	48	48	48	48	48	48	48	48	48	48	48	48	48	48	48
49	49	49	49	49	49	49	49	49	49	49	49	49	49	49	49
50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
51	51	51	51	51	51	51	51	51	51	51	51	51	51	51	51
52	52	52	52	52	52	52	52	52	52	52	52	52	52	52	52
53	53	53	53	53	53	53	53	53	53	53	53	53	53	53	53
54	54	54	54	54	54	54	54	54	54	54	54	54	54	54	54
55	55	55	55	55	55	55	55	55	55	55	55	55	55	55	55
56	56	56	56	56	56	56	56	56	56	56	56	56	56	56	56
57	57	57	57	57	57	57	57	57	57	57	57	57	57	57	57
58	58	58	58	58	58	58	58	58	58	58	58	58	58	58	58
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61	61	61	61	61	61	61	61	61	61	61	61	61	61	61	61
62	62	62	62	62	62	62	62	62	62	62	62	62	62	62	62
63	63	63	63	63	63	63	63	63	63	63	63	63	63	63	63
64	64	64	64	64	64	64	64	64	64	64	64	64	64	64	64
65	65	65	65	65	65	65	65	65	65	65	65	65	65	65	65
66	66	66	66	66	66	66	66	66	66	66	66	66	66	66	66
67	67	67	67	67	67	67	67	67	67	67	67	67	67	67	67
68	68	68	68	68	68	68	68	68	68	68	68	68	68	68	68
69	69	69	69	69	69	69	69	69	69	69	69	69	69	69	69
70	70	70	70	70	70	70	70	70	70	70	70	70	70	70	70

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		OC (dark) - acquired variance			
6		Read if value not within mean $\pm$ 10%			
7		0.99			
8		1.01			
9		1.01			
10		0.99			
11		1.00			
12		1.00			
13		1.01			
14		0.99			
15					
16					
17		0.97			
18		1.01			
19		1.01			
20		0.99			
21		0.93			
22		1.07			
23					
24					
25		1.03			
26		0.97			
27					
28					
29		0.99			
30		1.01			
31		0.98			
32		1.02			
33					
34					
35		1.01			
36		0.99			
37		1.01			
38		0.99			
39					
40		0.99			
41		1.01			
42		1.01			
43		1.01			
44		0.99			
45					
46					
47		1.00			
48		1.00			
49		0.99			
50		1.01			
51					
52		1.01			
53		0.99			
54		1.00			
55		1.00			
56		1.00			
57					
58					
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63					
64					
65		1.00			
66		1.00			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
		Assay Date	2/22/2007	Test Chemical	CR11613	# Concentrations tested	12595-13									
1				Assay ID	12595-4H	24FED007 P00										
2				Assay Ref.	12595-4H	24FED007 P00										
3				Technician ID	1	Positive Control	4-OH-ASDMS Pos. C03 TubesCode									
4																
5	Sample ID	Replicate	Aliq	Calculation	DPM in aliquot	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (mL)	Calculate netal ³ H ₂ O formed	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)	
6	CR11613	1-1	2	0.5	1	7177	14354	28714	0.1	183756	28610	0.0304	0.005	15	0.203	
7	CR11613	1-2	2	0.5	2	7260	14580	29140	0.1	187756	28944	0.0276	0.005	15	0.184	
8	CR11613	1-3	2	0.5	3	6509	13018	26036	0.1	187756	28944	0.0276	0.005	15	0.176	
9	CR11613	1-4	2	0.5	4	6555	13110	26220	0.1	187756	28944	0.0276	0.005	15	0.228	
10	CR11613	1-5	2	0.5	5	6144	12288	24576	0.1	187756	28944	0.0276	0.005	15	0.043	
11	CR11613	2-1	2	0.5	1	6397	12794	25588	0.1	187756	28944	0.0276	0.005	15	0.258	
12	CR11613	2-2	2	0.5	2	6177	12354	24708	0.1	187756	28944	0.0276	0.005	15	0.222	
13	CR11613	2-3	2	0.5	3	6111	12222	24444	0.1	187756	28944	0.0276	0.005	15	0.237	
14	CR11613	2-4	2	0.5	4	6207	12414	24828	0.1	187756	28944	0.0276	0.005	15	0.282	
15	CR11613	2-5	2	0.5	5	6135	12270	24540	0.1	187756	28944	0.0276	0.005	15	0.283	
16	CR11613	3-1	2	0.5	1	7031	14062	28124	0.1	187756	28944	0.0276	0.005	15	0.237	
17	CR11613	3-2	2	0.5	2	7080	14160	28320	0.1	187756	28944	0.0276	0.005	15	0.233	
18	CR11613	3-3	2	0.5	3	8512	17024	34048	0.1	187756	28944	0.0276	0.005	15	0.237	
19	CR11613	3-4	2	0.5	4	8486	16972	33944	0.1	187756	28944	0.0276	0.005	15	0.237	
20	CR11613	3-5	2	0.5	5	8474	16948	33896	0.1	187756	28944	0.0276	0.005	15	0.237	
21	CR11613	4-1	2	0.5	1	8271	16542	33084	0.1	187756	28944	0.0276	0.005	15	0.237	
22	CR11613	4-2	2	0.5	2	8320	16640	33280	0.1	187756	28944	0.0276	0.005	15	0.237	
23	CR11613	4-3	2	0.5	3	8474	16948	33896	0.1	187756	28944	0.0276	0.005	15	0.237	
24	CR11613	4-4	2	0.5	4	8512	17024	34048	0.1	187756	28944	0.0276	0.005	15	0.237	
25	CR11613	4-5	2	0.5	5	8486	16972	33944	0.1	187756	28944	0.0276	0.005	15	0.237	
26	CR11613	5-1	2	0.5	1	10029	20058	40116	0.1	187756	28944	0.0276	0.005	15	0.237	
27	CR11613	5-2	2	0.5	2	10181	20362	40724	0.1	187756	28944	0.0276	0.005	15	0.237	
28	CR11613	5-3	2	0.5	3	9943	19886	39772	0.1	187756	28944	0.0276	0.005	15	0.237	
29	CR11613	5-4	2	0.5	4	10237	20474	40948	0.1	187756	28944	0.0276	0.005	15	0.237	
30	CR11613	5-5	2	0.5	5	10288	20576	41152	0.1	187756	28944	0.0276	0.005	15	0.237	
31	CR11613	6-1	2	0.5	1	10311	20622	41244	0.1	187756	28944	0.0276	0.005	15	0.237	
32	CR11613	6-2	2	0.5	2	10311	20622	41244	0.1	187756	28944	0.0276	0.005	15	0.237	
33	CR11613	6-3	2	0.5	3	10311	20622	41244	0.1	187756	28944	0.0276	0.005	15	0.237	
34	CR11613	6-4	2	0.5	4	10311	20622	41244	0.1	187756	28944	0.0276	0.005	15	0.237	
35	CR11613	6-5	2	0.5	5	10311	20622	41244	0.1	187756	28944	0.0276	0.005	15	0.237	
36	CR11613	7-1	2	0.5	1	10545	21090	42180	0.1	187756	28944	0.0276	0.005	15	0.237	
37	CR11613	7-2	2	0.5	2	10545	21090	42180	0.1	187756	28944	0.0276	0.005	15	0.237	
38	CR11613	7-3	2	0.5	3	10545	21090	42180	0.1	187756	28944	0.0276	0.005	15	0.237	
39	CR11613	7-4	2	0.5	4	10545	21090	42180	0.1	187756	28944	0.0276	0.005	15	0.237	
40	CR11613	7-5	2	0.5	5	10545	21090	42180	0.1	187756	28944	0.0276	0.005	15	0.237	
41	CR11613	8-1	2	0.5	1	10736	21472	42944	0.1	187756	28944	0.0276	0.005	15	0.237	
42	CR11613	8-2	2	0.5	2	10736	21472	42944	0.1	187756	28944	0.0276	0.005	15	0.237	
43	CR11613	8-3	2	0.5	3	10736	21472	42944	0.1	187756	28944	0.0276	0.005	15	0.237	
44	CR11613	8-4	2	0.5	4	10736	21472	42944	0.1	187756	28944	0.0276	0.005	15	0.237	
45	CR11613	8-5	2	0.5	5	10736	21472	42944	0.1	187756	28944	0.0276	0.005	15	0.237	
46	CR11613	9-1	2	0.5	1	10945	21890	43780	0.1	187756	28944	0.0276	0.005	15	0.237	
47	CR11613	9-2	2	0.5	2	10945	21890	43780	0.1	187756	28944	0.0276	0.005	15	0.237	
48	CR11613	9-3	2	0.5	3	10945	21890	43780	0.1	187756	28944	0.0276	0.005	15	0.237	
49	CR11613	9-4	2	0.5	4	10945	21890	43780	0.1	187756	28944	0.0276	0.005	15	0.237	
50	CR11613	9-5	2	0.5	5	10945	21890	43780	0.1	187756	28944	0.0276	0.005	15	0.237	
51	CR11613	10-1	2	0.5	1	11155	22310	44620	0.1	187756	28944	0.0276	0.005	15	0.237	
52	CR11613	10-2	2	0.5	2	11155	22310	44620	0.1	187756	28944	0.0276	0.005	15	0.237	
53	CR11613	10-3	2	0.5	3	11155	22310	44620	0.1	187756	28944	0.0276	0.005	15	0.237	
54	CR11613	10-4	2	0.5	4	11155	22310	44620	0.1	187756	28944	0.0276	0.005	15	0.237	
55	CR11613	10-5	2	0.5	5	11155	22310	44620	0.1	187756	28944	0.0276	0.005	15	0.237	

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC Check - aliquot variance
5		
6		% RE
7		0.99
8		1.01
9		0.96
10		1.01
11		0.98
12		1.02
13		1.00
14		1.00
15		1.00
16		1.00
17		1.00
18		1.00
19		1.00
20		1.00
21		0.99
22		1.01
23		1.00
24		1.00
25		1.00
26		1.00
27		0.98
28		1.01
29		1.00
30		1.00
31		0.99
32		1.01
33		1.01
34		0.98
35		1.00
36		1.00
37		1.01
38		0.99
39		1.00
40		1.00
41		1.00
42		1.00
43		0.99
44		1.00
45		0.99
46		1.01
47		0.99
48		1.01
49		1.02
50		0.98
51		1.00
52		1.00
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	2/22/2007	Test Chemical ID	CR11613	# Concentrations tested					
2		NB Ref	12535-48	Counter data file name	23FE0007.P00	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
3		Technician ID	ase	Replicate #							
4	Control Type	Average	SD								
5	Full activity	0.289	0.019								
6	Background (uncorrected)	0.002	0.000								
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.002						
12	4-OH ASDN	1	2	-5.00	0.002						
13	4-OH ASDN	2	1	-6.00	0.019						
14	4-OH ASDN	2	2	-6.00	0.018						
15	4-OH ASDN	3	1	-6.30	0.033						
16	4-OH ASDN	3	2	-6.30	0.030						
17	4-OH ASDN	4	1	-7.00	0.113						
18	4-OH ASDN	4	2	-7.00	0.105						
19	4-OH ASDN	5	1	-7.30	0.161						
20	4-OH ASDN	5	2	-7.30	0.158						
21	4-OH ASDN	6	1	-8.00	0.239						
22	4-OH ASDN	6	2	-8.00	0.238						
23	4-OH ASDN	7	1	-9.00	0.257						
24	4-OH ASDN	7	2	-9.00	0.272						
25	4-OH ASDN	8	1	-10.00	0.252						
26	4-OH ASDN	8	2	-10.00	0.200						
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
38	CR11613	1	1	-3.00	0.203						
39	CR11613	1	2	-3.00	0.184						
40	CR11613	2	1	-3.00	0.176						
41	CR11613	2	2	-4.00	0.229						
42	CR11613	3	1	-4.00	0.258						
43	CR11613	3	2	-4.00	0.222						
44	CR11613	4	1	-5.00	0.239						
45	CR11613	4	2	-5.00	0.237						
46	CR11613	5	1	-6.00	0.233						
47	CR11613	5	2	-6.00	0.269						
48	CR11613	6	1	-6.00	0.266						
49	CR11613	6	2	-6.00	0.252						
50	CR11613	7	1	-7.00	0.284						
51	CR11613	7	2	-7.00	0.283						
52	CR11613	8	1	-7.00	0.289						
53	CR11613	8	2	-8.00	0.287						
54	CR11613	9	1	-8.00	0.280						
55	CR11613	9	2	-8.00	0.297						
56	CR11613	10	1	-8.00	0.282						
57	CR11613	10	2	-9.00	0.283						
58	CR11613	11	1	-9.00	0.295						
59	CR11613	11	2	-10.00	0.278						
60	CR11613	12	1	-10.00	0.276						
61	CR11613	12	2	-10.00	0.296						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/27/2007	Test Chemical ID	CR11613	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-51	Counter data filename	27FE2255.P00					
3	Technician	ase	Replicate #	2	Positive Control	4-OH.ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/27/2007	Test Chemical ID	CR11613	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-51	Counter data filename	27FE2255.P00					
3	Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0207		37562		1814589			
8	2	0.0206		37685		1829369			
9	3	0.0198		38888		1964040			
10	4	0.0205		40240		1962927			
11	5	0.0199		38600		1939698			
12						Average DPM/g soln		1902125	
13						SD		73990	
14						CV		3.89	
15						µCi/g soln		0.8568	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution							8.0842 g	
25	Mass of dilution B used in substrate prep							4.5389 g	
26	Concentration of nonradiolabeled ASDN in substrate soln.							0.56089175 µg/g	
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.010 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8568			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					=	0.5609	+	0.010	
37					=	0.571	µg ASDN/g soln.		
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=					1.500 µCi/µg ASDN			
41						9.535.E+05 dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	2/27/2007	Test Chemical ID	CR11613	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-51	Counter data filename	27FE2255.P00					
3			Technician ID	ase	Replicate #	2	Positive Control	4-QH ASDN	Pos Ctl	Tubes/Conc	2
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
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20											
21											
22											
23											
24											
25											
26											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	
	Test											
1	Ae 2/27/2007		Chemical ID		# Concentrations tested		8 Microsome ID 12535-13					
2	NI 12535-51		Counter data filename		27FE2255.P00							
3	Pos Ctrl											
4	D ase		Replicate # 2		Positive Control		4-OH ASDI Tubes/Conc				2	
5	Vial # Ttl DPM1				ISIE						Round DPM	
6	2	5	37561.64	15775.1	0	12.19	479.06				37562	
7	3	5	37685.11	18401.46	0	13.97	613.7				37685	
8	4	5	38887.53	18953.46	0	14.05	614.03				38888	
9	5	5	40240.31	19597.26	0	13.92	610.12				40240	
10	6	5	38599.61	18907.66	0	14	616.36				38600	
11	8	5	14205.75	6466.5	0	12.96	539.23				14206	
12	9	5	14261.87	6462.06	0	12.95	536.02				14262	
13	10	5	13545.45	6173.54	0	12.87	537.13				13545	
14	11	5	13658.36	6206.98	0	13.01	539.69				13658	
15	12	5	11665.43	5292.3	0	12.97	537.88				11665	
16	13	5	11375.45	5166.46	0	12.94	537.14				11375	
17	14	5	12348.72	5568.26	0	12.97	535.05				12349	
18	15	5	12539.21	5691.86	0	12.88	534.39				12539	
19	17	5	74.75	35.1	0	14.25	537.58				75	
20	18	5	59.41	28.3	0	11.78	536.46				59	
21	19	5	60.85	31.26	0	11.01	537.56				61	
22	20	5	56.44	27.26	0	13.87	537.53				56	
23	21	5	68.7	33.9	0	14.8	537.44				69	
24	22	5	56.31	30.5	0	10.88	533.99				56	
25	23	5	68.91	30.66	0	13.59	534.41				69	
26	24	5	65.89	31.06	0	12.67	536.91				66	
27	26	5	148.83	71.66	0	11.63	534.05				149	
28	27	5	166.93	77.5	0	12.81	535.26				167	
29	28	5	149.42	67.5	0	13.83	534.37				149	
30	29	5	164.62	75.7	0	13.27	535.65				165	
31												
32												
33	30	5	886.89	404.94	0	13	536.01				887	
34	31	5	886.68	403.46	0	13	535.19				887	
35	32	5	803.03	365.46	0	12.8	535.22				803	
36	33	5	849.74	394.46	0	12.68	532.53				850	
37												
38												
39	34	5	1543.22	701.3	0	12.88	534.38				1543	
40	35	5	1518.3	698.86	0	12.73	535.5				1518	
41	36	5	1550.66	708.1	0	12.88	538.92				1551	
42	37	5	1609.26	729.46	0	12.77	536.54				1609	
43												
44												
45	38	5	4973.4	2263.46	0	12.88	536.53				4973	
46	39	5	5267.07	2390.06	0	12.9	537.94				5267	
47	40	5	4947.34	2244.06	0	12.88	537.42				4947	
48	41	5	5153.54	2366.14	0	12.78	536.63				5154	
49												
50												
51	42	5	7234.7	3275.46	0	12.86	535.18				7235	
52	43	5	7339.49	3325.3	0	12.91	536.99				7339	
53	44	5	7151.4	3226.7	0	13.01	536.26				7151	
54	45	5	7375.94	3328.3	0	12.85	534.21				7376	
55												
56												
57	46	5	11661.19	5255.1	0	12.9	533.46				11661	
58	47	5	11692.01	5452.3	0	12.91	535.29				11692	
59	48	5	11105.28	5049.86	0	12.9	536.86				11105	
60	49	5	11013.85	5039.54	0	12.82	535.13				11014	
61												
62												
63	50	5	13312.62	6032.06	0	12.84	532.75				13313	
64	51	5	13491.35	6117.74	0	12.91	535.96				13491	

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	12387.6	5593.7	0	12.87	532.85				12388
66	53	5	12639.28	5702.34	0	12.96	533.65				12639
67											
68											
69	54	5	12754.12	5838.54	0	12.85	539.21				12754
70	55	5	13059.13	5964.46	0	12.95	539.41				13059
71	56	5	12796.21	5824.1	0	12.94	537.25				12796
72	57	5	12913.21	5879.06	0	12.93	535.96				12913
73											
74											
75	58	5	11182.27	5088.26	0	12.88	534.73				11182
76	60	5	11202.08	5091.46	0	12.86	537.4				11202
77	61	5	11953.95	5442.06	0	12.99	539.26				11954
78	62	5	12032.01	5440.46	0	12.92	533.49				12032
79	63	5	11393.35	5190.9	0	12.9	536.12				11393
80	64	5	11501.75	5230.86	0	12.89	536.65				11502
81	65	5	13246.08	5960.06	0	12.94	532.93				13246
82	66	5	13485.03	6117.1	0	12.9	536.82				13485
83	67	5	12211.95	5546.26	0	12.9	535.78				12212
84	68	5	12445.09	5655.06	0	12.97	535.62				12445
85	69	5	12191.99	5530.64	0	12.97	538.63				12192
86	70	5	12289.07	5539.9	0	12.91	535.01				12289
87	71	5	13749.7	6272.9	0	12.88	537.23				13750
88	72	5	13549.95	6139.7	0	12.92	534.71				13550
89	73	5	13971.72	6330.74	0	12.92	537.72				13972
90	74	5	14061.79	6389.1	0	12.97	538.15				14062
91	75	5	12779.09	5818.3	0	12.88	535.78				12779
92	76	5	13046.73	5922.66	0	12.85	532.89				13047
93	77	5	14559.01	6612.06	0	12.9	538.14				14559
94	78	5	14586.76	6612.46	0	12.93	536.6				14587
95	79	5	13786.51	6263.26	0	12.82	534.27				13787
96	80	5	13869.73	6259.7	0	12.94	534.76				13870
97	81	5	13672.69	6198.5	0	12.93	535.09				13673
98	82	5	13921.45	6303.06	0	12.9	536.4				13921
99	84	5	14579.99	6593.06	0	12.96	536.56				14580
100	85	5	14808.82	6701.1	0	12.87	533.89				14809
101	86	5	14144.86	6417.7	0	12.83	533.83				14145
102	87	5	14305.98	6509.06	0	12.92	536.71				14306
103	88	5	14222.17	6443.46	0	12.89	535.16				14222
104	89	5	14519.96	6573.14	0	12.92	535.79				14520
105	90	5	13928.99	6313.26	0	12.84	535.58				13929
106	91	5	13963.54	6315.86	0	12.84	533.8				13964
107	92	5	13765.68	6253.5	0	12.88	536.5				13766
108	93	5	13747.04	6232.46	0	12.86	536.07				13747
109	94	5	14475.18	6556.14	0	12.93	534.95				14475
110	95	5	14562.32	6631.86	0	12.88	538.21				14562
111	96	5	13225.67	6040.74	0	12.9	536.3				13226
112	97	5	13348.88	6056.94	0	12.92	535.12				13349
113	98	5	13504.48	6148.66	0	12.82	534.12				13504
114	99	5	13354.1	6034.5	0	12.84	534.24				13354
115	100	5	14562.62	6601.18	0	12.88	534.63				14563
116	101	5	14541.72	6580.86	0	12.9	533.65				14542
117	102	5	13523.42	6153.1	0	12.9	534.57				13523
118	103	5	14036.93	6354.58	0	12.93	533.91				14037
119	104	5	14283.98	6464.3	0	12.95	535.57				14284
120	105	5	14298.35	6477.66	0	12.9	533.56				14298
121	106	5	13429.43	6062.3	0	12.93	533.33				13429
122	107	5	13559.06	6127.06	0	12.96	535.09				13559
123	109	5	230692.02	53725.86	0	9.02	248.72				230692

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicat #	Nominal total volume (mL)	Aliq Volume (mL)	Aliq #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPH	Volume of substrate solution used/assay (mL)	total DPM as assay tube (mL)	Calculate DPM corrected for background (background value)	nmol ³ H ₂ O formed	Final protein in assay (mg/mL)	Inhibition area (nmol forming product/min)	Aromatase activity (nmol estrogen forming product/min)
1	1	2	0.5	1	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
2	2	2	0.5	2	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
3	3	2	0.5	3	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
4	4	2	0.5	4	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
5	5	2	0.5	5	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
6	6	2	0.5	6	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
7	7	2	0.5	7	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
8	8	2	0.5	8	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
9	9	2	0.5	9	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
10	10	2	0.5	10	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
11	11	2	0.5	11	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
12	12	2	0.5	12	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
13	13	2	0.5	13	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
14	14	2	0.5	14	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
15	15	2	0.5	15	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
16	16	2	0.5	16	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
17	17	2	0.5	17	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
18	18	2	0.5	18	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
19	19	2	0.5	19	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
20	20	2	0.5	20	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
21	21	2	0.5	21	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
22	22	2	0.5	22	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
23	23	2	0.5	23	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
24	24	2	0.5	24	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
25	25	2	0.5	25	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
26	26	2	0.5	26	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
27	27	2	0.5	27	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
28	28	2	0.5	28	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
29	29	2	0.5	29	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
30	30	2	0.5	30	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
31	31	2	0.5	31	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
32	32	2	0.5	32	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
33	33	2	0.5	33	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
34	34	2	0.5	34	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
35	35	2	0.5	35	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
36	36	2	0.5	36	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
37	37	2	0.5	37	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
38	38	2	0.5	38	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
39	39	2	0.5	39	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
40	40	2	0.5	40	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
41	41	2	0.5	41	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
42	42	2	0.5	42	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
43	43	2	0.5	43	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
44	44	2	0.5	44	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
45	45	2	0.5	45	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
46	46	2	0.5	46	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
47	47	2	0.5	47	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
48	48	2	0.5	48	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
49	49	2	0.5	49	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
50	50	2	0.5	50	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
51	51	2	0.5	51	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
52	52	2	0.5	52	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
53	53	2	0.5	53	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
54	54	2	0.5	54	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
55	55	2	0.5	55	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
56	56	2	0.5	56	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
57	57	2	0.5	57	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
58	58	2	0.5	58	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
59	59	2	0.5	59	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
60	60	2	0.5	60	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
61	61	2	0.5	61	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
62	62	2	0.5	62	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
63	63	2	0.5	63	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
64	64	2	0.5	64	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
65	65	2	0.5	65	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
66	66	2	0.5	66	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
67	67	2	0.5	67	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
68	68	2	0.5	68	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
69	69	2	0.5	69	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349
70	70	2	0.5	70	14206	28412	28408	56816	0.1	190212	5681	0.0584	0.005	15	0.349

Aromatase Assay Spreadsheet

	R	S	T	U	V
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5		QC check - adjust variance			
6		Read if values not within mean $\pm$ 10%			
7		1.00			
8		1.00			
9		1.00			
10		1.00			
11		1.01			
12		0.99			
13		0.99			
14		1.01			
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicates/Level	Nominal total volume (nL)	Aliq Volume (nL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (nL)	Total DPM in assay tube (initial)	Total DPM corrected for background (Background tubes)	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
1	CR11513 1-1	2	0.5	1	11182	22364	22364	44768	0.1	180212	44513	0.0467	0.005	15	0.311
2	CR11513 1-2	2	0.5	2	11202	22404	22404	44792	0.1	190212	47717	0.0500	0.005	15	0.334
3	CR11513 1-3	2	0.5	1	11954	23908	23908	47816	0.1	190212	47535	0.0478	0.005	15	0.318
4	CR11513 2-1	2	0.5	2	12032	24064	24064	48128	0.1	190212	48535	0.0558	0.005	15	0.372
5	CR11513 2-2	2	0.5	1	13047	26094	26094	52188	0.1	190212	52037	0.0577	0.005	15	0.385
6	CR11513 2-3	2	0.5	2	13459	26918	26918	53836	0.1	190212	49056	0.0515	0.005	15	0.343
7	CR11513 3-1	2	0.5	1	12112	24224	24224	48448	0.1	190212	48707	0.0511	0.005	15	0.341
8	CR11513 3-2	2	0.5	2	12268	24576	24576	49152	0.1	190212	48707	0.0511	0.005	15	0.341
9	CR11513 3-3	2	0.5	1	13550	27100	27300	54600	0.1	190212	54345	0.0570	0.005	15	0.380
10	CR11513 4-1	2	0.5	1	13872	27844	28034	56068	0.1	190212	55413	0.0585	0.005	15	0.390
11	CR11513 4-2	2	0.5	2	14062	28124	28626	5652	0.1	190212	51397	0.0559	0.005	15	0.359
12	CR11513 4-3	2	0.5	1	12779	25558	25826	51652	0.1	190212	58037	0.0609	0.005	15	0.406
13	CR11513 5-1	2	0.5	2	13711	27424	28146	56202	0.1	190212	58037	0.0609	0.005	15	0.406
14	CR11513 5-2	2	0.5	1	14857	29714	29714	59428	0.1	190212	58037	0.0609	0.005	15	0.406
15	CR11513 6-1	2	0.5	1	13787	27574	27657	55314	0.1	190212	56059	0.0577	0.005	15	0.385
16	CR11513 6-2	2	0.5	2	13670	27340	27594	55168	0.1	190212	54933	0.0576	0.005	15	0.384
17	CR11513 6-3	2	0.5	1	13673	27346	27594	55168	0.1	190212	54933	0.0576	0.005	15	0.384
18	CR11513 7-1	2	0.5	1	13921	27842	28398	56778	0.1	190212	56523	0.0614	0.005	15	0.409
19	CR11513 7-2	2	0.5	2	14390	28760	29398	58792	0.1	190212	56523	0.0614	0.005	15	0.409
20	CR11513 7-3	2	0.5	1	14509	29018	29518	59032	0.1	190212	56523	0.0614	0.005	15	0.409
21	CR11513 8-1	2	0.5	1	14145	28280	28451	56902	0.1	190212	56647	0.0584	0.005	15	0.385
22	CR11513 8-2	2	0.5	2	14306	28612	28742	57484	0.1	190212	56647	0.0584	0.005	15	0.385
23	CR11513 8-3	2	0.5	1	14222	28444	28742	57484	0.1	190212	56647	0.0584	0.005	15	0.385
24	CR11513 9-1	2	0.5	2	14520	29040	29258	58516	0.1	190212	57229	0.0600	0.005	15	0.400
25	CR11513 9-2	2	0.5	1	13923	27858	27893	55766	0.1	190212	55811	0.0582	0.005	15	0.388
26	CR11513 9-3	2	0.5	2	13514	27036	27513	55026	0.1	190212	55811	0.0582	0.005	15	0.388
27	CR11513 10-1	2	0.5	1	12719	25434	25753	51506	0.1	190212	54771	0.0574	0.005	15	0.383
28	CR11513 10-2	2	0.5	2	14475	28950	29057	58114	0.1	190212	54771	0.0574	0.005	15	0.383
29	CR11513 10-3	2	0.5	1	14582	29164	29164	58328	0.1	190212	54771	0.0574	0.005	15	0.383
30	CR11513 11-1	2	0.5	2	13226	26462	26575	53150	0.1	190212	57959	0.0607	0.005	15	0.405
31	CR11513 11-2	2	0.5	1	13226	26462	26575	53150	0.1	190212	57959	0.0607	0.005	15	0.405
32	CR11513 11-3	2	0.5	2	13349	26696	26868	53716	0.1	190212	57959	0.0607	0.005	15	0.405
33	CR11513 12-1	2	0.5	1	13504	27008	26858	53716	0.1	190212	57959	0.0607	0.005	15	0.405
34	CR11513 12-2	2	0.5	2	13653	27326	27105	54210	0.1	190212	57959	0.0607	0.005	15	0.405
35	CR11513 12-3	2	0.5	1	14542	29084	29125	58250	0.1	190212	57959	0.0607	0.005	15	0.405
36	CR11513 13-1	2	0.5	2	14542	29084	29125	58250	0.1	190212	57959	0.0607	0.005	15	0.405
37	CR11513 13-2	2	0.5	1	13523	27048	27950	55320	0.1	190212	54655	0.0575	0.005	15	0.384
38	CR11513 13-3	2	0.5	2	14037	28074	28582	57164	0.1	190212	54655	0.0575	0.005	15	0.384
39	CR11513 14-1	2	0.5	1	14294	28594	28582	57164	0.1	190212	54655	0.0575	0.005	15	0.384
40	CR11513 14-2	2	0.5	2	14294	28594	28582	57164	0.1	190212	54655	0.0575	0.005	15	0.384
41	CR11513 14-3	2	0.5	1	14294	28594	28582	57164	0.1	190212	54655	0.0575	0.005	15	0.384
42	CR11513 15-1	2	0.5	2	14294	28594	28582	57164	0.1	190212	54655	0.0575	0.005	15	0.384
43	CR11513 15-2	2	0.5	1	14294	28594	28582	57164	0.1	190212	54655	0.0575	0.005	15	0.384
44	CR11513 15-3	2	0.5	2	14294	28594	28582	57164	0.1	190212	54655	0.0575	0.005	15	0.384

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	2/27/2007	Test Chemical ID	CR11613	# Concentrations tested			8	Microsoma ID	12535-13
2		NB Ref	12535-51	Counter data							
3		Technician ID	ase	Replicate #	27FE2255-P00	2	Positive Control	4-OH ASDN		Pos Cal Tubes/Conc	2
4	Control Type	Average	SD								
5	Full activity	Overall	0.360		Bkg % of Fall						
6	Background (unconnected)	Overall	0.002		0.5 (Red if >1%)						
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	2	2	1.00E-05	-5.00	0.003					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.023					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.021					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.041					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.042					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.141					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.138					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.202					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.201					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.329					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.308					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.373					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.348					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.358					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.358					
27	4-OH ASDN										
28	4-OH ASDN										
29	4-OH ASDN										
30	4-OH ASDN										
31	4-OH ASDN										
32	4-OH ASDN										
33	4-OH ASDN										
34	4-OH ASDN										
35	4-OH ASDN										
36	4-OH ASDN										
37	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
38	CR11613	1	1	1.00E-05	-5.00	0.111					
39	CR11613	1	2	1.00E-05	-5.00	0.334					
40	CR11613	2	1	1.00E-05	-5.00	0.318					
41	CR11613	2	2	1.00E-06	-6.00	0.372					
42	CR11613	2	3	1.00E-06	-6.00	0.343					
43	CR11613	3	1	1.00E-06	-6.00	0.341					
44	CR11613	3	2	1.00E-07	-7.00	0.380					
45	CR11613	3	3	1.00E-07	-7.00	0.359					
46	CR11613	4	1	5.00E-08	-7.30	0.406					
47	CR11613	4	2	5.00E-08	-7.30	0.385					
48	CR11613	4	3	5.00E-08	-7.30	0.384					
49	CR11613	5	1	1.00E-08	-8.00	0.409					
50	CR11613	5	2	1.00E-08	-8.00	0.396					
51	CR11613	5	3	1.00E-08	-8.00	0.400					
52	CR11613	6	1	5.00E-09	-8.30	0.388					
53	CR11613	6	2	5.00E-09	-8.30	0.383					
54	CR11613	6	3	5.00E-09	-8.30	0.405					
55	CR11613	7	1	1.00E-09	-9.00	0.372					
56	CR11613	7	2	1.00E-09	-9.00	0.374					
57	CR11613	7	3	1.00E-09	-9.00	0.405					
58	CR11613	8	1	1.00E-10	-10.00	0.384					
59	CR11613	8	2	1.00E-10	-10.00	0.388					
60	CR11613	8	3	1.00E-10	-10.00	0.376					
61	CR11613										

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/28/2007	Test Chemical ID	CR11613	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-54	Counter data filename	28FE2301.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	2/26/2007	Test Chemical ID	CR11613	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-54	Counter data filename	28FE2301.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0209		39848		1906603			
8	2	0.0201		37419		1861642			
9	3	0.0199		38974		1958492			
10	4	0.0196		39775		2029337			
11	5	0.0198		41541		2098030			
12						Average DPM/g soln		1970821	
13						SD		94608	
14						CV		4.80	
15						µCi/g soln		0.8878	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.0	10		1000.00				
20	Dilution A			100	10.00				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1337 g			
25	Mass of dilution B used in substrate prep					4.5801 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56310166 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8878			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5631	+	0.011		
37				=	0.574	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=				1.547	µCi/µg ASDN			
41					9.835.E+05	dpm/nmol			

Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1		As	2/28/2007	Test Chemical ID	# Concentrations	CI	tested	8	Microsome ID	12535-13	
2		NI	12535-54	Counter data filename	28FE2301.P00						
3		T							Pos Ctl		
4		e									
5		c									
6		h									
7		ni									
8		ci									
9		a									
10		n									
11		i									
12		D	ase	Replicate # 3	Positive Control	4-OH ASD	Tubes/Conc		2		
13											
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64											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	13518.51	6078.58	0	13	533.6				13519
66	53	5	13724.13	6174.14	0	13.01	533.19				13724
67											
68											
69	54	5	13967.71	6269.98	0	13.14	533.18				13968
70	55	5	13979.07	6305.58	0	13.06	536.05				13979
71	56	5	13934.04	6277.54	0	13.07	535.28				13934
72	57	5	13986.36	6273.58	0	13.09	534.14				13986
73											
74											
75	59	5	11995.56	5398.98	0	13.07	538.8				11996
76	60	5	12228.76	5501.34	0	13.01	535.55				12229
77	61	5	12043.63	5434.18	0	13	533.07				12044
78	62	5	12269.9	5548.38	0	12.97	534.93				12270
79	63	5	12008.73	5439.98	0	13.03	537.34				12009
80	64	5	12343.67	5582.18	0	13.04	537.19				12344
81	65	5	13957.39	6246.58	0	13.13	535.66				13957
82	66	5	13837.88	6211.98	0	13.08	536.44				13838
83	67	5	12837.72	5792.58	0	13.08	534.09				12838
84	68	5	13024.77	5869.94	0	13.05	538.17				13025
85	69	5	12918.02	5815.14	0	13.12	536.51				12918
86	70	5	13175.5	5913.62	0	13.09	532.87				13176
87	71	5	14157.55	6357.38	0	13.11	534.68				14158
88	72	5	14408.56	6479.74	0	13.08	534.9				14409
89	73	5	17115.59	7687.46	0	13.08	534.97				17116
90	74	5	17346.66	7804.02	0	13.09	536.93				17347
91	75	5	13835.72	6249.02	0	13.01	536.27				13836
92	76	5	14090.07	6351.78	0	13	536.79				14090
93	77	5	15175.56	6829.78	0	13.14	537.74				15176
94	78	5	15620.98	6980.94	0	13.17	535.58				15621
95	79	5	13423.86	6099.74	0	13	539.77				13424
96	80	5	13800.81	6228.18	0	13.03	535.95				13801
97	81	5	12931.05	5801.14	0	13.1	538.02				12931
98	82	5	13275.63	5965.58	0	13.06	534.94				13276
99	84	5	13731.75	6162.34	0	13.03	533.75				13732
100	85	5	13901.85	6262.26	0	13.02	537.76				13902
101	86	5	11759.94	5334.82	0	13.02	537				11760
102	87	5	11800.16	5292.22	0	13.11	534.29				11800
103	88	5	12812.84	5731.34	0	13.07	534.25				12813
104	89	5	12856	5783.58	0	13.01	532.81				12856
105	90	5	13868.7	6254.94	0	13.04	534.5				13869
106	91	5	14186.62	6366.22	0	13.07	533.21				14187
107	92	5	13499.38	6076.78	0	13.02	534.91				13499
108	93	5	13720.95	6151.34	0	13.03	532.71				13721
109	94	5	12586.94	5673.06	0	13.08	537.04				12587
110	95	5	12859.85	5778.62	0	13.08	536.51				12860
111	96	5	14234.78	6426.22	0	13.01	536.45				14235
112	97	5	14268.06	6404.54	0	13.07	534.74				14268
113	98	5	13193.47	5922.22	0	13.08	533.95				13193
114	99	5	13377.06	6002.02	0	13.04	531.97				13377
115	100	5	13814.74	6208.66	0	13.04	532.27				13815
116	101	5	14090.72	6331.58	0	13.04	533.25				14091
117	102	5	14221.92	6379.74	0	13.12	534.52				14222
118	103	5	14680.26	6590.06	0	13.06	533.73				14680
119	104	5	13230.86	5969.02	0	13.09	537.3				13231
120	105	5	13473.54	6044.78	0	13.06	534.45				13474

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
121	106	5	13130.5	5917.18	0	13.01	536.75				13131
122	107	5	13659.61	6122.02	0	13.02	534.28				13660
123	109	5	233255.75	53553.02	0	9.15	249.47				233256

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
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Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
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Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested	Test Chemical ID	8	Microsoms ID	12458-13									
Assay Date	Chemical ID	# Concentrations tested													

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		OC check - aliquot			
5		valencies			
6		Red if value not			
7		within mean $\pm$			
8		10%			
9		0.98			
10		1.02			
11		1.00			
12		1.01			
13		0.99			
14		1.00			
15		1.00			
16		0.98			
17					
18					
19		1.01			
20		0.99			
21		0.92			
22		0.95			
23		0.98			
24		1.01			
25					
26					
27					
28					
29		1.00			
30		1.00			
31		1.02			
32		0.99			
33					
34		0.99			
35		1.01			
36		1.00			
37		1.00			
38		1.00			
39					
40					
41		1.00			
42		1.00			
43		0.99			
44					
45					
46		1.01			
47		0.99			
48		1.00			
49		1.00			
50					
51					
52					
53		0.99			
54		1.01			
55		1.01			
56		0.99			
57					
58		1.00			
59		1.00			
60		0.98			
61		1.01			
62					
63					
64		1.00			
65		1.00			
66		1.00			
67		1.00			
68					
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate Level	Assay Date	Chemical ID	Test	CR11613	# Compromissions tested	6 Microsome ID	17535-13							
7	GR11613	2/26/2007	22035-54	28F2301 P100	3	Positive Control	4-OH ASDN Free Oil Tubes/Conc	2							
8	GR11613	2	0.5	1	11906	23897	24225	48430	0.1	197082	48164	0.0490	0.005	15	0.326
9	GR11613	2	0.5	2	12229	24458	24014	48578	0.1	197082	48342	0.0492	0.005	15	0.326
10	GR11613	2	0.5	1	12044	24088	24014	48578	0.1	197082	48342	0.0492	0.005	15	0.326
11	GR11613	2	0.5	2	12270	24540	24353	48708	0.1	197082	48420	0.0492	0.005	15	0.326
12	GR11613	2	0.5	1	12009	24518	24353	48708	0.1	197082	48420	0.0492	0.005	15	0.326
13	GR11613	2	0.5	2	12344	24088	24353	48708	0.1	197082	48420	0.0492	0.005	15	0.326
14	GR11613	2	0.5	1	13657	27114	27796	55590	0.1	197082	55504	0.0502	0.005	15	0.375
15	GR11613	2	0.5	2	13838	27076	25863	51726	0.1	197082	51440	0.0523	0.005	15	0.349
16	GR11613	2	0.5	1	13533	26586	25863	51726	0.1	197082	51440	0.0523	0.005	15	0.349
17	GR11613	2	0.5	2	13076	26590	25863	51726	0.1	197082	51440	0.0523	0.005	15	0.349
18	GR11613	2	0.5	1	12918	25838	25863	51726	0.1	197082	51440	0.0523	0.005	15	0.349
19	GR11613	2	0.5	2	13176	26592	25863	51726	0.1	197082	51440	0.0523	0.005	15	0.349
20	GR11613	2	0.5	1	14158	28316	28397	57134	0.1	197082	56648	0.0578	0.005	15	0.385
21	GR11613	2	0.5	2	14409	28818	28397	57134	0.1	197082	56648	0.0578	0.005	15	0.385
22	GR11613	2	0.5	1	13876	27672	27626	55852	0.1	197082	55556	0.0555	0.005	15	0.377
23	GR11613	2	0.5	2	14000	29160	27626	55852	0.1	197082	55556	0.0555	0.005	15	0.377
24	GR11613	2	0.5	1	15176	30352	30717	61594	0.1	197082	61308	0.0623	0.005	15	0.416
25	GR11613	2	0.5	2	15021	31242	30717	61594	0.1	197082	61308	0.0623	0.005	15	0.416
26	GR11613	2	0.5	1	15021	31242	27725	54450	0.1	197082	54184	0.0551	0.005	15	0.367
27	GR11613	2	0.5	2	15021	31242	27725	54450	0.1	197082	54184	0.0551	0.005	15	0.367
28	GR11613	2	0.5	1	13951	26592	26207	52414	0.1	197082	52128	0.0530	0.005	15	0.353
29	GR11613	2	0.5	2	13776	26592	26207	52414	0.1	197082	52128	0.0530	0.005	15	0.353
30	GR11613	2	0.5	1	13732	27464	27534	55258	0.1	197082	54982	0.0559	0.005	15	0.373
31	GR11613	2	0.5	2	13902	27604	27534	55258	0.1	197082	54982	0.0559	0.005	15	0.373
32	GR11613	2	0.5	1	11760	23520	23590	47720	0.1	197082	46334	0.0476	0.005	15	0.317
33	GR11613	2	0.5	2	11800	23900	23590	47720	0.1	197082	46334	0.0476	0.005	15	0.317
34	GR11613	2	0.5	1	12813	25626	25609	51138	0.1	197082	51052	0.0519	0.005	15	0.346
35	GR11613	2	0.5	2	12856	25712	25609	51138	0.1	197082	51052	0.0519	0.005	15	0.346
36	GR11613	2	0.5	1	13669	27738	28056	58112	0.1	197082	55526	0.0558	0.005	15	0.378
37	GR11613	2	0.5	2	14187	28374	28056	58112	0.1	197082	55526	0.0558	0.005	15	0.378
38	GR11613	2	0.5	1	13494	26998	27720	54440	0.1	197082	54154	0.0561	0.005	15	0.367
39	GR11613	2	0.5	2	13721	27442	27720	54440	0.1	197082	54154	0.0561	0.005	15	0.367
40	GR11613	2	0.5	1	13887	28114	28447	50884	0.1	197082	50608	0.0515	0.005	15	0.343
41	GR11613	2	0.5	2	14250	28760	28447	50884	0.1	197082	50608	0.0515	0.005	15	0.343
42	GR11613	2	0.5	1	14260	28760	28503	57006	0.1	197082	56720	0.0577	0.005	15	0.384
43	GR11613	2	0.5	2	14260	28760	28503	57006	0.1	197082	56720	0.0577	0.005	15	0.384
44	GR11613	2	0.5	1	13191	26396	26570	53140	0.1	197082	52854	0.0537	0.005	15	0.353
45	GR11613	2	0.5	2	13191	26396	26570	53140	0.1	197082	52854	0.0537	0.005	15	0.353
46	GR11613	2	0.5	1	13815	28764	27806	55812	0.1	197082	55526	0.0556	0.005	15	0.376
47	GR11613	2	0.5	2	14051	29182	27806	55812	0.1	197082	55526	0.0556	0.005	15	0.376
48	GR11613	2	0.5	1	14222	28444	28902	57604	0.1	197082	57318	0.0585	0.005	15	0.380
49	GR11613	2	0.5	2	14680	29350	28902	57604	0.1	197082	57318	0.0585	0.005	15	0.380
50	GR11613	2	0.5	1	13331	26462	26705	53410	0.1	197082	53174	0.0540	0.005	15	0.360
51	GR11613	2	0.5	2	13474	26946	26705	53410	0.1	197082	53174	0.0540	0.005	15	0.360
52	GR11613	2	0.5	1	13131	26282	26791	53542	0.1	197082	53298	0.0542	0.005	15	0.361
53	GR11613	2	0.5	2	13131	26282	26791	53542	0.1	197082	53298	0.0542	0.005	15	0.361
54	GR11613	2	0.5	1	13660	27930									

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		QC Check - aliquot variance
5		
6		% RE
7		0.99
8		1.01
9		0.99
10		1.01
11		1.01
12		1.01
13		1.00
14		1.00
15		0.99
16		1.01
17		0.99
18		1.01
19		0.99
20		1.01
21		RVALUE
22		RVALUE
23		0.99
24		1.01
25		0.99
26		1.01
27		0.99
28		1.01
29		0.99
30		1.01
31		0.99
32		1.01
33		1.00
34		1.00
35		1.00
36		0.99
37		0.99
38		1.01
39		0.99
40		1.01
41		0.99
42		1.01
43		1.00
44		1.00
45		0.99
46		1.01
47		0.99
48		1.01
49		0.99
50		1.02
51		0.99
52		1.01
53		0.99
54		1.02

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	2/28/2007	Test Chemical ID	CR11613	# Concentrations tested					
2		NB Ref. Technician ID	12535-54	Counter data	28FEZ3D1P00				8	Microsome ID	12535-13
3				Replicate #		3	Positive Control	4-OH ASDN	Pos Ctrl Tubes/Conc		2
4	Control Type	Average	SD								
5	Full activity	0.357	0.016								
6	Background (uncorrected)	0.002	0.000								
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive CUI) M	Activity						
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.026					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.022					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.043					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.047					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.151					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.150					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.226					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.226					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.336					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.328					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.335					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.367					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.377					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.377					
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical) M	Activity						
38	CR11613	1	1	1.00E-05	-5.00	0.326					
39	CR11613	1	2	1.00E-05	-5.00	0.328					
40	CR11613	1	3	1.00E-05	-5.00	0.328					
41	CR11613	2	1	1.00E-06	-6.00	0.375					
42	CR11613	2	2	1.00E-06	-6.00	0.349					
43	CR11613	2	3	1.00E-06	-6.00	0.352					
44	CR11613	3	1	1.00E-07	-7.00	0.385					
45	CR11613	3	2	1.00E-07	-7.00	0.377					
46	CR11613	3	3	1.00E-07	-7.00	0.416					
47	CR11613	4	1	5.00E-08	-7.30	0.367					
48	CR11613	4	2	5.00E-08	-7.30	0.353					
49	CR11613	4	3	5.00E-08	-7.30	0.373					
50	CR11613	5	1	1.00E-08	-8.00	0.317					
51	CR11613	5	2	1.00E-08	-8.00	0.317					
52	CR11613	5	3	1.00E-08	-8.00	0.346					
53	CR11613	6	1	5.00E-09	-8.30	0.378					
54	CR11613	6	2	5.00E-09	-8.30	0.367					
55	CR11613	6	3	5.00E-09	-8.30	0.343					
56	CR11613	7	1	1.00E-09	-9.00	0.384					
57	CR11613	7	2	1.00E-09	-9.00	0.358					
58	CR11613	7	3	1.00E-09	-9.00	0.376					
59	CR11613	8	1	1.00E-10	-10.00	0.390					
60	CR11613	8	2	1.00E-10	-10.00	0.360					
61	CR11613	8	3	1.00E-10	-10.00	0.361					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
	Test # Concentrations								
1	Assay Date	3/9/2007	Chemical ID	CR11614	tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-66	Counter data filename	10MR0455.P00					
	Technician		Pos Ctl						
3	ID	ase	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K				
1	Assay Date	3/9/2007	Test Chemical ID	CR11614	# Concentrations tested		8	Microsome ID	12535-13				
2	NB Ref.	12535-66	Counter data filename	10MR0455.P00									
3	Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2				
4													
5													
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.							
7	1	0.0204		37025		1814851							
8	2	0.0199		37980		1908543							
9	3	0.0203		41544		2046502							
10	4	0.0210		40960		1950478							
11	5	0.0202		39760		1968317							
12						Average DPM/g soln		1937758					
13						SD		84935					
14						CV		4.38					
15						µCi/g soln		0.8729					
16													
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:												
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)								
19	Stock	10.02	10		1002.00								
20	Dilution A			100	10.02								
21	Dilution B			10	1.00								
22													
23	Calculation of concentration nonradiolabeled ASDN in substrate solution												
24	Total g substrate solution					8.1033 g							
25	Mass of dilution B used in substrate prep					4.5807 g							
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56641879 µg/g							
27	Calculation of Substrate Solution Specific Activity												
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.									
29				µg/g soln.									
30	a. µCi/g soln					0.8729							
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000							
32	c. Molecular wt of ASDN (mg/mmol)					286.4							
33	Formula=a/b*c												
34	2) Calculate total µg ASDN/g soln.												
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.												
36				=	0.5664	+	0.011						
37				=	0.577	µg ASDN/g soln.							
38	3) Calculate Solution Specific Activity												
39	= (µCi/g soln.)/(µg ASDN/g soln.)												
40				=	1.513	µCi/µg ASDN							
41					9.617.E+05	dpm/nmol							

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/9/2007	Test Chemical ID	CR11614	# Concentrations tested		8	Microsome ID	12535-13
2		NB Ref	12535-66	Counter data filename	10MR0455.P00					
3		Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4										
5	Microsome Dilution Details									
6										
7	Dilution A	0.12 mL microsome Stock used						Positive Control Concentrations		
8		2.4 mL total volume						Level		
9		20 dilution factor						1	1.00E-05	
10								2	1.00E-06	
11	Dilution B	1.5 mL microsome Dilution A used						3	5.00E-07	
12		60 mL total volume						4	1.00E-07	
13		40 dilution factor						5	5.00E-08	
14								6	1.00E-08	
15	Dilution C (if applicable)	mL microsome Dilution B used						7	1.00E-09	
16		mL total volume						8	1.00E-10	
17		dilution factor								
18	NA									
19		800 total dilution factor						Test Chemical Concentrations		
20								Level		
21	Protein Concentration (stock microsomes, mg/mL):			8.00				1	1.00E-03	
22	Protein Concentration (dilution added to assay, mg/mL):			0.01				2	1.00E-04	
23								3	1.00E-05	
24								4	1.00E-06	
25								5	1.00E-07	
26								6	1.00E-08	
								7	1.00E-09	
								8	1.00E-10	

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1	As	3/9/2007	Chemical ID CF	# Concentrations tested				B Microsome ID	12535-13	
2	NE	12535-66	Counter data filename	10MR0455.P00						
3	Te							Pos Ctl		
4	ch									
5	ni									
6	ci									
7	an									
8	ID ase	Replicate #	1	Positive Control	4-OH ASD/Tubes/Conc				2	
9										
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
68											
69	54	5	9906.25	4420.36	0	12.95	531.89				9906
70	55	5	9853.4	4405.2	0	12.97	531.94				9853
71	56	5	10520.35	4703.76	0	12.92	533.04				10520
72	57	5	10497.75	4695.48	0	12.98	537.31				10498
73											
74											
75	58	5	4956	2215.92	0	12.91	533.92				4956
76	60	5	5111.74	2279.76	0	12.82	533.09				5112
77	61	5	4704.53	2096.96	0	12.83	530.88				4705
78	62	5	4739.34	2102.76	0	13.01	532.02				4739
79	63	5	5224.55	2362.52	0	12.9	533.92				5225
80	64	5	5235.24	2350.52	0	12.9	531.65				5235
81	65	5	10356.8	4644.72	0	13.01	536.69				10357
82	66	5	10622.03	4740.58	0	13.04	536.78				10622
83	67	5	8299.9	3709.16	0	12.83	533.37				8300
84	68	5	8626.44	3848.52	0	13.06	535.62				8626
85	69	5	9590.43	4262.2	0	13	532.32				9590
86	70	5	9474.36	4222.16	0	12.89	530.66				9474
87	71	5	8642.11	3669.8	0	12.96	534.49				8642
88	72	5	8500	3804.16	0	12.88	531.97				8500
89	73	5	9744.14	4357.56	0	13.02	536.91				9744
90	74	5	9517.54	4259.92	0	12.9	532.59				9518
91	75	5	10780.35	4824.16	0	12.94	535.46				10780
92	76	5	10815.98	4812.6	0	13.01	530.73				10816
93	77	5	11394.34	5080.32	0	13.04	533.88				11394
94	78	5	11276.53	5023.36	0	12.98	529.9				11277
95	79	5	8821.3	3932.16	0	13	532.29				8821
96	80	5	9018.57	4032.6	0	12.94	533.28				9019
97	81	5	11390.87	5093	0	12.94	534.17				11391
98	82	5	11785.16	5243.8	0	12.97	532.68				11785
99	84	5	11322.09	5031.32	0	12.86	528.89				11322
100	85	5	11358.38	5096.72	0	12.93	533.89				11358
101	86	5	10357.19	4622.92	0	13.04	534.11				10357
102	87	5	10259.51	4674.72	0	12.97	532.27				10260
103	88	5	11287.27	5021.72	0	13.02	533.52				11287
104	89	5	11307.86	5021.52	0	13.01	531.12				11308
105	90	5	11387.89	5076.16	0	12.89	530.41				11388
106	91	5	11710.31	5181.76	0	13.01	531.09				11710
107	92	5	12175.03	5414.16	0	13.09	535.07				12175
108	93	5	12289.01	5464.96	0	13	530.75				12289
109	94	5	10438.35	4639.16	0	12.97	533.42				10438
110	95	5	10420.81	4650.32	0	12.87	531.26				10421
111	96	5	10237.85	4570.72	0	13	533.49				10238
112	97	5	10162.43	4540.96	0	12.87	531.46				10162
113	98	5	10913.47	4837.44	0	13.03	532.65				10913
114	99	5	10642.98	4758.52	0	12.96	534.19				10643
115	100	5	6479.77	2891.32	0	12.91	534.62				6480
116	101	5	6494.73	2901.72	0	12.99	534.45				6495
117	102	5	12021.32	5397.32	0	12.86	531.38				12021
118	103	5	12221.54	5444.92	0	12.93	531.18				12222
119	104	5	9692.93	4330	0	12.91	533.04				9693
120	105	5	9631.46	4317.28	0	13.02	534.56				9631
121	106	5	9779.11	4353.6	0	12.95	532.15				9779
122	107	5	9772.08	4338.96	0	12.89	530.6				9772
123	108	5	0	0.72	0	0.45	483.4				0
124	109	5	230835.66	33219.16	0	7.93	170.41				230836

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate	Volume (µl)	Adq. #	DPH/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (µL)	Total DPM (corrected for background)	Final protein in assay (mg/mL)	Final protein in assay (mg/mL)	Final protein in assay (mg/mL)	Final protein in assay (mg/mL)	Final protein in assay (mg/mL)	Final protein in assay (mg/mL)	Final protein in assay (mg/mL)
1	1	0.5	1	9141	18282	18336	0.1	36672	183776	183776	183776	183776	183776	183776	183776
2	2	0.5	2	9195	18390	18444	0.1	36780	184320	184320	184320	184320	184320	184320	184320
3	3	0.5	3	9219	18438	18492	0.1	36888	184776	184776	184776	184776	184776	184776	184776
4	4	0.5	4	9243	18486	18546	0.1	36996	185232	185232	185232	185232	185232	185232	185232
5	5	0.5	5	9267	18534	18588	0.1	37104	185688	185688	185688	185688	185688	185688	185688
6	6	0.5	6	9291	18582	18636	0.1	37212	186144	186144	186144	186144	186144	186144	186144
7	7	0.5	7	9315	18630	18684	0.1	37320	186600	186600	186600	186600	186600	186600	186600
8	8	0.5	8	9339	18678	18732	0.1	37428	187056	187056	187056	187056	187056	187056	187056
9	9	0.5	9	9363	18726	18780	0.1	37536	187512	187512	187512	187512	187512	187512	187512
10	10	0.5	10	9387	18774	18828	0.1	37644	187968	187968	187968	187968	187968	187968	187968
11	11	0.5	11	9411	18822	18882	0.1	37752	188424	188424	188424	188424	188424	188424	188424
12	12	0.5	12	9435	18870	18936	0.1	37860	188880	188880	188880	188880	188880	188880	188880
13	13	0.5	13	9459	18918	18990	0.1	37968	189336	189336	189336	189336	189336	189336	189336
14	14	0.5	14	9483	18966	19044	0.1	38076	189792	189792	189792	189792	189792	189792	189792
15	15	0.5	15	9507	19014	19098	0.1	38184	190248	190248	190248	190248	190248	190248	190248
16	16	0.5	16	9531	19062	19146	0.1	38292	190704	190704	190704	190704	190704	190704	190704
17	17	0.5	17	9555	19110	19194	0.1	38400	191160	191160	191160	191160	191160	191160	191160
18	18	0.5	18	9579	19158	19242	0.1	38508	191616	191616	191616	191616	191616	191616	191616
19	19	0.5	19	9603	19206	19290	0.1	38616	192072	192072	192072	192072	192072	192072	192072
20	20	0.5	20	9627	19254	19338	0.1	38724	192528	192528	192528	192528	192528	192528	192528
21	21	0.5	21	9651	19302	19386	0.1	38832	192984	192984	192984	192984	192984	192984	192984
22	22	0.5	22	9675	19350	19434	0.1	38940	193440	193440	193440	193440	193440	193440	193440
23	23	0.5	23	9699	19398	19482	0.1	39048	193896	193896	193896	193896	193896	193896	193896
24	24	0.5	24	9723	19446	19530	0.1	39156	194352	194352	194352	194352	194352	194352	194352
25	25	0.5	25	9747	19494	19578	0.1	39264	194808	194808	194808	194808	194808	194808	194808
26	26	0.5	26	9771	19542	19626	0.1	39372	195264	195264	195264	195264	195264	195264	195264
27	27	0.5	27	9795	19590	19674	0.1	39480	195720	195720	195720	195720	195720	195720	195720
28	28	0.5	28	9819	19638	19722	0.1	39588	196176	196176	196176	196176	196176	196176	196176
29	29	0.5	29	9843	19686	19770	0.1	39696	196632	196632	196632	196632	196632	196632	196632
30	30	0.5	30	9867	19734	19818	0.1	39804	197088	197088	197088	197088	197088	197088	197088
31	31	0.5	31	9891	19782	19866	0.1	39912	197544	197544	197544	197544	197544	197544	197544
32	32	0.5	32	9915	19830	19914	0.1	40020	198000	198000	198000	198000	198000	198000	198000
33	33	0.5	33	9939	19878	19962	0.1	40128	198456	198456	198456	198456	198456	198456	198456
34	34	0.5	34	9963	19926	20010	0.1	40236	198912	198912	198912	198912	198912	198912	198912
35	35	0.5	35	9987	19974	20058	0.1	40344	199368	199368	199368	199368	199368	199368	199368
36	36	0.5	36	10011	20022	20106	0.1	40452	199824	199824	199824	199824	199824	199824	199824
37	37	0.5	37	10035	20070	20154	0.1	40560	200280	200280	200280	200280	200280	200280	200280
38	38	0.5	38	10059	20118	20202	0.1	40668	200736	200736	200736	200736	200736	200736	200736
39	39	0.5	39	10083	20166	20250	0.1	40776	201192	201192	201192	201192	201192	201192	201192
40	40	0.5	40	10107	20214	20298	0.1	40884	201648	201648	201648	201648	201648	201648	201648
41	41	0.5	41	10131	20262	20346	0.1	40992	202104	202104	202104	202104	202104	202104	202104
42	42	0.5	42	10155	20310	20394	0.1	41100	202560	202560	202560	202560	202560	202560	202560
43	43	0.5	43	10179	20358	20442	0.1	41208	203016	203016	203016	203016	203016	203016	203016
44	44	0.5	44	10203	20406	20490	0.1	41316	203472	203472	203472	203472	203472	203472	203472
45	45	0.5	45	10227	20454	20538	0.1	41424	203928	203928	203928	203928	203928	203928	203928
46	46	0.5	46	10251	20502	20586	0.1	41532	204384	204384	204384	204384	204384	204384	204384
47	47	0.5	47	10275	20550	20634	0.1	41640	204840	204840	204840	204840	204840	204840	204840
48	48	0.5	48	10299	20598	20682	0.1	41748	205296	205296	205296	205296	205296	205296	205296
49	49	0.5	49	10323	20646	20730	0.1	41856	205752	205752	205752	205752	205752	205752	205752
50	50	0.5	50	10347	20694	20778	0.1	41964	206208	206208	206208	206208	206208	206208	206208
51	51	0.5	51	10371	20742	20826	0.1	42072	206664	206664	206664	206664	206664	206664	206664
52	52	0.5	52	10395	20790	20874	0.1	42180	207120	207120	207120	207120	207120	207120	207120
53	53	0.5	53	10419	20838	20922	0.1	42288	207576	207576	207576	207576	207576	207576	207576
54	54	0.5	54	10443	20886	20970	0.1	42396	208032	208032	208032	208032	208032	208032	208032
55	55	0.5	55	10467	20934	21018	0.1	42504	208488	208488	208488	208488	208488	208488	208488
56	56	0.5	56	10491	20982	21066	0.1	42612	208944	208944	208944	208944	208944	208944	208944
57	57	0.5	57	10515	21030	21114	0.1	42720	209400	209400	209400	209400	209400	209400	209400
58	58	0.5	58	10539	21078	21162	0.1	42828	209856	209856	209856	209856	209856	209856	209856
59	59	0.5	59	10563	21126	21210	0.1	42936	210312	210312	210312	210312	210312	210312	210312
60	60	0.5	60	10587	21174	21258	0.1	43044	210768	210768	210768	210768	210768	210768	210768
61	61	0.5	61	10611	21222	21306	0.1	43152	211224	211224	211224	211224	211224	211224	211224
62	62	0.5	62	10635	21270	21354	0.1	43260	211680	211680	211680	211680	211680	211680	211680
63	63	0.5	63	10659	21318	21402	0.1	43368	212136	212136	212136	212136	212136	212136	212136
64	64	0.5	64	10683	21366	21450	0.1	43476	212592	212592	212592	212592	212592	212592	212592
65	65	0.5	65	10707	21414	21498	0.1	43584	213048	213048	213048	213048	213048	213048	213048
66	66	0.5	66	10731	21462	21546	0.1	43692	213504	213504	213504	213504	213504	213504	213504
67	67	0.5	67	10755	21510	21594	0.1	43800	213960	213960	213960	213960	213960	213960	213960
68	68	0.5	68	10779	21558	21642	0.1	43908	214416	214416	214416	214416	214416	214416	214416
69	69	0.5	69	10803	21606	21690	0.1	44016	214872	214872	214872	214872	214872	214872	214872
70	70	0.5	70	10827	21654	21738	0.1	44124	215328	215328	215328	215328	215328	215328	215328

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot reference			
6		Read if calibrator within mean $\pm$ 10%			
7		1.00			
8		1.00			
9		0.99			
10		1.01			
11		1.00			
12		1.00			
13		1.01			
14		0.99			
15		1.04			
16		0.94			
17		1.00			
18		1.00			
19		0.99			
20		0.98			
21		1.02			
22		0.91			
23		1.09			
24		1.02			
25		0.98			
26					
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate Level	Normal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/μl	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	Total DPM in assay tube (initial)	Calculate nmd ³ H ₂ O formed	nmd ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmd estrogen formed)/protein/mg
1	GR11614	1-1	2	0.5	1	4836	9672	20736	0.1	193776	193776	0.0207	0.005	15	0.138
2	GR11614	1-2	2	0.5	2	5112	10224	18888	0.1	193776	193776	0.0184	0.005	15	0.139
3	GR11614	1-3	2	0.5	1	4705	9410	18888	0.1	193776	193776	0.0215	0.005	15	0.143
4	GR11614	2-1	2	0.5	2	5235	10470	20920	0.1	193776	193776	0.0434	0.005	15	0.289
5	GR11614	2-2	2	0.5	1	10351	20702	41948	0.1	193776	193776	0.0350	0.005	15	0.243
6	GR11614	2-3	2	0.5	2	18624	37248	74496	0.1	193776	193776	0.0344	0.005	15	0.263
7	GR11614	3-1	2	0.5	1	9474	18948	37896	0.1	193776	193776	0.0354	0.005	15	0.236
8	GR11614	3-2	2	0.5	2	1642	3284	6568	0.1	193776	193776	0.0398	0.005	15	0.285
9	GR11614	3-3	2	0.5	1	9744	19488	38976	0.1	193776	193776	0.0447	0.005	15	0.288
10	GR11614	4-1	2	0.5	1	10780	21560	43102	0.1	193776	193776	0.0468	0.005	15	0.313
11	GR11614	4-2	2	0.5	2	11277	22554	45117	0.1	193776	193776	0.0468	0.005	15	0.246
12	GR11614	4-3	2	0.5	1	11351	22702	45402	0.1	193776	193776	0.0469	0.005	15	0.320
13	GR11614	5-1	2	0.5	1	11321	22644	45350	0.1	193776	193776	0.0469	0.005	15	0.313
14	GR11614	5-2	2	0.5	2	10357	20714	41524	0.1	193776	193776	0.0426	0.005	15	0.284
15	GR11614	5-3	2	0.5	1	11287	22574	45190	0.1	193776	193776	0.0458	0.005	15	0.312
16	GR11614	6-1	2	0.5	2	11306	22616	45232	0.1	193776	193776	0.0478	0.005	15	0.319
17	GR11614	6-2	2	0.5	1	11710	23420	46840	0.1	193776	193776	0.0508	0.005	15	0.338
18	GR11614	6-3	2	0.5	2	12288	24576	49152	0.1	193776	193776	0.0431	0.005	15	0.288
19	GR11614	7-1	2	0.5	1	10431	20862	41718	0.1	193776	193776	0.0422	0.005	15	0.281
20	GR11614	7-2	2	0.5	2	10182	20364	40728	0.1	193776	193776	0.0445	0.005	15	0.297
21	GR11614	7-3	2	0.5	1	10643	21286	42572	0.1	193776	193776	0.0457	0.005	15	0.318
22	GR11614	8-1	2	0.5	2	6495	12990	25980	0.1	193776	193776	0.0502	0.005	15	0.335
23	GR11614	8-2	2	0.5	1	12021	24042	48084	0.1	193776	193776	0.0490	0.005	15	0.286
24	GR11614	8-3	2	0.5	2	12222	24444	48888	0.1	193776	193776	0.0464	0.005	15	0.289
25	GR11614	9-1	2	0.5	1	9631	19262	38524	0.1	193776	193776	0.0464	0.005	15	0.289
26	GR11614	9-2	2	0.5	2	8779	17558	35116	0.1	193776	193776	0.0464	0.005	15	0.289
27	GR11614	9-3	2	0.5	1	9772	19544	39088	0.1	193776	193776	0.0464	0.005	15	0.289

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		0.98
8		1.02
9		1.00
10		1.00
11		1.00
12		1.00
13		0.99
14		0.99
15		1.02
16		1.01
17		0.99
18		1.01
19		0.99
20		1.01
21		1.01
22		0.99
23		1.00
24		1.00
25		1.01
26		0.99
27		0.99
28		0.98
29		1.02
30		1.00
31		1.00
32		1.00
33		1.00
34		1.00
35		1.00
36		1.00
37		0.99
38		1.01
39		1.00
40		1.00
41		1.00
42		1.00
43		1.00
44		1.00
45		1.01
46		0.99
47		1.00
48		1.00
49		0.99
50		1.01
51		1.00
52		1.00
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/9/2007	Test Chemical ID	CR11614	# Concentrations tested			6	Microsome ID	12535-13
2		NB Ref.	12535-66	Counter data file name	10MR0455.P00						
3		Technician ID	ase	Replicate #		1	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4											
5	Control Type	Average	SD								
6	Full activity	0.254	0.038								
7	Background (uncorrected)	0.002	0.000								
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.004					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.025					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.015					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.045					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.038					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.148					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.141					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.171					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.195					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.263					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.248					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.266					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.235					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.272					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.290					
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical) M	Activity						
38	CR11614	1	1	1.00E-03	-3.00	0.138					
39	CR11614	1	2	1.00E-03	-3.00	0.129					
40	CR11614	1	3	1.00E-03	-3.00	0.143					
41	CR11614	2	1	1.00E-04	-4.00	0.289					
42	CR11614	2	2	1.00E-04	-4.00	0.233					
43	CR11614	2	3	1.00E-04	-4.00	0.263					
44	CR11614	3	1	1.00E-05	-5.00	0.298					
45	CR11614	3	2	1.00E-05	-5.00	0.265					
46	CR11614	3	3	1.00E-05	-5.00	0.298					
47	CR11614	4	1	1.00E-06	-6.00	0.313					
48	CR11614	4	2	1.00E-06	-6.00	0.246					
49	CR11614	4	3	1.00E-06	-6.00	0.320					
50	CR11614	5	1	1.00E-07	-7.00	0.313					
51	CR11614	5	2	1.00E-07	-7.00	0.284					
52	CR11614	5	3	1.00E-07	-7.00	0.312					
53	CR11614	6	1	1.00E-08	-8.00	0.319					
54	CR11614	6	2	1.00E-08	-8.00	0.338					
55	CR11614	6	3	1.00E-08	-8.00	0.288					
56	CR11614	7	1	1.00E-09	-9.00	0.281					
57	CR11614	7	2	1.00E-09	-9.00	0.297					
58	CR11614	7	3	1.00E-09	-9.00	0.178					
59	CR11614	8	1	1.00E-10	-10.00	0.335					
60	CR11614	8	2	1.00E-10	-10.00	0.266					
61	CR11614	8	3	1.00E-10	-10.00	0.269					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
	Test # Concentrations								
1	Assay Date	3/13/2007	Chemical ID	CR11614	tested	8 Microsome ID 12535-13			
2	NB Ref.	12535-69	Counter data filename	13MR2110.P00					
	Technician								Pos Ctl
3	ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/13/2007	Test Chemical ID	CR11614	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-69	Counter data filename	13MR2110.P00					
3	Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0203		43284		2132217			
8	2	0.0197		44979		2283198			
9	3	0.0196		42969		2192296			
10	4	0.0202		46828		2318218			
11	5	0.0204		42883		2107010			
12						Average DPM/g soln	2206588		
13						SD	92170		
14						CV	4.18		
15						µCi/g soln	0.9940		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0481 g			
25	Mass of dilution B used in substrate prep					4.5062 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56046846 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.012 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.9940			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5605	+	0.012		
37				=	0.573	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39		= (µCi/g soln.)/(µg ASDN/g soln.)							
40		= 1.736 µCi/µg ASDN							
41		1.104.E+06 dpm/nmol							

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/13/2007	Test Chemical ID	CR11614	# Concentrations tested	8	Microsome ID	12535-13	
2			NB Ref	12535-69	Counter data filename	13MR2110.P00					
3			Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4											
5			Microsome Dilution Details								
6											
7			Dilution A								
8			0.12 mL microsome Stock used								
9			2.4 mL total volume								
10			20 dilution factor								
11			Dilution B								
12			1.5 mL microsome Dilution A used								
13			60 mL total volume								
14			40 dilution factor								
15			Dilution C (if applicable)								
16			ml microsome Dilution B used								
17			ml total volume								
18			dilution factor								
19			800 total dilution factor								
20											
21			Protein Concentration (stock microsomes, mg/mL):								
22			Protein Concentration (dilution added to assay, mg/mL):								
23											
24											
25											
26											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	
	Test			#							
1	Assay Date	3/13/2007	Chemical ID	CR11614	Concentrations tested			8 Microsome ID 12535-13			
2	NB Ref.	12535-69	Counter data filename	13MR2110.P00							
3	Technician		Replicate		Positive		Pos Ctl				
4	ID	ase	#	2	Control	4-OH ASDI	Tubes/Conc	2			
5	Vial	Time (min)	DPM1	ISIE				Round DPM			
6	2	5	43283.6	20183.32	0	13.63	579.78	43284			
7	3	5	44979.01	20352.36	0	13.22	547.63	44979			
8	4	5	42969.27	19578.88	0	13.3	557.9	42969			
9	5	5	46828.32	21124.16	0	13.09	542	46828			
10	6	5	42982.99	19340.12	0	13.07	541.31	42983			
11	8	5	9673.63	4302.08	0	13.01	532.76	9674			
12	9	5	9605.08	4285.38	0	13.01	537.34	9605			
13	10	5	10607.95	4757.32	0	12.93	532.68	10608			
14	11	5	10624.84	4762.08	0	12.85	532.22	10625			
15	12	5	12631.12	5656.48	0	12.92	535.02	12631			
16	13	5	12505.23	5615.16	0	12.89	533.48	12505			
17	14	5	7781.85	3450.12	0	12.95	530.47	7782			
18	15	5	7783.85	3483.08	0	12.87	534.43	7784			
19	17	5	57.92	27.88	0	10.1	529.93	58			
20	18	5	52.98	27.56	0	11.67	531.16	53			
21	19	5	57.15	24.82	0	12.64	533.73	57			
22	20	5	66.32	29.76	0	11.4	532.58	66			
23	21	5	72.12	31.32	0	12.22	529.74	72			
24	22	5	76.19	32.28	0	13.47	532.59	76			
25	23	5	80.17	26.08	0	13.09	536.27	80			
26	24	5	57.18	27.32	0	11.67	533.34	57			
27	26	5	183.48	81.32	0	11.8	534.38	183			
28	27	5	190.18	82.28	0	12.81	531.93	190			
29	28	5	163.87	75.08	0	12.18	531.67	164			
30	29	5	184.72	83	0	13.19	534.71	185			
31											
32											
33	30	5	1134.93	500.32	0	12.85	536.56	1135			
34	31	5	1145.42	514.32	0	12.87	532.94	1145			
35	32	5	1051.22	467.28	0	12.93	531.09	1051			
36	33	5	1103.62	490.52	0	12.55	530.72	1104			
37											
38											
39	34	5	1834.51	820.48	0	12.98	532.74	1835			
40	35	5	1871	836.72	0	12.7	532.36	1871			
41	36	5	1792.49	800.4	0	12.73	533.72	1792			
42	37	5	1857.94	834.28	0	12.89	531.96	1858			
43											
44											
45	38	5	5416.97	2438.92	0	12.86	532.94	5417			
46	39	5	5579.41	2488.88	0	12.86	531.5	5579			
47	40	5	5264.81	2338.28	0	12.96	531.74	5265			
48	41	5	5251.84	2355.12	0	12.89	534.31	5252			
49											
50											
51	42	5	7745.14	3455.92	0	12.87	528.59	7745			
52	43	5	7798.57	3503.68	0	12.95	535.67	7799			
53	44	5	7183.18	3193.08	0	12.88	530.86	7183			
54	45	5	7221.85	3204.08	0	12.92	530.29	7222			
55											
56											
57	48	5	7887.21	3535.08	0	12.8	532.96	7887			
58	47	5	7897.48	3505	0	12.92	530.56	7897			
59	48	5	9201.11	4078.52	0	12.95	529.42	9201			
60	49	5	9347.14	4178.72	0	12.92	533.94	9347			
61											
62											
63	50	5	10668.38	4728.36	0	12.97	529.85	10668			
64	51	5	10724.54	4767.92	0	12.88	530.38	10725			
65	52	5	9896.95	4412.52	0	12.92	529.16	9897			
66	53	5	9796.03	4345.68	0	12.9	529.78	9796			
67											
68											
69	54	5	11265.89	5021.92	0	12.82	527.02	11266			
70	55	5	11415.48	5082.56	0	12.87	528.2	11415			
71	56	5	10670.8	4761.72	0	12.87	531.87	10671			
72	57	5	10669.43	4785.12	0	12.88	531.24	10669			
73											
74											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
75	59	5	10745.28	4799.52		0	12.87	530.89			10745
76	60	5	10804.8	4817.28		0	12.87	530.21			10805
77	61	5	9711.96	4388.88		0	12.82	530.39			9712
78	62	5	9883.64	4428.72		0	13	537.94			9884
79	63	5	9927.59	4454.16		0	12.91	534.31			9928
80	64	5	10137.24	4547.68		0	12.97	537.48			10137
81	65	5	11315.06	5047.08		0	12.83	528.34			11315
82	66	5	11432.67	5115.92		0	12.85	531.72			11433
83	67	5	9067.63	4007.88		0	12.91	527.31			9068
84	68	5	9032.59	4019.56		0	12.88	530.89			9033
85	69	5	7813.8	3479.28		0	12.97	532.13			7814
86	70	5	7946.31	3524.2		0	13.01	529.42			7946
87	71	5	7342.86	3278.68		0	12.92	534.71			7343
88	72	5	7424.23	3352.92		0	12.94	540.01			7424
89	73	5	11482.62	5078.92		0	12.94	527.7			11483
90	74	5	11507.56	5158.92		0	12.81	529.66			11508
91	75	5	9983.34	4467		0	12.92	534.72			9983
92	76	5	10187.65	4527.36		0	12.98	531.61			10188
93	77	5	10042.69	4498.32		0	12.94	534.05			10043
94	78	5	10127.63	4516.32		0	12.92	532.13			10128
95	79	5	11029.55	4941.52		0	12.91	532.66			11030
96	80	5	11325.85	5071.88		0	12.89	530.51			11326
97	81	5	7983.35	3577.52		0	12.85	533.23			7983
98	82	5	7988.21	3581.08		0	12.79	532.57			7988
99	84	5	9720.64	4328.08		0	12.88	532.81			9721
100	85	5	9913.17	4430.72		0	12.91	531.6			9913
101	86	5	9830.44	4376.36		0	12.89	533.14			9830
102	87	5	9845.22	4395.16		0	12.86	531.14			9845
103	88	5	10507.45	4691.32		0	12.88	531.69			10507
104	89	5	10901.12	4690.28		0	12.93	529.67			10901
105	90	5	8339.49	3716.56		0	12.92	531.97			8339
106	91	5	8524.26	3823.28		0	12.92	533.28			8524
107	92	5	10979.33	4877.28		0	12.93	533.02			10979
108	93	5	11017.61	4897.88		0	13	533.29			11018
109	94	5	11706.14	5199.28		0	12.93	531.09			11706
110	95	5	11889.85	5203.52		0	12.92	532.11			11890
111	96	5	11007.69	4906.28		0	12.95	531.16			11008
112	97	5	11220.69	4982.12		0	13.01	532.83			11221
113	98	5	11179.76	4984.12		0	12.99	533.93			11180
114	99	5	11453.44	5101.92		0	12.89	533.03			11453
115	100	5	10784.77	4825.28		0	12.94	531.79			10785
116	101	5	11262.07	4977.92		0	12.96	530.06			11262
117	102	5	11287.62	5030.36		0	12.9	531.5			11288
118	103	5	11301.29	5063.16		0	12.89	533.15			11301
119	104	5	6986.78	3144.96		0	12.91	532.93			6987
120	105	5	6865.72	3099.28		0	12.9	534.92			6866
121	106	5	11339.49	5053.56		0	12.86	531.15			11339
122	107	5	11308.65	5074.36		0	12.96	534.4			11309
123	109	5	229317.75	33792.08		0	7.92	172.57			229318

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Sample type	Replicate	Nominal total volume (mL)	Aliq. #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPM	Volume of substrate solution used assay tube (mL)	Total DPM in assay tube (fmol)	Total DPM corrected for background (Tubes)	nmol ³ H ₂ O formed	Final [protein] in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/protein/min)
1	Full negative control	1	2	1	9674	19279	38558	38558	0.1	220659	38558	0.0347	0.005	15	0.231
2	2	2	2	2	9605	19210	38558	38558	0.1	220659	42217	0.0362	0.005	15	0.256
3	3	2	2	2	10605	21233	42466	42466	0.1	220659	50272	0.0453	0.005	15	0.302
4	4	2	2	2	10625	21250	42466	42466	0.1	220659	50272	0.0453	0.005	15	0.302
5	5	2	2	2	12631	25136	50272	50272	0.1	220659	50272	0.0453	0.005	15	0.302
6	6	2	2	2	12505	25010	50272	50272	0.1	220659	50272	0.0453	0.005	15	0.302
7	7	2	2	2	7784	15566	31132	31132	0.1	220659	30863	0.0290	0.005	15	0.187
8	8	2	2	2	7784	15566	31132	31132	0.1	220659	30863	0.0290	0.005	15	0.187
9	9	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
10	10	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
11	11	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
12	12	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
13	13	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
14	14	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
15	15	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
16	16	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
17	17	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
18	18	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
19	19	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
20	20	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
21	21	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
22	22	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
23	23	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
24	24	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
25	25	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
26	26	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
27	27	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
28	28	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
29	29	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
30	30	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
31	31	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
32	32	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
33	33	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
34	34	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
35	35	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
36	36	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
37	37	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
38	38	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
39	39	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
40	40	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
41	41	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
42	42	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
43	43	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
44	44	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
45	45	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
46	46	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
47	47	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
48	48	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
49	49	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
50	50	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
51	51	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
52	52	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
53	53	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
54	54	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
55	55	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
56	56	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
57	57	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
58	58	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
59	59	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
60	60	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
61	61	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
62	62	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
63	63	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
64	64	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
65	65	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
66	66	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
67	67	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
68	68	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
69	69	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000
70	70	2	2	2	58	111	222	222	0.1	220659	28	0.0040	0.005	15	0.000

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - aliquot variance			
6		Red if value not within mean +/- 10%			
7		1.00			
8		1.00			
9		1.00			
10		1.00			
11		1.01			
12		0.99			
13		1.00			
14		1.00			
15		1.05			
16		0.95			
17		0.93			
18		1.07			
19		0.97			
20		1.03			
21		1.03			
22		0.97			
23		0.98			
24		0.98			
25		0.94			
26		1.06			
27					
28					
29		1.00			
30		1.00			
31		0.98			
32		1.02			
33					
34					
35		0.99			
36		1.01			
37		0.99			
38		1.02			
39					
40					
41		0.99			
42		1.01			
43		1.00			
44		1.00			
45					
46					
47		1.00			
48		1.00			
49		1.00			
50		1.00			
51					
52					
53		1.00			
54		1.00			
55		0.99			
56		1.01			
57					
58					
59		1.00			
60		1.00			
61		1.00			
62		0.99			
63					
64					
65		0.99			
66		1.01			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Sample type	Replicate	Assay Date	Chemical	Test	Chemical	Conc	# Concentrations tested	B	Microsome ID	12335-13						
7	CR11614	1-1	2	0.5	1	107425	21490	21540	43100	220659	220659	0.0388	0.005	15	0.259	
8	CR11614	1-2	2	0.5	1	109035	21810	19596	39192	220659	220659	0.0553	0.005	15	0.235	
9	CR11614	1-3	2	0.5	1	97172	19424	19596	39192	220659	220659	0.0381	0.005	15	0.241	
10	CR11614	1-4	2	0.5	1	98748	18169	20065	40130	220659	220659	0.0410	0.005	15	0.213	
11	CR11614	1-5	2	0.5	1	101177	20724	22748	45496	220659	220659	0.0410	0.005	15	0.213	
12	CR11614	1-6	2	0.5	1	113155	22630	22748	45496	220659	220659	0.0410	0.005	15	0.213	
13	CR11614	1-7	2	0.5	1	14433	22668	18101	36702	220659	220659	0.0326	0.005	15	0.217	
14	CR11614	1-8	2	0.5	1	90031	18366	15760	31520	220659	220659	0.0283	0.005	15	0.189	
15	CR11614	1-9	2	0.5	1	7814	15628	15760	31520	220659	220659	0.0283	0.005	15	0.189	
16	CR11614	1-10	2	0.5	1	7946	15892	14767	29534	220659	220659	0.0285	0.005	15	0.177	
17	CR11614	1-11	2	0.5	1	7343	14596	45982	220659	220659	220659	0.0414	0.005	15	0.276	
18	CR11614	1-12	2	0.5	1	11493	22865	22991	45982	220659	220659	0.0383	0.005	15	0.242	
19	CR11614	1-13	2	0.5	1	7424	14548	40342	40342	220659	220659	0.0383	0.005	15	0.242	
20	CR11614	1-14	2	0.5	1	11508	22018	20171	40342	220659	220659	0.0383	0.005	15	0.242	
21	CR11614	1-15	2	0.5	1	9983	19985	20171	40342	220659	220659	0.0383	0.005	15	0.242	
22	CR11614	1-16	2	0.5	1	10185	20378	20171	40342	220659	220659	0.0383	0.005	15	0.242	
23	CR11614	1-17	2	0.5	1	10543	20588	22336	44112	220659	220659	0.0403	0.005	15	0.269	
24	CR11614	1-18	2	0.5	1	10738	20258	22336	44112	220659	220659	0.0403	0.005	15	0.269	
25	CR11614	1-19	2	0.5	1	11330	22650	15971	31942	220659	220659	0.0287	0.005	15	0.181	
26	CR11614	1-20	2	0.5	1	7963	15966	1834	39268	220659	220659	0.0354	0.005	15	0.236	
27	CR11614	1-21	2	0.5	1	7698	15976	1834	39268	220659	220659	0.0354	0.005	15	0.236	
28	CR11614	1-22	2	0.5	1	9721	19442	1834	39268	220659	220659	0.0354	0.005	15	0.236	
29	CR11614	1-23	2	0.5	1	5913	19226	1834	39268	220659	220659	0.0354	0.005	15	0.236	
30	CR11614	1-24	2	0.5	1	8830	19660	1834	39268	220659	220659	0.0354	0.005	15	0.236	
31	CR11614	1-25	2	0.5	1	8945	19650	21108	42216	220659	220659	0.0380	0.005	15	0.253	
32	CR11614	1-26	2	0.5	1	10507	21014	21108	42216	220659	220659	0.0380	0.005	15	0.253	
33	CR11614	1-27	2	0.5	1	10031	21202	15963	33126	220659	220659	0.0380	0.005	15	0.253	
34	CR11614	1-28	2	0.5	1	8339	16776	15963	33126	220659	220659	0.0380	0.005	15	0.253	
35	CR11614	1-29	2	0.5	1	8524	17105	15963	33126	220659	220659	0.0380	0.005	15	0.253	
36	CR11614	1-30	2	0.5	1	10934	21946	21997	43894	220659	220659	0.0386	0.005	15	0.264	
37	CR11614	1-31	2	0.5	1	10934	21946	21997	43894	220659	220659	0.0386	0.005	15	0.264	
38	CR11614	1-32	2	0.5	1	11708	23412	23396	46192	220659	220659	0.0422	0.005	15	0.281	
39	CR11614	1-33	2	0.5	1	11690	23390	22228	44458	220659	220659	0.0401	0.005	15	0.267	
40	CR11614	1-34	2	0.5	1	11008	22016	22228	44458	220659	220659	0.0408	0.005	15	0.272	
41	CR11614	1-35	2	0.5	1	11803	22360	22843	45285	220659	220659	0.0387	0.005	15	0.265	
42	CR11614	1-36	2	0.5	1	11463	22326	22047	44084	220659	220659	0.0387	0.005	15	0.265	
43	CR11614	1-37	2	0.5	1	10785	21570	22047	44084	220659	220659	0.0387	0.005	15	0.265	
44	CR11614	1-38	2	0.5	1	11262	22524	22859	45178	220659	220659	0.0407	0.005	15	0.271	
45	CR11614	1-39	2	0.5	1	11286	22576	22859	45178	220659	220659	0.0407	0.005	15	0.271	
46	CR11614	1-40	2	0.5	1	11301	22692	13953	27706	220659	220659	0.0409	0.005	15	0.186	
47	CR11614	1-41	2	0.5	1	9987	19974	13953	27706	220659	220659	0.0409	0.005	15	0.186	
48	CR11614	1-42	2	0.5	1	6950	13732	22648	45296	220659	220659	0.0408	0.005	15	0.272	
49	CR11614	1-43	2	0.5	1	11339	22078	22648	45296	220659	220659	0.0408	0.005	15	0.272	
50	CR11614	1-44	2	0.5	1	11359	22078	22648	45296	220659	220659	0.0408	0.005	15	0.272	

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC Check - aliquot variance
6		% RE
7		1.00
8		1.00
9		0.99
10		1.01
11		0.99
12		1.00
13		0.99
14		1.01
15		1.00
16		1.00
17		0.99
18		1.01
19		0.99
20		1.01
21		1.00
22		1.00
23		0.99
24		1.01
25		1.00
26		1.00
27		0.99
28		1.01
29		1.00
30		1.00
31		0.99
32		1.01
33		1.00
34		1.00
35		1.00
36		1.00
37		0.99
38		1.01
39		1.00
40		1.00
41		1.00
42		0.99
43		1.01
44		0.99
45		1.01
46		0.99
47		1.02
48		1.00
49		1.00
50		1.01
51		0.99
52		1.00
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/13/2007	Test Chemical ID	CR11614	# Concentrations tested			8 Microsome ID	12535-13	
2		NE Ref. technician ID	12535-69	flourensia		2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
3				Replicate #							
4											
5	Control Type	Average	SD								
6	Full activity	Overall	0.048								
7	Background (unrecorded)	Overall	0.002								
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	-5.00	0.003						
12	4-OH ASDN	1	2	-5.00	0.003						
13	4-OH ASDN	2	1	-6.00	0.026						
14	4-OH ASDN	2	2	-6.00	0.025						
15	4-OH ASDN	3	1	-6.30	0.043						
16	4-OH ASDN	3	2	-6.30	0.043						
17	4-OH ASDN	4	1	-7.00	0.131						
18	4-OH ASDN	4	2	-7.00	0.126						
19	4-OH ASDN	5	1	-7.30	0.165						
20	4-OH ASDN	5	2	-7.30	0.173						
21	4-OH ASDN	6	1	-8.00	0.189						
22	4-OH ASDN	6	2	-8.00	0.223						
23	4-OH ASDN	7	1	-9.00	0.257						
24	4-OH ASDN	7	2	-9.00	0.236						
25	4-OH ASDN	8	1	-10.00	0.272						
26	4-OH ASDN	8	2	-10.00	0.256						
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
38	CR11614	1	1	-4.00	0.259						
39	CR11614	1	2	-4.00	0.235						
40	CR11614	1	3	-4.00	0.241						
41	CR11614	2	1	-5.00	0.273						
42	CR11614	2	2	-5.00	0.217						
43	CR11614	2	3	-5.00	0.189						
44	CR11614	3	1	-6.00	0.177						
45	CR11614	3	2	-6.00	0.276						
46	CR11614	3	3	-6.00	0.242						
47	CR11614	4	1	-6.30	0.242						
48	CR11614	4	2	-6.30	0.269						
49	CR11614	4	3	-6.30	0.191						
50	CR11614	5	1	-7.00	0.236						
51	CR11614	5	2	-7.00	0.236						
52	CR11614	5	3	-7.00	0.253						
53	CR11614	6	1	-8.00	0.202						
54	CR11614	6	2	-8.00	0.264						
55	CR11614	6	3	-8.00	0.281						
56	CR11614	7	1	-9.00	0.267						
57	CR11614	7	2	-9.00	0.272						
58	CR11614	7	3	-9.00	0.255						
59	CR11614	8	1	-10.00	0.271						
60	CR11614	8	2	-10.00	0.166						
61	CR11614	8	3	-10.00	0.272						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/14/2007	Test Chemical ID	CR11614	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-72	Counter data filename	14MR2107.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/14/2007	Test Chemical ID	CR11614	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-72	Counter data filename	14MR2107.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0211		41713		1976919			
8	2	0.0198		40321		2036414			
9	3	0.0199		44370		2229648			
10	4	0.0199		48370		2430653			
11	5	0.0207		44103		2130580			
12						Average DPM/g soln	2160843		
13						SD	178716		
14						CV	8.27		
15						µCi/g soln	0.9734		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.01	10		1001.00				
20	Dilution A			100	10.01				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0224 g			
25	Mass of dilution B used in substrate prep					4.4868 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.55984329 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.012 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.9734			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5598	+	0.012		
37				=	0.572 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=	1.703	µCi/µg ASDN						
41		1.082.E+06	dpm/nmol						

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/14/2007	Test Chemical ID	CR11614	# Concentrations tested			8 Microsome ID	12535-13
2			NB Ref	12535-72	Counter data filename	14MR2107.P00					
3			Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN		Pos Ctrl Tubes/Conc	2
4											
5	Microsome Dilution Details										
6											
7											
8											
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10											
11											
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
			Test #							
1		Chemical	Concentrations							
	A:	3/14/2007	ID Cl	tested		8	Microsome ID	12535-13		
2		Counter data								
	Ni	12535-72	filename	14MR2107.P00						
3		Replicate	Positive	Pos Ctl						
	Dase	#	3	Control	4-OH ASDI Tubes/Conc				2	
4										
5	Vial	Ti	DPM1		TSIE					Round DPM
6	2	5	41713.45	18317.9	0	12.73	516.88			41713
7	3	5	40320.82	17588.08	0	12.71	514.98			40321
8	4	5	44369.64	19802.14	0	12.98	533.92			44370
9	5	5	48370.24	21849.78	0	13.1	544.72			48370
10	6	5	44103.31	19680.1	0	12.99	533.76			44103
11	8	5	12504.36	5574.5	0	12.87	529.69			12504
12	9	5	12413.81	5565.46	0	12.87	531.06			12414
13	10	5	8560.23	3823.18	0	12.91	529.4			8560
14	11	5	8608.94	3840.1	0	12.9	530.73			8609
15	12	5	10451.88	4883.26	0	12.87	532.61			10452
16	13	5	10252.85	4580.9	0	12.91	530.16			10253
17	14	5	9657.74	4312.06	0	12.82	530.14			9658
18	15	5	9430.48	4235.5	0	12.92	534.68			9430
19	17	5	87.83	35.66	0	12.15	531.06			88
20	18	5	73.98	31.3	0	10.15	530.21			74
21	19	5	67.29	28.46	0	12.38	534.17			67
22	20	5	63.68	28.26	0	13.08	530.93			64
23	21	5	55.22	23.28	0	11.52	531.22			55
24	22	5	65.24	28.5	0	11.09	533.36			65
25	23	5	59.31	27.88	0	10.87	529.53			59
26	24	5	79.48	33.48	0	12.29	530.34			79
27	26	5	160.27	73.46	0	11.94	528.99			160
28	27	5	182.65	79.5	0	13.08	529.89			183
29	28	5	162.54	72.08	0	11.8	531.13			163
30	29	5	170.07	79.86	0	11.3	532.47			170
31										
32										
33	30	5	994.42	437.66	0	12.68	529.74			994
34	31	5	982.24	439.88	0	12.64	531.16			982
35	32	5	921.47	413.9	0	12.7	529.66			921
36	33	5	944.36	420.86	0	12.94	530.92			944
37										
38										
39	34	5	1767.84	786.14	0	12.9	529.31			1768
40	35	5	1807.6	815.1	0	12.92	529.71			1808
41	36	5	1708.19	762.26	0	12.8	528.84			1708
42	37	5	1652.45	743.86	0	12.66	530.94			1652
43										
44										
45	38	5	5195.02	2318.3	0	12.82	530.41			5195
46	39	5	5402	2394.86	0	12.93	533.22			5402
47	40	5	5385.48	2406.86	0	12.88	531.7			5385
48	41	5	5288.92	2377.28	0	12.89	529.44			5289
49										
50										
51	42	5	7532.9	3377.88	0	12.81	529.81			7533
52	43	5	7711.67	3441.9	0	12.87	527.59			7712
53	44	5	7326.57	3249.3	0	12.85	525.67			7327
54	45	5	7181.03	3184.46	0	12.8	527.73			7181
55										
56										
57	46	5	10146.82	4518.5	0	12.79	526.81			10146
58	47	5	10348.09	4632.14	0	12.87	530.87			10349
59	48	5	10819.96	4756.7	0	12.84	529.38			10620
60	49	5	11060.45	4916.86	0	12.84	528.26			11060
61										
62										
63	50	5	11071.63	4908.46	0	12.83	529.24			11072
64	51	5	11316.25	5041.9	0	12.87	528.79			11316

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
66	52	5	10421.19	4622.54	0	12.87	528.65				10421
66	53	5	10330.35	4634.9	0	12.79	527.91				10330
67											
68											
69	54	5	11352.97	5035.66	0	12.84	528.11				11353
70	55	5	11232.92	4995.66	0	12.81	526.89				11233
71	56	5	10916.37	4854.66	0	12.77	524.94				10916
72	57	5	10929.71	4856.06	0	12.83	526.83				10930
73											
74											
75	59	5	10203.67	4499.7	0	12.93	526				10204
76	60	5	10307.11	4578.3	0	12.85	527.39				10307
77	61	5	9078.39	4060.7	0	12.8	527.96				9078
78	62	5	9108.91	4057.06	0	12.92	529.16				9107
79	63	5	10200.78	4528.7	0	12.88	528.98				10201
80	64	5	10217.93	4548.26	0	12.88	530.27				10218
81	65	5	9218.3	4111.86	0	12.72	526.11				9218
82	66	5	9612.91	4263.46	0	12.84	526.53				9613
83	67	5	11326.67	5092.86	0	12.81	531.69				11327
84	68	5	11569.05	5149.3	0	12.84	527.33				11569
85	69	5	10532.42	4695.94	0	12.82	527.95				10532
86	70	5	10554.64	4713.1	0	12.89	529.26				10555
87	71	5	11141.63	4953.26	0	12.94	528.89				11142
88	72	5	11167.43	4995.26	0	12.88	530.89				11167
89	73	5	11894.05	5291.06	0	12.72	526.83				11894
90	74	5	11938.47	5302.06	0	12.86	528.03				11938
91	75	5	11908.53	5305.5	0	12.88	528.57				11908
92	76	5	12173.52	5396.22	0	12.86	527.4				12174
93	77	5	11169.76	4976.3	0	12.85	528.19				11170
94	78	5	11440.59	5060.1	0	12.84	526.87				11441
95	79	5	8938.98	3959.86	0	12.87	527.21				8939
96	80	5	8971.82	4024.7	0	12.82	529.85				8972
97	81	5	10921.17	4854.26	0	12.81	526.95				10921
98	82	5	10923.51	4870.34	0	12.86	530.24				10924
99	84	5	11278.02	5038.1	0	12.88	530.7				11278
100	85	5	11219.53	5017.94	0	12.82	529.69				11220
101	86	5	11919.39	5315.1	0	12.81	528.91				11919
102	87	5	12092.25	5406.5	0	12.87	531.54				12092
103	88	5	10129.63	4518.3	0	12.89	530.76				10130
104	89	5	10368.12	4594.46	0	12.93	529.34				10368
105	90	5	11659.53	5178.26	0	12.85	528.93				11660
106	91	5	11763.66	5221.26	0	12.78	525.74				11764
107	92	5	11538.27	5148.54	0	12.83	527.21				11538
108	93	5	11698.71	5230.46	0	12.87	529.21				11699
109	94	5	8941.28	3985.1	0	12.96	530.51				8941
110	95	5	9112.47	4052.9	0	12.92	532.29				9112
111	96	5	10835.78	4825.86	0	12.89	530.12				10836
112	97	5	10912.07	4878.34	0	12.95	532.49				10912
113	98	5	10886.17	4875.74	0	12.87	533.73				10888
114	99	5	10974.66	4889.46	0	12.83	530.16				10975
115	100	5	9407.24	4188.66	0	12.89	529.46				9407
116	101	5	9473.61	4217.1	0	12.93	529.01				9474
117	102	5	10773.49	4798.9	0	12.85	531				10773
118	103	5	11006.76	4898.26	0	12.88	528.95				11007
119	104	5	8243.79	3663.5	0	12.94	529.57				8244
120	105	5	8424.91	3756.3	0	12.87	529.32				8425
121	106	5	6088.38	2712.54	0	12.9	531.96				6088
122	107	5	8124.71	2736.54	0	12.82	529.8				8125
123	109	5	233474.97	33639.9	0	7.92	170.97				233475

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Chemical ID	Test ID	Assay Date	Chemical ID	Test ID	Assay Date	Chemical ID	Test ID	Assay Date	Chemical ID	Test ID	Assay Date	Chemical ID	Test ID	Assay Date
3/14/2017	CRI-1614	14MR2107_P00	3/14/2017	CRI-1614	14MR2107_P00	3/14/2017	CRI-1614	14MR2107_P00	3/14/2017	CRI-1614	14MR2107_P00	3/14/2017	CRI-1614	14MR2107_P00	3/14/2017
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
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1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
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Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		QC Check - aliquot			
5		variance			
6		Rec'd J value not			
7		within mean $\pm$			
8		10%			
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1				Assay Date	Test Chemical	GR11614	# Concentrations tested			Microsome ID	12535-13					
2				MB Rpt	12535-12	14HR2107 P90										
3				Technician ID base	Replicate #	3	Positive Control	4-OH-ASDAN	Pos CH Tubes/Comp	2						
4																
5	Sample ID				Calculate DPM in aqueous portion after extraction											
6	Sample type	Replicate Level	Nonnaest total volume (mL)	Aliq. Volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (fmol)	Cellulite nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Final protein (in assay (mg/mL))	Inactivation time (min)	Acetylcholinesterase activity (nmol acetylcholinesterase/min/mg protein)
7	GR11614	1-1	2	0.5	1	10234	20409	20511	41022	0.1	216094	40747	0.0176	0.005	15	0.251
8	GR11614	1-2	2	0.5	2	10307	20614	18155	36370	0.1	216094	36095	0.0333	0.005	15	0.222
9	GR11614	1-3	2	0.5	1	9078	18156	18155	36370	0.1	216094	36095	0.0333	0.005	15	0.222
10	GR11614	1-4	2	0.5	2	9107	18214	18155	36370	0.1	216094	36095	0.0333	0.005	15	0.222
11	GR11614	1-5	2	0.5	1	10201	20402	20419	40953	0.1	216094	40747	0.0176	0.005	15	0.251
12	GR11614	1-6	2	0.5	2	10218	20436	20419	40953	0.1	216094	40747	0.0176	0.005	15	0.251
13	GR11614	1-7	2	0.5	1	10218	20436	18631	37662	0.1	216094	37387	0.0345	0.005	15	0.240
14	GR11614	1-8	2	0.5	2	10218	20436	18631	37662	0.1	216094	37387	0.0345	0.005	15	0.240
15	GR11614	1-9	2	0.5	1	10218	20436	22096	45732	0.1	216094	45517	0.0420	0.005	15	0.280
16	GR11614	1-10	2	0.5	2	10218	20436	22096	45732	0.1	216094	45517	0.0420	0.005	15	0.280
17	GR11614	1-11	2	0.5	1	10532	21064	21087	42174	0.1	216094	41899	0.0387	0.005	15	0.268
18	GR11614	1-12	2	0.5	2	10532	21064	21087	42174	0.1	216094	41899	0.0387	0.005	15	0.268
19	GR11614	1-13	2	0.5	1	11142	22324	22309	44616	0.1	216094	44343	0.0410	0.005	15	0.273
20	GR11614	1-14	2	0.5	2	11142	22324	22309	44616	0.1	216094	44343	0.0410	0.005	15	0.273
21	GR11614	1-15	2	0.5	1	11894	23468	23832	47664	0.1	216094	47399	0.0436	0.005	15	0.292
22	GR11614	1-16	2	0.5	2	11894	23468	23832	47664	0.1	216094	47399	0.0436	0.005	15	0.292
23	GR11614	1-17	2	0.5	1	11909	23816	24093	48166	0.1	216094	47891	0.0442	0.005	15	0.295
24	GR11614	1-18	2	0.5	2	12174	24348	24093	48166	0.1	216094	47891	0.0442	0.005	15	0.295
25	GR11614	1-19	2	0.5	1	11770	23540	22611	45222	0.1	216094	44947	0.0415	0.005	15	0.277
26	GR11614	1-20	2	0.5	2	11441	22892	22611	45222	0.1	216094	44947	0.0415	0.005	15	0.277
27	GR11614	1-21	2	0.5	1	10921	21842	21945	43690	0.1	216094	43415	0.0401	0.005	15	0.267
28	GR11614	1-22	2	0.5	2	10921	21842	21945	43690	0.1	216094	43415	0.0401	0.005	15	0.267
29	GR11614	1-23	2	0.5	1	11278	22596	22498	44986	0.1	216094	44721	0.0413	0.005	15	0.275
30	GR11614	1-24	2	0.5	2	11278	22596	22498	44986	0.1	216094	44721	0.0413	0.005	15	0.275
31	GR11614	1-25	2	0.5	1	11919	23938	24011	48022	0.1	216094	47747	0.0441	0.005	15	0.294
32	GR11614	1-26	2	0.5	2	12062	24184	24011	48022	0.1	216094	47747	0.0441	0.005	15	0.294
33	GR11614	1-27	2	0.5	1	10130	20360	20398	40996	0.1	216094	40721	0.0376	0.005	15	0.251
34	GR11614	1-28	2	0.5	2	10398	20796	20398	40996	0.1	216094	40721	0.0376	0.005	15	0.251
35	GR11614	1-29	2	0.5	1	11860	23820	23424	46848	0.1	216094	46573	0.0430	0.005	15	0.287
36	GR11614	1-30	2	0.5	2	11860	23820	23424	46848	0.1	216094	46573	0.0430	0.005	15	0.287
37	GR11614	1-31	2	0.5	1	11764	23428	23237	46374	0.1	216094	46199	0.0427	0.005	15	0.285
38	GR11614	1-32	2	0.5	2	11538	23016	23237	46374	0.1	216094	46199	0.0427	0.005	15	0.285
39	GR11614	1-33	2	0.5	1	10941	21862	18653	36106	0.1	216094	36931	0.0331	0.005	15	0.221
40	GR11614	1-34	2	0.5	2	10941	21862	18653	36106	0.1	216094	36931	0.0331	0.005	15	0.221
41	GR11614	1-35	2	0.5	1	11412	18224	18653	36106	0.1	216094	36931	0.0331	0.005	15	0.221
42	GR11614	1-36	2	0.5	2	11412	18224	18653	36106	0.1	216094	36931	0.0331	0.005	15	0.221
43	GR11614	1-37	2	0.5	1	10836	21672	21748	43496	0.1	216094	43221	0.0399	0.005	15	0.266
44	GR11614	1-38	2	0.5	2	10836	21672	21748	43496	0.1	216094	43221	0.0399	0.005	15	0.266
45	GR11614	1-39	2	0.5	1	10812	21624	21661	43722	0.1	216094	43417	0.0401	0.005	15	0.269
46	GR11614	1-40	2	0.5	2	10812	21624	21661	43722	0.1	216094	43417	0.0401	0.005	15	0.269
47	GR11614	1-41	2	0.5	1	10975	21850	18981	37762	0.1	216094	37487	0.0348	0.005	15	0.231
48	GR11614	1-42	2	0.5	2	10975	21850	18981	37762	0.1	216094	37487	0.0348	0.005	15	0.231
49	GR11614	1-43	2	0.5	1	10474	18948	18981	37762	0.1	216094	37487	0.0348	0.005	15	0.231
50	GR11614	1-44	2	0.5	2	10474	18948	18981	37762	0.1	216094	37487	0.0348	0.005	15	0.231
51	GR11614	1-45	2	0.5	1	10773	21546	21760	43690	0.1	216094	43295	0.0400	0.005	15	0.287
52	GR11614	1-46	2	0.5	2	11067	22014	21760	43690	0.1	216094	43295	0.0400	0.005	15	0.287
53	GR11614	1-47	2	0.5	1	10344	18488	16669	33338	0.1	216094	33063	0.0305	0.005	15	0.204
54	GR11614	1-48	2	0.5	2	10344	18488	16669	33338	0.1	216094	33063	0.0305	0.005	15	0.204
55	GR11614	1-49	2	0.5	1	10425	18550	12213	24426	0.1	216094	24151	0.0223	0.005	15	0.149
56	GR11614	1-50	2	0.5	2	10425	18550	12213	24426	0.1	216094	24151	0.0223	0.005	15	0.149

Aromatase Assay Spreadsheet

	R	S
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2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		0.99
8		1.01
9		1.00
10		1.00
11		1.00
12		1.00
13		0.99
14		1.01
15		0.99
16		1.01
17		1.00
18		1.00
19		1.00
20		1.00
21		1.00
22		1.00
23		0.99
24		1.01
25		0.99
26		1.01
27		1.00
28		1.00
29		1.00
30		1.00
31		1.00
32		1.00
33		0.99
34		1.01
35		0.99
36		1.01
37		1.00
38		1.00
39		0.99
40		1.01
41		0.99
42		1.01
43		1.00
44		1.00
45		1.00
46		1.00
47		1.00
48		1.00
49		0.99
50		1.01
51		0.99
52		1.01
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/14/2007	Test Chemical ID	CR11614	# Concentrations tested					
2		Assay Date	12535-72	Counter date	14MR2107 P00				8	Microsome ID	12535-13
3		Technician ID	ase	Replicate #		3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4	Control Type	Average	SD								
5	Full activity	0.250	0.041		Bkg % of Full						
6	Background (unconnected)	0.002	0.000			0.7 (Red if >1%)					
7											
8											
9											
10	Positive Control	Level	Replicate	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002					
13	4-OH ASDN	2	1	1.00E-06	-6.00	0.023					
14	4-OH ASDN	2	2	1.00E-06	-6.00	0.021					
15	4-OH ASDN	3	1	5.00E-07	-6.30	0.042					
16	4-OH ASDN	3	2	5.00E-07	-6.30	0.040					
17	4-OH ASDN	4	1	1.00E-07	-7.00	0.126					
18	4-OH ASDN	4	2	1.00E-07	-7.00	0.130					
19	4-OH ASDN	5	1	5.00E-08	-7.30	0.186					
20	4-OH ASDN	5	2	5.00E-08	-7.30	0.177					
21	4-OH ASDN	6	1	1.00E-08	-8.00	0.251					
22	4-OH ASDN	6	2	1.00E-08	-8.00	0.265					
23	4-OH ASDN	7	1	1.00E-09	-9.00	0.274					
24	4-OH ASDN	7	2	1.00E-09	-9.00	0.254					
25	4-OH ASDN	8	1	1.00E-10	-10.00	0.277					
26	4-OH ASDN	8	2	1.00E-10	-10.00	0.267					
27											
28											
29											
30											
31											
32											
33											
34											
35											
36											
37	Test Chemical	Level	Replicate	Log(test chemical)	Activity						
38	CR11614	1	1	1.00E-04	-4.00	0.251					
39	CR11614	1	2	1.00E-04	-4.00	0.222					
40	CR11614	2	1	1.00E-04	-4.00	0.250					
41	CR11614	2	2	1.00E-05	-5.00	0.230					
42	CR11614	2	3	1.00E-05	-5.00	0.280					
43	CR11614	3	1	1.00E-06	-6.00	0.256					
44	CR11614	3	2	1.00E-06	-6.00	0.273					
45	CR11614	3	3	1.00E-06	-6.00	0.292					
46	CR11614	4	1	5.00E-07	-6.30	0.295					
47	CR11614	4	2	5.00E-07	-6.30	0.277					
48	CR11614	4	3	5.00E-07	-6.30	0.219					
49	CR11614	5	1	1.00E-07	-7.00	0.267					
50	CR11614	5	2	1.00E-07	-7.00	0.275					
51	CR11614	5	3	1.00E-07	-7.00	0.294					
52	CR11614	6	1	1.00E-08	-8.00	0.281					
53	CR11614	6	2	1.00E-08	-8.00	0.285					
54	CR11614	6	3	1.00E-08	-8.00	0.221					
55	CR11614	7	1	1.00E-09	-9.00	0.266					
56	CR11614	7	2	1.00E-09	-9.00	0.268					
57	CR11614	7	3	1.00E-09	-9.00	0.231					
58	CR11614	8	1	1.00E-10	-10.00	0.267					
59	CR11614	8	2	1.00E-10	-10.00	0.204					
60	CR11614	8	3	1.00E-10	-10.00	0.145					
61	CR11614										

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
	Test # Concentrations									
1	Assay Date	3/16/2007	Chemical ID	CR11615	tested	8 Microsome ID 12535-13				
2	NB Ref.	12535-75	Counter data filename	16MR2033.P00						
	Technician								Pos Ctl	
3	ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc		2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/16/2007	Test Chemical ID	CR11615	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-75	Counter data filename	16MR2033.P00				Pos Ctrl Tubes/Conc	
3	Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN			2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0192		36269		1889010			
8	2	0.0191		38669		2024555			
9	3	0.0190		38688		2036211			
10	4	0.0190		39160		2061053			
11	5	0.0202		40058		1983069			
12						Average DPM/g soln		1998780	
13						SD		67527	
14						CV		3.38	
15						µCi/g soln		0.9004	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution				8.1007 g				
25	Mass of dilution B used in substrate prep				4.6091 g				
26	Concentration of nonradiolabeled ASDN in substrate soln.				0.56840655 µg/g				
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln				0.9004				
31	b. Specific activity of [³ H]ASDN (µCi/nmol)				23500000				
32	c. Molecular wt of ASDN (mg/nmol)				286.4				
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln.= µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36					= 0.5684 + 0.011				
37					= 0.579 µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=	1.554	µCi/µg ASDN						
41		9.880.E+05	dpm/nmol						

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/16/2007	Test Chemical ID	CR11615	# Concentrations tested			8	Microsome ID 12535-13
2			NB Ref	12535-75	Counter data filename	16MR2033.P00					
3			Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
5	Microsome Dilution Details										
6											
7	Dilution A			0.12 mL microsome Stock used							
8				2.4 mL total volume							
9				20 dilution factor							
10											
11	Dilution B			1.5 mL microsome Dilution A used							
12				60 mL total volume							
13				40 dilution factor							
14											
15	Dilution C (if applicable)			mL microsome Dilution B used							
16				mL total volume							
17		NA		dilution factor							
18											
19				800 total dilution factor							
20											
21	Protein Concentration (stock microsomes, mg/mL):				8.00						
22	Protein Concentration (dilution added to assay, mg/mL):				0.01						
23											
24											
25											
26											

Positive Control Concentrations	
Level	Final Concentration (M)
1	1.00E-05
2	1.00E-06
3	5.00E-07
4	1.00E-07
5	5.00E-08
6	1.00E-08
7	1.00E-09
8	1.00E-10

Test Chemical Concentrations	
Level	Final Concentration (M)
1	1.00E-03
2	1.00E-04
3	1.00E-05
4	1.00E-06
5	1.00E-07
6	1.00E-08
7	1.00E-09
8	1.00E-10

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1				Test #							
				Chemical ID	Concentrations						
2				As: 3/18/2007	C	tested	8	Microsome ID	12535-13		
3				Counter data							
4				filename	16MR2033.P00						
5				Te							
6				ch							
7				nic							
8				ian							
9				ID PRP	Replicate # 1	Positive Control	Pos Ctl	4-OH ASD Tubes/Conc	2		
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	69	5	7052.08	3011.96	0	13.8	548.01				7052
72	70	5	7003.99	3005.44	0	13.61	550.04				7004
73	71	5	7004.09	2975.36	0	13.62	546.51				7004
74	72	5	6289.44	2899	0	13.72	551.36				6289
75	73	5	6860.97	2952.56	0	13.6	548.81				6861
76	74	5	6840.81	2942.36	0	13.79	551.99				6841
77	75	5	10458.15	4448.52	0	13.67	547.52				10456
78	76	5	10463.2	4483.84	0	13.63	548.14				10463
79	77	5	10917.96	4670.76	0	13.64	548.91				10918
80	78	5	10883.86	4653.48	0	13.66	547.38				10884
81	79	5	10055.88	4310.2	0	13.65	548.72				10056
82	80	5	10049.03	4306.76	0	13.69	548.44				10049
83	81	5	9962.49	4300.6	0	13.53	547.98				9962
84	82	5	9910.68	4267.8	0	13.61	550.82				9911
85	83	5	10795.83	4605.6	0	13.7	547.41				10796
86	84	5	10552.29	4501.56	0	13.63	547.1				10552
87	85	5	11074.07	4747.2	0	13.68	549.02				11074
88	86	5	11095.11	4729.4	0	13.65	547.24				11095
89	87	5	9638.17	4135.24	0	13.65	549.02				9638
90	88	5	9916.24	4232.76	0	13.64	548.97				9916
91	89	5	10768.52	4601.04	0	13.76	547.86				10769
92	90	5	10531.17	4484.6	0	13.69	548.82				10531
93	91	5	9352.75	4003.4	0	13.64	548.02				9353
94	92	5	9365.8	4013	0	13.65	547.06				9366

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
95	93	5	10787.91	4608.4	0	13.71	548.3				10788
96	94	5	10758.42	4607.56	0	13.86	545.83				10758
97	95	5	10318.24	4415.56	0	13.81	549.94				10318
98	96	5	10315.63	4436.16	0	13.73	549.83				10316
99	97	5	10379.18	4429.16	0	13.64	546.8				10379
100	98	5	10324.14	4434.56	0	13.7	549.23				10324
101	99	5	10961.45	4693.44	0	13.67	547.81				10961
102	100	5	10812	4641.64	0	13.64	547.39				10812
103	101	5	11009.43	4718.24	0	13.58	547.35				11009
104	102	5	10870.62	4647.4	0	13.84	545.39				10871
105	103	5	10129.31	4329.6	0	13.66	545.54				10129
106	104	5	10165.21	4347.48	0	13.65	546.48				10165
107	106	5	235425.59	108227.52	0	14.97	621.19				235426
108											0
109											0
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1					Test Chemical ID	CR11615	# Concentrations tested	12535-13								
2					Assay Date	3/19/2007										
3					NE Ref. Technician	12535-75	19MR2033 P00									
4					PRP	12535-75	19MR2033 P00									
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Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1					Test Chemical ID	CR11615	# Concentrations tested			B. Microsome ID.	12535-13					
2					NB Ref.	12535-75	16MR2033.P00									
3					Technician ID	RRP	Replicate #		1	Positive Control	4-OH ASDN Piv. CH Tubes Conc	2				
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Aromatase Assay Spreadsheet

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	QC check - aligned variance	
6		% RE
7		1.01
8		0.98
9		1.00
10		1.00
11		1.01
12		0.99
13		1.01
14		0.99
15		1.00
16		1.00
17		1.00
18		1.00
19		1.00
20		1.00
21		1.05
22		0.95
23		1.00
24		1.00
25		1.00
26		1.00
27		1.00
28		1.00
29		1.00
30		1.00
31		1.00
32		1.00
33		1.01
34		0.99
35		1.00
36		1.00
37		0.99
38		1.01
39		1.01
40		0.99
41		1.00
42		1.00
43		1.00
44		1.00
45		1.00
46		1.00
47		1.00
48		1.00
49		1.01
50		0.99
51		1.01
52		0.99
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/16/2007	Test Chemical ID	CR11615	# Concentrations			8	Microsome ID	12535-13
2		NB Ref.	12535-75	Counter data filename	16MR2033.P00						
3		Technician ID	PRP	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctl Tubos/Conic		2
4											
5	Control Type	Portion	Average	SD							
6	Full activity	Overall	0.262	0.065							
7	Background (uncorrected)	Overall	0.002	0.000							
8					Bkg % of Full						
9											
10	Positive Control	Level	Replicate	[Positive Ctl] M	Log[Positive Ctl]	Activity					
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.003					
12	4-OH ASDN	2	2	1.00E-05	-5.00	0.002					
13											
14	4-OH ASDN	2	1	1.00E-06	-6.00	0.024					
15	4-OH ASDN	2	2	1.00E-06	-6.00	0.022					
16											
17	4-OH ASDN	3	1	5.00E-07	-6.30	0.044					
18	4-OH ASDN	3	2	5.00E-07	-6.30	0.041					
19											
20	4-OH ASDN	4	1	1.00E-07	-7.00	0.140					
21	4-OH ASDN	4	2	1.00E-07	-7.00	0.129					
22											
23	4-OH ASDN	5	1	5.00E-08	-7.30	0.188					
24	4-OH ASDN	5	2	5.00E-08	-7.30	0.161					
25											
26	4-OH ASDN	6	1	1.00E-08	-8.00	0.252					
27	4-OH ASDN	6	2	1.00E-08	-8.00	0.261					
28											
29	4-OH ASDN	7	1	1.00E-09	-9.00	0.300					
30	4-OH ASDN	7	2	1.00E-09	-9.00	0.289					
31											
32	4-OH ASDN	8	1	1.00E-10	-10.00	0.169					
33	4-OH ASDN	8	2	1.00E-10	-10.00	0.263					
34											
35											
36											
37	Test Chemical	Level	Replicate	Test chemical [M]	Log[est chemical]	Activity					
38	CR11615	1	1	1.00E-03	-3.00	0.097					
39	CR11615	2	2	1.00E-03	-3.00	0.098					
40	CR11615	1	3	1.00E-03	-3.00	0.098					
41	CR11615	2	1	1.00E-04	-4.00	0.133					
42	CR11615	2	2	1.00E-04	-4.00	0.127					
43	CR11615	3	1	1.00E-04	-4.00	0.132					
44	CR11615	3	2	1.00E-05	-5.00	0.178					
45	CR11615	3	3	1.00E-05	-5.00	0.183					
46	CR11615	4	1	1.00E-05	-5.00	0.280					
47	CR11615	4	2	1.00E-06	-6.00	0.292					
48	CR11615	4	3	1.00E-06	-6.00	0.270					
49	CR11615	5	1	1.00E-07	-7.00	0.298					
50	CR11615	5	2	1.00E-07	-7.00	0.285					
51	CR11615	5	3	1.00E-07	-7.00	0.287					
52	CR11615	6	1	1.00E-08	-8.00	0.262					
53	CR11615	6	2	1.00E-08	-8.00	0.265					
54	CR11615	6	3	1.00E-08	-8.00	0.251					
55	CR11615	7	1	1.00E-09	-9.00	0.289					
56	CR11615	7	2	1.00E-09	-9.00	0.277					
57	CR11615	7	3	1.00E-09	-9.00	0.278					
58	CR11615	8	1	1.00E-10	-10.00	0.292					
59	CR11615	8	2	1.00E-10	-10.00	0.293					
60	CR11615	8	3	1.00E-10	-10.00	0.272					
61	CR11615										

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11615	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-79	Counter data filename	20MR2049.P00					
3	Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2	

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11615	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-79	Counter data filename	20MR2049.P00				Pos Ctrl	
3	Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN		Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0190		34276		1804000			
8	2	0.0193		34541		1789689			
9	3	0.0197		36417		1848579			
10	4	0.0189		41148		2177143			
11	5	0.0195		39187		2009590			
12						Average DPM/g soln		1925800	
13						SD		165468	
14						CV		8.59	
15						µCi/g soln		0.8675	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.03	10		1003.00				
20	Dilution A			100	10.03				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.1545 g			
25	Mass of dilution B used in substrate prep					4.5816 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56353483 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.8675			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36						0.5635	+	0.011	
37						0.574		µg ASDN/g soln.	
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=					1.511 µCi/µg ASDN			
41						9.607.E+05 dpm/nmol			

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11615	# Concentrations tested	8	Microsome ID	12535-13			
2	NB Ref	12535-79	Counter data filename	20MR2049.P00							
3	Technician ID	PRP	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc	2			
4											
5	Microsome Dilution Details										
6											
7	Dilution A										
8	0.12 mL microsome Stock used										
9	2.4 mL total volume										
10	20 dilution factor										
11	Dilution B										
12	1.5 mL microsome Dilution A used										
13	60 mL total volume										
14	40 dilution factor										
15	Dilution C (if applicable)										
16	mL microsome Dilution B used										
17	mL total volume										
18	dilution factor										
19	800 total dilution factor										
20											
21	Protein Concentration (stock microsomes, mg/mL):										
22	Protein Concentration (dilution added to assay, mg/mL):										
23	8.00										
24	0.01										
25											
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
	Test # Chemical Concentrations As: 3/20/2007 ID C tested 8 Microsome ID 12535-13										
1											
2	Counter data NB 12535-79 filename 20MR2049.P00										
	Te ch nic ian ID PRP Replicate # 2 Positive Control 4-OH ASD Tubes/Conc 2										
3											
4											
5	Vial	Tin	DPM1	ISIE							Round DPM
6	2	5	34276.42	13455.1	0	12.74	483.76				34276
7	3	5	34541.41	13559.3	0	12.82	486.93				34541
8	4	5	36417.12	14349.74	0	12.75	484.89				36417
9	5	5	41148.41	16316.7	0	12.84	490.78				41148
10	6	5	39187.13	15516.34	0	12.86	490.54				39187
11	8	5	12909.88	5508.54	0	13.71	549.92				12810
12	9	5	12677.81	5430.94	0	13.72	549.28				12678
13	10	5	12151.11	5183.7	0	13.74	547.56				12151
14	11	5	12004.86	5160.7	0	13.73	551.68				12005
15	12	5	12569.7	5389.14	0	13.77	551.65				12570
16	13	5	12564.54	5388.14	0	13.82	553.99				12565
17	14	5	12231.57	5214.98	0	13.71	547.49				12232
18	15	5	12195.8	5234.34	0	13.79	554.53				12196
19	16	5	57.78	25.14	0	14.43	548.41				58
20	17	5	73.54	31.34	0	18.11	553.16				74
21	18	5	56.37	24.7	0	12.85	550.27				56
22	19	5	64.78	29.74	0	13.77	551.46				65
23	20	5	87.43	28.9	0	13.93	551.77				87
24	21	5	68.29	30.3	0	14.21	548.97				68
25	22	5	208.32	84.98	0	12.81	520.05				208
26	23	5	204.36	85.94	0	13.81	525.62				204
27	25	5	85.2	38.1	0	14.33	574.08				85
28	26	5	64.04	30.7	0	13.53	574.76				64
29	27	5	78.92	34.78	0	15.62	574.08				79
30	28	5	81.99	35.9	0	14.15	573.75				82
31	29	5	834.99	359.9	0	13.82	551.08				835
32	30	5	813.05	341.3	0	13.66	548.33				813
33	31	5	783.82	340.14	0	13.81	554.06				784
34	32	5	794.55	345.74	0	13.43	550.35				795
35	33	5	1495.16	633.54	0	13.88	551.73				1495
36	34	5	1491.78	648.34	0	13.82	553.67				1492
37	35	5	1373.84	581.58	0	13.9	551.21				1374
38	36	5	1413.02	604.54	0	13.79	550.41				1413
39	37	5	5001.4	2146.34	0	13.82	552.93				5001
40	38	5	5051.3	2154.54	0	13.77	548.45				5051
41	39	5	4537.75	1941.74	0	13.87	551.83				4538
42	40	5	4446.23	1886.34	0	13.79	549.79				4446
43	41	5	7208.59	3102.7	0	13.78	553.74				7209
44	42	5	7287.6	3117.94	0	13.7	549.9				7288
45	43	5	6887.49	2950.54	0	13.82	554.98				6887
46	44	5	6912.83	2957.58	0	13.78	551.81				6913
47	45	5	11280.54	4855.94	0	13.7	554.07				11281
48	46	5	10602.44	4640.5	0	13.78	552.52				10602
49	47	5	10722.41	4563.62	0	13.71	550.44				10722
50	48	5	10483.05	4464.7	0	13.76	548.55				10483
51	49	5	11957.83	5132.14	0	13.74	550.91				11958
52	50	5	11885.46	5078.22	0	13.72	548.4				11885
53	51	5	12312.32	5307.74	0	13.74	554.35				12312
54	52	5	12383.77	5299.5	0	13.8	549.91				12364
55	53	5	12150.33	5225.5	0	13.75	550.89				12150
56	54	5	12194.78	5251.5	0	13.78	553.28				12195
57	55	5	12490.6	5348.9	0	13.81	550.74				12491
58	56	5	12669.78	5398.54	0	13.81	550.67				12670
59	58	5	4943.85	2119.5	0	13.71	548.37				4944
60	59	5	4935.01	2110.26	0	13.75	548.69				4935
61	60	5	5383.97	2289.74	0	13.83	548.69				5384
62	61	5	5365.41	2304.54	0	13.7	551.22				5365
63	62	5	5224.87	2221.1	0	13.86	549.02				5225
64	63	5	5186.78	2223.34	0	13.82	550.18				5187
65	64	5	5640.18	2435.62	0	13.81	552.54				5640
66	65	5	5814.48	2397.94	0	13.66	549.2				5614
67	66	5	5618.53	2399.1	0	13.86	554.08				5619
68	67	5	5673.6	2421.74	0	13.8	550.76				5674
69	68	5	5641.56	2407.7	0	13.72	548.55				5642
70	69	5	5465.57	2333.14	0	13.75	549.79				5466

Aromalase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	6924.06	2943.3	0	13.79	550.08				6924
72	71	5	6968.98	2985.5	0	13.78	551				6969
73	72	5	7300.92	3129.78	0	13.72	552.17				7301
74	73	5	7431.99	3174.1	0	13.86	550.74				7432
75	74	5	7122.21	3044.5	0	13.63	549.42				7122
76	75	5	7062.95	3048.66	0	13.82	555.87				7063
77	76	5	9071.57	3900.18	0	13.83	552.88				9072
78	77	5	9169.59	3933.14	0	13.74	552.38				9170
79	78	5	9129.76	3900.1	0	13.81	550.04				9130
80	79	5	9018.94	3845.34	0	13.81	549.94				9019
81	80	5	8584.19	3682.58	0	13.72	548.73				8584
82	81	5	8639.91	3693.34	0	13.8	552.03				8640
83	82	5	10943.36	4694.3	0	13.75	552.12				10943
84	83	5	10760.04	4631.74	0	13.78	552.25				10760
85	84	5	10696.61	4585.34	0	13.78	551.57				10697
86	85	5	10696.5	4589.78	0	13.76	553.02				10697
87	86	5	10471.57	4483.98	0	13.78	552.12				10472
88	87	5	10294.79	4417.18	0	13.76	551.22				10295
89	88	5	12820.12	5482.34	0	13.79	550.86				12820
90	89	5	12803.88	5486.34	0	13.79	549.77				12804

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
91	90	5	11441.55	4890.74	0	13.82	551.82				11442
92	91	5	11434.21	4877.18	0	13.72	549.09				11434
93	92	5	11844.65	5084.9	0	13.77	551.59				11845
94	93	5	11878.86	5082.74	0	13.77	550.21				11879
95	94	5	12886.96	5493.18	0	13.78	549				12887
96	95	5	12770.81	5477.3	0	13.76	551.33				12771
97	96	5	12602.09	5373.74	0	13.77	548.82				12602
98	97	5	12371.43	5318.14	0	13.75	551.71				12371
99	98	5	12104.54	5196.74	0	13.74	551.56				12105
100	99	5	11914.57	5138.82	0	13.67	550.81				11915
101	100	5	12527.49	5402.9	0	13.7	552.03				12527
102	101	5	12573.81	5364.38	0	13.75	550.05				12574
103	102	5	12727.38	5437.14	0	13.75	550.62				12727
104	103	5	12746.32	5442.14	0	13.76	547.27				12746
105	104	5	12349.02	5332.54	0	13.73	552.64				12349
106	105	5	12156.39	5207.7	0	13.72	550.39				12156
107	106	5	234620.23	108158.86	0	15.07	626.23				234620
108											0
109											0
110											0
111											0
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Chemical ID	Test ID	GR11615	# Concentrations tested	6. Microsome ID	12935-13									
Assay Date	Chemical ID	Test ID	GR11615	# Concentrations tested	6. Microsome ID	12935-13									
Assay Date	Chemical ID	Test ID	GR11615	# Concentrations tested	6. Microsome ID	12935-13									
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Assay Date	Chemical ID	Test ID	GR11615	# Concentrations tested	6. Microsome ID	12935-13					</				

Aromatase Assay Spreadsheet

	R	S	T	U	V
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Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	3/20/2007	Test	CR11615	# Concentrations tested									
2		NB Ref	12535-79	Over date	20MR040 P00										
3		Technician ID	PRP	Replicate #	2	Positive Control	4-DH ASDN	Pos Ctl Tubes/Conc							
4															
5	Sample ID														
6	Sample type	Replicate Level	Aliq Volume (mL)	Aliq #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPM	Volume of substrate solution (mL)	Total DPM in assay tube (initial)	Calculate nmol ³ H ₂ O formed (background Tubes)	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)
7	CR11615	1-1	2	0.5	4934	9868	9879	19758	0.1	192580	19358	0.0201	0.005	15	0.134
8		1-2	2	0.5	4935	9870	10749	21488	0.1	192580	21098	0.0220	0.005	15	0.146
9		1-3	2	0.5	5384	10768	10749	21488	0.1	192580	20474	0.0213	0.005	15	0.142
10		1-4	2	0.5	5385	10730	10412	20824	0.1	192580	22108	0.0230	0.005	15	0.153
11		2-1	2	0.5	5612	11224	11284	22568	0.1	192580	22185	0.0231	0.005	15	0.154
12		2-2	2	0.5	5614	11228	11293	22585	0.1	192580	21815	0.0227	0.005	15	0.151
13		2-3	2	0.5	5642	11284	11108	22216	0.1	192580	27385	0.0285	0.005	15	0.190
14		3-1	2	0.5	5944	13948	13951	27885	0.1	192580	28066	0.0291	0.005	15	0.202
15		3-2	2	0.5	7301	14602	14733	29466	0.1	192580	27970	0.0291	0.005	15	0.194
16		3-3	2	0.5	7432	14864	14185	28370	0.1	192580	36084	0.0378	0.005	15	0.250
17		4-1	2	0.5	7083	14126	14242	28484	0.1	192580	35898	0.0374	0.005	15	0.249
18		4-2	2	0.5	8172	16344	16145	32288	0.1	192580	34048	0.0354	0.005	15	0.236
19		4-3	2	0.5	8190	16380	16145	32288	0.1	192580	43016	0.0448	0.005	15	0.298
20		5-1	2	0.5	8640	17280	17224	34448	0.1	192580	42888	0.0441	0.005	15	0.294
21		5-2	2	0.5	10943	21886	21703	43406	0.1	192580	41134	0.0426	0.005	15	0.285
22		5-3	2	0.5	10697	21394	21394	42788	0.1	192580	50448	0.0529	0.005	15	0.353
23		6-1	2	0.5	10472	20944	20767	41534	0.1	192580	47048	0.0472	0.005	15	0.315
24		6-2	2	0.5	10295	20590	20524	41048	0.1	192580	50618	0.0530	0.005	15	0.353
25		6-3	2	0.5	12602	25204	24973	49946	0.1	192580	49346	0.0516	0.005	15	0.344
26		7-1	2	0.5	12371	24742	24742	49484	0.1	192580	47640	0.0496	0.005	15	0.351
27		7-2	2	0.5	12105	24210	24020	48040	0.1	192580	49802	0.0518	0.005	15	0.346
28		7-3	2	0.5	11915	23830	25101	50202	0.1	192580	50546	0.0526	0.005	15	0.351
29		8-1	2	0.5	13574	27148	25101	50202	0.1	192580	48010	0.0506	0.005	15	0.337
30		8-2	2	0.5	12727	25484	25473	50946	0.1	192580					
31		8-3	2	0.5	12146	24292	24565	49010	0.1	192580					
32			2	0.5	12349	24688									
33			2	0.5	12189	24312									

Aromatase Assay Spreadsheet

	R	S
1		
2		
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4		
5		OC Check - aliquot variance
6		% RE
7		1.00
8		1.00
9		1.00
10		1.00
11		1.00
12		1.00
13		1.00
14		1.00
15		1.00
16		1.00
17		1.02
18		0.98
19		1.00
20		1.00
21		0.99
22		1.01
23		1.00
24		1.00
25		0.99
26		1.01
27		1.01
28		0.98
29		1.00
30		1.00
31		1.01
32		0.99
33		1.00
34		1.00
35		1.01
36		0.99
37		1.00
38		1.00
39		1.00
40		1.00
41		1.00
42		1.00
43		1.00
44		1.00
45		1.01
46		0.99
47		1.01
48		0.99
49		1.00
50		1.00
51		1.00
52		1.00
53		1.01
54		0.99

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Data	3/20/2007	Test Chemical ID	CR11615	# Concentrations					
2		NB Ref	12535-79	Counter data filename	20MR2049.P00	2	Positive Control	4-OH ASDN	8	Microsome ID	12535-13
3		Technician ID	PRP	Replicate #						Pos Ctl Tubes/Conc	2
4											
5	Control Type	Average	SD								
6	Full activity	0.341	0.009								
7	Background (unconnected)	0.003	0.002								
8											
9											
10	Positive Control	Level	Replicate	[Positive Ctl] M	Log(Positive Ctl)	Activity					
11	4-OH ASDN	1	1	1.00E-05	-5.00	0.001					
12	4-OH ASDN	1	2	1.00E-05	-5.00	0.002					
13											
14	4-OH ASDN	2	1	1.00E-06	-6.00	0.020					
15	4-OH ASDN	2	2	1.00E-06	-6.00	0.019					
16											
17	4-OH ASDN	3	1	5.00E-07	-6.30	0.039					
18	4-OH ASDN	3	2	5.00E-07	-6.30	0.036					
19											
20	4-OH ASDN	4	1	1.00E-07	-7.00	0.137					
21	4-OH ASDN	4	2	1.00E-07	-7.00	0.122					
22											
23	4-OH ASDN	5	1	5.00E-08	-7.30	0.198					
24	4-OH ASDN	5	2	5.00E-08	-7.30	0.189					
25											
26	4-OH ASDN	6	1	1.00E-08	-8.00	0.304					
27	4-OH ASDN	6	2	1.00E-08	-8.00	0.292					
28											
29	4-OH ASDN	7	1	1.00E-09	-9.00	0.328					
30	4-OH ASDN	7	2	1.00E-09	-9.00	0.340					
31											
32	4-OH ASDN	8	1	1.00E-10	-10.00	0.336					
33	4-OH ASDN	8	2	1.00E-10	-10.00	0.346					
34											
35											
36											
37	Test Chemical	Level	Replicate	[Test chemical] M	Log[Test Chemical]	Activity					
38	CR11615	1	1	5.00E-05	-4.30	0.134					
39	CR11615	1	2	5.00E-05	-4.30	0.146					
40	CR11615	1	3	5.00E-05	-4.30	0.142					
41	CR11615	2	1	2.50E-05	-4.60	0.153					
42	CR11615	2	2	2.50E-05	-4.60	0.164					
43	CR11615	2	3	2.50E-05	-4.60	0.151					
44	CR11615	3	1	1.00E-05	-5.00	0.190					
45	CR11615	3	2	1.00E-05	-5.00	0.202					
46	CR11615	3	3	1.00E-05	-5.00	0.194					
47	CR11615	4	1	5.00E-06	-5.30	0.250					
48	CR11615	4	2	5.00E-06	-5.30	0.249					
49	CR11615	4	3	5.00E-06	-5.30	0.236					
50	CR11615	5	1	2.50E-06	-5.60	0.298					
51	CR11615	5	2	2.50E-06	-5.60	0.294					
52	CR11615	5	3	2.50E-06	-5.60	0.286					
53	CR11615	6	1	1.00E-06	-6.00	0.353					
54	CR11615	6	2	1.00E-06	-6.00	0.315					
55	CR11615	6	3	1.00E-06	-6.00	0.326					
56	CR11615	7	1	1.00E-07	-7.00	0.353					
57	CR11615	7	2	1.00E-07	-7.00	0.344					
58	CR11615	7	3	1.00E-07	-7.00	0.331					
59	CR11615	8	1	1.00E-08	-8.00	0.346					
60	CR11615	8	2	1.00E-08	-8.00	0.351					
61	CR11615	8	3	1.00E-08	-8.00	0.337					

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/21/2007	Test Chemical ID	CR11615	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-83	Counter data filename	21MR2059.P00				Pos Ctl	
3	ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/21/2007	Test Chemical ID	CR11615	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-83	Counter data filename	21MR2059.P00					
3	Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0178		33863		1902416			
8	2	0.0198		36869		1862071			
9	3	0.0194		39193		2020258			
10	4	0.0200		34722		1736100			
11	5	0.0200		42383		2119150			
12						Average DPM/g soln		1927999	
13						SD		147374	
14						CV		7.64	
15						µCi/g soln		0.8685	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0990 g			
25	Mass of dilution B used in substrate prep					4.5760 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.56444302 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =				0.011 µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8685			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5644	+	0.011		
37				=	0.575	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	= 1.510 µCi/µg ASDN								
41	9.603.E+05 dpm/nmol								

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K
1		Assay Date	3/21/2007	Test Chemical ID	CR11615	# Concentrations tested			8. Microsome ID	12535-13
2		NB Ref	12535-83	Counter data filename	21MR2059.P00					
3		Technician ID	PRP	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4										
5	Microsome Dilution Details									
6										
7			0.12 mL microsome Stock used							
8			2.4 mL total volume							
9			20 dilution factor							
10										
11			1.5 mL microsome Dilution A used							
12			60 mL total volume							
13			40 dilution factor							
14										
15			mL microsome Dilution B used							
16			mL total volume							
17			dilution factor							
18		NA								
19			800 total dilution factor							
20										
21			Protein Concentration (stock microsomes, mg/mL):	8.00						
22			Protein Concentration (dilution added to assay, mg/mL):	0.01						
23										
24										
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Test								
			Chemical								
2			ID	C	Concentrations						
			As: 3/21/2007		tested		8	Microsome ID	12535-13		
3			Counter data								
			file name	21MR2059.P00							
4			Te								
			ch								
			nic								
			ian								
5			ID	PRP	Replicate # 3	Positive	Control	4-OH ASD	Tubes/Conc	2	
6											
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Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
71	70	5	7120.7	3035.5	0	13.74	545.24				7121
72	71	5	7053.84	2996.3	0	13.67	542.89				7054
73	72	5	7310.11	3097.7	0	13.73	544.56				7310
74	73	5	7376.06	3135.46	0	13.74	545.55				7376
75	74	5	7272.97	3100.5	0	13.82	547.27				7273
76	75	5	7393.9	3148.98	0	13.82	547.74				7394
77	76	5	9080.52	3659.26	0	13.69	544.59				9081
78	77	5	9013.53	3814.9	0	13.83	547.06				9014
79	78	5	8803.56	3744.86	0	13.71	544.2				8804
80	79	5	8797.18	3715.26	0	13.87	543.45				8797
81	80	5	8768.82	3708.74	0	13.7	541.89				8769
82	81	5	8762.63	3737.88	0	13.7	546.73				8763
83	82	5	5290.28	2306.48	0	14.22	569.8				5290
84	83	5	5346.65	2340.5	0	14.14	571.82				5347
85	84	5	5174.67	2252.46	0	14.18	570.39				5175
86	85	5	5192.41	2254.48	0	14.14	566.77				5192

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
87	86	5	10736.67	4511.34	0	13.81	542.42				10737
88	87	5	10518.49	4481.06	0	13.77	546.78				10518
89	88	5	11470.02	4831.74	0	13.8	545.41				11470
90	89	5	11497.06	4875.02	0	13.87	547.29				11497
91	90	5	11400.22	4834.06	0	13.8	547.68				11400
92	91	5	11239.82	4770.86	0	13.8	545.69				11240
93	92	5	11528.65	4883.66	0	13.81	544.98				11529
94	93	5	11353.51	4800.1	0	13.88	545.91				11354
95	94	5	12469.51	5282.3	0	13.78	545.23				12470
96	95	5	12250.57	5221.06	0	13.85	547.03				12251
97	96	5	12073.47	5126.3	0	13.78	545.86				12073
98	97	5	12073.4	5126.46	0	13.77	545.98				12073
99	98	5	12564.83	5356.58	0	13.77	546.1				12565
100	99	5	12397.41	5224.7	0	13.77	544.59				12397
101	100	5	12428.97	5326.94	0	13.7	549.55				12429
102	101	5	12552.93	5304.5	0	13.89	545.41				12553
103	102	5	12344.76	5246.74	0	13.68	542.7				12345
104	103	5	12451.32	5301.5	0	13.73	546.21				12451
105	104	5	12549.01	5290.1	0	13.82	543.8				12549
106	105	5	12343.36	5231.94	0	13.81	544.81				12343
107	106	5	236022.68	108434.27	0	15.13	624.31				236023
108											0
109											0
110											0
111											0
112											0
113											0
114											0
115											0
116											0
117											0
118											0
119											0
120											0
121											0
122											0
123											0
124											0

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
1		Assay Date	3/7/2007		Test Chemical ID	CR11615	# Concentrations tested	8	Microsome ID	12535-13						
2		NB Ref. Technician	12535-83		Conc. data. Reagents	21MP2059-P00			4-OH ASDN For Ch TubesConc	2						
3					PPP	3		Positive Control								
4																
5	Sample ID															
6	Sample type	Protein volume (µl)	Nonprotein volume (µl)	Aliq Volume (µl)	Aliq #	DPH/mL	DPH/µl	Ave DPH/mL	Total DPM	Volume of substrate solution (µl)	total DPM in assay tube (final)	total DPM in assay tube (background)	nmol ³ H ₂ O formed	Protein in assay (mg/mL)	Inhibition (assay tube final)	Aromatase activity (nmol ³ H ₂ O formed/mg protein/h)
7	Full activity control	1	2	0.5	2	13287	26574	26837	53148	0.1	192800	53448	0.0557	0.005	15	0.371
8	1	2	0.5	2	13250	26500	26268	26268	52558	0.1	192800	52110	0.0545	0.005	15	0.363
9	2	2	0.5	2	13153	26306	26115	26115	52630	0.1	192800	49690	0.0517	0.005	15	0.345
10	3	2	0.5	2	12973	25946	26138	26138	52770	0.1	192800	49862	0.0489	0.005	15	0.326
11	4	2	0.5	2	11892	23784	26104	26104	47208	0.1	192800	-12	0.0400	0.005	15	0.300
12	Background control	1	2	0.5	2	11112	22224	107	214	0.1	192800	-72	-0.0091	0.005	15	0.000
13	1	2	0.5	2	54	108	84	84	164	0.1	192800	-58	-0.0091	0.005	15	0.000
14	2	2	0.5	2	41	82	149	149	282	0.1	192800	72	0.0091	0.005	15	0.000
15	3	2	0.5	2	78	156	152	152	224	0.1	192800	-2	0.0091	0.005	15	0.000
16	4	2	0.5	2	62	124	112	112	224	0.1	192800	1222	0.0013	0.005	15	0.006
17	4-OH ASDN	1-1	2	0.25	2	180	360	724	1448	0.1	192800	1070	0.0011	0.005	15	0.007
18	1-2	2	0.25	2	169	338	648	648	1296	0.1	192800			0.005	15	
19	1-3	2	0.25	2	164	328	656	656		0.1	192800			0.005	15	
20	2-1	2	0.5	2	555	1110	1710	1710	3420	0.1	192800	3234	0.0034	0.005	15	0.022
21	2-2	2	0.5	2	878	1756	1602	1602	3100	0.1	192800	2874	0.0030	0.005	15	0.020
22	2-3	2	0.5	2	748	1496	1498	1498		0.1	192800			0.005	15	
23	3-1	2	0.5	2	1572	3144	3107	3107	6214	0.1	192800	5988	0.0062	0.005	15	0.042
24	3-2	2	0.5	2	1535	3070	2942	2942	5884	0.1	192800	1658	0.0059	0.005	15	0.039
25	3-3	2	0.5	2	1496	2992	2972	2972		0.1	192800			0.005	15	
26	4-1	2	0.5	2	5327	10654	10605	10605	21210	0.1	192800	20984	0.0219	0.005	15	0.148
27	4-2	2	0.5	2	5278	10556	9476	9476	18952	0.1	192800	18726	0.0186	0.005	15	0.130
28	4-3	2	0.5	2	4746	9492	9460	9460		0.1	192800			0.005	15	
29	5-1	2	0.5	2	7424	14848	14887	14887	29774	0.1	192800	29468	0.0304	0.005	15	0.202
30	5-2	2	0.5	2	7273	14546	14238	14238	28476	0.1	192800	28252	0.0284	0.005	15	0.196
31	5-3	2	0.5	2	7019	14038				0.1	192800			0.005	15	
32	6-1	2	0.5	2	10850	21700	21561	21561	43102	0.1	192800	43076	0.0449	0.005	15	0.299
33	6-2	2	0.5	2	10729	21458	21012	21012	42024	0.1	192800	41786	0.0435	0.005	15	0.290
34	6-3	2	0.5	2	10492	20984				0.1	192800			0.005	15	
35	7-1	2	0.5	2	12116	24232	23778	23778	47556	0.1	192800	47330	0.0482	0.005	15	0.328
36	7-2	2	0.5	2	11662	23324	24372	24372	48744	0.1	192800	48676	0.0508	0.005	15	0.338
37	7-3	2	0.5	2	12315	24630				0.1	192800			0.005	15	
38	8-1	2	0.5	2	12885	25770	25277	25277	50554	0.1	192800	50328	0.0524	0.005	15	0.349
39	8-2	2	0.5	2	12592	25184	24301	24301	48602	0.1	192800	48576	0.0504	0.005	15	0.336
40	8-3	2	0.5	2	12207	24414				0.1	192800			0.005	15	

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
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4					
5		QC check - aliquot variance			
6		Rep if value not within mean $\pm$ 10%			
7		1.01			
8		0.99			
9		1.00			
10		1.00			
11		1.01			
12		0.99			
13		1.01			
14		0.99			
15		0.99			
16		1.01			
17		1.02			
18		0.98			
19		1.02			
20		1.02			
21		1.02			
22		1.02			
23		1.02			
24		1.02			
25		0.99			
26		1.01			
27		1.01			
28		0.99			
29		0.99			
30		1.01			
31		1.02			
32		0.98			
33		1.01			
34		1.01			
35		0.99			
36		0.99			
37		1.02			
38		0.98			
39		1.01			
40		1.00			
41		1.00			
42		1.00			
43		1.00			
44		1.00			
45		1.00			
46		1.01			
47		1.01			
48		0.99			
49		1.01			
50		0.99			
51		1.00			
52		1.00			
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57		1.00			
58		1.02			
59		0.99			
60		1.00			
61		1.00			
62		1.00			
63		1.00			
64		1.00			
65		1.00			
66		1.00			
67		1.00			
68		1.00			
69		1.00			
70		1.00			

Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q		
Assay Date		3/21/2007	Chemical ID	CR11615	# Concentrations tested	12335-13													
NS Ref.		12633-43	Counter data filename	21MR2059 P10															
Technician ID		ERP	Replicates #	3	Positive Control	4-OH-ASPM Pos Ctrl Tubes/Conc													
Sample ID		Calculate DPM in aqueous portion after extraction										Calculate nmol ³ H ₂ O formed							
Sample Type	Replicate Level	Nominal total volume (mL)	Aliq Volume (mL)	Aliq #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of substrate solution used/assay tube (mL)	total DPM in assay tube (initial)	nmol ³ H ₂ O formed	Final (protein) in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/mg protein/min)					
6	CR11615	1-1	2	0.5	5506	11212	11188	22386	0.1	192800	22170	0.0211	0.005	15	0.021	0.134			
7																			
8																			
9	1-2	2	0.5	5582	11184	10385	10347	20694	0.1	192800	20468	0.0213	0.005	15	0.021	0.142			
10																			
11	1-3	2	0.5	5153	10396	10396	10827	21654	0.1	192800	21428	0.0223	0.005	15	0.023	0.149			
12																			
13	2-1	2	0.5	5574	10748	11046	11046	22092	0.1	192800	21868	0.0228	0.005	15	0.023	0.152			
14																			
15	2-2	2	0.5	5493	10465	11046	11046	22092	0.1	192800	21868	0.0228	0.005	15	0.023	0.152			
16																			
17	2-3	2	0.5	5283	10368	11046	11046	22092	0.1	192800	21868	0.0228	0.005	15	0.023	0.154			
18																			
19	3-1	2	0.5	5598	11208	11208	11208	22416	0.1	192800	22190	0.0231	0.005	15	0.023	0.154			
20																			
21	3-2	2	0.5	7121	14242	14175	14175	28350	0.1	192800	28124	0.0263	0.005	15	0.026	0.195			
22																			
23	3-3	2	0.5	7310	14620	14688	14688	29376	0.1	192800	29150	0.0284	0.005	15	0.028	0.202			
24																			
25	4-1	2	0.5	7594	14768	14546	14667	29334	0.1	192800	29106	0.0303	0.005	15	0.030	0.202			
26																			
27	4-2	2	0.5	9014	18162	18095	18095	36190	0.1	192800	35864	0.0375	0.005	15	0.037	0.250			
28																			
29	4-3	2	0.5	8803	17608	17601	17601	35202	0.1	192800	34076	0.0364	0.005	15	0.036	0.243			
30																			
31	5-1	2	0.5	8765	17568	17592	17592	35084	0.1	192800	34638	0.0363	0.005	15	0.036	0.242			
32																			
33	5-2	2	0.5	5247	21348	21274	21274	42548	0.1	192800	42322	0.0441	0.005	15	0.044	0.294			
34																			
35	5-3	2	0.5	5162	20788	20734	20734	41468	0.1	192800	41242	0.0429	0.005	15	0.042	0.296			
36																			
37	6-1	2	0.5	10519	21032	21474	21283	42506	0.1	192800	42280	0.0440	0.005	15	0.044	0.294			
38																			
39	6-2	2	0.5	11497	22894	22840	22967	45934	0.1	192800	45708	0.0476	0.005	15	0.047	0.317			
40																			
41	6-3	2	0.5	11526	23068	22840	22840	45680	0.1	192800	45454	0.0469	0.005	15	0.046	0.313			
42																			
43	7-1	2	0.5	12470	24940	24721	24842	49684	0.1	192800	49418	0.0474	0.005	15	0.047	0.316			
44																			
45	7-2	2	0.5	12073	24146	24146	24146	48292	0.1	192800	48065	0.0513	0.005	15	0.051	0.342			
46																			
47	7-3	2	0.5	12585	25130	24862	24862	49724	0.1	192800	49598	0.0518	0.005	15	0.051	0.334			
48																			
49	8-1	2	0.5	12397	24594	24594	24594	49188	0.1	192800	48962	0.0518	0.005	15	0.051	0.345			
50																			
51	8-2	2	0.5	12553	25105	24788	24788	49572	0.1	192800	49366	0.0514	0.005	15	0.051	0.343			
52																			
53	8-3	2	0.5	12443	24886	24882	24882	49764	0.1	192800	49558	0.0515	0.005	15	0.051	0.344			
54																			

Aromatase Assay Spreadsheet

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Aromatase Assay Spreadsheet

A		B	C	D	E	F	G	H	I	J	K	L
Assay Date		3/21/2007	Test Chemical ID	CR11615								
NB Ref.		12535-63	Counter data filename	21MRZ059.P00								
Technician ID		PRP	Replicate #									
							3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc		
Control Type		Average	SD									
Full activity		0.351	0.020									
Background (unconnected)		0.002	0.000									

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K	
1	Assay Date	3/16/2007	Test Chemical ID	CR11616	# Concentrations tested			8	Microsome ID	12535-13
2	NB Ref.	12535-76	Counter data filename	16MR2052.P00						
3	Technician		Replicate #	1	Positive Control	4-OH ASDN	Tubes/Conc			2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/16/2007	Test Chemical ID	CR11616	# Concentrations tested		8	Microsome ID	12535-13
2	NB Ref.	12535-76	Counter data filename	16MR2052.P00					
3	Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0201		41031		2041343			
8	2	0.0194		41543		2141392			
9	3	0.0197		41961		2130000			
10	4	0.0197		42649		2164924			
11	5	0.0202		42216		2089901			
12						Average DPM/g soln		2113512	
13						SD		48626	
14						CV		2.30	
15						µCi/g soln		0.9520	
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0108 g			
25	Mass of dilution B used in substrate prep					4.4647 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.55679166 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.012 µg/g soln.					
29				µg/g soln.					
30	a. µCi/g soln					0.9520			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36			=	0.5568	+	0.012			
37			=	0.568	µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=		1.675	µCi/µg ASDN					
41			1.065.E+06	dpm/nmol					

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/16/2007	Test Chemical ID	CR11616	# Concentrations tested		8	Microsome ID	12535-13
2			INB Ref	12535-76	Counter data filename	16MR2052.P00					
3			Technician ID	ase	Replicate #	1	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
5	Microsome Dilution Details							Positive Control Concentrations			
6								Level	Final Concentration (M)		
7	Dilution A			0.12 mL microsome Stock used				1	1.00E-05		
8				2.4 mL total volume				2	1.00E-06		
9				20 dilution factor				3	5.00E-07		
10								4	1.00E-07		
11	Dilution B			1.5 mL microsome Dilution A used				5	5.00E-08		
12				60 mL total volume				6	1.00E-08		
13				40 dilution factor				7	1.00E-09		
14								8	1.00E-10		
15	Dilution C (if applicable)			mL microsome Dilution B used							
16				mL total volume							
17		NA		dilution factor				Test Chemical Concentrations			
18								Level	Final Concentration (M)		
19				800 total dilution factor				1	1.00E-03		
20								2	1.00E-04		
21	Protein Concentration (stock microsomes, mg/mL):				8			3	1.00E-05		
22	Protein Concentration (dilution added to assay, mg/mL):				0.01			4	1.00E-06		
23								5	1.00E-07		
24								6	1.00E-08		
25								7	1.00E-09		
26								8	1.00E-10		

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Test #								
			Chemical Concentrations								
2			NI 12535-76	Counter data filename 16MR2052.P00							
3			Replicate # 1	Positive Control							
4			Pos Ctl 4-OH ASDI Tubes/Conc								
5	Vial	TI	DPM1								Round DPM
6	2	5	41030.57	16772.8	0	12.01	468.78				41031
7	3	5	41543.23	18460.8	0	12.93	528.83				41543
8	4	5	41960.59	18689.04	0	12.98	532.76				41961
9	5	5	42648.97	19280.24	0	13.19	548.73				42649
10	6	5	42215.66	18937.24	0	12.98	536.36				42216
11	8	5	13429.59	6041.36	0	13.02	538.21				13430
12	9	5	13225.19	5949.56	0	13.04	539.01				13225
13	10	5	12076.29	5428.8	0	13.11	541.13				12076
14	11	5	11990.2	5407.64	0	13.15	543.02				11990
15	12	5	9494.83	4263.04	0	13.02	541.26				9495
16	13	5	9559.55	4275.4	0	13.1	539.4				9560
17	14	5	6933.15	3109.8	0	13.11	539.2				6933
18	15	5	6945.49	3108.08	0	13.13	542.84				6945
19	17	5	68.82	31.56	0	13.21	538.46				69
20	18	5	55.77	25	0	11.12	536.14				56
21	19	5	68.65	30.6	0	12.17	535.7				69
22	20	5	77.43	31.2	0	12.13	536.99				77
23	21	5	59.06	27.16	0	14.66	535.59				59
24	22	5	72.38	31.8	0	13.46	532.02				72
25	23	5	52.93	23.66	0	11.56	535.46				53
26	24	5	63.59	29.76	0	13.1	534.84				64
27	26	5	197.62	88.28	0	12.52	534.37				198
28	27	5	195.19	87.36	0	12.69	533.84				195
29	28	5	182.51	82.84	0	13.27	533.99				183
30	29	5	173.77	79.56	0	12.11	538.66				174
31											
32											
33	30	5	1113.54	496.16	0	13.09	533.83				1114
34	31	5	1084.25	489.2	0	12.68	535.44				1084
35	32	5	1059.16	475.16	0	12.78	533.9				1059
36	33	5	1078.35	480.76	0	13.05	533.08				1078
37											
38											
39	34	5	1898.81	847.36	0	13.01	533.38				1899
40	35	5	1898.11	845.4	0	12.9	532.18				1896
41	36	5	1776.19	800.98	0	12.9	533.8				1776
42	37	5	1742.51	782.36	0	12.93	534.07				1743
43											
44											
45	38	5	5899.36	2631.36	0	13.03	536.96				5899
46	39	5	5792.71	2598.48	0	12.84	531.94				5793
47	40	5	5680.43	2526.6	0	12.96	534.19				5660
48	41	5	5626.26	2509.84	0	12.92	533.32				5626
49											
50											
51	42	5	7183.89	3195.4	0	12.92	533.41				7184
52	43	5	7383.73	3314.36	0	12.94	534.96				7384
53	44	5	6823.52	3050.48	0	12.89	533.46				6824
54	45	5	6797.05	3055.56	0	12.9	532.93				6797
55											
56											
57	48	5	11306.84	5064.76	0	12.96	534.52				11307
58	47	5	11428.75	5136.76	0	12.94	534.99				11429
59	48	5	9301.26	4118.6	0	13.06	532.45				9301
60	49	5	9259.21	4136.48	0	13	532.34				9259
61											
62											
63	50	5	12207.06	5462	0	12.98	535.18				12207
64	51	5	12167.19	5436.8	0	12.95	535.13				12167

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
66	52	5	8932.32	3996.24	0	13.04	534.94				8932
68	53	5	8833.15	3937.16	0	12.97	532.61				8833
67											
68											
69	54	5	11567.43	5156.16	0	12.97	533.03				11557
70	55	5	11813.1	5288.16	0	13.03	534.54				11813
71	56	5	11534.67	5183.6	0	12.93	534.85				11535
72	57	5	11316.98	5083.84	0	12.92	534.02				11317
73											
74											
75	59	5	9213.24	4121.4	0	12.95	535.9				9213
76	60	5	9306.78	4170.4	0	12.99	534.27				9307
77	61	5	11407.13	5120.16	0	12.89	532.23				11407
78	62	5	11754.47	5267.84	0	12.97	534.22				11754
79	63	5	11147.97	4964.4	0	12.88	532.61				11148
80	64	5	11344.24	5069.4	0	12.93	529.95				11344
81	65	5	9582.33	4289.96	0	12.96	533.19				9582
82	66	5	9787.9	4375.8	0	12.99	536.1				9788
83	67	5	11913.69	5322.76	0	13.1	536.05				11914
84	68	5	12136.89	5399.76	0	12.99	533.84				12137
85	69	5	11013.12	4904.56	0	13.03	534.43				11013
86	70	5	11326.69	5069.76	0	13.06	541.12				11327
87	71	5	9279.73	4145.4	0	12.93	535.89				9280
88	72	5	9459.08	4203.96	0	13.02	531.46				9459
89	73	5	11310.39	5075.64	0	12.93	535.07				11310
90	74	5	11377.93	5074.56	0	13.01	533.91				11378
91	75	5	11773.04	5252.96	0	12.93	532.74				11773
92	76	5	11794.68	5289.36	0	12.87	535.02				11795
93	77	5	11842.27	5305.24	0	12.93	532.57				11842
94	78	5	11975.13	5361.4	0	12.88	532.89				11975
95	79	5	10048.29	4465.64	0	12.91	531.62				10048
96	80	5	10065.55	4516.6	0	12.95	535.95				10066
97	81	5	10368.78	4661.4	0	12.89	535.27				10369
98	82	5	10846.17	4723.16	0	13.02	531.07				10846
99	84	5	11244.19	5048.8	0	12.89	534.16				11244
100	85	5	11333.4	5067.4	0	13	535.66				11333
101	86	5	11041	4946.48	0	12.86	531.79				11041
102	87	5	10902.19	4867.8	0	13	532.52				10902
103	88	5	10741.49	4840.16	0	13.11	543.48				10741
104	89	5	10947.15	4890.56	0	12.97	535.87				10947
105	90	5	12178.47	5434.4	0	13.09	536.13				12178
106	91	5	11868.63	5285.76	0	12.87	530.37				11869
107	92	5	6947.95	3084.56	0	12.98	535.95				6948
108	93	5	6922.34	3107.6	0	12.91	535.46				6922
109	94	5	10146.43	4527.76	0	12.93	530.85				10146
110	95	5	10298.38	4597.56	0	12.99	536.84				10298
111	96	5	11813.83	5231.96	0	12.96	532.16				11814
112	97	5	12006.5	5372.08	0	12.91	533.32				12007
113	98	5	11280.8	5062	0	12.94	533.33				11281
114	99	5	11420.46	5127.76	0	12.98	532.77				11420
115	100	5	10153.01	4529.36	0	12.96	533.2				10153
116	101	5	10285.98	4607.24	0	12.87	533				10286
117	102	5	11261.7	5032.36	0	12.85	532.14				11262
118	103	5	11321.97	5059.36	0	12.92	534.05				11322
119	104	5	9164.14	4106.36	0	12.93	534.89				9164
120	105	5	9400.02	4183.56	0	13.11	534.02				9400
121	106	5	7501.26	3350	0	12.93	533.37				7501
122	107	5	7567.56	3379.44	0	12.88	531.49				7568
123	108	5	230939.8	33603.76	0	7.95	172.15				230940
124											#REF!
125											#REF!
126											#REF!
127											#REF!
128											#REF!
129											#REF!
130											#REF!
131											#REF!

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
				Test Chemical ID			# Concentrations tested		8. Microsome ID	12355-13					
	Assay Date			CFR14616											
	NE Ref. Technician ID			12355-75											
				346			1	Positive Control	4-CH-ASDN	Pos Ctrl Tubes/Conc	2				
Sample ID	Replicate	Nonfinal total volume (mL)	Aliq. #	DPM/aliq	DPM/mL	Ave DPM/mL	Total DPM	Volume of solvent solution used in assay (mL)	Total DPM in assay tube (final)	Final protein in assay (mg/mL)	Incubation time (min)	Amperase activity			
6	1	2	0.5	13430	26860	26865	53310	0.1	211351	0.005	15	0.332			
7	2	2	0.5	13226	26450	26455	52910	0.1	211351	0.005	15	0.332			
8	3	2	0.5	12078	24152	24085	48132	0.1	211351	0.005	15	0.332			
9	4	2	0.5	11980	23940	23940	47920	0.1	211351	0.005	15	0.332			
10	5	2	0.5	9495	18990	19005	38110	0.1	211351	0.005	15	0.332			
11	6	2	0.5	6933	13866	13878	27756	0.1	211351	0.005	15	0.332			
12	7	2	0.5	6945	13890	13890	27780	0.1	211351	0.005	15	0.332			
13	8	2	0.5	69	138	138	276	0.1	211351	0.005	15	0.332			
14	9	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
15	10	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
16	11	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
17	12	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
18	13	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
19	20	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
20	21	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
21	22	2	0.5	59	118	118	236	0.1	211351	0.005	15	0.332			
22	23	2	0.5	165	330	330	660	0.1	211351	0.005	15	0.332			
23	24	2	0.5	165	330	330	660	0.1	211351	0.005	15	0.332			
24	25	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
25	26	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
26	27	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
27	28	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
28	29	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
29	30	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
30	31	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
31	32	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
32	33	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
33	34	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
34	35	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
35	36	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
36	37	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
37	38	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
38	39	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
39	40	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
40	41	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
41	42	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
42	43	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
43	44	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
44	45	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
45	46	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
46	47	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
47	48	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
48	49	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
49	50	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
50	51	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
51	52	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
52	53	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
53	54	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
54	55	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
55	56	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
56	57	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
57	58	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
58	59	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
59	60	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
60	61	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
61	62	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
62	63	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
63	64	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
64	65	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
65	66	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
66	67	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
67	68	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
68	69	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
69	70	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
70	71	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
71	72	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
72	73	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
73	74	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
74	75	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
75	76	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
76	77	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
77	78	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
78	79	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
79	80	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
80	81	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
81	82	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
82	83	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
83	84	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
84	85	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
85	86	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
86	87	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
87	88	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
88	89	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
89	90	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
90	91	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
91	92	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
92	93	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
93	94	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
94	95	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
95	96	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
96	97	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
97	98	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
98	99	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
99	100	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			
100	101	2	0.5	174	348	348	696	0.1	211351	0.005	15	0.332			

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4					
5		QC check - atleast variance			
6		Red if value not within mean +/- 10%			
7					
8		1.01			
9		0.99			
10		1.00			
11		1.00			
12		1.00			
13		1.00			
14		1.00			
15					
16					
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Aromatase Assay Spreadsheet

[illegible]

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC Check - aliquot variance
6		% RE
7		0.99
8		1.01
9		0.99
10		1.01
11		0.99
12		0.99
13		0.99
14		1.01
15		0.99
16		1.01
17		0.99
18		1.01
19		0.99
20		1.01
21		1.00
22		1.00
23		1.00
24		1.00
25		0.99
26		1.01
27		1.00
28		0.99
29		1.01
30		1.00
31		1.00
32		1.01
33		1.01
34		0.99
35		0.99
36		1.01
37		1.01
38		0.99
39		1.00
40		1.00
41		0.99
42		1.01
43		0.99
44		1.01
45		0.99
46		1.01
47		0.99
48		1.01
49		1.00
50		1.00
51		0.99
52		1.01
53		1.00
54		1.00

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1	Assay Date	Assay Ref.	Assay ID	Test Chemical ID	CR111816	# Concentrations tested					
2	3/16/2007	12535-76	16MR2052 P00	CR111816	16MR2052 P00	1	Positive Control	4-OH ASDN	8 Microsome ID	12535-13	
3											
4											
5	Control Type	Average	SD								
6	Full activity	D.260	D.071								
7	Background (uncorrected)	0.002	0.000								
8											
9											
10	Positive Control	Replicate	Positive Ctl M	Log(Positive Ctl)	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.003						
12	4-OH ASDN	2	1.00E-06	-6.00	0.003						
13	4-OH ASDN	1	1.00E-06	-6.00	0.026						
14	4-OH ASDN	2	1.00E-06	-6.00	0.025						
15	4-OH ASDN	1	1.00E-06	-6.00	0.025						
16	4-OH ASDN	2	1.00E-06	-6.00	0.025						
17	4-OH ASDN	1	5.00E-07	-6.30	0.046						
18	4-OH ASDN	2	5.00E-07	-6.30	0.042						
19	4-OH ASDN	1	1.00E-07	-7.00	0.145						
20	4-OH ASDN	2	1.00E-07	-7.00	0.140						
21	4-OH ASDN	1	5.00E-08	-7.30	0.161						
22	4-OH ASDN	2	5.00E-08	-7.30	0.169						
23	4-OH ASDN	1	1.00E-08	-8.00	0.263						
24	4-OH ASDN	2	1.00E-08	-8.00	0.231						
25	4-OH ASDN	1	1.00E-08	-8.00	0.263						
26	4-OH ASDN	2	1.00E-08	-8.00	0.231						
27	4-OH ASDN	1	1.00E-09	-9.00	0.304						
28	4-OH ASDN	2	1.00E-09	-9.00	0.221						
29	4-OH ASDN	1	1.00E-10	-10.00	0.251						
30	4-OH ASDN	2	1.00E-10	-10.00	0.284						
31	4-OH ASDN	1	1.00E-10	-10.00	0.251						
32	4-OH ASDN	2	1.00E-10	-10.00	0.284						
33	4-OH ASDN	1	1.00E-10	-10.00	0.251						
34	4-OH ASDN	2	1.00E-10	-10.00	0.284						
35											
36											
37	Test Chemical	Replicate	Test chemical M	Log(test chemical)	Activity						
38	CR111616	1	1.00E-03	-3.00	0.230						
39	CR111616	2	1.00E-03	-3.00	0.286						
40	CR111616	1	1.00E-03	-3.00	0.280						
41	CR111616	2	1.00E-04	-4.00	0.241						
42	CR111616	1	1.00E-04	-4.00	0.259						
43	CR111616	2	1.00E-04	-4.00	0.278						
44	CR111616	1	1.00E-05	-5.00	0.233						
45	CR111616	2	1.00E-05	-5.00	0.282						
46	CR111616	1	1.00E-06	-6.00	0.293						
47	CR111616	2	1.00E-06	-6.00	0.297						
48	CR111616	1	1.00E-06	-6.00	0.250						
49	CR111616	2	1.00E-06	-6.00	0.261						
50	CR111616	1	1.00E-07	-7.00	0.281						
51	CR111616	2	1.00E-07	-7.00	0.273						
52	CR111616	1	1.00E-07	-7.00	0.270						
53	CR111616	2	1.00E-08	-8.00	0.269						
54	CR111616	1	1.00E-08	-8.00	0.172						
55	CR111616	2	1.00E-08	-8.00	0.254						
56	CR111616	1	1.00E-09	-9.00	0.297						
57	CR111616	2	1.00E-09	-9.00	0.283						
58	CR111616	1	1.00E-09	-9.00	0.283						
59	CR111616	2	1.00E-10	-10.00	0.281						
60	CR111616	1	1.00E-10	-10.00	0.231						
61	CR111616	2	1.00E-10	-10.00	0.187						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11616	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-80	Counter data filename	20MR2221.P00					
3	Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/20/2007	Test Chemical ID	CR11616	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-80	Counter data filename	20MR2221.P00					
3	Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0203		39160		1929064			
8	2	0.0201		40757		2027711			
9	3	0.0197		41515		2107360			
10	4	0.0201		41244		2051940			
11	5	0.0200		40432		2021600			
12						Average DPM/g soln	2027535		
13						SD	64613		
14						CV	3.19		
15						µCi/g soln	0.9133		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	[ASDN] in solution (µg/mL)				
19	Stock	10.03	10		1003.00				
20	Dilution A			100	10.03				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0118 g			
25	Mass of dilution B used in substrate prep					4.4600 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.55834894 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.9133			
31	b. Specific activity of [³ H]ASDN (µCi/mmol)					23500000			
32	c. Molecular wt of ASDN (mg/mmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				=	0.5583	+	0.011		
37				=	0.569	µg ASDN/g soln.			
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=		1.604	µCi/µg ASDN					
41			1.020.E+06	dpm/nmol					

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1			Assay Date	3/20/2007	Test Chemical ID	CR11616	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-80	Counter data filename	20MR2221.P00					
3			Technician ID	ase	Replicate #	2	Positive Control	4-OH ASDN	Pos Ctl	Tubes/Conc	2
4											
5	Microsome Dilution Details										
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											
19											
20											
21											
22											
23											
24											
25											
26											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
1				Test #							
				Chemical Concentrations							
2				At: 3/20/2007	ID	Cl	tested	8	Microsome ID	12535-13	
3				Counter data							
4				NI 12535-80	filename	20MR2221.P00					
				T							
				e							
				c							
				h							
				ni							
				cl							
				a							
				n							
				l							
3				Replicate	Positive	Pos Ctl					
4				Dase # 2	Control	4-OH ASDI Tubes/Conc				2	
5											
6				Vial	TI	DPM1					Round DPM
7				2	5	39160.07	17323.36	0	12.79	523.95	39160
8				3	5	40756.93	18050.38	0	12.83	523.98	40757
9				4	5	41514.61	18484.32	0	12.86	529.93	41515
10				5	5	41244.1	18703	0	13.28	552.27	41244
11				6	5	40431.94	18104.84	0	13.03	535.92	40432
12				8	5	15990.96	7173.4	0	12.84	531.23	15991
13				9	5	15718.72	7038.84	0	12.93	534.53	15719
14				10	5	15549.89	6917.8	0	12.91	530.6	15550
15				11	5	15420.49	6886.96	0	12.95	532.57	15420
16				12	5	14082.02	6265.44	0	12.91	530.31	14082
17				13	5	13780.46	6140.2	0	12.92	531.75	13780
18				14	5	11920.96	5328.96	0	12.92	530.26	11921
19				15	5	12027.9	5375.8	0	12.87	531.4	12028
20				17	5	65.99	28.2	0	14.38	531.43	66
21				18	5	56.47	25	0	11.33	531.04	58
22				19	5	70.87	29.16	0	12.88	531.98	71
23				20	5	67.23	27.44	0	15.96	531.38	67
24				21	5	65.93	30.96	0	11	530.85	66
25				22	5	69.2	27	0	14.48	532.94	69
26				23	5	72.56	31.6	0	13.83	533	73
27				24	5	63.08	28.38	0	12.85	531.35	63
28				26	5	175.8	77.8	0	12.84	532.44	176
29				27	5	171.22	76.8	0	12.29	532.83	171
30				28	5	152.78	68.48	0	13.08	531.09	153
31				29	5	163.1	72.56	0	13.44	531.14	163
32											
33				30	5	933.74	417.4	0	12.88	532.6	934
34				31	5	936.57	414.84	0	12.9	531.84	937
35				32	5	923.21	415	0	13.07	530.02	923
36				33	5	954.03	424.2	0	12.83	531.32	954
37											
38											
39				34	5	1672.51	750.76	0	12.98	533.07	1673
40				35	5	1720.29	775.84	0	12.93	532.87	1720
41				36	5	1754.16	779.66	0	12.83	533.12	1754
42				37	5	1718.07	774	0	12.81	533.61	1718
43											
44											
45				38	5	5713.25	2562.2	0	12.86	533.26	5713
46				39	5	5751.78	2552.36	0	12.95	528.79	5752
47				40	5	5129.89	2287.16	0	12.87	531.88	5130
48				41	5	5273.95	2346.24	0	13	531.48	5274
49											
50											
51				42	5	8450.18	3753.64	0	12.98	533.15	8450
52				43	5	8647.56	3854.48	0	12.86	530.1	8648
53				44	5	7800.27	3479.92	0	12.94	529.62	7800
54				45	5	7809.66	3477.44	0	12.89	530.92	7810
55											
56											
57				46	5	13019.56	5814.8	0	12.97	534.2	13020
58				47	5	13145.29	5897.36	0	12.91	532.54	13145
59				48	5	12256.1	5472.44	0	12.91	532.02	12256
60				49	5	12360.69	5521.98	0	12.92	534.17	12361
61											
62											
63				50	5	14092.87	6325.44	0	12.86	533.48	14093
64				51	5	14446.69	6461.84	0	12.88	533.66	14446

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	13502.87	6035.76	0	12.91	529.63				13503
66	53	5	13663.26	6081.44	0	12.94	530.3				13663
67											
68											
69	54	5	14326.07	6405.88	0	12.86	529.64				14326
70	55	5	14422.88	6418.56	0	12.93	531.04				14423
71	56	5	13671.02	6103.08	0	12.91	532.01				13671
72	57	5	13804.52	6128.8	0	12.98	529.57				13805
73											
74											
75	59	5	14956.5	6652.64	0	12.95	530.25				14957
76	60	5	15194.95	6776.76	0	12.94	529.67				15195
77	61	5	13821.7	6203.6	0	12.93	534.06				13822
78	62	5	14280.6	6342	0	12.92	528.87				14281
79	63	5	13997.85	6247.48	0	12.97	534.16				13998
80	64	5	14229.52	6356.36	0	12.95	532.07				14230
81	65	5	14326.27	6411.76	0	12.99	534.38				14326
82	66	5	14480.54	6481.6	0	12.94	533.67				14481
83	67	5	13980.41	6214.16	0	12.95	529.85				13980
84	68	5	14379.84	6400.16	0	12.91	531.54				14380
85	69	5	13784.14	6165.24	0	12.92	533.57				13784
86	70	5	14008.79	6258.76	0	12.95	531.15				14009
87	71	5	14449.4	6473.6	0	12.96	532.15				14449
88	72	5	14381.58	6413.8	0	13.03	533.24				14382
89	73	5	12227.09	5474.6	0	12.93	532.2				12227
90	74	5	12292.6	5496.96	0	12.86	529.42				12293
91	75	5	14468.86	6473.56	0	12.95	530.94				14469
92	76	5	14363.11	6422.44	0	12.88	531.65				14363
93	77	5	14050.96	6288.64	0	12.85	531.68				14051
94	78	5	14644.55	6520.56	0	12.97	532.41				14645
95	79	5	13970.09	6268	0	12.96	534.9				13970
96	80	5	14043.29	6277.4	0	12.92	531.77				14043
97	81	5	14257.97	6347.56	0	12.95	532.53				14258
98	82	5	14510.28	6489.48	0	12.91	531.91				14510
99	84	5	14104.25	6300.24	0	12.92	530.81				14104
100	85	5	14394.04	6395.38	0	12.9	530.05				14394
101	86	5	13702.19	6094.76	0	12.88	530.67				13702
102	87	5	13729.2	6088.36	0	12.93	529.72				13729
103	88	5	14295.63	6419.36	0	12.85	532.47				14296
104	89	5	15123.74	6715.84	0	12.96	530.49				15124
105	90	5	14321.72	6431.96	0	12.83	532.65				14322
106	91	5	14307.37	6409.4	0	12.96	531.47				14307
107	92	5	13680.27	6096.96	0	12.88	527.93				13680
108	93	5	14022.38	6239.4	0	12.88	529.59				14022
109	94	5	14107.98	6315.6	0	12.93	533.85				14108
110	95	5	14738.48	6548.04	0	12.97	530.34				14738
111	96	5	13339.25	5936.2	0	12.91	531.01				13339
112	97	5	13442.53	6028.2	0	12.91	533.68				13443
113	98	5	13525.06	6024.24	0	12.86	529.84				13525
114	99	5	13749.18	6165.52	0	12.86	533.65				13749
115	100	5	14181.1	6314.32	0	12.91	530.97				14181
116	101	5	14539.94	6486.84	0	12.94	530.91				14540
117	102	5	13271.79	5906.16	0	12.97	530.57				13272
118	103	5	13468.18	6014.96	0	12.94	531.7				13468
119	104	5	14565.71	6509.56	0	12.94	534.66				14566
120	105	5	15093.22	6714.8	0	12.88	529.64				15093
121	106	5	16221.67	7203.56	0	12.94	531.39				16222
122	107	5	16535.84	7390	0	12.9	533.56				16536
123	109	5	231349.48	33784.2	0	7.98	173.11				231349
124											#REF!
125											#REF!
126											#REF!
127											#REF!
128											#REF!
129											#REF!
130											#REF!
131											#REF!
132											#REF!
133											231349
134											#REF!
135											#REF!
136											#REF!
137											#REF!
138											#REF!
139											#REF!
140											#REF!

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate level	Nonfinal volume (mL)	Assay volume (mL)	Allo. #	DPM/assay	DPM/mL	Ave. DPM/mL	Total DPM	Volume of substrate added (assay tube, µL)	total DPM in assay tube (total)	Calculate nmol ³ H ₂ O formed (total DPM converted for background (Background tubes))	nmol ³ H ₂ O formed	Final (pmol) in assay (mg/mL)	incubation time (min)	Aromatase activity for each condition (pmol/min)
1	Full activity control	2	0.5	1	15991	31982	31710	63420	0.1	202754	63155	0.0618	0.005	15	0.413
2	1	2	0.5	2	15716	31436	20970	61940	0.1	202754	61675	0.0605	0.005	15	0.403
3	2	2	0.5	2	15560	31100	20970	61940	0.1	202754	61675	0.0605	0.005	15	0.403
4	3	2	0.5	2	14082	28164	27852	55724	0.1	202754	55459	0.0544	0.005	15	0.363
5	4	2	0.5	2	13680	27360	27360	54720	0.1	202754	54459	0.0531	0.005	15	0.353
6	Background control	1	0.5	1	11821	23642	23849	47698	0.1	202754	47533	0.0467	0.005	15	0.311
7	1	2	0.5	2	12026	24056	122	244	0.1	202754	22	0.0000	0.005	15	0.000
8	2	2	0.5	2	56	112	138	276	0.1	202754	11	0.0000	0.005	15	0.000
9	3	2	0.5	2	67	134	135	270	0.1	202754	5	0.0000	0.005	15	0.000
10	4	2	0.5	2	83	166	136	272	0.1	202754	7	0.0000	0.005	15	0.000
11	4-OH ASON	1	0.5	1	176	352	347	694	0.1	202754	429	0.0004	0.005	15	0.003
12	1	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
13	2	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
14	3	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
15	4	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
16	Background control	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
17	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
18	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
19	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
20	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
21	4-OH ASON	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
22	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
23	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
24	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
25	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
26	Background control	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
27	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
28	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
29	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
30	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
31	4-OH ASON	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
32	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
33	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
34	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
35	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
36	Background control	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
37	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
38	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
39	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
40	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
41	4-OH ASON	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
42	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
43	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
44	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
45	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
46	Background control	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
47	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
48	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
49	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
50	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
51	4-OH ASON	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
52	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
53	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
54	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
55	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
56	Background control	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
57	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
58	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
59	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
60	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
61	4-OH ASON	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
62	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
63	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
64	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
65	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
66	Background control	1	0.5	1	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002
67	1	2	0.5	2	163	326	376	752	0.1	202754	429	0.0004	0.005	15	0.003
68	2	2	0.5	2	183	366	376	752	0.1	202754	429	0.0004	0.005	15	0.003
69	3	2	0.5	2	171	342	347	694	0.1	202754	429	0.0004	0.005	15	0.003
70	4	2	0.5	2	153	306	316	632	0.1	202754	367	0.0004	0.005	15	0.002

Aromatase Assay Spreadsheet

	R	S	T	U	V
1					
2					
3					
4		QC check - aliquot			
5		variance			
6		Rec'd if value not			
7		within 10%			
8		1.01			
9		1.99			
10		1.00			
11		1.01			
12		0.99			
13		1.00			
14		1.00			
15		1.03			
16		0.92			
17		1.03			
18		0.97			
19		0.98			
20		1.01			
21		0.97			
22		0.93			
23		1.01			
24		0.99			
25		0.97			
26		1.03			
27					
28					
29					
30					
31					
32					
33					
34					
35		0.99			
36		1.01			
37					
38		0.99			
39					
40					
41		1.00			
42		1.00			
43		0.98			
44		1.01			
45					
46					
47		0.99			
48		1.01			
49		1.00			
50		1.00			
51					
52		1.00			
53		1.00			
54		1.00			
55		1.00			
56		1.00			
57					
58					
59		0.99			
60		1.01			
61		0.99			
62		1.01			
63					
64					
65		1.00			
66					
67		1.00			
68					
69		1.00			
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Assay Date	Assay Ref	Technician	ID	Assay Date	Assay Ref	Technician	ID	Assay Date	Assay Ref	Technician	ID	Assay Date	Assay Ref	Technician	ID
3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80
OR111616	20M22221 P00	2	Positive Control	OR111616	20M22221 P00	2	Positive Control	OR111616	20M22221 P00	2	Positive Control	OR111616	20M22221 P00	2	Positive Control
Sample ID	Replicate	Volume (mL)	Assay Date	Assay Ref	Technician <td>ID</td> <td>Assay Date</td> <td>Assay Ref</td> <td>Technician<td>ID</td><td>Assay Date</td><td>Assay Ref</td><td>Technician<td>ID</td><td>Assay Date</td></td></td>	ID	Assay Date	Assay Ref	Technician <td>ID</td> <td>Assay Date</td> <td>Assay Ref</td> <td>Technician<td>ID</td><td>Assay Date</td></td>	ID	Assay Date	Assay Ref	Technician <td>ID</td> <td>Assay Date</td>	ID	Assay Date
1	1-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
2	1-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
3	1-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
4	2-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
5	2-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
6	2-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
7	3-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
8	3-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
9	3-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
10	4-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
11	4-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
12	4-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
13	5-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
14	5-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
15	5-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
16	6-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
17	6-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
18	6-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
19	7-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
20	7-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
21	7-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
22	8-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
23	8-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
24	8-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
25	9-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
26	9-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
27	9-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
28	10-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
29	10-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
30	10-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
31	11-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
32	11-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
33	11-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
34	12-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
35	12-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
36	12-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
37	13-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
38	13-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
39	13-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
40	14-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
41	14-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
42	14-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
43	15-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
44	15-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
45	15-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
46	16-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
47	16-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
48	16-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
49	17-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
50	17-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
51	17-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
52	18-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
53	18-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
54	18-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
55	19-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
56	19-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
57	19-3	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
58	20-1	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
59	20-2	2	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	3/20/2007	12535-80	12535-80	12535-80	12535-80
60	20-3	2	3/20/20												

Aromatase Assay Spreadsheet

	R	S
1		
2		
3		
4		
5		QC check - aliquot variance
6		% RE
7		0.99
8		1.01
9		0.98
10		1.02
11		0.99
12		1.01
13		0.99
14		1.01
15		0.99
16		1.01
17		0.98
18		1.01
19		1.00
20		1.00
21		1.00
22		1.00
23		1.00
24		1.00
25		0.98
26		1.02
27		1.00
28		1.00
29		0.99
30		1.01
31		0.99
32		1.01
33		1.00
34		1.00
35		0.97
36		1.03
37		1.00
38		1.00
39		0.98
40		1.01
41		0.99
42		1.02
43		1.00
44		1.00
45		0.99
46		1.01
47		0.99
48		1.01
49		0.99
50		1.01
51		0.99
52		1.02
53		0.99
54		1.01

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Date	3/20/2007	Test Chemical ID	CR111616	# Concentrations tested			8	Microsome ID	12535-13
2		NE Ref. Technician ID	12535-80	Counter data filename	20MR2221.P00	2	Positive Control	4-OH ASDN		Pos Ctl Tubes/Conc	2
3				Replicate #							
4	Control Type	Average	SD								
5	Full activity	0.373	0.046								
6	Background (uncorrected)	0.002	0.000								
7											
8											
9											
10	Positive Control	Level	Replicate	Log[Positive Ctl]	Activity						
11	4-OH ASDN	1	1	-5.00	0.003						
12	4-OH ASDN	1	2	-5.00	0.002						
13											
14	4-OH ASDN	2	1	1.00E-06	0.023						
15	4-OH ASDN	2	2	1.00E-06	0.023						
16											
17	4-OH ASDN	3	1	5.00E-07	0.043						
18	4-OH ASDN	3	2	5.00E-07	0.044						
19											
20	4-OH ASDN	4	1	1.00E-07	0.148						
21	4-OH ASDN	4	2	1.00E-07	0.134						
22											
23	4-OH ASDN	5	1	5.00E-08	0.222						
24	4-OH ASDN	5	2	5.00E-08	0.202						
25											
26	4-OH ASDN	6	1	1.00E-08	0.340						
27	4-OH ASDN	6	2	1.00E-08	0.320						
28											
29	4-OH ASDN	7	1	1.00E-09	0.371						
30	4-OH ASDN	7	2	1.00E-09	0.353						
31											
32	4-OH ASDN	8	1	1.00E-10	0.374						
33	4-OH ASDN	8	2	1.00E-10	0.358						
34											
35											
36											
37	Test Chemical	Level	Replicate	Log[Test chemical]	Activity						
38	CR111616	1	1	1.00E-04	0.393						
39	CR111616	1	2	1.00E-04	0.366						
40	CR111616	1	3	1.00E-04	0.367						
41	CR111616	2	1	1.00E-05	0.375						
42	CR111616	2	2	1.00E-05	0.369						
43	CR111616	2	3	1.00E-05	0.362						
44	CR111616	3	1	1.00E-06	0.375						
45	CR111616	3	2	1.00E-06	0.319						
46	CR111616	3	3	1.00E-06	0.375						
47	CR111616	4	1	5.00E-07	0.373						
48	CR111616	4	2	5.00E-07	0.365						
49	CR111616	4	3	5.00E-07	0.374						
50	CR111616	5	1	1.00E-07	0.371						
51	CR111616	5	2	1.00E-07	0.357						
52	CR111616	5	3	1.00E-07	0.383						
53	CR111616	6	1	1.00E-08	0.373						
54	CR111616	6	2	1.00E-08	0.360						
55	CR111616	6	3	1.00E-08	0.375						
56	CR111616	7	1	1.00E-09	0.349						
57	CR111616	7	2	1.00E-09	0.359						
58	CR111616	7	3	1.00E-09	0.374						
59	CR111616	8	1	1.00E-10	0.348						
60	CR111616	8	2	1.00E-10	0.366						
61	CR111616	8	3	1.00E-10	0.427						

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/21/2007	Test Chemical ID	CR11616	# Concentrations tested			8	Microsome ID 12535-13
2	NB Ref.	12535-84	Counter data filename	21MR2105.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Tubes/Conc		2

Aromatase Assay Spreadsheet

	C	D	E	F	G	H	I	J	K
1	Assay Date	3/21/2007	Test Chemical ID	CR11616	# Concentrations tested	8	Microsome ID	12535-13	
2	NB Ref.	12535-84	Counter data filename	21MR2105.P00					
3	Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN	Pos Ctl Tubes/Conc	2	
4									
5									
6	Aliquot #	Weight of aliquot (g)		DPM/Aliq.		DPM/g soln.			
7	1	0.0193		34646		1795130			
8	2	0.0194		36803		1897062			
9	3	0.0201		41829		2081045			
10	4	0.0197		39361		1998020			
11	5	0.0194		41377		2132835			
12						Average DPM/g soln	1980818		
13						SD	136881		
14						CV	6.91		
15						µCi/g soln	0.8923		
16									
17	Calculation of actual concentration of nonradiolabeled ASDN in solution used to prepare substrate solution:								
18	ASDN solution	mg ASDN added	total volume (mL)	dilution factor	{ASDN} in solution (µg/mL)				
19	Stock	9.99	10		999.00				
20	Dilution A			100	9.99				
21	Dilution B			10	1.00				
22									
23	Calculation of concentration nonradiolabeled ASDN in substrate solution								
24	Total g substrate solution					8.0508 g			
25	Mass of dilution B used in substrate prep					4.5045 g			
26	Concentration of nonradiolabeled ASDN in substrate soln.					0.5589501 µg/g			
27	Calculation of Substrate Solution Specific Activity								
28	1) Calculate µg [³ H]ASDN/g soln. =			0.011	µg/g soln.				
29					µg/g soln.				
30	a. µCi/g soln					0.8923			
31	b. Specific activity of [³ H]ASDN (µCi/nmol)					23500000			
32	c. Molecular wt of ASDN (mg/nmol)					286.4			
33	Formula=a/b*c								
34	2) Calculate total µg ASDN/g soln.								
35	µg ASDN/g soln. = µg cold ASDN/g soln. + µg [³ H]ASDN/g soln.								
36				0.5590	+	0.011			
37				0.570	µg ASDN/g soln.				
38	3) Calculate Solution Specific Activity								
39	= (µCi/g soln.)/(µg ASDN/g soln.)								
40	=	1.566	µCi/µg ASDN						
41		9.956.E+05	dpm/nmol						

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
					Test Chemical ID						
1			Assay Date	3/21/2007	Counter data filename	CR11616	# Concentrations tested		8	Microsome ID	12535-13
2			NB Ref	12535-84		21MR2105.P00					
3			Technician ID	ase	Replicate #	3	Positive Control	4-OH ASDN		Pos Ctl	
4											2
5	Microsome Dilution Details										
6											
7	Dilution A			0.12 mL microsome Stock used						Positive Control Concentrations	
8				2.4 mL total volume						Level	
9				20 dilution factor						1	1.00E-05
10										2	1.00E-06
11	Dilution B			1.5 mL microsome Dilution A used						3	5.00E-07
12				60 mL total volume						4	1.00E-07
13				40 dilution factor						5	5.00E-08
14										6	1.00E-08
15	Dilution C (if applicable)			mL microsome Dilution B used						7	1.00E-09
16				mL total volume						8	1.00E-10
17		NA		dilution factor							
18											
19				800 total dilution factor							
20											
21	Protein Concentration (stock microsomes, mg/mL):				8						
22	Protein Concentration (dilution added to assay, mg/mL):				0.01						
23											
24											
25											
26											

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
				Test #							
				Chemical Concentrations							
1			A: 3/21/2007	ID C1	tested			8	Microsome ID	12535-13	
2			Counter data	filename	21MR2105.P00						
			T								
			e								
			c								
			h								
			n								
			i								
			c								
			a								
			n								
			l								
3				Replicate	Positive			Pos C11			
			Dase	#	3	Control		4-OH ASDI Tubes/Conc		2	
4											
5			Vial	Ti	DPM1	ISIE					Round DPM
6			2	5	34646.43	15537.54	0	13.07	536.42		34646
7			3	5	36803.29	16520.54	0	13.07	538.61		36803
8			4	5	41828.67	18726.38	0	12.97	532.94		41829
9			5	5	39360.74	17812.5	0	13.12	546.11		39361
10			6	5	41376.75	18587.7	0	13.1	540.67		41377
11			8	5	17056.94	7580.34	0	12.97	531.64		17057
12			9	5	16644.14	7424.22	0	12.94	530.74		16644
13			10	5	18434.09	7301.14	0	12.95	531.21		16434
14			11	5	16275.78	7225.62	0	12.89	529.57		16278
15			12	5	14950.63	6650.9	0	12.95	528.61		14951
16			13	5	15039.14	6701.14	0	12.95	529.69		15039
17			14	5	15948.69	7113.7	0	12.99	535.28		15949
18			15	5	15950.81	7124.54	0	12.97	530.98		15951
19			17	5	84.73	37.34	0	15.5	530.64		85
20			18	5	98.21	42.3	0	13.87	532.13		96
21			19	5	93.95	42.54	0	13.65	533.11		94
22			20	5	101.47	44.94	0	13.89	531.49		101
23			21	5	101.34	44.62	0	14.64	530.13		101
24			22	5	94.92	43.9	0	14.06	533.39		95
25			23	5	92.07	42.18	0	13.04	531.22		92
26			24	5	122.93	52.54	0	14	530.85		123
27			26	5	183.75	85.9	0	13.22	530		184
28			27	5	195.55	86.98	0	13.79	529.02		196
29			28	5	195.24	85.54	0	14.09	533.47		195
30			29	5	185.09	86.02	0	12.82	532.51		185
31											
32											
33			30	5	1091.22	485.9	0	12.81	530.54		1091
34			31	5	1104.31	491.54	0	12.81	531.19		1104
35			32	5	1084.44	494.78	0	12.65	530.69		1084
36			33	5	1124.84	502.34	0	12.83	529.99		1125
37											
38											
39			34	5	2069.21	920.3	0	12.86	529.29		2069
40			35	5	2098.16	924.1	0	13.14	531.25		2098
41			36	5	1904.37	846.7	0	12.93	531.93		1904
42			37	5	1926.7	862.54	0	12.99	529.32		1927
43											
44											
45			38	5	6972.31	3081.7	0	13.01	530.52		6972
46			39	5	6942.75	3100.38	0	12.88	531.78		6943
47			40	5	6215.62	2776.3	0	12.86	531.35		6216
48			41	5	6205.99	2772.78	0	12.92	532.8		6206
49											
50											
51			42	5	9560.74	4260.3	0	12.89	532.24		9561
52			43	5	9571.37	4233.7	0	13.03	528.66		9571
53			44	5	9611.21	4246.74	0	12.96	527.33		9611
54			45	5	9669.32	4296.5	0	12.94	531.03		9669
55											
56											
57			46	5	14820	6804.02	0	12.96	531.97		14820
58			47	5	14842.26	6598.98	0	12.95	529.69		14842
59			48	5	13881.51	6199.74	0	12.92	532.86		13882
60			49	5	13859.54	6138.1	0	13	529.46		13860
61											
62											
63			50	5	15936.7	7041.7	0	12.91	527.41		15937
64			51	5	15879.03	7066.54	0	12.95	530.64		15879

Aromatase Assay Spreadsheet

	A	B	C	D	E	F	G	H	I	J	K
65	52	5	15740.68	6975.1	0	12.96	529.81				15741
66	53	5	15638.82	6955.1	0	12.94	531.15				15639
67											
68											
69	54	5	16480.75	7335.7	0	12.94	530.37				16481
70	55	5	16398.62	7280.78	0	12.94	529.94				16399
71	56	5	15900.56	7119.78	0	12.89	531.43				15901
72	57	5	15921.09	7122.54	0	12.84	530.07				15921
73											
74											
75	59	5	15993.3	7138.14	0	12.95	531.27				15993
76	60	5	16447.44	7308.5	0	12.93	528.43				16447
77	61	5	16285.54	7262.34	0	12.93	532.07				16286
78	62	5	16379.81	7296.54	0	12.92	529.46				16380
79	63	5	15905.45	7090.22	0	12.96	530.84				15905
80	64	5	16057.81	7150.1	0	12.93	530.3				16058
81	65	5	16562.3	7401.98	0	12.93	532.83				16562
82	66	5	16620.85	7422.9	0	12.95	531.73				16621
83	67	5	17048.41	7620.98	0	12.92	532.29				17046
84	68	5	17046.24	7552.3	0	13	530.5				17046
85	69	5	17442.88	7759.38	0	12.92	530.67				17443
86	70	5	17283.17	7729.42	0	12.91	531.03				17283
87	71	5	16865.41	7497.78	0	12.96	530.52				16865
88	72	5	16666.79	7424.58	0	12.89	529.43				16667
89	73	5	16932.29	7510.7	0	12.95	530.35				16932
90	74	5	16993.27	7561.18	0	12.93	531.57				16993
91	75	5	16560.59	7413.98	0	12.88	533.05				16561
92	76	5	16848.75	7493.7	0	12.89	530.59				16849
93	77	5	16300.71	7181.74	0	13	528.53				16301
94	78	5	16455.88	7292.5	0	12.96	529.7				16456
95	79	5	15927	7073.18	0	12.86	527.82				15927
96	80	5	16430.23	7265.34	0	12.96	528.38				16430
97	81	5	15881.61	7064.98	0	12.94	529.74				15882
98	82	5	16058.83	7127.7	0	12.95	529.47				16059
99	84	5	15660.23	6989.22	0	12.88	530.55				15660
100	85	5	15742.42	7033.7	0	12.9	530.82				15742
101	86	5	16023.06	7190.1	0	12.86	533.49				16023
102	87	5	16101.84	7189.74	0	12.96	531.36				16102
103	88	5	17414.45	7788.7	0	12.95	531.23				17414
104	89	5	17563.93	7805.5	0	12.95	529.92				17564
105	90	5	16873.68	7498.34	0	12.9	527.56				16874
106	91	5	17128.13	7576.54	0	12.98	529.06				17128
107	92	5	16414.14	7330.38	0	12.94	530.6				16414
108	93	5	16788.55	7506.14	0	12.93	531.19				16789
109	94	5	17322.56	7684.7	0	12.94	529.2				17323
110	95	5	17402.93	7786.82	0	12.89	530.2				17403
111	96	5	16504.85	7396.98	0	12.87	530.99				16505
112	97	5	16841.22	7504.74	0	13	532.65				16841
113	98	5	15633.22	6987.3	0	12.88	531.83				15633
114	99	5	15970.98	7069.78	0	12.9	529.1				15971
115	100	5	16812.19	7395.7	0	12.92	530.49				16812
116	101	5	16940.2	7527.22	0	12.92	527.84				16940
117	102	5	16143.81	7168.9	0	13	530.68				16144
118	103	5	16250.04	7217.18	0	12.94	529.41				16250
119	104	5	16456.7	7296.14	0	12.96	529.45				16457
120	105	5	16772.51	7426.98	0	12.98	528.11				16773
121	106	5	16647.6	7359.9	0	12.95	528.01				16648
122	107	5	16744.3	7463.58	0	12.92	530.19				16744
123	109	5	228748.8	33738.94	0	7.97	173.59				228749
124											
125											
126											
127											
128											
129											
130											
131											

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
Sample ID	Replicate	Assay Date	Chemical ID	Test Chemical ID	CR11616	# Concentrations tested	B Microsome ID									
1		3/21/2007			CR11616		12535-13									
2		12/3/94			21MR210S.P00											
3					3	Positive Control	4-OH ANDEN									
4																
Calculate DPM in aqueous portion after extraction																
Sample ID	Replicate	Assay Date	Chemical ID	Test Chemical ID	CR11616	# Concentrations tested	B Microsome ID									
6	1	3/21/2007			CR11616		12535-13									
7	2	3/21/2007			CR11616		12535-13									
8	3	3/21/2007			CR11616		12535-13									
9	4	3/21/2007			CR11616		12535-13									
10	5	3/21/2007			CR11616		12535-13									
11	6	3/21/2007			CR11616		12535-13									
12	7	3/21/2007			CR11616		12535-13									
13	8	3/21/2007			CR11616		12535-13									
14	9	3/21/2007			CR11616		12535-13									
15	10	3/21/2007			CR11616		12535-13									
16	11	3/21/2007			CR11616		12535-13									
17	12	3/21/2007			CR11616		12535-13									
18	13	3/21/2007			CR11616		12535-13									
19	14	3/21/2007			CR11616		12535-13									
20	15	3/21/2007			CR11616		12535-13									
21	16	3/21/2007			CR11616		12535-13									
22	17	3/21/2007			CR11616		12535-13									
23	18	3/21/2007			CR11616		12535-13									
24	19	3/21/2007			CR11616		12535-13									
25	20	3/21/2007			CR11616		12535-13									
26	21	3/21/2007			CR11616		12535-13									
27	22	3/21/2007			CR11616		12535-13									
28	23	3/21/2007			CR11616		12535-13									
29	24	3/21/2007			CR11616		12535-13									
30	25	3/21/2007			CR11616		12535-13									
31	26	3/21/2007			CR11616		12535-13									
32	27	3/21/2007			CR11616		12535-13									
33	28	3/21/2007			CR11616		12535-13									
34	29	3/21/2007			CR11616		12535-13									
35	30	3/21/2007			CR11616		12535-13									
36	31	3/21/2007			CR11616		12535-13									
37	32	3/21/2007			CR11616		12535-13									
38	33	3/21/2007			CR11616		12535-13									
39	34	3/21/2007			CR11616		12535-13									
40	35	3/21/2007			CR11616		12535-13									
41	36	3/21/2007			CR11616		12535-13									
42	37	3/21/2007			CR11616		12535-13									
43	38	3/21/2007			CR11616		12535-13									
44	39	3/21/2007			CR11616		12535-13									
45	40	3/21/2007			CR11616		12535-13									
46	41	3/21/2007			CR11616		12535-13									
47	42	3/21/2007			CR11616		12535-13									
48	43	3/21/2007			CR11616		12535-13									
49	44	3/21/2007			CR11616		12535-13									
50	45	3/21/2007			CR11616		12535-13									
51	46	3/21/2007			CR11616		12535-13									
52	47	3/21/2007			CR11616		12535-13									
53	48	3/21/2007			CR11616		12535-13									
54	49	3/21/2007			CR11616		12535-13									
55	50	3/21/2007			CR11616		12535-13									
56	51	3/21/2007			CR11616		12535-13									
57	52	3/21/2007			CR11616		12535-13									
58	53	3/21/2007			CR11616		12535-13									
59	54	3/21/2007			CR11616		12535-13									
60	55	3/21/2007			CR11616		12535-13									
61	56	3/21/2007			CR11616		12535-13									
62	57	3/21/2007			CR11616		12535-13									
63	58	3/21/2007			CR11616		12535-13									
64	59	3/21/2007			CR11616		12535-13									
65	60	3/21/2007			CR11616		12535-13									
66	61	3/21/2007			CR11616		12535-13									
67	62	3/21/2007			CR11616		12535-13									
68	63	3/21/2007			CR11616		12535-13									
69	64	3/21/2007			CR11616		12535-13									
70	65	3/21/2007			CR11616		12535-13									

Aromatase Assay Spreadsheet

	P	S	T	U	V
1					
2					
3					
4		QC check - aliquot variance			
5		Rec'd value not within mean $\pm$ 10%			
6					
7		1.01			
8		0.99			
9		1.00			
10		1.00			
11		1.00			
12		1.00			
13		1.00			
14		1.00			
15		0.94			
16		1.06			
17		0.96			
18		1.04			
19		1.00			
20		0.97			
21					
22					
23		0.97			
24		1.03			
25		1.03			
26		0.97			
27					
28					
29		0.99			
30		1.01			
31		0.99			
32		1.02			
33					
34					
35		0.99			
36		1.01			
37		0.99			
38		1.01			
39					
40					
41		1.00			
42		1.00			
43		1.00			
44		1.00			
45					
46					
47		1.00			
48		1.00			
49		1.00			
50		1.00			
51					
52					
53		1.00			
54		1.00			
55		1.00			
56					
57					
58		1.00			
59		1.00			
60		1.00			
61		1.00			
62		1.00			
63					
64		1.00			
65		1.00			
66		1.00			
67		1.00			
68		1.00			
69					
70					

Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Sample ID	Replicate Level	Assay Date	Volume (mL)	Aliq #	DPH/mL	DPH/mL	Ave DPH/mL	Total DPH	Volume of substrate solution used/assay tube (mL)	Total DPH in assay tube (mL)	Calculate nmol ³ H ₂ O formed	nmol ³ H ₂ O formed	Fuel fraction in assay (mg/mL)	Incubation time (min)	Aromatase activity (nmol estrogen formed/protein/min)
6	1	3/21/2007	0.5	1	15923	31986	32440	54890	0.1	159092	Total DPM corrected for background (Background tubes)	64187	0.0548	15	0.432
7	2	3/21/2007	0.5	2	16447	32894	32896	65792	0.1	190052	64399	0.0552	0.005	15	0.435
8	3	3/21/2007	0.5	3	16366	32572	32572	65144	0.1	190052	65259	0.0549	0.005	15	0.425
9	4	3/21/2007	0.5	4	16380	32760	31963	63976	0.1	190052	65973	0.0563	0.005	15	0.442
10	5	3/21/2007	0.5	5	15939	31910	31963	63976	0.1	190052	67791	0.0581	0.005	15	0.454
11	6	3/21/2007	0.5	6	15958	32110	31963	63976	0.1	190052	68019	0.0582	0.005	15	0.452
12	7	3/21/2007	0.5	7	16271	32542	34022	68164	0.1	190052	68019	0.0582	0.005	15	0.446
13	8	3/21/2007	0.5	8	16271	32542	34022	68164	0.1	190052	68019	0.0582	0.005	15	0.452
14	9	3/21/2007	0.5	9	17046	34092	34022	68164	0.1	190052	68019	0.0582	0.005	15	0.452
15	10	3/21/2007	0.5	10	17443	34526	34526	69042	0.1	190052	68019	0.0582	0.005	15	0.446
16	11	3/21/2007	0.5	11	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
17	12	3/21/2007	0.5	12	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
18	13	3/21/2007	0.5	13	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
19	14	3/21/2007	0.5	14	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
20	15	3/21/2007	0.5	15	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
21	16	3/21/2007	0.5	16	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
22	17	3/21/2007	0.5	17	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
23	18	3/21/2007	0.5	18	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
24	19	3/21/2007	0.5	19	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
25	20	3/21/2007	0.5	20	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
26	21	3/21/2007	0.5	21	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
27	22	3/21/2007	0.5	22	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
28	23	3/21/2007	0.5	23	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
29	24	3/21/2007	0.5	24	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
30	25	3/21/2007	0.5	25	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
31	26	3/21/2007	0.5	26	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
32	27	3/21/2007	0.5	27	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
33	28	3/21/2007	0.5	28	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
34	29	3/21/2007	0.5	29	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
35	30	3/21/2007	0.5	30	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
36	31	3/21/2007	0.5	31	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
37	32	3/21/2007	0.5	32	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
38	33	3/21/2007	0.5	33	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
39	34	3/21/2007	0.5	34	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
40	35	3/21/2007	0.5	35	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
41	36	3/21/2007	0.5	36	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
42	37	3/21/2007	0.5	37	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
43	38	3/21/2007	0.5	38	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
44	39	3/21/2007	0.5	39	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
45	40	3/21/2007	0.5	40	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
46	41	3/21/2007	0.5	41	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
47	42	3/21/2007	0.5	42	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
48	43	3/21/2007	0.5	43	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
49	44	3/21/2007	0.5	44	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
50	45	3/21/2007	0.5	45	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
51	46	3/21/2007	0.5	46	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
52	47	3/21/2007	0.5	47	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
53	48	3/21/2007	0.5	48	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
54	49	3/21/2007	0.5	49	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
55	50	3/21/2007	0.5	50	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
56	51	3/21/2007	0.5	51	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
57	52	3/21/2007	0.5	52	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
58	53	3/21/2007	0.5	53	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446
59	54	3/21/2007	0.5	54	16965	33730	33532	67064	0.1	190052	68019	0.0582	0.005	15	0.446

Aromatase Assay Spreadsheet

	K	S
1		
2		
3		
4		Observed - aliquot
5		variance
6		% RE
7		0.99
8		1.01
9		1.00
10		1.00
11		1.00
12		1.00
13		1.00
14		1.00
15		1.00
16		1.00
17		1.00
18		0.99
19		1.01
20		0.99
21		1.00
22		1.00
23		0.99
24		1.01
25		1.00
26		1.00
27		0.99
28		1.01
29		0.99
30		1.01
31		1.00
32		1.00
33		1.00
34		1.00
35		1.00
36		1.00
37		0.99
38		1.01
39		0.99
40		1.01
41		1.00
42		1.00
43		0.99
44		1.01
45		0.99
46		1.01
47		0.99
48		1.01
49		1.00
50		1.00
51		0.99
52		1.01
53		1.00
54		1.00

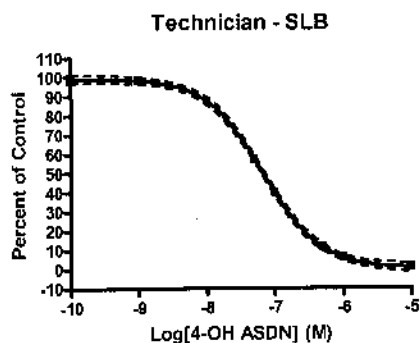
Aromatase Assay Spreadsheet

A	B	C	D	E	F	G	H	I	J	K	L
1		Assay Data	3/21/2007	Chemical ID	CR11616						
2		NB Ref	12535-84	Counter data	21MP2105 P00				8	Microsome ID	12535-13
3		Technician ID	ase	Replicate #	3	Positive Control		4-OH ASDN	Pos Ctrl Tubes/Conc		2
4	Control Type	Portion	SD								
5	Full activity	Average	0.021								
6	Background (uncorrected)	Overall	0.003								
7											
8											
9											
10	Positive Control	Level	[Positive Ctrl] M	Log[Positive Ctrl]	Activity						
11	4-OH ASDN	1	1.00E-05	-5.00	0.002						
12	4-OH ASDN	2	1.00E-05	-5.00	0.002						
13	4-OH ASDN	1	1.00E-06	-6.00	0.027						
14	4-OH ASDN	2	1.00E-06	-6.00	0.027						
15	4-OH ASDN	1	1.00E-07	-7.00	0.053						
16	4-OH ASDN	2	1.00E-07	-7.00	0.049						
17	4-OH ASDN	1	1.00E-08	-8.00	0.184						
18	4-OH ASDN	2	1.00E-08	-8.00	0.164						
19	4-OH ASDN	1	1.00E-09	-9.00	0.254						
20	4-OH ASDN	2	1.00E-09	-9.00	0.256						
21	4-OH ASDN	1	1.00E-10	-10.00	0.395						
22	4-OH ASDN	2	1.00E-10	-10.00	0.369						
23	4-OH ASDN	1	1.00E-09	-9.00	0.423						
24	4-OH ASDN	2	1.00E-09	-9.00	0.419						
25	4-OH ASDN	1	1.00E-10	-10.00	0.438						
26	4-OH ASDN	2	1.00E-10	-10.00	0.424						
27	4-OH ASDN	1	1.00E-10	-10.00							
28	4-OH ASDN	2	1.00E-10	-10.00							
29	4-OH ASDN	1	1.00E-10	-10.00							
30	4-OH ASDN	2	1.00E-10	-10.00							
31	4-OH ASDN	1	1.00E-10	-10.00							
32	4-OH ASDN	2	1.00E-10	-10.00							
33	4-OH ASDN	1	1.00E-10	-10.00							
34	4-OH ASDN	2	1.00E-10	-10.00							
35	4-OH ASDN	1	1.00E-10	-10.00							
36	4-OH ASDN	2	1.00E-10	-10.00							
37	Test Chemical	Level	[Test chemical] M	Log[Test chemical]	Activity						
38	CR11616	1	1.00E-04	-4.00	0.432						
39	CR11616	2	1.00E-04	-4.00	0.435						
40	CR11616	1	1.00E-05	-5.00	0.425						
41	CR11616	2	1.00E-05	-5.00	0.442						
42	CR11616	1	1.00E-06	-6.00	0.454						
43	CR11616	2	1.00E-06	-6.00	0.462						
44	CR11616	1	1.00E-07	-7.00	0.446						
45	CR11616	2	1.00E-07	-7.00	0.452						
46	CR11616	1	1.00E-08	-8.00	0.445						
47	CR11616	2	1.00E-08	-8.00	0.436						
48	CR11616	1	1.00E-09	-9.00	0.431						
49	CR11616	2	1.00E-09	-9.00	0.425						
50	CR11616	1	1.00E-10	-10.00	0.418						
51	CR11616	2	1.00E-10	-10.00	0.428						
52	CR11616	1	1.00E-07	-7.00	0.466						
53	CR11616	2	1.00E-07	-7.00	0.453						
54	CR11616	1	1.00E-08	-8.00	0.442						
55	CR11616	2	1.00E-08	-8.00	0.462						
56	CR11616	1	1.00E-09	-9.00	0.444						
57	CR11616	2	1.00E-09	-9.00	0.421						
58	CR11616	1	1.00E-10	-10.00	0.447						
59	CR11616	2	1.00E-10	-10.00	0.431						
60	CR11616	1	1.00E-10	-10.00	0.442						
61	CR11616	2	1.00E-10	-10.00	0.445						

APPENDIX F

Concentration Response Curves Positive Control Assays

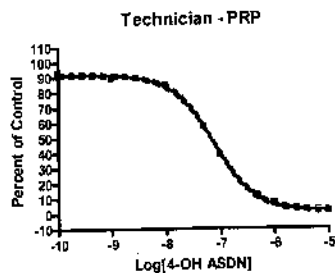
Technician - 1



Log[4-OH ASDN]	Percent of Control		
	Y1	Y2	Y3
-5.0	0.7	0.6	0.5
-6.0	6.6	5.7	5.3
-6.3	11.4	11.5	10.5
-7.0	42.3	39.1	37.1
-7.3	58.3	56.6	56.2
-8.0	89.4	88.8	84.2
-9.0	102.2	98.6	94.2
-10.0	102.2	100.5	94.2

	Percent of Control
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	0.212
TOP	99.4
LOGEC50	-7.18
HILLSLOPE	-1.04
EC50	6.67e-008
Std. Error	
BOTTOM	1.20
TOP	0.986
LOGEC50	0.0213
HILLSLOPE	0.0568
Lower 95% conf. limit	
BOTTOM	-2.30
TOP	97.3
LOGEC50	-7.22
HILLSLOPE	-1.16
EC50	6.02e-008
Upper 95% conf. limit	
BOTTOM	2.72
TOP	101
LOGEC50	-7.13
HILLSLOPE	-0.924
EC50	7.39e-008
Goodness of Fit	
Degrees of Freedom	20
R ²	0.997
Absolute Sum of Squares	103
Sy.x	2.27
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

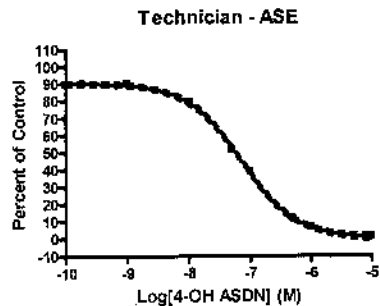
Technician - 2



Log[4-OH ASDN]	Percent of Control		
	Y1	Y2	Y3
-5.0	0.8	0.7	0.8
-6.0	6.7	6.2	6.1
-6.3	12.3	11.4	11.0
-7.0	41.0	37.4	36.5
-7.3	59.2	54.5	57.4
-8.0	83.6	83.3	86.9
-9.0	87.1	90.7	89.9
-10.0	92.0	95.0	91.3

	Percent of Control
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.356
TOP	91.76
LOGEC50	-7.124
HILLSLOPE	-1.142
EC50	7.508e-008
Std. Error	
BOTTOM	0.9792
TOP	0.7828
LOGEC50	0.01785
HILLSLOPE	0.05851
Lower 95% conf. limit	
BOTTOM	-0.6868
TOP	90.12
LOGEC50	-7.162
HILLSLOPE	-1.264
EC50	6.891e-008
Upper 95% conf. limit	
BOTTOM	3.398
TOP	93.39
LOGEC50	-7.087
HILLSLOPE	-1.020
EC50	8.180e-008
Goodness of Fit	
Degrees of Freedom	20
R ²	0.9977
Absolute Sum of Squares	72.07
Sy.x	1.898
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

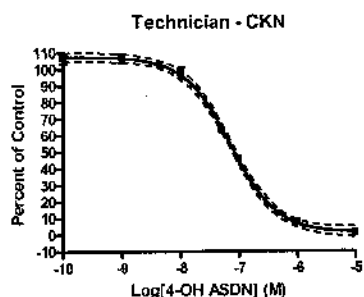
Technician - 3



Log[4-OH ASDN]	Percent of Control		
	Y1	Y2	Y3
-5.0	0.7	0.5	0.6
-6.0	6.3	5.9	6.1
-6.3	11.7	11.1	11.3
-7.0	39.2	37.2	39.6
-7.3	53.5	52.5	47.7
-8.0	82.5	80.4	74.9
-9.0	90.0	91.1	88.7
-10.0	87.8	90.3	89.5

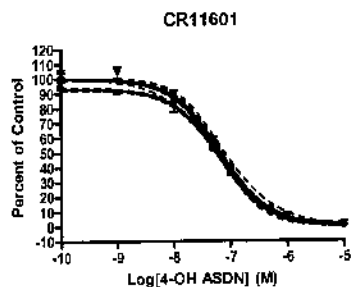
	Percent of Control
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	-0.1361
TOP	90.40
LOGEC50	-7.152
HILLSLOPE	-0.9759
EC50	7.052e-008
Std. Error	
BOTTOM	1.104
TOP	0.8630
LOGEC50	0.02166
HILLSLOPE	0.04947
Lower 95% conf. limit	
BOTTOM	-2.439
TOP	88.60
LOGEC50	-7.197
HILLSLOPE	-1.079
EC50	6.355e-008
Upper 95% conf. limit	
BOTTOM	2.167
TOP	92.20
LOGEC50	-7.107
HILLSLOPE	-0.8727
EC50	7.825e-008
Goodness of Fit	
Degrees of Freedom	20
R ²	0.9974
Absolute Sum of Squares	76.06
Sy.x	1.950
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0

Technician - 4



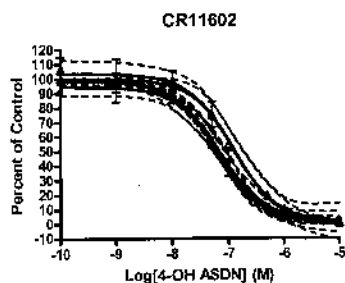
Log[4-OH ASDN]	Percent of Control		
	Y1	Y2	Y3
-5.0	0.9	0.8	0.8
-6.0	9.1	6.9	6.8
-6.3	13.5	12.1	11.7
-7.0	45.0	44.6	45.9
-7.3	66.2	62.4	59.7
-8.0	104.1	95.8	94.0
-9.0	112.2	103.8	104.1
-10.0	111.7	101.6	106.3

Percent of Control	
Sigmoidal dose-response (variable slope)	
Best-fit values	
BOTTOM	1.20
TOP	108
LOGEC50	-7.15
HILLSLOPE	-1.09
EC50	7.06e-008
Std. Error	
BOTTOM	1.67
TOP	1.35
LOGEC50	0.0286
HILLSLOPE	0.0793
Lower 95% conf. limit	
BOTTOM	-2.28
TOP	105
LOGEC50	-7.21
HILLSLOPE	-1.26
EC50	6.22e-008
Upper 95% conf. limit	
BOTTOM	4.87
TOP	110
LOGEC50	-7.10
HILLSLOPE	-0.928
EC50	8.02e-008
Goodness of Fit	
Degrees of Freedom	20
R ²	0.995
Absolute Sum of Squares	205
Sy.x	3.20
Data	
Number of X values	8
Number of Y replicates	3
Total number of values	24
Number of missing values	0



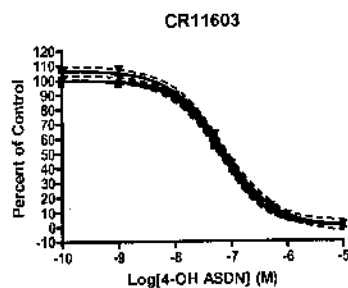
4-OH ASDN	CR11601_R1		CR11601_R2		CR11601_R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	0.5	0.6	0.7	0.6	0.7	0.6
-6.0	5.3	5.5	6.2	6.4	5.6	5.8
-6.3	10.3	9.8	12.0	10.8	10.4	11.0
-7.0	35.6	35.8	39.2	37.9	39.3	37.6
-7.3	52.4	52.3	60.8	55.1	58.7	55.4
-8.0	83.3	81.2	92.3	77.1	89.2	87.1
-9.0	91.8	90.2	108.2	100.4	100.0	97.8
-10.0	94.6	92.7	104.3	103.3	104.5	98.4

	CR11601_R1	CR11601_R2	CR11601_R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	P=0.0001	0.1385	0.5315
Conclusion (alpha = 0.05)	Reject null hypothesis	Do not reject null hypothesis	Do not reject null hypothesis
Predicted model	Equation 1	Equation 2	Equation 2
F (D.F., D.Fd)	95.6 (2,12)	2.34 (2,12)	0.667 (2,12)
Equation 1			
Best-fit values			
BOTTOM	0.432	-1.10	0.266
TOP	93.1	106	101
LOGEC50	-7.20	-7.23	-7.20
HILLSLOPE	-1.08	-0.903	-1.05
EC50	8.35e-006	8.85e-006	8.33e-006
Std. Error			
BOTTOM	0.570	2.84	1.06
TOP	0.485	2.35	0.897
LOGEC50	0.0108	0.0509	0.0166
HILLSLOPE	0.0304	0.0994	0.0501
Lower 95% conf. limit			
BOTTOM	-0.823	-7.30	-2.10
TOP	92.0	99.8	99.0
LOGEC50	-7.22	-7.34	-7.24
HILLSLOPE	-1.13	-1.12	-1.15
EC50	8.01e-006	4.53e-006	5.77e-006
Upper 95% conf. limit			
BOTTOM	1.60	5.09	2.51
TOP	94.2	110	103
LOGEC50	-7.17	-7.12	-7.16
HILLSLOPE	-0.993	-0.886	-0.936
EC50	8.71e-006	7.56e-006	8.89e-006
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	1.00	0.992	0.999
Absolute Sum of Squares	6.98	187	33.0
Syx	0.912	4.08	1.66
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	109	100
LOGEC50	-7.28	-7.19	-7.19
HILLSLOPE	-0.880	-1.01	-1.08
EC50	6.22e-006	6.51e-006	6.52e-006
Std. Error			
LOGEC50	0.0308	0.0356	0.0126
HILLSLOPE	0.0579	0.0874	0.0344
Lower 95% conf. limit			
LOGEC50	-7.35	-7.28	-7.21
HILLSLOPE	-1.02	-1.20	-1.13
EC50	4.40e-006	5.45e-006	6.12e-006
Upper 95% conf. limit			
LOGEC50	-7.22	-7.11	-7.18
HILLSLOPE	-0.774	-0.824	-0.885
EC50	6.08e-006	7.76e-006	6.84e-006
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.992	0.990	0.999
Absolute Sum of Squares	169	275	36.7
Syx	3.48	4.43	1.62
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



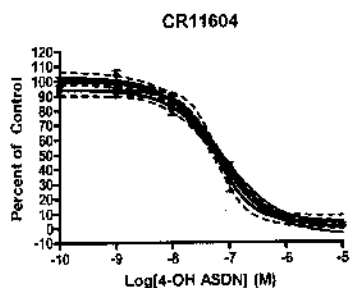
4-OH ASDN	CR11602_R4		CR11602-R5		CR11602_R1	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	0.9	0.7	0.7	1.1	1.0	0.6
-6.0	6.7	6.0	9.7	10.4	8.4	5.0
-6.3	11.2	10.4	20.5	18.4	12.9	10.0
-7.0	40.5	36.3	54.6	55.1	50.3	33.0
-7.3	52.3	54.6	84.0	66.3	59.1	51.6
-8.0	91.4	88.0	105.2	92.2	89.8	82.9
-9.0	97.2	95.7	113.6	84.0	90.7	103.8
-10.0	98.0	97.3	97.5	115.0	80.3	90.2

	CR11602_R4	CR11602-R5	CR11602_R1
Comparison of Fit			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.2133	0.6607	0.1933
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 2
F (DFn, DFd)	1.76 (2, 12)	0.428 (2, 12)	1.89 (2, 12)
Equation 1			
Best-fit values			
BOTTOM	1.65	0.740	0.863
TOP	98.3	104	84.5
LOGEC50	-7.21	-6.83	-7.13
HILLSLOPE	-1.12	-1.08	-1.09
EC50	6.21e-008	1.17e-007	7.48e-008
Std. Error			
BOTTOM	1.24	0.37	3.81
TOP	1.08	4.18	2.85
LOGEC50	0.0222	0.102	0.0847
HILLSLOPE	0.0710	0.249	0.150
Lower 95% conf. limit			
BOTTOM	-1.05	-13.1	-7.20
TOP	95.9	94.5	88.2
LOGEC50	-7.26	-7.16	-7.27
HILLSLOPE	-1.28	-1.82	-1.51
EC50	5.55e-008	6.89e-008	5.39e-008
Upper 95% conf. limit			
BOTTOM	4.34	14.8	8.53
TOP	101	113	101
LOGEC50	-7.16	-6.71	-6.99
HILLSLOPE	-0.958	-0.545	-0.677
EC50	6.94e-008	1.95e-007	1.05e-007
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.998	0.995	0.984
Absolute Sum of Squares	51.0	900	368
Syx	2.06	8.66	5.55
Equation 2			
Best-fit values			
BOTTOM	0.8	0.0	0.0
TOP	100	100	100
LOGEC50	-7.21	-6.89	-7.18
HILLSLOPE	-1.04	-1.13	-0.988
EC50	8.20e-008	1.30e-007	8.55e-008
Std. Error			
LOGEC50	0.0472	0.0838	0.0488
HILLSLOPE	0.0448	0.168	0.126
Lower 95% conf. limit			
LOGEC50	-7.24	-7.02	-7.29
HILLSLOPE	-1.14	-1.49	-1.20
EC50	5.70e-008	8.47e-008	5.14e-008
Upper 95% conf. limit			
LOGEC50	-7.17	-6.75	-7.08
HILLSLOPE	-0.943	-0.785	-0.734
EC50	6.75e-008	1.76e-007	8.33e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.997	0.964	0.978
Absolute Sum of Squares	66.0	964	488
Syx	2.17	8.30	5.88
Constants			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



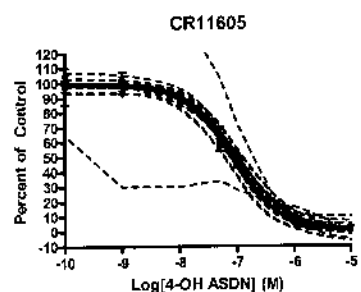
	4-OH ASDN	CR11603_R1		CR11603_R2		CR11603_R3	
		Y1	Y2	Y1	Y2	Y1	Y2
■ CR11603_R1	-5.0	0.9	0.7	0.8	0.7	0.8	0.9
▲ CR11603_R2	-6.0	6.7	6.0	6.4	5.8	7.8	7.1
▼ CR11603_R3	-6.3	11.2	10.4	11.3	11.1	14.0	13.3
	-7.0	40.5	36.3	41.4	39.0	41.6	43.1
	-7.3	52.3	54.6	58.4	59.4	65.3	58.9
	-8.0	91.4	88.0	91.3	87.7	87.8	91.3
	-9.0	97.2	95.7	104.5	98.1	103.5	109.7
	-10.0	98.0	97.3	99.6	105.9	108.6	102.0

	CR11603_R1	CR11603_R2	CR11603_R3
Comparison of fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.2133	0.0702	0.0029
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 1
F (DFn, DFd)	1.76 (2, 12)	3.34 (2, 12)	10.3 (2, 12)
Equation 1			
Best-fit values			
BOTTOM	1.65	0.138	-0.328
TOP	88.3	103	107
LOGEC50	-7.21	-7.18	-7.17
HILLSLOPE	-1.12	-1.03	-0.936
EC50	6.21e-008	6.56e-008	6.70e-008
Std. Error			
BOTTOM	1.24	1.34	1.98
TOP	1.08	1.10	1.55
LOGEC50	0.0222	0.0230	0.0334
HILLSLOPE	0.0710	0.0600	0.0700
Lower 95% conf. limit			
BOTTOM	-1.05	-2.78	-4.60
TOP	95.9	100	103
LOGEC50	-7.26	-7.23	-7.25
HILLSLOPE	-1.25	-1.16	-1.05
EC50	5.55e-008	5.84e-008	5.86e-008
Upper 95% conf. limit			
BOTTOM	4.34	3.05	3.95
TOP	101	105	110
LOGEC50	-7.16	-7.13	-7.10
HILLSLOPE	-0.989	-0.900	-0.784
EC50	6.94e-008	7.35e-008	7.62e-008
Goodness of fit			
Degrees of Freedom	12	12	12
R ²	0.998	0.998	0.997
Absolute Sum of Squares	51.0	50.8	82.5
Syx	2.06	2.05	2.75
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.21	-7.15	-7.18
HILLSLOPE	-1.04	-1.08	-1.05
EC50	6.20e-008	7.03e-008	7.88e-008
Std. Error			
LOGEC50	0.0172	0.0182	0.0329
HILLSLOPE	0.0449	0.0517	0.0877
Lower 95% conf. limit			
LOGEC50	-7.24	-7.19	-7.17
HILLSLOPE	-1.14	-1.19	-1.24
EC50	5.70e-008	6.43e-008	6.85e-008
Upper 95% conf. limit			
LOGEC50	-7.17	-7.11	-7.03
HILLSLOPE	-0.943	-0.869	-0.868
EC50	6.75e-008	7.69e-008	9.25e-008
Goodness of fit			
Degrees of Freedom	14	14	14
R ²	0.997	0.997	0.991
Absolute Sum of Squares	58.0	78.8	251
Syx	2.17	2.37	4.24
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



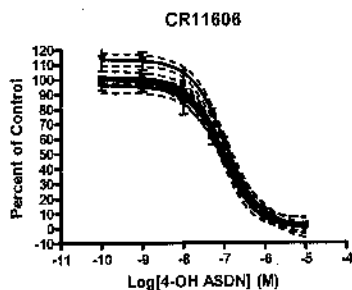
	4-OH ASDN	CR11604_R1		CR11604_R2		CR11604_R3	
		Y1	Y2	Y1	Y2	Y1	Y2
■ CR11604_R1	-5.0	0.9	0.8	0.7	0.9	0.8	0.7
▼ CR11604_R2	-6.0	6.1	5.9	6.4	6.6	7.0	5.9
◆ CR11604_R3	-6.3	12.1	10.7	11.6	11.3	10.8	11.4
	-7.0	39.1	24.4	43.4	39.9	41.2	39.1
	-7.3	51.1	56.5	54.4	52.6	58.6	53.7
	-8.0	90.8	87.5	89.5	76.5	86.8	80.9
	-9.0	96.0	89.2	90.0	91.6	107.2	100.9
	-10.0	95.3	92.3	96.6	93.9	102.9	97.0

	CR11604_R1	CR11604_R2	CR11604_R3
Comparison of fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.0402	0.0177	0.3616
Conclusion (alpha = 0.05)	Reject null hypothesis	Reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 1	Equation 1	Equation 2
F (Df1, Df2)	4.25 (2, 12)	5.75 (2, 12)	1.11 (2, 12)
Equation 1			
Best-fit values			
BOTTOM	3.81	-0.410	-0.884
TOP	94.1	84.1	105
LOGEC50	-7.23	-7.14	-7.22
HILLSLOPE	-1.41	-0.984	-0.613
EC50	5.89e-008	7.38e-008	6.69e-008
Std. Error			
BOTTOM	2.14	2.28	2.25
TOP	2.06	1.79	1.83
LOGEC50	0.0384	0.0428	0.0408
HILLSLOPE	0.208	0.0947	0.0812
Lower 95% conf. limit			
BOTTOM	-0.656	-5.38	-5.78
TOP	99.6	90.3	99.7
LOGEC50	-7.31	-7.23	-7.30
HILLSLOPE	-1.68	-1.17	-1.09
EC50	4.85e-008	5.66e-008	4.91e-008
Upper 95% conf. limit			
BOTTOM	5.48	4.58	4.01
TOP	98.8	97.9	107
LOGEC50	-7.15	-7.04	-7.13
HILLSLOPE	-0.865	-0.758	-0.738
EC50	7.13e-008	9.04e-008	7.47e-008
Goodness of fit			
Degrees of Freedom	12	12	12
R ²	0.981	0.994	0.986
Absolute Sum of Squares	212	125	123
Sy ²	4.20	3.23	3.20
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.23	-7.22	-7.19
HILLSLOPE	-1.07	-0.890	-0.976
EC50	5.60e-008	5.96e-008	5.41e-008
Std. Error			
LOGEC50	0.0400	0.0375	0.0268
HILLSLOPE	0.110	0.0849	0.0603
Lower 95% conf. limit			
LOGEC50	-7.34	-7.30	-7.25
HILLSLOPE	-1.31	-0.998	-1.11
EC50	4.59e-008	4.97e-008	5.62e-008
Upper 95% conf. limit			
LOGEC50	-7.12	-7.14	-7.14
HILLSLOPE	-0.832	-0.721	-0.847
EC50	8.82e-008	7.20e-008	7.31e-008
Goodness of fit			
Degrees of Freedom	14	14	14
R ²	0.994	0.989	0.994
Absolute Sum of Squares	362	245	148
Sy ²	5.08	4.18	3.22
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



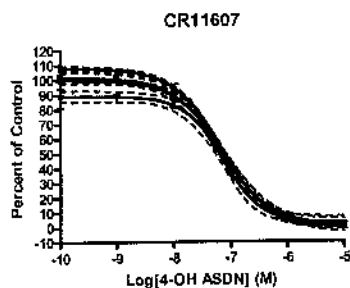
4-OH ASDN	CR11605-R1		CR11605-R2		CR11605-R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	0.9	1.0	1.3	1.0	0.9	0.9
-6.0	6.3	7.5	10.1	9.0	7.8	7.4
-6.3	13.6	13.9	17.6	17.5	13.9	13.0
-7.0	42.9	45.1	47.8	51.3	46.3	43.9
-7.3	61.9	63.6	65.5	70.9	63.8	61.9
-8.0	92.7	90.7	93.7	84.4	92.4	92.0
-9.0	107.1	93.0	97.6	87.8	104.6	97.2
-10.0	109.9	85.6	100.0	106.0	94.4	101.5

	CR11605-R1	CR11605-R2	CR11605-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.0458	0.7885	0.7942
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 2
F (DfN, DfD)	0.0582 (2,12)	0.242 (2,12)	0.235 (2,12)
Equation 1			
Best-fit values			
BOTTOM	1.37	-0.262	1.11
TOP	100	98.5	100
LOGEC50	-7.14	-6.97	-7.09
HILLSLOPE	-1.05	-0.975	-1.10
EC50	7.32e-008	1.08e-007	8.16e-008
Std Error			
BOTTOM	4.21	3.37	1.89
TOP	3.31	2.23	1.28
LOGEC50	0.0732	0.0583	0.0283
HILLSLOPE	0.195	0.118	0.0824
Lower 95% conf. limit			
BOTTOM	-7.93	-7.61	-2.56
TOP	92.9	93.7	97.3
LOGEC50	-7.29	-7.10	-7.15
HILLSLOPE	-1.47	-1.23	-1.28
EC50	5.07e-008	7.84e-008	7.08e-008
Upper 95% conf. limit			
BOTTOM	10.5	7.08	4.79
TOP	107	103	103
LOGEC50	-6.98	-6.85	-7.03
HILLSLOPE	-0.821	-0.717	-0.919
EC50	1.08e-007	1.43e-007	9.40e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.981	0.990	0.997
Absolute Sum of Squares	479	229	77.2
Syx	6.32	4.36	2.54
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.12	-6.99	-7.07
HILLSLOPE	-1.01	-0.963	-1.07
EC50	7.64e-008	1.01e-007	8.43e-008
Std Error			
LOGEC50	0.0469	0.0342	0.0185
HILLSLOPE	0.114	0.0701	0.0499
Lower 95% conf. limit			
LOGEC50	-7.22	-7.07	-7.11
HILLSLOPE	-1.26	-1.10	-1.18
EC50	5.05e-008	8.55e-008	7.85e-008
Upper 95% conf. limit			
LOGEC50	-7.02	-6.92	-7.03
HILLSLOPE	-0.767	-0.802	-0.981
EC50	9.85e-008	1.20e-007	9.23e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.990	0.990	0.997
Absolute Sum of Squares	483	238	80.2
Syx	5.86	4.12	2.39
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



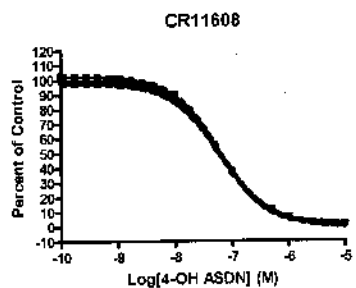
4-OH ASDN	CR11606-R1		CR11606-R2		CR11606-R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	1.0	1.1	1.4	1.5	1.1	1.0
-6.0	8.8	7.4	11.4	10.0	10.3	9.4
-6.3	10.0	13.9	20.1	18.7	17.0	15.6
-7.0	45.8	47.5	57.8	59.2	52.4	53.8
-7.3	56.1	65.6	79.7	76.7	65.2	63.8
-8.0	98.3	95.5	104.6	103.9	93.7	76.5
-9.0	103.8	96.4	106.8	118.3	97.1	91.6
-10.0	96.6	102.3	120.7	106.1	102.7	93.1

	CR11606-R1	CR11606-R2	CR11606-R3
Comparison of Fit			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.5024	P=0.0001	0.2588
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Reject null hypothesis	Do not reject null hypothesis
Prefere model	Equation 2	Equation 1	Equation 2
F (Dfn, DFe)	0.729 (2,12)	27.9 (2,12)	1.53 (2,12)
Equation 1			
Best-fit values			
BOTTOM	2.00	0.462	-1.40
TOP	101	113	96.2
LOGEC50	-7.10	-6.87	-6.84
HILLSLOPE	-1.11	-1.03	-0.930
EC50	7.95e-008	1.08e-007	1.14e-007
Std. Error			
BOTTOM	2.54	2.80	3.72
TOP	1.96	1.94	2.37
LOGEC50	0.0423	0.0428	0.0858
HILLSLOPE	0.127	0.0982	0.119
Lower 95% conf. limit			
BOTTOM	-3.52	-5.80	-8.50
TOP	97.2	109	91.0
LOGEC50	-7.19	-7.07	-7.88
HILLSLOPE	-1.39	-1.25	-1.19
EC50	6.43e-008	8.57e-008	8.22e-008
Upper 95% conf. limit			
BOTTOM	7.57	5.76	6.70
TOP	106	118	101
LOGEC50	-7.01	-6.86	-6.80
HILLSLOPE	-0.852	-0.821	-0.672
EC50	9.64e-008	1.32e-007	1.59e-007
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.993	0.994	0.868
Absolute Sum of Squares	160	181	251
Syx	3.86	3.89	4.58
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.06	-6.84	-7.01
HILLSLOPE	-1.06	-1.22	-0.884
EC50	8.71e-008	1.45e-007	9.73e-008
Std. Error			
LOGEC50	0.0292	0.0941	0.0412
HILLSLOPE	0.0787	0.188	0.0733
Lower 95% conf. limit			
LOGEC50	-7.12	-6.88	-7.10
HILLSLOPE	-1.25	-1.63	-1.04
EC50	7.54e-008	1.05e-007	7.94e-008
Upper 95% conf. limit			
LOGEC50	-7.00	-6.70	-6.92
HILLSLOPE	-0.805	-0.622	-0.727
EC50	1.01e-007	1.89e-007	1.19e-007
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.802	0.967	0.886
Absolute Sum of Squares	202	1022	315
Syx	3.80	8.55	4.75
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	6	8	6
Number of Y replicates	2	2	2
Total number of values	18	16	18
Number of missing values	0	0	0



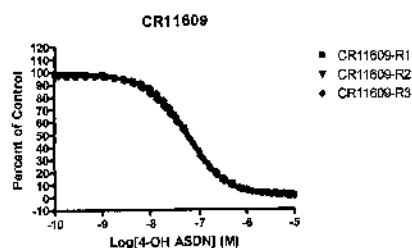
4-OH ASDN	CR11607_R1		CR11607_R2		CR11607_R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	8.9	0.9	0.7	0.7	0.7	0.7
-6.0	7.3	7.1	6.9	6.9	6.8	6.6
-6.3	11.5	13.3	12.1	11.8	12.0	11.8
-7.0	41.1	41.0	42.6	41.0	34.2	36.3
-7.3	60.5	57.3	63.1	59.7	55.6	54.1
-8.0	97.0	82.3	96.1	93.3	87.9	81.5
-9.0	102.6	98.0	105.8	105.7	87.1	89.5
-10.0	104.1	98.8	106.5	110.4	94.1	81.3

	CR11607_R1	CR11607_R2	CR11607_R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.6834	P<0.0001	0.0003
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Reject null hypothesis	Reject null hypothesis
Preferred model	Equation 2	Equation 1	Equation 1
F (Df1, Df2)	0.602 (2,12)	71.2 (2,12)	17.8 (2,12)
Equation 1			
Best-fit values			
BOTTOM	0.698	0.518	2.99
TOP	102	108	85.0
LOGEC50	-7.17	-7.19	-7.15
HILLSLOPE	-1.02	-1.04	-1.28
EC50	6.79e-008	6.43e-008	7.92e-008
Std Error			
BOTTOM	2.27	0.670	2.83
TOP	1.63	0.734	1.74
LOGEC50	0.0384	0.0143	0.0375
HILLSLOPE	0.101	0.0381	0.164
Lower 95% conf. limit			
BOTTOM	-4.24	-7.38	-1.44
TOP	97.7	108	85.2
LOGEC50	-7.25	-7.22	-7.24
HILLSLOPE	-1.25	-1.12	-1.64
EC50	6.57e-008	5.99e-008	5.89e-008
Upper 95% conf. limit			
BOTTOM	8.63	2.41	7.42
TOP	108	110	85.5
LOGEC50	-7.08	-7.18	-7.07
HILLSLOPE	-0.853	-0.956	-0.819
EC50	8.26e-008	9.91e-008	8.47e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.994	0.999	0.992
Absolute Sum of Squares	141	22.0	151
Syx	3.43	1.35	3.54
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.14	-7.11	-7.25
HILLSLOPE	-1.04	-1.17	-0.861
EC50	7.22e-008	7.78e-008	5.83e-008
Std Error			
LOGEC50	0.0082	0.0325	0.0574
HILLSLOPE	0.0689	0.111	0.197
Lower 95% conf. limit			
LOGEC50	-7.20	-7.18	-7.37
HILLSLOPE	-1.18	-1.41	-1.12
EC50	6.34e-008	6.63e-008	4.24e-008
Upper 95% conf. limit			
LOGEC50	-7.09	-7.04	-7.13
HILLSLOPE	-0.863	-0.934	-0.882
EC50	8.21e-008	9.14e-008	7.47e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.984	0.990	0.971
Absolute Sum of Squares	155	28.0	581
Syx	3.33	4.49	6.50
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



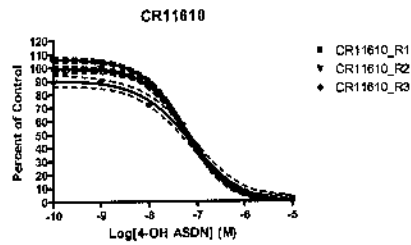
	4-OH ASDN	CR11608_R1		CR11608_R2		CR11608_R3	
		Y1	Y2	Y1	Y2	Y1	Y2
■ CR11608_R1	-5.0	0.8	0.7	0.7	0.7	0.8	0.5
▼ CR11608_R2	-6.0	5.9	6.0	6.0	5.6	5.5	5.4
◆ CR11608_R3	-6.3	11.1	10.6	10.7	10.2	11.1	9.8
	-7.0	37.1	37.6	36.3	35.4	35.4	35.9
	-7.3	52.9	57.0	52.7	51.6	54.1	56.6
	-8.0	90.0	89.0	85.3	83.8	88.2	84.5
	-9.0	101.8	99.5	96.1	98.2	99.3	95.2
	-10.0	104.8	100.3	95.4	97.5	100.1	98.5

	CR11608_R1	CR11608_R2	CR11608_R3
Comparison of Fit			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.0103	0.0047	0.0285
Conclusion (alpha = 0.05)	Reject null hypothesis	Reject null hypothesis	Do not reject null hypothesis
Predicted model	Equation 1	Equation 1	Equation 2
F (DFs, DFs)	6.85 (2,12)	8.86 (2,12)	0.810 (2,12)
Equation 1			
Best-fit values			
BOTTOM	0.874	0.759	0.584
TOP	103	97.7	99.9
LOGEC50	-7.24	-7.24	-7.22
HILLSLOPE	-1.05	-1.04	-1.07
EC50	5.77e-008	5.78e-008	6.01e-008
Std. Error			
BOTTOM	0.811	0.637	0.908
TOP	0.799	0.569	0.767
LOGEC50	0.0103	0.0120	0.0185
HILLSLOPE	0.0444	0.0317	0.0468
Lower 95% conf. limit			
BOTTOM	-4.11	-0.029	-1.39
TOP	101	96.5	97.2
LOGEC50	-7.27	-7.27	-7.26
HILLSLOPE	-1.15	-1.11	-1.17
EC50	5.32e-008	5.42e-008	5.54e-008
Upper 95% conf. limit			
BOTTOM	2.86	2.15	2.57
TOP	104	98.9	101
LOGEC50	-7.20	-7.21	-7.19
HILLSLOPE	-0.958	-0.968	-0.909
EC50	6.26e-008	6.12e-008	6.53e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.989	0.999	0.989
Absolute Sum of Squares	28.3	12.6	26.1
Syx	1.48	1.02	1.47
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	109	100	100
LOGEC50	-7.20	-7.28	-7.23
HILLSLOPE	-1.08	-0.973	-1.03
EC50	5.29e-008	5.91e-008	5.94e-008
Std. Error			
LOGEC50	0.0155	0.0124	0.0117
HILLSLOPE	0.041	0.0278	0.0300
Lower 95% conf. limit			
LOGEC50	-7.23	-7.29	-7.25
HILLSLOPE	-1.17	-1.03	-1.10
EC50	5.62e-008	5.18e-008	5.50e-008
Upper 95% conf. limit			
LOGEC50	-7.17	-7.23	-7.20
HILLSLOPE	-0.984	-0.913	-0.967
EC50	6.79e-008	5.86e-008	6.29e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.999	0.999	0.999
Absolute Sum of Squares	56.3	30.8	30.0
Syx	2.01	1.46	1.46
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	5	5	5
Number of Y replicates	2	2	2
Total number of values	10	10	10
Number of missing values	0	0	0



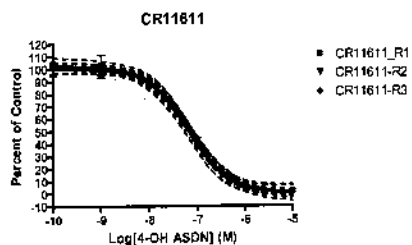
4-OH ASDN	CR11609-R1		CR11609-R2		CR11609-R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	0.7	0.8	0.8	0.7	0.5	0.3
-6.0	5.8	5.6	5.9	5.4	5.6	5.9
-6.3	10.4	11.2	10.6	9.8	10.5	10.7
-7.0	35.7	35.5	34.9	33.0	35.2	30.0
-7.3	53.8	55.2	53.7	50.0	54.0	53.4
-8.0	85.7	85.8	86.1	80.2	86.9	87.2
-9.0	95.0	98.6	97.0	97.4	94.8	98.2
-10.0	98.4	97.5	100.1	94.2	96.1	97.1

	CR11609-R1	CR11609-R2	CR11609-R3
Comparison of Fit			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.0130	0.2367	0.9321
Conclusion (alpha = 0.05)	Reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Preferred model	Equation 1	Equation 2	Equation 1
F (DfN, DfD)	6.37 (2,12)	1.82 (2,12)	4.84 (2,12)
Equation 1			
Best-fit values			
BOTTOM	0.925	0.852	2.01
TOP	98.1	98.0	97.2
LOGEC50	-7.22	-7.27	-7.26
HILLSLOPE	-1.07	-1.02	-1.19
EC50	5.89e-008	5.43e-008	5.67e-008
Std. Error			
BOTTOM	0.623	1.24	1.08
TOP	0.524	1.10	0.979
LOGEC50	0.0111	0.0237	0.0185
HILLSLOPE	0.0314	0.0804	0.0702
Lower 95% conf. limit			
BOTTOM	-0.388	-2.00	-0.298
TOP	97.0	95.8	95.0
LOGEC50	-7.25	-7.32	-7.25
HILLSLOPE	-1.14	-1.15	-1.34
EC50	5.64e-008	4.82e-008	5.14e-008
Upper 95% conf. limit			
BOTTOM	2.24	3.39	4.31
TOP	99.3	100	99.3
LOGEC50	-7.20	-7.21	-7.29
HILLSLOPE	-0.999	-0.891	-1.04
EC50	6.30e-008	6.11e-008	6.25e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	1.00	0.998	0.998
Absolute Sum of Squares	11.5	48.0	42.8
Syx	0.980	2.00	1.98
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.24	-7.28	-7.24
HILLSLOPE	-1.00	-0.865	-1.05
EC50	5.81e-008	5.22e-008	5.58e-008
Std. Error			
LOGEC50	0.0108	0.0177	0.0185
HILLSLOPE	0.0255	0.0387	0.0494
Lower 95% conf. limit			
LOGEC50	-7.26	-7.32	-7.30
HILLSLOPE	-1.06	-1.05	-1.16
EC50	5.51e-008	4.78e-008	5.06e-008
Upper 95% conf. limit			
LOGEC50	-7.21	-7.24	-7.22
HILLSLOPE	-0.948	-0.882	-0.948
EC50	6.12e-008	5.70e-008	5.98e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.999	0.997	0.997
Absolute Sum of Squares	23.8	61.0	75.8
Syx	1.30	2.09	2.33
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



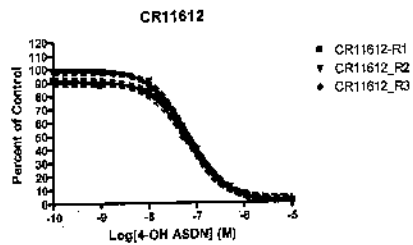
4-OH ASDN	CR11610_R1		CR11610_R2		CR11610_R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	0.7	0.7	0.8	0.7	1.0	0.7
-6.0	6.1	6.0	6.3	5.9	7.7	7.1
-6.3	12.0	11.0	11.2	10.5	13.2	11.6
-7.0	41.1	38.1	39.7	37.6	42.1	37.6
-7.3	59.2	54.6	53.8	55.9	54.9	51.4
-8.0	90.3	88.1	89.8	83.6	86.5	77.6
-9.0	104.8	103.7	100.0	98.3	93.4	87.3
-10.0	108.3	104.2	94.3	100.9	88.7	90.5

	CR11610_R1	CR11610_R2	CR11610_R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	P < 0.001	0.7442	9.3029
Conclusion (alpha = 0.05)	Reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Preferred model	Equation 1	Equation 2	Equation 1
F (DFn, DFd)	31.5 (2, 12)	0.303 (2, 12)	9.88 (2, 12)
Equation 1			
Best-fit values			
BOTTOM	-4.408	0.832	-2.11
TOP	108	98.1	90.8
LOGEC50	-7.24	-7.26	-7.13
HILLSLOPE	-0.851	-1.04	-0.606
EC50	5.79e-008	6.27e-008	7.38e-008
Std. Error			
BOTTOM	1.01	1.39	2.93
TOP	0.854	1.17	2.12
LOGEC50	0.0177	0.0291	0.0562
HILLSLOPE	0.0367	0.0607	0.0897
Lower 95% conf. limit			
BOTTOM	-2.82	-2.89	-3.49
TOP	106	96.8	88.1
LOGEC50	-7.28	-7.28	-7.29
HILLSLOPE	-1.04	-1.18	-1.00
EC50	5.30e-008	5.83e-008	5.48e-008
Upper 95% conf. limit			
BOTTOM	1.80	3.56	4.28
TOP	108	102	95.4
LOGEC50	-7.20	-7.15	-7.00
HILLSLOPE	-0.887	-0.882	-0.811
EC50	6.33e-008	7.12e-008	9.93e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.998	0.998	0.982
Absolute Sum of Squares	27.5	57.8	151
Syx	1.51	2.18	3.55
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	9.9
TOP	109	100	100
LOGEC50	-7.17	-7.21	-7.33
HILLSLOPE	-1.07	-1.01	-0.683
EC50	8.75e-008	6.22e-008	4.57e-008
Std. Error			
LOGEC50	0.0270	0.0167	0.0585
HILLSLOPE	0.0760	0.0407	0.0608
Lower 95% conf. limit			
LOGEC50	-7.23	-7.24	-7.45
HILLSLOPE	-1.24	-1.09	-0.812
EC50	5.90e-008	5.73e-008	3.54e-008
Upper 95% conf. limit			
LOGEC50	-7.11	-7.17	-7.21
HILLSLOPE	-0.911	-0.828	-0.553
EC50	7.71e-008	6.75e-008	6.18e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.994	0.998	0.978
Absolute Sum of Squares	472	50.9	400
Syx	3.50	2.07	5.34
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Date			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



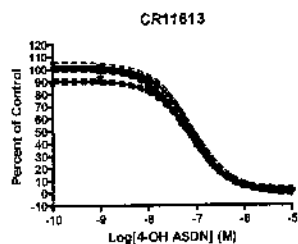
4-OH ASDN	CR11611-R1		CR11611-R2		CR11611-R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	1.6	0.3	0.8	0.7	0.8	0.8
-6.0	6.3	5.2	6.4	5.8	6.9	6.6
-6.3	10.0	10.0	11.3	11.1	12.8	11.3
-7.0	44.7	36.2	41.4	39.0	40.4	41.3
-7.3	57.4	54.7	58.4	59.4	62.2	58.5
-8.0	87.4	90.0	91.3	87.7	86.5	92.4
-9.0	93.0	111.0	104.5	98.1	101.4	95.1
-10.0	94.5	109.4	99.6	105.9	109.7	95.7

	CR11611-R1	CR11611-R2	CR11611-R3
Comparison of Fit			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.6221	0.8702	0.7729
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 2
F (Df _h , Df _e)	0.497 (2,12)	3.34 (2,12)	0.284 (2,12)
Equation 1			
Best-fit values			
BOTTOM	-0.0719	0.136	8.459
TOP	103	103	101
LOGEC50	-7.21	-7.18	-7.15
HILLSLOPE	-1.02	-1.03	-1.04
EC50	8.21e-008	6.55e-008	7.01e-008
Std. Error			
BOTTOM	3.39	1.34	2.39
TOP	2.84	1.19	1.91
LOGEC50	0.6993	0.0230	0.0412
HILLSLOPE	0.150	0.0600	0.109
Lower 95% conf. limit			
BOTTOM	-7.45	-2.78	-4.76
TOP	96.9	100	87.0
LOGEC50	-7.34	-7.22	-7.24
HILLSLOPE	-1.34	-1.16	-1.28
EC50	1.61e-008	5.04e-008	5.71e-008
Upper 95% conf. limit			
BOTTOM	7.31	3.05	5.87
TOP	109	105	105
LOGEC50	-7.08	-7.13	-7.08
HILLSLOPE	-0.690	-0.900	-0.902
EC50	8.29e-008	7.38e-008	8.82e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.988	0.898	0.984
Absolute Sum of Squares	329	50.6	157
Syx	5.23	2.05	3.62
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.18	-7.16	-7.14
HILLSLOPE	-1.07	-1.08	-1.05
EC50	6.64e-008	7.03e-008	7.22e-008
Std. Error			
LOGEC50	0.8391	0.0182	0.0267
HILLSLOPE	0.108	0.0517	0.0711
Lower 95% conf. limit			
LOGEC50	-7.26	-7.19	-7.19
HILLSLOPE	-1.30	-1.19	-1.20
EC50	6.47e-008	6.43e-008	6.41e-008
Upper 95% conf. limit			
LOGEC50	-7.09	-7.11	-7.08
HILLSLOPE	-0.636	-0.969	-0.699
EC50	6.05e-008	7.89e-008	8.35e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.988	0.987	0.993
Absolute Sum of Squares	356	78.9	164
Syx	5.04	2.37	3.43
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



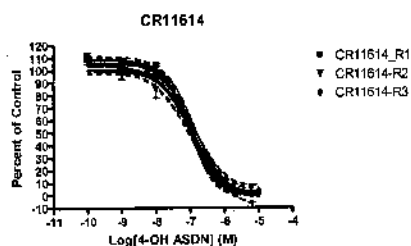
4-OH ASDN	CR11612_R1		CR11612_R2		CR11612_R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	0.8	0.7	0.8	0.7	0.8	0.7
-6.0	6.1	6.0	6.2	6.0	6.0	5.8
-6.3	11.7	10.2	11.5	10.9	10.9	10.3
-7.0	39.1	37.0	39.6	38.4	38.0	35.7
-7.3	58.2	55.9	58.3	55.0	47.3	50.1
-8.0	87.1	79.0	92.0	89.7	86.6	81.2
-9.0	84.8	89.7	97.8	97.4	88.9	88.5
-10.0	92.0	91.2	97.5	95.8	90.5	91.1

	CR11612_R1	CR11612_R2	CR11612_R3
Comparison of Fit			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	P=0.0001	0.0142	0.0001
Conclusion (alpha = 0.05)	Reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Predicted model	Equation 1	Equation 2	Equation 1
F (DFn, DFd)	29.3 (2,12)	3.28 (2,12)	20.8 (2,12)
Equation 1			
Best-fit values			
BOTTOM	1.17	1.77	1.54
TOP	90.0	98.1	91.1
LOGEC50	-7.11	-7.18	-7.20
HILLSLOPE	-1.16	-1.13	-1.11
EC50	7.75e-008	6.89e-008	6.29e-008
Std. Error			
BOTTOM	1.55	1.00	1.55
TOP	1.25	0.898	1.34
LOGEC50	0.0284	0.0173	0.0282
HILLSLOPE	0.0960	0.0621	0.0929
Lower 95% conf. limit			
BOTTOM	-2.20	-0.410	-1.85
TOP	87.4	96.2	88.2
LOGEC50	-7.17	-7.21	-7.27
HILLSLOPE	-1.37	-1.31	-1.31
EC50	8.70e-008	8.13e-008	5.41e-008
Upper 95% conf. limit			
BOTTOM	4.84	3.96	4.93
TOP	92.7	100	94.8
LOGEC50	-7.05	-7.14	-7.14
HILLSLOPE	-0.951	-1.04	-0.903
EC50	8.81e-008	7.29e-008	7.02e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.999	0.999	0.998
Absolute Sum of Squares	72.1	34.0	78.1
Syx	2.45	1.86	2.55
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.23	-7.18	-7.20
HILLSLOPE	-0.880	-1.08	-0.872
EC50	5.95e-008	6.58e-008	5.05e-008
Std. Error			
LOGEC50	0.0488	0.0148	0.0450
HILLSLOPE	0.0852	0.0423	0.0794
Lower 95% conf. limit			
LOGEC50	-7.33	-7.25	-7.38
HILLSLOPE	-1.07	-1.17	-1.04
EC50	4.67e-008	8.21e-008	4.04e-008
Upper 95% conf. limit			
LOGEC50	-7.12	-7.14	-7.20
HILLSLOPE	-0.880	-0.898	-0.791
EC50	7.57e-008	7.19e-008	5.30e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.979	0.998	0.982
Absolute Sum of Squares	424	52.4	347
Syx	5.50	1.94	4.98
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



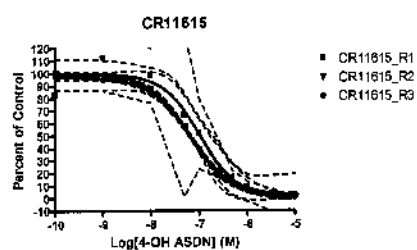
4-OH ASDN	CR11613_R1		CR11613_R2		CR11613_R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	0.8	0.7	0.7	0.7	0.7	0.6
-6.0	6.6	6.2	6.4	5.9	7.4	6.2
-6.3	11.6	10.5	11.4	11.8	12.0	13.2
-7.0	39.2	36.4	39.2	38.7	42.4	42.1
-7.3	55.6	54.8	56.1	55.9	63.2	63.3
-8.0	82.9	82.2	91.3	85.3	94.1	91.9
-9.0	89.0	94.2	103.5	96.6	93.8	102.9
-10.0	87.1	89.0	99.7	99.3	105.6	105.5

	CR11613_R1	CR11613_R2	CR11613_R3
Comparison of Fit			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	P<0.0001	0.6621	0.1422
Conclusion (alpha = 0.05)	Reject null hypothesis	Do not reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 1	Equation 2	Equation 2
F (DFn, DFe)	48.2 (2,12)	0.265 (2,12)	2.31 (2,12)
Equation 1			
Best-fit values			
BOTTOM	-1.27	0.677	0.713
TOP	80.6	101	103
LOGEC50	-7.14	-7.20	-7.13
HILLSLOPE	-1.13	-1.04	-1.09
EC50	7.33e-008	6.28e-008	7.40e-008
Std. Error			
BOTTOM	1.17	1.27	1.80
TOP	0.939	1.97	1.43
LOGEC50	0.0217	0.0227	0.0228
HILLSLOPE	0.0692	0.0603	0.0872
Lower 95% conf. limit			
BOTTOM	-1.27	-2.10	-3.21
TOP	86.5	96.3	99.7
LOGEC50	-7.16	-7.25	-7.20
HILLSLOPE	-1.24	-1.17	-1.28
EC50	8.57e-008	5.82e-008	6.38e-008
Upper 95% conf. limit			
BOTTOM	3.82	3.45	4.84
TOP	82.6	103	106
LOGEC50	-7.09	-7.15	-7.07
HILLSLOPE	-0.875	-0.908	-0.896
EC50	6.17e-008	7.05e-008	6.58e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.998	0.999	0.998
Absolute Sum of Squares	40.8	47.7	52.4
Syx	1.84	1.99	2.78
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-7.24	-7.19	-7.16
HILLSLOPE	-0.980	-1.03	-1.12
EC50	5.75e-008	8.51e-008	8.03e-008
Std. Error			
LOGEC50	0.0447	0.0151	0.0225
HILLSLOPE	0.0610	0.0369	0.0662
Lower 95% conf. limit			
LOGEC50	-7.34	-7.22	-7.14
HILLSLOPE	-0.95	-1.12	-1.27
EC50	4.61e-008	8.04e-008	7.19e-008
Upper 95% conf. limit			
LOGEC50	-7.14	-7.15	-7.05
HILLSLOPE	-0.708	-0.949	-0.976
EC50	7.17e-008	7.91e-008	9.98e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.983	0.995	0.995
Absolute Sum of Squares	355	50.8	128
Syx	5.03	1.91	3.02
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



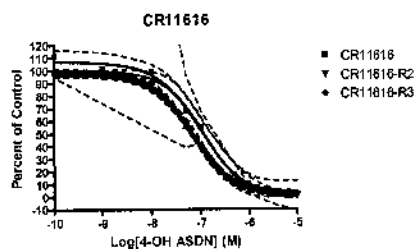
4-OH ASDN	CR11614-R1		CR11614-R2		CR11614-R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	1.3	1.5	1.2	1.1	1.0	1.0
-6.0	10.0	5.8	10.7	10.1	9.0	8.5
-6.3	17.8	14.8	17.7	17.5	16.9	15.8
-7.0	58.4	55.3	53.9	51.5	51.4	51.8
-7.3	67.3	76.9	76.4	70.8	74.3	70.6
-8.0	103.4	97.7	77.6	91.3	100.1	106.0
-9.0	104.6	92.6	105.4	97.0	109.4	101.4
-10.0	107.2	114.1	111.8	105.1	110.4	106.8

	CR11614-R1	CR11614-R2	CR11614-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.1148	0.4305	0.0001
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Related model	Equation 2	Equation 2	Equation 1
F (DFs, DF4)	2.80 (2, 12)	0.805 (2, 12)	21.3 (2, 12)
Equation 1			
Best-fit values			
BOTTOM	0.124	-3.17	1.86
TOP	106	104	106
LOGEC50	-6.95	-6.96	-7.03
HILLSLOPE	-1.10	-0.839	-1.14
EC50	1.08e-007	1.10e-007	9.23e-008
Std. Error			
BOTTOM	3.77	9.12	1.90
TOP	2.35	3.22	1.38
LOGEC50	0.0585	0.0849	0.0299
HILLSLOPE	0.153	0.127	0.0893
Lower 95% conf. limit			
BOTTOM	-8.10	-14.3	-2.46
TOP	106	97.4	105
LOGEC50	-7.08	-7.14	-7.10
HILLSLOPE	-1.44	-1.12	-1.34
EC50	8.07e-008	7.17e-008	7.99e-008
Upper 95% conf. limit			
BOTTOM	8.35	7.97	5.79
TOP	111	111	111
LOGEC50	-6.64	-6.77	-6.87
HILLSLOPE	-0.769	-0.562	-0.992
EC50	1.45e-007	1.65e-007	1.07e-007
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.955	0.964	0.997
Absolute Sum of Squares	335	409	95.7
Syx	5.27	5.84	2.82
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-6.81	-6.94	-6.84
HILLSLOPE	-1.20	-0.978	-1.23
EC50	1.23e-007	1.14e-007	1.14e-007
Std. Error			
LOGEC50	0.0436	0.0476	0.0407
HILLSLOPE	0.131	0.100	0.133
Lower 95% conf. limit			
LOGEC50	-7.00	-7.64	-7.03
HILLSLOPE	-1.48	-1.19	-1.51
EC50	9.95e-008	9.04e-008	9.35e-008
Upper 95% conf. limit			
LOGEC50	-6.82	-6.84	-6.85
HILLSLOPE	-0.816	-0.763	-0.942
EC50	1.53e-007	1.45e-007	1.40e-007
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.993	0.991	0.985
Absolute Sum of Squares	476	471	435
Syx	5.84	5.80	5.57
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Other			
Number of X values	5	5	5
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



4-OH ASDN	CR11615_R1		CR11615_R2		CR11615_R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	1.1	1.0	0.4	0.5	2.4	2.1
-6.0	9.0	8.4	5.9	5.6	6.4	5.7
-6.3	16.6	15.7	11.3	10.5	11.8	11.2
-7.0	53.5	49.1	40.0	35.7	41.5	37.0
-7.3	71.6	61.3	58.1	55.3	57.6	55.8
-8.0	86.2	99.6	86.9	85.4	85.1	82.6
-9.0	114.4	110.2	96.1	99.5	93.5	96.6
-10.0	64.2	100.2	98.1	101.5	99.4	95.6

	CR11615_R1	CR11615_R2	CR11615_R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.9424	0.8789	0.0180
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Parameter model	Equation 2	Equation 2	Equation 1
F (DFn, DFd)	0.0586 (2,12)	0.133 (2,12)	5.95 (2,12)
Equation 1			
Best-fit values			
BOTTOM	2.42	0.248	0.832
TOP	86.6	86.5	86.8
LOGEC50	-7.00	-7.19	-7.12
HILLSLOPE	-1.21	-1.05	-1.02
EC50	1.00e-007	8.40e-008	6.72e-008
Std. Error			
BOTTOM	8.04	1.07	1.12
TOP	5.73	0.896	0.904
LOGEC50	0.133	0.0191	0.0205
HILLSLOPE	0.437	0.0516	0.0521
Lower 95% conf. limit			
BOTTOM	-15.1	-2.09	-1.50
TOP	86.3	97.6	94.8
LOGEC50	-7.29	-7.24	-7.22
HILLSLOPE	-2.19	-1.18	-1.13
EC50	5.14e-008	5.81e-008	6.06e-008
Upper 95% conf. limit			
BOTTOM	19.9	2.58	3.36
TOP	111	101	98.7
LOGEC50	-6.71	-7.15	-7.13
HILLSLOPE	-0.255	-0.934	-0.908
EC50	1.95e-007	7.04e-008	7.44e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.939	0.999	0.998
Absolute Sum of Squares	1743	33.8	34.2
Syx	12.1	1.88	1.89
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-4.98	-7.20	-7.20
HILLSLOPE	-1.12	-1.03	-0.851
EC50	1.01e-007	6.36e-008	6.27e-008
Std. Error			
LOGEC50	0.0850	0.0125	0.0188
HILLSLOPE	0.240	0.0320	0.0304
Lower 95% conf. limit			
LOGEC50	-7.18	-7.22	-7.24
HILLSLOPE	-1.63	-1.10	-1.01
EC50	6.88e-008	5.86e-008	5.71e-008
Upper 95% conf. limit			
LOGEC50	-6.80	-7.17	-7.16
HILLSLOPE	-0.805	-0.962	-0.849
EC50	1.59e-007	6.77e-008	6.88e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.832	0.999	0.987
Absolute Sum of Squares	1760	34.8	38.1
Syx	11.2	1.97	2.21
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	6	3	6
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0



4-OH ASDN	CR11616		CR11616-R2		CR11616-R3	
	Y1	Y2	Y1	Y2	Y1	Y2
-5.0	1.3	1.1	0.8	0.6	2.4	2.1
-6.0	10.0	9.7	8.1	6.1	6.4	5.7
-6.3	17.6	16.3	11.4	11.7	11.8	11.2
-7.0	55.6	53.7	39.8	36.1	41.5	37.0
-7.3	69.4	64.9	59.5	54.3	57.6	55.8
-8.0	108.8	88.7	91.4	85.9	85.1	82.0
-9.0	116.7	84.9	99.7	94.9	93.5	98.0
-10.0	111.8	109.3	100.4	98.0	99.4	95.6

	CR11616	CR11616-R2	CR11616-R3
Completion of F-Test			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.2578	0.4370	0.0160
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 1
F (DFn, DFe)	1.47 (2,12)	0.389 (2,12)	5.96 (2,12)
Equation 1			
Best-fit values			
BOTTOM	-0.272	1.26	0.932
TOP	107	98.6	86.8
LOGEC50	-7.02	-7.49	-7.17
HILLSLOPE	-0.989	-1.11	-1.02
EC50	9.62e-008	6.53e-008	5.72e-008
Std Error			
BOTTOM	6.33	1.43	1.12
TOP	4.36	1.21	0.904
LOGEC50	0.102	0.0262	0.0205
HILLSLOPE	0.231	0.0782	0.0521
Lower 95% conf. limit			
BOTTOM	-14.1	-4.83	-1.50
TOP	97.8	95.9	94.8
LOGEC50	-7.24	-7.24	-7.22
HILLSLOPE	-1.43	-1.28	-1.13
EC50	5.78e-008	5.75e-008	6.08e-008
Upper 95% conf. limit			
BOTTOM	13.5	4.38	3.36
TOP	117	101	88.7
LOGEC50	-6.80	-7.13	-7.13
HILLSLOPE	-0.810	-0.939	-0.908
EC50	5.60e-007	7.40e-008	7.44e-008
Goodness of Fit			
Degrees of Freedom	12	12	12
R ²	0.970	0.997	0.998
Absolute Sum of Squares	836	64.4	34.2
Syx	8.36	2.32	1.69
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100	100	100
LOGEC50	-6.94	-7.18	-7.23
HILLSLOPE	-1.09	-1.04	-0.991
EC50	1.14e-007	6.50e-008	6.27e-008
Std Error			
LOGEC50	0.0670	0.0182	0.0168
HILLSLOPE	0.173	0.0475	0.0384
Lower 95% conf. limit			
LOGEC50	-7.09	-7.23	-7.24
HILLSLOPE	-1.46	-1.14	-1.01
EC50	9.16e-008	6.85e-008	6.71e-008
Upper 95% conf. limit			
LOGEC50	-6.80	-7.15	-7.16
HILLSLOPE	-0.716	-0.939	-0.849
EC50	1.58e-007	7.11e-008	6.85e-008
Goodness of Fit			
Degrees of Freedom	14	14	14
R ²	0.963	0.997	0.997
Absolute Sum of Squares	1045	73.9	66.1
Syx	8.64	2.30	2.21
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100	TOP = 100	TOP = 100
Data			
Number of X values	8	8	8
Number of Y replicates	2	2	2
Total number of values	16	16	16
Number of missing values	0	0	0

APPENDIX G

Reference Chemical Percent of Control
Values for Each Replicate

Percent of Control Values by Chemical and Replicate

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Vinclozolin CR11601	1	-3.0	83.69	85.95	82.03	83.89	1.96	1.13	2.34
		-4.0	86.53	86.17	92.61	88.44	3.62	2.09	4.09
		-5.0	91.06	94.25	96.01	93.78	2.51	1.45	2.67
		-6.0	93.79	94.71	99.26	95.92	2.93	1.69	3.06
		-7.0	95.36	94.79	96.07	95.40	0.64	0.37	0.67
		-8.0	98.27	95.54	97.25	97.02	1.38	0.80	1.42
		-9.0	96.40	97.14	94.69	96.08	1.25	0.72	1.30
		-10.0	95.36	96.23	94.25	95.28	0.99	0.57	1.04
	2	-4.0	105.63	103.87	88.09	99.20	9.66	5.58	9.74
		-5.0	109.24	110.17	94.11	104.51	9.02	5.21	8.63
		-6.0	109.10	100.86	92.56	100.84	8.27	4.77	8.20
		-6.3	107.32	96.40	96.94	100.22	6.15	3.55	6.14
		-7.0	108.00	93.80	91.12	97.64	9.07	5.24	9.29
		-8.0	108.38	97.69	93.36	99.81	7.73	4.46	7.75
		-9.0	111.76	91.34	95.46	99.52	10.80	6.23	10.85
		-10.0	105.82	91.98	106.61	101.47	8.23	4.75	8.11
	3	-4.0	98.30	98.11	100.82	99.08	1.51	0.87	1.52
		-5.0	108.24	105.54	106.06	106.61	1.43	0.83	1.34
		-6.0	104.01	102.81	109.07	105.30	3.32	1.92	3.16
		-6.3	108.56	106.36	108.66	107.86	1.30	0.75	1.20
		-7.0	107.29	105.06	92.16	101.50	8.17	4.72	8.05
		-8.0	106.78	107.94	97.09	103.94	5.96	3.44	5.73
		-9.0	105.82	112.35	93.93	104.03	9.34	5.39	8.98
		-10.0	101.92	106.64	95.76	101.44	5.46	3.15	5.38
Bisphenol A CR11602	1	-3.0	3.60	3.40	2.70	3.23	0.47	0.27	14.62
		-4.0	59.30	58.30	59.80	59.13	0.76	0.44	1.29
		-5.0	91.80	87.50	83.40	87.57	4.20	2.43	4.80
		-6.0	86.40	94.50	89.20	90.03	4.11	2.38	4.57
		-7.0	89.50	96.00	91.00	92.17	3.40	1.96	3.69
		-8.0	94.10	95.60	87.20	92.30	4.48	2.59	4.85
		-9.0	93.60	91.90	94.30	93.27	1.23	0.71	1.32
		-10.0	95.00	89.80	94.00	92.93	2.76	1.59	2.97
	4	-3.0	5.30	3.40	5.30	4.67	1.10	0.63	23.51
		-3.3	14.50	23.40	20.20	19.37	4.51	2.60	23.28
		-3.6	37.80	44.20	44.90	42.30	3.91	2.26	9.25
		-4.0	64.70	68.70	62.70	65.37	3.06	1.76	4.67
		-4.3	74.10	99.50	93.60	89.07	13.29	7.67	14.92
		-4.6	94.30	63.10	88.90	82.10	16.67	9.63	20.31
		-5.0	100.80	108.90	83.70	97.80	12.87	7.43	13.15
		-6.0	95.60	74.70	91.80	87.37	11.13	6.43	12.74

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Bisphenol A CR11602	5	-3.0	4.32	4.86	4.58	4.59	0.27	0.16	5.86
		-3.3	20.61	21.45	20.48	20.85	0.53	0.31	2.53
		-3.6	43.67	42.52	44.31	43.50	0.90	0.52	2.08
		-4.0	73.02	76.46	69.87	73.12	3.30	1.90	4.51
		-4.3	97.71	83.68	90.70	90.70	7.02	4.05	7.73
		-4.6	98.28	95.43	98.00	97.24	1.57	0.91	1.61
		-5.0	118.19	93.54	100.79	104.18	12.67	7.31	12.16
		-6.0	114.41	111.76	114.13	113.43	1.45	0.84	1.28
Tributyltin CR11603	1	-3.0	82.57	73.44	78.07	78.03	4.56	2.63	5.85
		-4.0	81.28	85.64	82.83	83.25	2.21	1.27	2.65
		-5.0	99.98	89.83	89.03	92.95	6.11	3.53	6.57
		-6.0	97.58	100.89	98.10	98.86	1.78	1.03	1.80
		-7.0	100.44	96.72	89.30	95.49	5.67	3.27	5.94
		-8.0	96.65	92.90	92.14	93.89	2.42	1.39	2.57
		-9.0	92.22	88.21	96.48	92.30	4.14	2.39	4.48
		-10.0	96.79	86.68	96.13	93.20	5.66	3.27	6.07
	2	-3.0	90.13	77.86	74.87	80.95	8.09	4.67	9.99
		-4.0	84.36	80.89	79.85	81.70	2.36	1.36	2.89
		-5.0	91.68	96.43	96.43	94.85	2.74	1.58	2.89
		-6.0	99.99	98.73	103.53	100.75	2.49	1.44	2.47
		-7.0	85.74	98.87	99.45	94.69	7.75	4.48	8.19
		-8.0	92.24	99.90	90.25	94.13	5.09	2.94	5.41
		-9.0	97.36	98.65	97.26	97.76	0.77	0.45	0.79
		-10.0	95.83	98.36	103.54	99.24	3.93	2.27	3.96
	3	-3.0	88.47	74.23	83.59	82.10	7.24	4.18	8.81
		-4.0	77.00	81.86	92.79	83.89	8.09	4.67	9.64
		-5.0	95.47	90.91	84.63	90.34	5.44	3.14	6.03
		-6.0	103.08	99.28	97.25	99.87	2.96	1.71	2.96
		-7.0	90.37	83.40	96.86	90.21	6.73	3.89	7.46
		-8.0	92.56	100.21	103.46	98.74	5.60	3.23	5.67
		-9.0	102.43	89.67	93.85	95.32	6.51	3.76	6.82
		-10.0	91.57	91.16	96.19	92.97	2.79	1.61	3.00
Diethylhexyl phthalate CR11604	1	-3.0	108.14	104.48	108.69	107.10	2.29	1.32	2.14
		-4.0	107.77	109.26	110.61	109.21	1.42	0.82	1.30
		-5.0	113.26	104.45	109.89	109.20	4.45	2.57	4.07
		-6.0	93.32	102.67	97.53	97.84	4.68	2.70	4.79
		-7.0	104.14	99.70	90.48	98.11	6.97	4.02	7.10
		-8.0	97.96	102.35	89.63	96.65	6.46	3.73	6.69
		-9.0	98.62	100.31	101.76	100.23	1.57	0.91	1.57
		-10.0	99.95	97.87	90.01	95.94	5.25	3.03	5.47

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Diethylhexyl phthalate CR11604	2	-4.0	106.92	100.22	95.58	100.91	5.70	3.29	5.65
		-5.0	109.86	101.97	107.23	106.35	4.02	2.32	3.78
		-6.0	98.61	100.38	92.10	97.03	4.36	2.52	4.50
		-6.3	105.49	101.08	96.47	101.01	4.51	2.60	4.46
		-7.0	94.22	98.20	94.17	95.53	2.31	1.33	2.42
		-8.0	96.52	93.97	90.17	93.55	3.20	1.85	3.42
		-9.0	94.14	88.97	96.99	93.37	4.06	2.35	4.35
		-10.0	102.07	99.83	97.24	99.72	2.41	1.39	2.42
	3	-4.0	116.00	108.29	107.28	110.52	4.77	2.76	4.32
		-5.0	105.58	112.04	113.81	110.48	4.33	2.50	3.92
		-6.0	96.55	106.59	104.15	102.43	5.23	3.02	5.11
		-6.3	99.28	107.97	102.44	103.23	4.40	2.54	4.26
		-7.0	106.72	109.21	99.81	105.25	4.87	2.81	4.63
		-8.0	98.79	99.94	98.76	99.16	0.67	0.39	0.68
		-9.0	103.55	104.54	97.73	101.94	3.68	2.12	3.61
		-10.0	100.20	102.04	104.91	102.38	2.37	1.37	2.32
Methoxychlor CRr11605	1	-3.0	77.36	83.92	82.20	81.16	3.40	1.96	4.19
		-4.0	86.77	97.06	97.19	93.68	5.98	3.45	6.38
		-5.0	91.37	98.75	98.66	96.26	4.24	2.45	4.40
		-6.0	90.56	100.14	102.33	97.67	6.26	3.61	6.41
		-7.0	100.48	79.32	94.06	91.29	10.85	6.26	11.88
		-8.0	98.14	97.09	105.68	100.30	4.68	2.70	4.67
		-9.0	108.61	105.10	102.52	105.41	3.06	1.76	2.90
		-10.0	102.80	101.09	103.28	102.39	1.15	0.67	1.13
	2	-5.0	110.63	107.79	109.58	109.33	1.44	0.83	1.32
		-6.0	106.59	108.13	112.55	109.09	3.09	1.79	2.84
		-7.0	114.68	116.96	118.31	116.65	1.84	1.06	1.57
		-7.3	93.88	116.26	110.83	106.99	11.68	6.74	10.91
		-8.0	116.53	114.32	107.41	112.76	4.76	2.75	4.22
		-8.3	97.60	119.68	114.57	110.62	11.55	6.67	10.45
		-9.0	116.20	121.25	115.35	117.60	3.19	1.84	2.71
		-10.0	112.92	116.53	119.05	116.17	3.08	1.78	2.65
	3	-5.0	84.65	104.46	92.92	94.01	9.95	5.74	10.58
		-6.0	110.30	104.56	100.57	105.14	4.89	2.83	4.65
		-7.0	100.25	101.80	109.31	103.79	4.85	2.80	4.67
		-7.3	78.35	100.11	104.71	94.39	14.08	8.13	14.92
		-8.0	95.60	91.94	110.72	99.42	9.95	5.75	10.01
		-8.3	103.98	102.56	103.47	103.34	0.72	0.41	0.69
		-9.0	110.62	91.40	104.74	102.25	9.85	5.68	9.63
		-10.0	104.22	97.76	105.99	102.66	4.33	2.50	4.22

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Aldicarb CR11606	1	-3.0	90.53	92.53	75.82	86.29	9.12	5.27	10.57
		-4.0	97.33	92.69	92.17	94.06	2.84	1.64	3.02
		-5.0	96.47	89.50	95.57	93.84	3.79	2.19	4.04
		-6.0	77.78	97.76	75.43	83.66	12.27	7.09	14.67
		-7.0	95.94	86.73	96.36	93.01	5.45	3.14	5.85
		-8.0	89.07	94.45	94.84	92.79	3.22	1.86	3.47
		-9.0	100.61	94.45	97.99	97.68	3.09	1.78	3.16
		-10.0	89.75	71.91	85.73	82.46	9.36	5.40	11.35
	2	-3.0	108.26	101.54	109.09	106.30	4.14	2.39	3.89
		-4.0	120.42	123.85	105.67	116.65	9.66	5.58	8.28
		-5.0	104.65	115.65	119.60	113.30	7.74	4.47	6.83
		-6.0	124.85	101.32	113.98	113.38	11.78	6.80	10.39
		-7.0	119.50	118.67	127.27	121.81	4.75	2.74	3.90
		-8.0	115.32	113.42	118.24	115.66	2.43	1.40	2.10
		-9.0	115.42	104.59	123.55	114.52	9.51	5.49	8.30
		-10.0	123.77	112.24	117.55	117.85	5.77	3.33	4.90
	3	-3.0	91.60	74.74	88.40	84.92	8.95	5.17	10.54
		-4.0	106.82	110.64	100.73	106.06	4.99	2.88	4.71
		-5.0	105.34	99.40	95.73	100.16	4.85	2.80	4.84
		-6.0	108.02	99.54	96.83	101.46	5.84	3.37	5.76
		-7.0	67.97	106.48	111.21	95.22	23.72	13.69	24.91
		-8.0	104.03	79.14	103.28	95.49	14.16	8.17	14.83
		-9.0	106.39	82.93	95.48	94.93	11.74	6.78	12.37
		-10.0	87.08	107.34	100.86	98.42	10.35	5.97	10.51
Flavone CR11607	1	-3.0	1.75	1.86	2.05	1.88	0.15	0.09	8.01
		-4.0	25.14	23.02	24.99	24.39	1.18	0.68	4.85
		-5.0	81.68	77.42	71.57	76.89	5.07	2.93	6.60
		-6.0	109.00	97.93	86.22	97.72	11.39	6.58	11.66
		-7.0	98.10	100.74	94.38	97.74	3.19	1.84	3.26
		-8.0	104.44	93.27	98.82	98.84	5.58	3.22	5.65
		-9.0	100.60	96.66	97.16	98.14	2.15	1.24	2.19
		-10.0	104.17	100.74	97.73	100.88	3.22	1.86	3.20
	2	-3.6	11.53	11.17	11.05	11.25	0.25	0.14	2.21
		-4.0	25.01	25.87	24.83	25.24	0.56	0.32	2.21
		-4.3	43.10	41.37	40.32	41.60	1.41	0.81	3.38
		-4.6	58.91	58.69	56.04	57.88	1.60	0.92	2.76
		-5.0	77.65	76.29	78.29	77.41	1.02	0.59	1.32
		-5.3	89.53	85.56	86.71	87.27	2.04	1.18	2.34
		-6.0	99.58	97.24	97.78	98.20	1.23	0.71	1.25
		-7.0	102.78	104.35	97.10	101.41	3.82	2.20	3.76

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Flavone CR11607	3	-3.6	11.03	10.69	10.98	10.90	0.18	0.11	1.70
		-4.0	26.10	25.53	27.05	26.23	0.77	0.44	2.94
		-4.3	41.17	40.56	41.20	40.98	0.36	0.21	0.87
		-4.6	57.98	56.86	58.77	57.87	0.96	0.56	1.67
		-5.0	77.46	76.16	74.62	76.08	1.43	0.82	1.87
		-5.3	89.12	72.24	85.93	82.43	8.97	5.18	10.88
		-6.0	93.40	98.33	90.81	94.18	3.82	2.21	4.06
		-7.0	99.28	98.05	91.32	96.22	4.29	2.48	4.46
Triadimefon CR11608	1	-3.0	0.20	0.18	0.09	0.16	0.06	0.03	37.60
		-4.0	2.04	2.12	2.11	2.09	0.04	0.03	2.12
		-5.0	16.74	16.45	15.89	16.36	0.43	0.25	2.65
		-6.0	65.67	63.58	64.97	64.74	1.06	0.61	1.64
		-7.0	98.21	89.15	95.63	94.33	4.67	2.69	4.95
		-8.0	104.90	102.60	100.85	102.78	2.03	1.17	1.97
		-9.0	100.37	102.92	99.88	101.06	1.63	0.94	1.62
		-10.0	99.67	97.09	100.79	99.18	1.89	1.09	1.91
	2	-4.0	1.75	1.88	1.86	1.83	0.07	0.04	3.77
		-4.3	3.89	3.71	3.68	3.76	0.12	0.07	3.13
		-5.0	15.48	14.84	14.23	14.85	0.62	0.36	4.20
		-5.3	25.07	25.01	24.87	24.98	0.10	0.06	0.40
		-5.6	40.40	39.93	37.56	39.30	1.52	0.88	3.87
		-6.0	60.30	58.44	58.46	59.06	1.07	0.62	1.80
		-7.0	90.93	90.58	90.43	90.65	0.26	0.15	0.29
		-8.0	96.95	96.10	96.94	96.66	0.49	0.28	0.51
	3	-4.0	2.15	2.07	2.18	2.14	0.06	0.03	2.63
		-4.3	4.14	4.02	4.39	4.19	0.19	0.11	4.55
		-5.0	16.96	16.49	16.81	16.75	0.24	0.14	1.43
		-5.3	27.60	27.55	27.85	27.67	0.16	0.09	0.59
		-5.6	42.18	42.36	42.54	42.36	0.18	0.10	0.43
		-6.0	63.63	64.25	63.58	63.82	0.37	0.21	0.58
		-7.0	97.28	96.81	93.09	95.73	2.29	1.32	2.40
		-8.0	99.26	102.01	100.94	100.73	1.38	0.80	1.37
Imazalil CR11609	1	-3.0	0.12	0.11	0.20	0.15	0.05	0.03	33.73
		-4.0	0.08	0.14	0.00	0.07	0.07	0.04	93.86
		-5.0	0.18	0.12	0.12	0.14	0.03	0.02	22.62
		-6.0	1.12	1.02	1.22	1.12	0.10	0.06	8.79
		-7.0	8.97	8.94	8.94	8.95	0.02	0.01	0.19
		-8.0	46.27	46.37	46.24	46.29	0.07	0.04	0.15
		-9.0	90.55		90.94	90.75	0.28	0.16	0.30
		-10.0	99.33		97.92	98.62	1.00	0.58	1.01

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Imazalil CR11609	2	-5.0	0.28	0.25	0.25	0.26	0.02	0.01	6.00
		-6.0	1.85	1.82	1.67	1.78	0.10	0.05	5.34
		-7.0	15.06	15.21	14.51	14.93	0.36	0.21	2.44
		-7.6	40.83	40.51	39.41	40.25	0.74	0.43	1.85
		-8.0	62.25	62.00	60.96	61.73	0.69	0.40	1.11
		-8.6	85.57	87.04	84.94	85.85	1.08	0.62	1.25
		-9.0	93.58	95.34	90.98	93.30	2.19	1.27	2.35
		-10.0	98.63	99.97	97.90	98.84	1.05	0.61	1.06
	3	-5.0	0.07	-0.11	0.01	-0.01	0.09	0.05	-862.16
		-6.0	1.49	1.01	0.92	1.14	0.30	0.18	26.73
		-7.0	14.84	9.79	9.98	11.54	2.87	1.65	24.84
		-7.6	35.92	25.67	29.24	30.28	5.21	3.01	17.20
		-8.0	62.69	46.73	46.59	52.00	9.26	5.34	17.80
		-8.6	87.43	72.62	72.64	77.56	8.55	4.93	11.02
		-9.0	95.19	80.02	77.66	84.29	9.51	5.49	11.29
		-10.0	99.48	86.32	85.54	90.45	7.83	4.52	8.66
Apigenin CR11610	1	-3.0	7.88	6.87	6.83	7.19	0.59	0.34	8.27
		-4.0	17.16	16.68	16.18	16.67	0.49	0.28	2.94
		-5.0	51.39	51.24	50.64	51.09	0.40	0.23	0.78
		-6.0	94.15	93.60	91.60	93.11	1.34	0.78	1.44
		-7.0	102.61	103.19	101.16	102.32	1.05	0.60	1.02
		-8.0	100.96	104.15	102.26	102.46	1.60	0.92	1.56
		-9.0	102.38	100.96	96.62	99.99	3.00	1.73	3.00
		-10.0	102.37	102.50	96.95	100.61	3.17	1.83	3.15
	2	-4.3	18.47	20.22	17.36	18.68	1.44	0.83	7.72
		-4.6	32.78	31.73	31.06	31.86	0.87	0.50	2.73
		-5.0	52.27	52.31	52.76	52.45	0.27	0.16	0.52
		-5.3	72.17	67.49	69.00	69.55	2.39	1.38	3.44
		-5.6	81.46	82.99	78.45	80.97	2.31	1.33	2.86
		-6.0	93.29	90.56	93.06	92.30	1.51	0.87	1.64
		-7.0	97.62	97.68	97.41	97.57	0.14	0.08	0.15
		-8.0	104.33	100.53	97.37	100.74	3.48	2.01	3.46
	3	-4.3	12.57	12.94	12.54	12.69	0.22	0.13	1.76
		-4.6	22.63	22.32	20.34	21.77	1.24	0.72	5.71
		-5.0	36.74	39.57	40.14	38.82	1.82	1.05	4.68
		-5.3	55.89	53.47	55.37	54.91	1.27	0.73	2.32
		-5.6	66.21	67.22	67.01	66.81	0.53	0.31	0.80
		-6.0	79.25	85.83	83.85	82.97	3.38	1.95	4.07
		-7.0	91.04	95.70	98.23	94.99	3.65	2.11	3.84
		-8.0	90.82	92.98	92.46	92.09	1.13	0.65	1.23

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Ronidazole Cr11611	1	-3.0	111.62	105.66	109.52	108.93	3.03	1.75	2.78
		-4.0	114.46	114.40	110.66	113.17	2.18	1.26	1.92
		-5.0	116.38	114.43	112.51	114.44	1.94	1.12	1.69
		-6.0	112.91	111.14	110.53	111.53	1.24	0.72	1.11
		-7.0	113.27	109.78	100.26	107.77	6.73	3.89	6.25
		-8.0	112.27	113.75	109.89	111.97	1.95	1.12	1.74
		-9.0	111.18	109.94	102.53	107.88	4.67	2.70	4.33
		-10.0	111.73	114.62	107.62	111.33	3.52	2.03	3.16
	2	-5.0	105.89	102.35	98.39	102.21	3.75	2.16	3.67
		-6.0	105.37	101.61	97.70	101.56	3.84	2.22	3.78
		-7.0	106.86	103.51	94.06	101.48	6.64	3.83	6.55
		-7.3	104.03	98.72	106.63	103.13	4.03	2.33	3.91
		-8.0	101.59	92.89	96.85	97.11	4.36	2.51	4.48
		-8.3	99.97	95.08	94.16	96.40	3.12	1.80	3.24
		-9.0	103.14	103.97	99.14	102.09	2.58	1.49	2.53
		-10.0	103.01	100.44	94.54	99.33	4.34	2.51	4.37
	3	-5.0	100.34	100.58	93.82	98.25	3.83	2.21	3.90
		-6.0	104.46	99.73	96.18	100.12	4.15	2.40	4.15
		-7.0	105.25	97.88	99.63	100.92	3.85	2.22	3.82
		-7.3	100.80	45.56	98.55	81.64	31.27	18.05	38.30
		-8.0	100.00	97.87	94.61	97.49	2.71	1.57	2.78
		-8.3	74.70	100.22	99.87	91.60	14.64	8.45	15.98
		-9.0	99.23	101.59	94.86	98.56	3.41	1.97	3.46
		-10.0	103.86	105.76	98.07	102.56	4.01	2.31	3.91
Genistein CR11612	1	-3.0	59.73	58.75	59.78	59.42	0.58	0.33	0.98
		-4.0	84.21	87.89	88.80	86.97	2.43	1.40	2.79
		-5.0	91.58	94.22	96.31	94.04	2.37	1.37	2.52
		-6.0	99.62	92.98	97.58	96.72	3.40	1.96	3.52
		-7.0	98.74	95.53	98.16	97.48	1.71	0.99	1.75
		-8.0	100.18	96.12	100.64	98.98	2.49	1.43	2.51
		-9.0	102.31	92.54	96.99	97.28	4.89	2.82	5.03
		-10.0	98.24	92.76	101.00	97.33	4.20	2.42	4.31
	2	-3.3	75.35	74.44	67.62	72.47	4.22	2.44	5.83
		-3.6	82.92	81.00	84.79	82.90	1.89	1.09	2.29
		-4.0	91.66	89.80	94.00	91.82	2.10	1.21	2.29
		-5.0	101.66	98.17	101.77	100.53	2.04	1.18	2.03
		-6.0	98.55	94.82	100.14	97.84	2.73	1.58	2.79
		-7.0	100.80	96.78	98.43	98.67	2.02	1.17	2.05
		-8.0	97.69	93.69	98.14	96.51	2.45	1.42	2.54
		-9.0	97.08	100.66	102.47	100.07	2.74	1.58	2.74

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Genistein CR11612	3	-3.3	74.69	74.40	73.18	74.09	0.80	0.46	1.07
		-3.6	84.15	80.74	87.72	84.20	3.49	2.02	4.15
		-4.0	82.93	88.19	85.01	85.38	2.65	1.53	3.10
		-5.0	82.84	81.63	84.16	82.87	1.27	0.73	1.53
		-6.0	86.16	84.13	84.03	84.77	1.20	0.69	1.42
		-7.0	87.16	86.66	93.95	89.26	4.07	2.35	4.56
		-8.0	86.35	84.76	93.65	88.25	4.74	2.74	5.37
		-9.0	97.84	94.76	96.93	96.51	1.58	0.91	1.64
p,p'-DDE CR11613	1	-3.0	70.19	63.76	60.76	64.90	4.82	2.78	7.42
		-4.0	79.15	89.31	76.87	81.78	6.62	3.82	8.10
		-5.0	82.63	81.95	80.67	81.75	1.00	0.58	1.22
		-6.0	93.19	92.11	87.29	90.86	3.14	1.82	3.46
		-7.0	98.36	98.08	100.01	98.82	1.04	0.60	1.06
		-8.0	99.30	96.80	102.68	99.59	2.95	1.70	2.96
		-9.0	97.69	98.06	102.11	99.29	2.45	1.42	2.47
		-10.0	95.35	95.44	103.10	97.96	4.45	2.57	4.54
	2	-5.0	86.36	92.57	88.34	89.09	3.18	1.83	3.56
		-6.0	103.23	95.18	94.50	97.63	4.86	2.80	4.97
		-7.0	105.43	108.28	99.71	104.48	4.36	2.52	4.18
		-7.3	112.60	106.82	106.57	108.66	3.41	1.97	3.14
		-8.0	113.54	109.90	111.03	111.49	1.86	1.08	1.67
		-8.3	107.70	106.26	112.25	108.74	3.13	1.81	2.88
		-9.0	102.62	103.72	112.44	106.26	5.38	3.11	5.06
		-10.0	106.44	110.41	104.22	107.02	3.13	1.81	2.93
	3	-5.0	91.46	91.80	91.94	91.73	0.25	0.14	0.27
		-6.0	105.02	97.68	98.56	100.42	4.01	2.31	3.99
		-7.0	107.95	NS	105.51	106.73	1.72	0.99	1.61
		-7.3	116.42	102.85	98.99	106.09	9.15	5.29	8.63
		-8.0	104.41	88.93	96.94	96.76	7.74	4.47	8.00
		-8.3	106.01	102.83	96.10	101.65	5.06	2.92	4.98
		-9.0	107.71	100.37	105.44	104.50	3.76	2.17	3.60
		-10.0	109.22	100.88	101.20	103.77	4.73	2.73	4.55
Alachlor CR11614	1	-3.0	54.33	50.93	56.47	53.91	2.80	1.61	5.19
		-4.0	113.88	91.76	103.43	103.02	11.07	6.39	10.74
		-5.0	92.94	104.51	117.25	104.90	12.16	7.02	11.59
		-6.0	123.12	96.75	125.87	115.25	16.08	9.28	13.95
		-7.0	123.17	111.91	122.70	119.26	6.37	3.68	5.34
		-8.0	125.45	132.90	113.23	123.86	9.93	5.73	8.02
		-9.0	110.72	117.03	70.20	99.32	25.41	14.67	25.59
		-10.0	131.70	104.85	106.09	114.21	15.16	8.75	13.27

Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Alachlor CR11614	2	-4.0	106.18	96.49	98.82	100.50	5.06	2.92	5.03
		-5.0	112.11	89.09	77.48	92.89	17.63	10.18	18.97
		-6.0	72.56	113.32	99.34	95.07	20.71	11.96	21.78
		-6.3	99.34	110.17	78.53	96.01	16.08	9.28	16.75
		-7.0	96.68	96.89	103.99	99.18	4.16	2.40	4.19
		-8.0	82.95	108.39	115.33	102.22	17.05	9.84	16.68
		-9.0	109.54	111.59	108.64	109.93	1.51	0.87	1.38
		-10.0	111.33	68.03	111.62	96.99	25.08	14.48	25.86
	3	-4.0	100.20	88.76	99.75	96.24	6.48	3.74	6.73
		-5.0	91.94	111.93	103.04	102.30	10.02	5.78	9.79
		-6.0	109.05	116.54	117.77	114.45	4.72	2.73	4.13
		-6.3	110.53	87.41	106.76	101.57	12.40	7.16	12.21
		-7.0	109.97	117.42	100.14	109.18	8.67	5.00	7.94
		-8.0	114.53	113.61	88.11	105.42	14.99	8.66	14.22
		-9.0	106.29	106.84	92.18	101.77	8.31	4.80	8.16
		-10.0	106.44	81.31	59.39	82.38	23.55	13.59	28.58
Nitrofen CR11615	1	-3.0	37.19	37.36	37.89	37.48	0.36	0.21	0.97
		-4.0	53.02	48.35	50.41	50.59	2.34	1.35	4.63
		-5.0	71.68	67.76	69.86	69.77	1.97	1.14	2.82
		-6.0	107.02	111.57	102.83	107.14	4.37	2.52	4.08
		-7.0	101.63	109.23	113.46	108.11	5.99	3.46	5.54
		-8.0	99.99	108.98	95.69	101.55	6.78	3.92	6.68
		-9.0	110.25	105.55	105.91	107.24	2.61	1.51	2.44
		-10.0	111.42	111.97	103.80	109.06	4.56	2.63	4.18
	2	-4.3	39.34	42.88	41.51	41.24	1.78	1.03	4.32
		-4.6	44.93	45.09	44.34	44.79	0.40	0.23	0.89
		-5.0	55.66	59.07	56.85	57.19	1.73	1.00	3.03
		-5.3	73.34	72.96	69.20	71.83	2.29	1.32	3.18
		-5.6	87.40	86.15	83.60	85.72	1.94	1.12	2.26
		-6.0	103.34	92.17	95.62	97.04	5.72	3.30	5.89
		-7.0	103.48	100.70	96.82	100.33	3.34	1.93	3.33
		-8.0	101.22	102.73	98.79	100.91	1.98	1.15	1.97
	3	-4.3	43.81	40.44	42.34	42.20	1.69	0.97	4.00
		-4.6	43.21	43.35	43.85	43.47	0.34	0.19	0.77
		-5.0	55.57	57.60	57.52	56.90	1.15	0.66	2.02
		-5.3	71.06	69.11	68.84	69.67	1.21	0.70	1.74
		-5.6	83.63	81.49	83.54	82.89	1.21	0.70	1.46
		-6.0	90.32	89.03	89.99	89.78	0.67	0.39	0.75
		-7.0	97.25	94.98	98.20	96.81	1.66	0.96	1.71
		-8.0	98.28	97.55	97.93	97.92	0.37	0.21	0.38

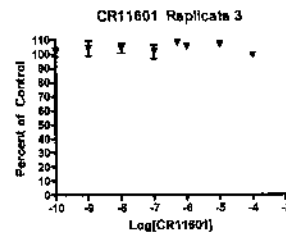
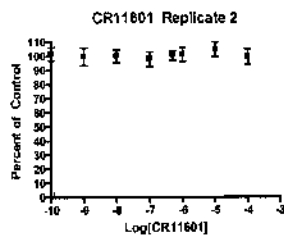
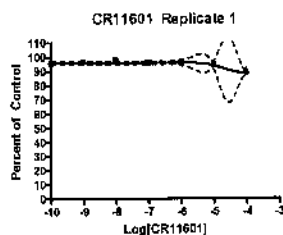
Test Chemical	Replicate	Log[RC] (M)	Percent of Control			Mean	±SD	SEM	CV
			Tube 1	Tube 2	Tube 3				
Trifluralin CR11616	1	-3.0	88.48	110.81	107.60	102.30	12.07	6.97	11.80
		-4.0	92.57	115.10	106.86	104.84	11.40	6.58	10.87
		-5.0	89.54	108.54	112.77	103.62	12.37	7.14	11.94
		-6.0	113.97	96.15	100.49	103.54	9.29	5.36	8.97
		-7.0	108.00	104.95	103.73	105.56	2.20	1.27	2.09
		-8.0	115.08	66.11	97.74	92.98	24.83	14.33	26.70
		-9.0	113.99	108.60	97.62	106.74	8.34	4.82	7.82
		-10.0	108.04	88.70	71.88	89.54	18.09	10.45	20.21
	2	-4.0	105.37	98.18	98.61	100.72	4.03	2.33	4.00
		-5.0	100.65	99.08	97.09	98.94	1.78	1.03	1.80
		-6.0	100.73	85.60	100.73	95.69	8.74	5.04	9.13
		-6.3	100.26	97.86	100.51	99.54	1.46	0.84	1.47
		-7.0	99.56	95.82	102.80	99.39	3.49	2.02	3.51
		-8.0	100.02	96.77	100.78	99.19	2.13	1.23	2.15
		-9.0	93.54	95.27	100.35	96.38	3.54	2.04	3.67
		-10.0	93.39	103.64	114.52	103.85	10.56	6.10	10.17
	3	-4.0	101.14	101.85	99.65	100.88	1.13	0.65	1.12
		-5.0	103.48	106.33	108.25	106.02	2.40	1.39	2.27
		-6.0	104.57	105.80	104.19	104.85	0.84	0.49	0.81
		-6.3	102.14	100.88	99.58	100.87	1.28	0.74	1.27
		-7.0	97.89	100.16	109.11	102.38	5.93	3.42	5.79
		-8.0	106.04	103.54	108.32	105.97	2.39	1.38	2.25
		-9.0	103.99	98.52	104.63	102.38	3.36	1.94	3.28
		-10.0	101.00	103.62	104.13	102.92	1.68	0.97	1.63

Concentrations with ***bold, italicized*** type were insoluble in the reaction mixture and data obtained are excluded from modeling.
 NS – No sample due to experimental mishap.

APPENDIX H

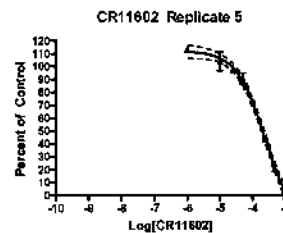
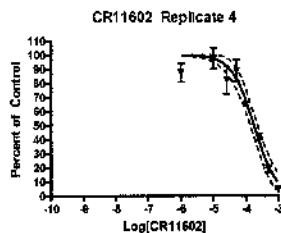
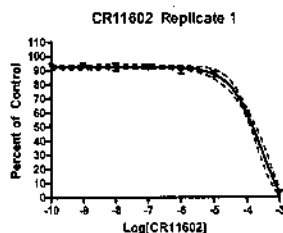
Concentration Response Curves Reference Chemical Assays

CR11601 Results



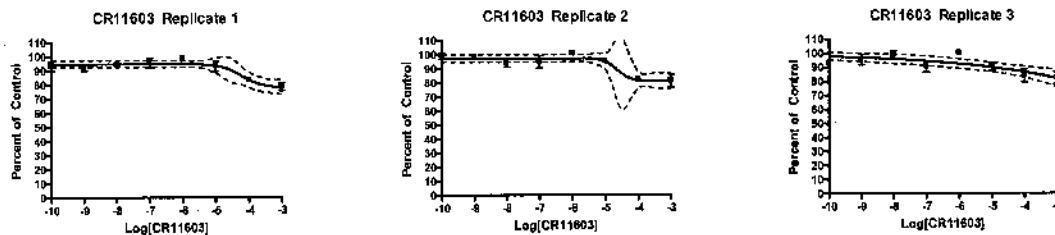
	CR11601-R1	CR11601-R2	CR11601-R3
Comparison of Fits		Does not converge.	Does not converge.
Null hypothesis	Equation 2		
Alternative hypothesis	Equation 1		
P value	0.6150		
Conclusion (alpha = 0.05)	Reject null hypothesis		
Preferred model	Equation 1		
F (DFn, DFd)	5.433 (2,17)		
Equation 1			
Best fit values			
BOTTOM	88.04		
TOP	95.95		
LOGEC50	-4.752		
HILLSLOPE	-1.688		
EC50	1.772e-005		
Std. Error			
BOTTOM	10.01		
TOP	0.5058		
LOGEC50	1.740		
HILLSLOPE	7.603		
Lower 95% conf. limit			
BOTTOM	66.93		
TOP	94.69		
LOGEC50	-8.424		
HILLSLOPE	-17.73		
EC50	3.771e-009		
Upper 95% conf. limit			
BOTTOM	109.2		
TOP	97.22		
LOGEC50	-1.080		
HILLSLOPE	14.35		
EC50	0.06327		
Goodness of Fit			
Degrees of Freedom	17		
R ²	0.6970		
Absolute Sum of Squares	71.38		
Syx	2.049		
Equation 2			
Best fit values			
BOTTOM	0.0		
TOP	100.0		
LOGEC50	4.929		
HILLSLOPE	-0.1089		
EC50	94841		
Std. Error			
LOGEC50	2.623		
HILLSLOPE	0.02722		
Lower 95% conf. limit			
LOGEC50	-0.5607		
HILLSLOPE	-0.1669		
EC50	0.2750		
Upper 95% conf. limit			
LOGEC50	10.42		
HILLSLOPE	-0.05194		
EC50	2.617e+010		
Goodness of Fit			
Degrees of Freedom	19		
R ²	0.4542		
Absolute Sum of Squares	117.0		
Syx	2.481		
Constraints			
BOTTOM	BOTTOM = 0.0		
TOP	TOP = 100.0		
Data			
Number of X values	9		
Number of Y replicates	3		
Total number of values	21		
Number of missing values	6		

CR11602 Results



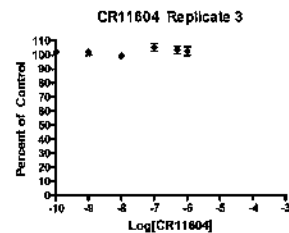
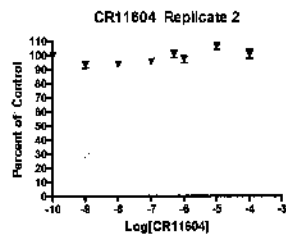
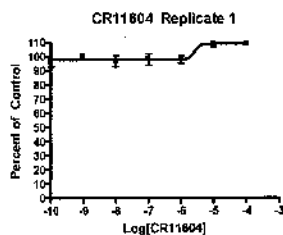
	CR11602-R1	CR11602-R2	CR11602-R3	CR11602-R4	CR11602-R5
Comparison of Fits					
Null hypothesis	Equation 2		Equation 2	Equation 2	
Alternative hypothesis	Equation 1		Equation 1	Equation 1	
P value	P<0.0001		0.1162	0.0003	
Conclusion (alpha = 0.05)	Reject null hypothesis		Do not reject null hypothesis	Reject null hypothesis	
Preferred model	Equation 1		Equation 2	Equation 1	
F (DFn, DFd)	70.71 (2,20)		2.351 (2,20)	12.25 (2,20)	
Equation 1					
Best-fit values					
BOTTOM	-22.92		-6.355		-23.66
TOP	92.37		91.92		112.1
LOGEC50	-3.576		-3.622		-3.604
HILLSLOPE	-0.8282		-1.418		-0.9766
EC50	0.0002650		0.0002390		0.0002491
Std. Error					
BOTTOM	16.63		15.50		13.79
TOP	0.7697		4.265		2.808
LOGEC50	0.1855		0.1303		0.1035
HILLSLOPE	0.1801		0.4367		0.1341
Lower 95% conf. limit					
BOTTOM	-58.03		-36.68		-52.64
TOP	90.72		83.04		106.2
LOGEC50	-3.962		-3.893		-3.819
HILLSLOPE	-1.302		-2.229		-1.285
EC50	0.0001091		0.0001278		0.0001515
Upper 95% conf. limit					
BOTTOM	12.19		25.87		4.909
TOP	94.02		100.8		117.9
LOGEC50	-3.188		-3.360		-3.388
HILLSLOPE	-0.5505		-0.5074		-0.8888
EC50	0.0008485		0.0004470		0.0004094
Goodness of Fit					
Degrees of Freedom	20		20		20
R ²	0.9922		0.6231		0.9856
Absolute Sum of Squares	164.8		2097		507.5
Syx	2.872		10.24		5.037
Equation 2					
Best-fit values					
BOTTOM	0.0		0.0		0.0
TOP	100.0		100.0		100.0
LOGEC50	-3.912		-3.759		-3.689
HILLSLOPE	-1.104		-1.280		-1.578
EC50	0.0001224		0.0001742		0.0002049
Std. Error					
LOGEC50	0.06684		0.06248		0.03136
HILLSLOPE	0.1876		0.1738		0.1531
Lower 95% conf. limit					
LOGEC50	-4.061		-3.866		-3.764
HILLSLOPE	-1.514		-1.840		-1.897
EC50	8.896e-005		0.0001358		0.0001764
Upper 95% conf. limit					
LOGEC50	-3.774		-3.650		-3.824
HILLSLOPE	-0.8942		-0.9191		-1.261
EC50	0.0001686		0.0002235		0.0002379
Goodness of Fit					
Degrees of Freedom	22		22		22
R ²	0.9373		0.9048		0.9680
Absolute Sum of Squares	1331		2596		1131
Syx	7.779		10.86		7.168
Constraints					
BOTTOM	BOTTOM = 0.0		BOTTOM = 0.0		BOTTOM = 0.0
TOP	TOP = 100.0		TOP = 100.0		TOP = 100.0
Data					
Number of X values	12		8		8
Number of Y replicates	3		3		3
Total number of values	24		24		24
Number of missing values	12		0		0

CR11603 Results



	CR11603-R1	CR11603-R2	CR11603-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	P < 0.0001	0.0376	0.1737
Conclusion (alpha = 0.05)	Reject null hypothesis	Reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 1	Equation 1	Equation 2
F (DFn, DFd)	23.32 (2,20)	3.883 (2,20)	1.913 (2,20)
Equation 1			
Best-fit values			
BOTTOM	77.73	81.05	82.98
TOP	94.89	97.31	96.42
LOGEC50	-4.234	-4.859	-4.961
HILLSLOPE	-1.387	-2.230	-6.315
EC50	5.834e-005	2.193e-005	1.045e-005
Std. Error			
BOTTOM	2.931	2.841	3.316
TOP	1.158	1.258	1.847
LOGEC50	0.2712	0.5537	4.8877
HILLSLOPE	1.099	3.267	2.117e+007
Lower 95% conf. limit			
BOTTOM	71.48	75.13	76.07
TOP	82.27	94.68	91.98
LOGEC50	-4.800	-5.814	-102000
HILLSLOPE	-3.678	-9.046	-44160000
EC50	1.558e-005	1.535e-005	
Upper 95% conf. limit			
BOTTOM	83.97	86.98	89.91
TOP	97.11	99.93	98.86
LOGEC50	-3.666	-3.504	101953
HILLSLOPE	0.9081	4.585	4.416e+007
EC50	0.0002147	0.0003134	
Goodness of Fit			
Degrees of Freedom	20	20	20
R ²	0.6969	0.7009	0.4672
Absolute Sum of Squares	388.1	472.5	762.5
Syx	4.405	4.860	6.174
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100.0	100.0	100.0
LOGEC50	-4.0790000	-0.4193	1.870
HILLSLOPE	2.483e-008	-0.2286	-0.1418
EC50	0.0	0.3608	46.74
Std. Error			
LOGEC50	6.267e+013	0.6910	1.953
HILLSLOPE	0.03789	0.05791	0.04469
Lower 95% conf. limit			
LOGEC50	-130000000000000	-2.267	-2.380
HILLSLOPE	-0.07867	-0.3487	-0.2345
EC50		0.005405	0.004164
Upper 95% conf. limit			
LOGEC50	1.300e+014	1.428	5.720
HILLSLOPE	0.07867	-0.1085	-0.04911
EC50		26.63	524741
Goodness of Fit			
Degrees of Freedom	22	22	22
R ²	-1.627e-007	0.5848	0.3652
Absolute Sum of Squares	1293	666.0	906.3
Syx	7.967	6.460	6.425
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100.0	TOP = 100.0	TOP = 100.0
Data			
Number of X values	8	8	8
Number of Y replicates	3	3	3
Total number of values	24	24	24
Number of missing values	0	0	0

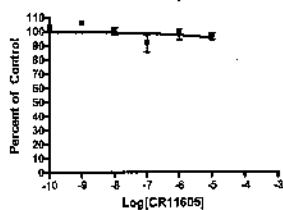
CR11604 Results



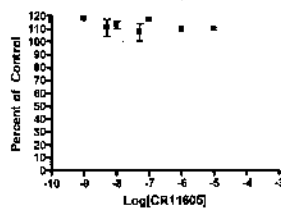
	CR11604-R1	CR11604-R2	CR11604-R3
Comparison of Fit	Equation 2	Does not converge.	Does not converge.
Null hypothesis	Equation 1		
Alternative hypothesis	Equation 1		
P value	0.0004		
Conclusion (alpha = 0.05)	Reject null hypothesis		
Predicted model	Equation 1		
F (DFn, DFd)	12.55 (2,17)		
Equation 1			
Best-fit values			
BOTTOM	97.73		
TOP	109.2		
LOGEC50	-5.591		
HILLSLOPE	4.929		
EC50	2.564e-008		
Std. Error			
BOTTOM	1.329		
TOP	2.658		
LOGEC50	7.478		
HILLSLOPE	84.05		
Lower 95% conf. limit			
BOTTOM	94.93		
TOP	103.6		
LOGEC50	-21.37		
HILLSLOPE	-172.4		
EC50	4.244e-022		
Upper 95% conf. limit			
BOTTOM	100.6		
TOP	114.8		
LOGEC50	10.19		
HILLSLOPE	182.3		
EC50	1.649e+010		
Goodness of Fit			
Degrees of Freedom	17		
R ²	0.6084		
Absolute Sum of Squares	380.4		
Syx	4.804		
Equation 2			
Best-fit values			
BOTTOM	0.0		
TOP	100.0		
LOGEC50	-13.51		
HILLSLOPE	0.3997		
EC50	3.090e-014		
Std. Error			
LOGEC50	8.115		
HILLSLOPE	0.8660		
Lower 95% conf. limit			
LOGEC50	-30.50		
HILLSLOPE	-1.411		
EC50	3.198e-031		
Upper 95% conf. limit			
LOGEC50	3.475		
HILLSLOPE	2.210		
EC50	2988		
Goodness of Fit			
Degrees of Freedom	19		
R ²	0.03250		
Absolute Sum of Squares	892.8		
Syx	8.854		
Constraints			
BOTTOM	BOTTOM = 0.0		
TOP	TOP = 100.0		
Data			
Number of X values	9		
Number of Y replicates	3		
Total number of values	27		
Number of missing values	6		

CR11605 Results

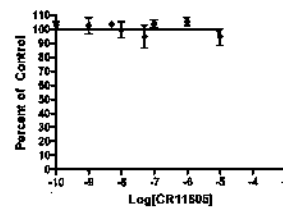
CR11605 Replicate 1



CR11605 Replicate 2

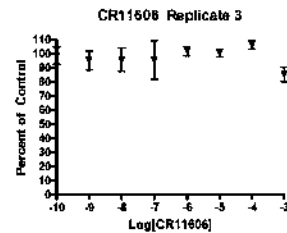
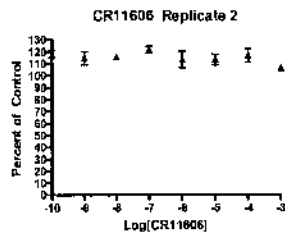
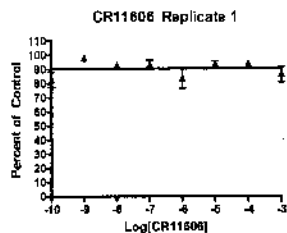


CR11605 Replicate 3



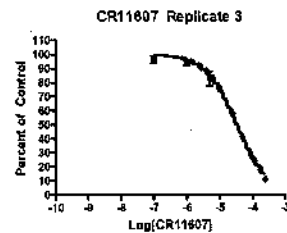
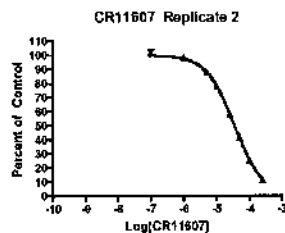
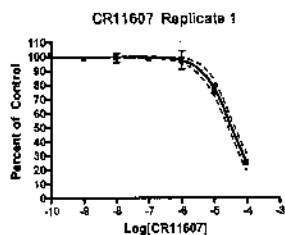
	CR11605-R1	CR11605-R2	CR11605-R3
Comparison of Fits		Does not converge	
Null hypothesis	Equation 2		Equation 2
Alternative hypothesis	Equation 1		Equation 1
P value	0.0879		Not Necessary
Conclusion (alpha = 0.05)	Do not reject null hypothesis		Other model didn't converge
Preferred model	Equation 2		Equation 2
F (Df1, Df2)	2.908 (2,14)		
Equation 1			Didn't Converge
Best-fit values			
BOTTOM	96.97		
TOP	103.9		
LOGEC50	-7.981		
HILLSLOPE	-8.661		
EC50	1.044e-008		
Std. Error			
BOTTOM	2.971		
TOP	2.599		
LOGEC50	123448		
HILLSLOPE	5.566e+007		
Lower 95% conf. limit			
BOTTOM	89.99		
TOP	98.32		
LOGEC50	-284800		
HILLSLOPE	-121900000		
EC50			
Upper 95% conf. limit			
BOTTOM	100.2		
TOP	109.5		
LOGEC50	264784		
HILLSLOPE	1.215e+008		
EC50			
Goodness of Fit			
Degrees of Freedom	14		
R ²	0.3970		
Absolute Sum of Squares	486.0		
Syx	5.952		
Equation 2			
Best-fit values			
BOTTOM	0.0		0.0
TOP	100.0		100.0
LOGEC50	0.5212		-4.835
HILLSLOPE	-0.2396		-7.238
EC50	3.321		1.405e-005
Std. Error			
LOGEC50	8.658		122537
HILLSLOPE	0.3328		5.359e+005
Lower 95% conf. limit			
LOGEC50	-17.83		-264100
HILLSLOPE	-0.9411		-11120000
EC50	1.489e-013		
Upper 95% conf. limit			
LOGEC50	15.88		294138
HILLSLOPE	0.4899		1.112e+007
EC50	7.503e+015		
Goodness of Fit			
Degrees of Freedom	18		22
R ²	0.1041		0.06527
Absolute Sum of Squares	702.1		1408
Syx	8.624		7.999
Constraints			
BOTTOM	BOTTOM = 0.0		BOTTOM = 0.0
TOP	TOP = 100.0		TOP = 100.0
Data			
Number of X values	10		10
Number of Y replicates	3		3
Total number of values	18		24
Number of missing values	12		6

CR11606 Results



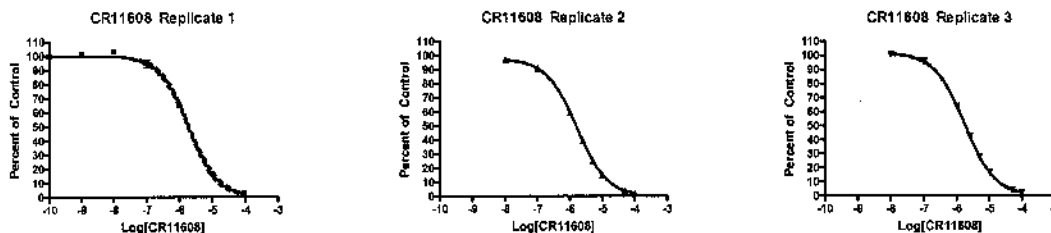
	CR11606-R1	CR11606-R2	CR11606-R3
Comparison of fits		Does not converge.	Does not converge.
Null hypothesis	Equation 2		
Alternative hypothesis	Equation 1		
P value	Not Necessary		
Conclusion (alpha = 0.05)	Other model didn't converge		
Preferred model	Equation 2		
F (Df1, Df2)			
Equation 1	Didn't Converge		
Best-fit values			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Std. Error			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
Lower 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Upper 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Goodness of Fit			
Degrees of Freedom			
R ²			
Absolute Sum of Squares			
Syx			
Equation 2			
Best-fit values			
BOTTOM	0.0		
TOP	100.0		
LOGEC50	-649.6		
HILLSLOPE	0.001800		
EC50	0.0		
Std. Error			
LOGEC50	10039		
HILLSLOPE	0.03627		
Lower 95% conf. limit			
LOGEC50	-23240		
HILLSLOPE	-0.07341		
EC50			
Upper 95% conf. limit			
LOGEC50	22139		
HILLSLOPE	0.07731		
EC50			
Goodness of Fit			
Degrees of Freedom	22		
R ²	9.438e-005		
Absolute Sum of Squares	1435		
Syx	8.077		
Constraints			
BOTTOM	BOTTOM = 0.0		
TOP	TOP = 100.0		
Data			
Number of X values	8		
Number of Y replicates	3		
Total number of values	24		
Number of missing values	0		

CR11607 Results



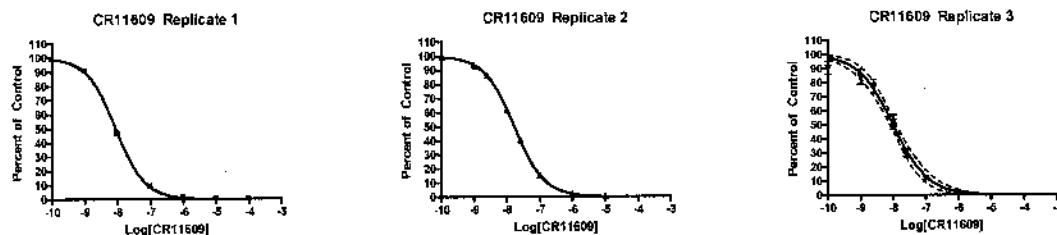
	CR11607-R1	CR11607-R2	CR11607-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.7977	0.1090	0.0584
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 2
F (DfN, DfD)	0.2381 (2,17)	2.628 (2,20)	3.331 (2,20)
Equation 1			
Best-fit values			
BOTTOM	14.85	-3.973	-6.247
TOP	98.94	101.8	95.75
LOGEC50	-4.686	-4.438	-4.370
HILLSLOPE	-1.342	-0.9307	-0.9122
EC50	2.151e-005	3.645e-005	4.269e-005
Std. Error			
BOTTOM	30.09	2.940	7.333
TOP	1.512	0.8584	1.637
LOGEC50	0.4426	0.03116	0.07910
HILLSLOPE	1.147	0.04608	0.1054
Lower 95% conf. limit			
BOTTOM	-46.65	-10.13	-21.54
TOP	95.75	100.0	92.92
LOGEC50	-6.599	-4.603	-4.536
HILLSLOPE	-3.763	-1.027	-1.132
EC50	2.519e-006	3.134e-005	2.920e-005
Upper 95% conf. limit			
BOTTOM	78.35	2.179	9.061
TOP	102.1	103.8	100.6
LOGEC50	-3.732	-4.373	-4.205
HILLSLOPE	1.079	-0.8346	-0.6923
EC50	0.0001855	4.233e-005	6.242e-005
Goodness of Fit			
Degrees of Freedom	17	20	20
R ²	0.9683	0.9978	0.9886
Absolute Sum of Squares	443.6	54.01	246.9
Syx	5.108	1.843	3.514
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100.0	100.0	100.0
LOGEC50	-4.484	-4.461	-4.482
HILLSLOPE	-1.015	-1.014	-0.9194
EC50	3.278e-005	3.457e-005	3.293e-005
Std. Error			
LOGEC50	0.04702	0.009548	0.02234
HILLSLOPE	0.09129	0.02289	0.04534
Lower 95% conf. limit			
LOGEC50	-4.583	-4.461	-4.529
HILLSLOPE	-1.206	-1.061	-1.043
EC50	2.614e-005	3.503e-005	2.950e-005
Upper 95% conf. limit			
LOGEC50	-4.396	-4.461	-4.436
HILLSLOPE	-0.8242	-0.9661	-0.8283
EC50	4.112e-005	3.518e-005	3.994e-005
Goodness of Fit			
Degrees of Freedom	19	22	22
R ²	0.9685	0.9972	0.9946
Absolute Sum of Squares	455.5	67.66	329.1
Syx	4.886	1.754	3.868
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100.0	TOP = 100.0	TOP = 100.0
Data			
Number of X values	12	9	9
Number of Y replicates	3	3	3
Total number of values	21	24	24
Number of missing values	15	3	3

CR11608 Results



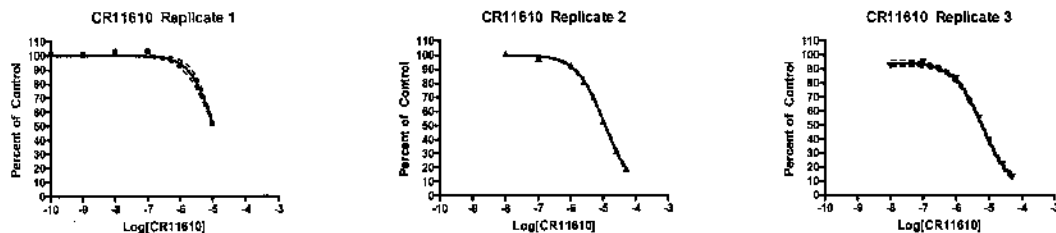
	CR11608-R1	CR11608-R2	CR11608-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.4240	P=0.0001	0.0033
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Reject null hypothesis	Reject null hypothesis
Preferred model	Equation 2	Equation 1	Equation 1
F (DFn, DFd)	0.9029 (2,17)	22.47 (2,20)	7.884 (2,20)
Equation 1			
Best-fit values			
BOTTOM	-0.06823	-0.2917	0.002184
TOP	101.0	97.55	101.5
LOGEC50	-6.740	-5.791	-5.757
HILLSLOPE	-0.9571	-0.9236	-0.8381
EC50	1.819e-008	1.618e-008	1.752e-008
Std. Error			
BOTTOM	1.798	0.4058	0.5301
TOP	0.7803	0.3968	0.4998
LOGEC50	0.03409	0.007580	0.009421
HILLSLOPE	0.08154	0.01680	0.02095
Lower 95% conf. limit			
BOTTOM	-3.892	-1.144	-1.104
TOP	99.38	95.72	100.8
LOGEC50	-5.612	-5.807	-5.776
HILLSLOPE	-1.088	-0.9588	-0.9829
EC50	1.542e-008	1.580e-008	1.677e-008
Upper 95% conf. limit			
BOTTOM	3.696	0.5610	1.108
TOP	102.7	99.38	102.8
LOGEC50	-5.558	-5.775	-5.738
HILLSLOPE	-0.8257	-0.8888	-0.8954
EC50	2.146e-008	1.678e-008	1.830e-008
Goodness of Fit			
Degrees of Freedom	17	20	20
R ²	0.9971	0.9987	0.9995
Absolute Sum of Squares	96.96	8.484	15.85
Syx	2.388	0.6866	0.6909
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100.0	100.0	100.0
LOGEC50	-5.728	-5.829	-5.734
HILLSLOPE	-0.9801	-0.8581	-0.9601
EC50	1.899e-008	1.482e-008	1.844e-008
Std. Error			
LOGEC50	0.02254	0.008924	0.007379
HILLSLOPE	0.04330	0.01568	0.01688
Lower 95% conf. limit			
LOGEC50	-5.775	-5.847	-5.748
HILLSLOPE	-1.071	-0.9190	-1.004
EC50	1.677e-008	1.422e-008	1.781e-008
Upper 95% conf. limit			
LOGEC50	-5.681	-5.811	-5.719
HILLSLOPE	-0.8895	-0.8532	-0.9338
EC50	2.034e-008	1.544e-008	1.911e-008
Goodness of Fit			
Degrees of Freedom	19	22	22
R ²	0.9988	0.9989	0.9991
Absolute Sum of Squares	107.3	30.80	28.08
Syx	2.376	1.182	1.129
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100.0	TOP = 100.0	TOP = 100.0
Data			
Number of X values	11	9	9
Number of Y replicates	3	3	3
Total number of values	21	24	24
Number of missing values	12	3	3

CR11609 Results



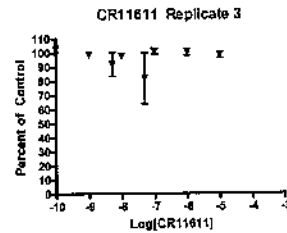
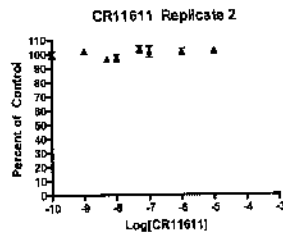
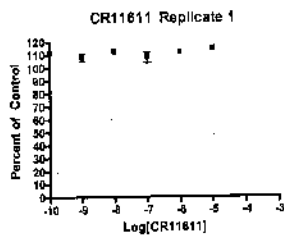
	CR11609-R1	CR11609-R2	CR11609-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.2510	0.6059	0.0688
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 2
F (Dfn, Dfd)	1.518 (2,15)	0.5138 (2,20)	3.050 (2,20)
Equation 1			
Best-fit values			
BOTTOM	0.2596	-0.03275	0.2208
TOP	100.4	99.51	91.06
LOGEC50	-8.054	-7.776	-7.882
HILLSLOPE	-0.9940	-0.9668	-1.017
EC50	8.634e-009	1.673e-008	1.312e-008
Std. Error			
BOTTOM	0.2045	0.4121	2.553
TOP	0.4607	0.4980	3.299
LOGEC50	0.007813	0.008387	0.08455
HILLSLOPE	0.01893	0.01967	0.1445
Lower 95% conf. limit			
BOTTOM	-0.1763	-0.8923	-5.054
TOP	99.43	98.47	84.19
LOGEC50	-8.080	-7.795	-8.017
HILLSLOPE	-1.034	-1.008	-1.318
EC50	9.305e-009	1.600e-008	9.625e-009
Upper 95% conf. limit			
BOTTOM	0.8954	0.9268	5.505
TOP	101.4	100.5	97.94
LOGEC50	-8.047	-7.757	-7.747
HILLSLOPE	-0.9538	-0.9248	-0.7153
EC50	8.971e-009	1.751e-008	1.789e-008
Goodness of Fit			
Degrees of Freedom	15	20	20
R ²	0.9998	0.9995	0.9771
Absolute Sum of Squares	5.189	18.88	701.2
Syx	0.5882	0.9128	5.921
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100.0	100.0	100.0
LOGEC50	-8.056	-7.783	-8.005
HILLSLOPE	-0.9647	-0.9566	-0.8179
EC50	8.738e-009	1.648e-008	9.875e-009
Std. Error			
LOGEC50	0.005684	0.006052	0.04779
HILLSLOPE	0.01388	0.01265	0.07221
Lower 95% conf. limit			
LOGEC50	-8.068	-7.795	-8.105
HILLSLOPE	-1.024	-0.9318	-0.9876
EC50	8.550e-009	1.602e-008	7.850e-009
Upper 95% conf. limit			
LOGEC50	-8.044	-7.770	-7.806
HILLSLOPE	-0.9655	-0.9254	-0.6681
EC50	9.035e-009	1.697e-008	1.241e-008
Goodness of Fit			
Degrees of Freedom	17	22	22
R ²	0.9995	0.9995	0.9701
Absolute Sum of Squares	6.239	17.52	915.1
Syx	0.6065	0.8924	6.450
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100.0	TOP = 100.0	TOP = 100.0
Data			
Number of X values	10	10	10
Number of Y replicates	3	3	3
Total number of values	19	24	24
Number of missing values	11	6	5

CR11610 Results



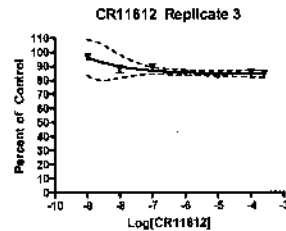
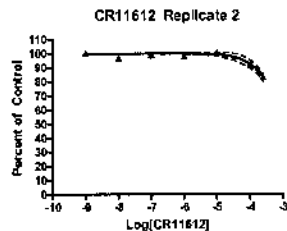
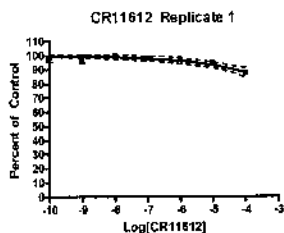
	CR11610-R1	CR11610-R2	CR11610-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.0642	0.9886	0.0001
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 1
F (DFn, DFd)	3.383 (2,14)	0.01148 (2,20)	14.51 (2,20)
Equation 1			
Best-fit values			
BOTTOM	50.95	0.3750	2.478
TOP	101.3	98.58	94.31
LOGEC50	-5.793	-4.948	-5.188
HILLSLOPE	-3.274	-0.9888	-0.9968
EC50	1.647e-006	1.126e-006	6.488e-008
Std. Error			
BOTTOM	37.23	4.516	3.488
TOP	0.7078	0.5278	1.058
LOGEC50	7.209	0.04832	0.04250
HILLSLOPE	103.6	0.06673	0.07645
Lower 95% conf. limit			
BOTTOM	-25.91	-9.254	-4.788
TOP	99.83	98.15	92.10
LOGEC50	-21.26	-5.051	-5.277
HILLSLOPE	-225.5	-1.128	-1.156
EC50	5.652e-022	8.866e-006	5.200e-006
Upper 95% conf. limit			
BOTTOM	130.5	10.00	9.754
TOP	102.9	101.5	95.52
LOGEC50	9.650	-4.845	-5.099
HILLSLOPE	219.0	-0.8495	-0.6373
EC50	4.791e+009	1.428e-006	7.857e-006
Goodness of Fit			
Degrees of Freedom	14	20	20
R ²	0.9597	0.9985	0.9949
Absolute Sum of Squares	63.07	70.52	105.6
Syx	2.123	1.876	2.309
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100.0	100.0	100.0
LOGEC50	-4.894	-4.948	-5.239
HILLSLOPE	-1.148	-0.9821	-0.9940
EC50	1.036e-005	1.133e-006	5.899e-006
Std. Error			
LOGEC50	0.02149	0.01043	0.02079
HILLSLOPE	0.1027	0.02318	0.03914
Lower 95% conf. limit			
LOGEC50	-5.030	-4.987	-5.272
HILLSLOPE	-1.366	-1.030	-0.9452
EC50	9.332e-008	1.080e-005	5.341e-006
Upper 95% conf. limit			
LOGEC50	-4.939	-4.925	-5.166
HILLSLOPE	-0.9300	-0.9340	-0.7828
EC50	1.151e-005	1.190e-005	6.514e-008
Goodness of Fit			
Degrees of Freedom	16	22	22
R ²	0.9848	0.9985	0.9975
Absolute Sum of Squares	93.37	70.50	261.3
Syx	2.416	1.791	3.447
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100.0	TOP = 100.0	TOP = 100.0
Data			
Number of X values	12	10	10
Number of Y replicates	3	3	3
Total number of values	18	24	24
Number of missing values	18	6	6

CR11611 Results



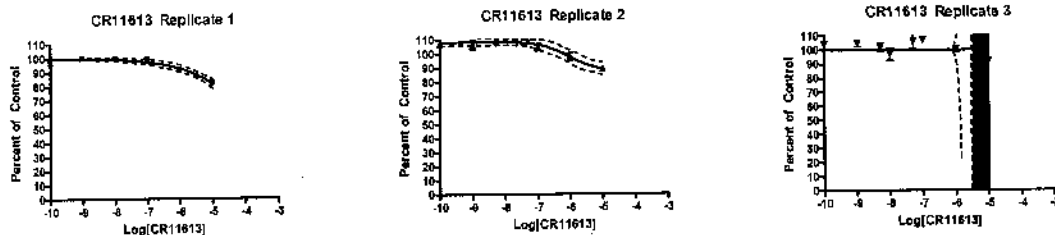
	CR11611-R1	CR11611-R2	CR11611-R3
Comparison of Fits	Does not converge.	Does not converge.	Does not converge.
Null hypothesis			
Alternative hypothesis			
P value			
Conclusion (alpha = 0.05)			
Preferred model			
F (DFn, DFd)			
Equation 1			
Best-fit values			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Std. Error			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
Lower 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Upper 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Goodness of fit			
Degrees of Freedom			
R ²			
Absolute Sum of Squares			
Syx			
Equation 2			
Best-fit values			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Std. Error			
LOGEC50			
HILLSLOPE			
Lower 95% conf. limit			
LOGEC50			
HILLSLOPE			
EC50			
Upper 95% conf. limit			
LOGEC50			
HILLSLOPE			
EC50			
Goodness of fit			
Degrees of Freedom			
R ²			
Absolute Sum of Squares			
Syx			
Constraints			
BOTTOM			
TOP			
Data			
Number of X values			
Number of Y replicates			
Total number of values			
Number of missing values			

CR11612 Results



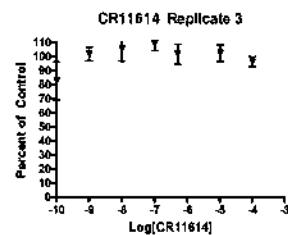
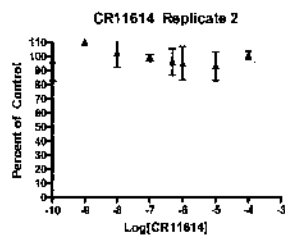
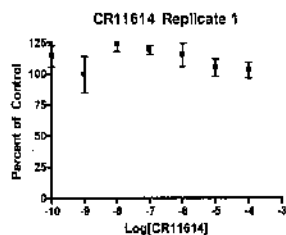
	CR11612-R1	CR11612-R2	CR11612-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.2198	0.1320	0.0172
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Do not reject null hypothesis	Reject null hypothesis
Preferred model	Equation 2	Equation 2	Equation 1
F (Dfa, Dfb)	1.658 (2,17)	2.287 (2,17)	5.212 (2,17)
Equation 1			
Best-fit values			
BOTTOM	75.41	81.98	84.08
TOP	97.81	98.72	109.7
LOGEC50	-3.858	-3.958	-11.52
HILLSLOPE	-0.6602	-3.491	-0.3524
EC50	0.0001102	0.0001107	3.021e-012
Std. Error			
BOTTOM	92.51	160.2	2.047
TOP	1.050	0.7239	2281
LOGEC50	5.208	4.215	35.04
HILLSLOPE	1.190	160.5	0.6796
Lower 95% conf. limit			
BOTTOM	-119.8	-268.0	79.77
TOP	95.60	97.20	-4590
LOGEC50	-14.95	-12.84	-85.45
HILLSLOPE	-3.172	-342.4	-1.788
EC50	1.133e-015	1.431e-013	
Upper 95% conf. limit			
BOTTOM	270.6	420.0	88.41
TOP	100.0	100.3	4960
LOGEC50	7.031	4.933	52.41
HILLSLOPE	1.652	335.4	1.082
EC50	1.073e+007	86649	
Goodness of Fit			
Degrees of Freedom	17	17	17
R ²	0.5545	0.8629	0.6735
Absolute Sum of Squares	153.5	105.9	167.9
Syx	3.006	2.507	3.142
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100.0	100.0	100.0
LOGEC50	-0.7823	-2.906	6.274
HILLSLOPE	-0.2648	-0.8923	-0.08876
EC50	0.1651	0.001241	1.881e+006
Std. Error			
LOGEC50	0.9348	0.1936	3.186
HILLSLOPE	0.08761	0.2383	0.01871
Lower 95% conf. limit			
LOGEC50	-2.844	-3.311	-0.3638
HILLSLOPE	-0.4081	-1.481	-0.1079
EC50	0.001433	0.0004881	0.4040
Upper 95% conf. limit			
LOGEC50	1.279	-2.501	12.94
HILLSLOPE	-0.1235	-0.4835	-0.02961
EC50	19.02	0.003155	8.765e+012
Goodness of Fit			
Degrees of Freedom	19	19	19
R ²	0.5872	0.8260	0.4733
Absolute Sum of Squares	183.4	135.6	270.8
Syx	3.107	2.671	3.775
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100.0	TOP = 100.0	TOP = 100.0
Data			
Number of X values	10	9	9
Number of Y replicates	3	3	3
Total number of values	21	21	21
Number of missing values	9	6	6

CR11613 Results



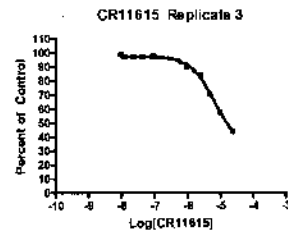
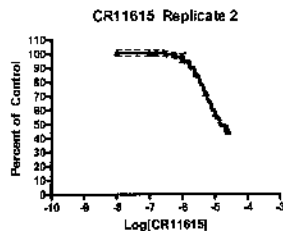
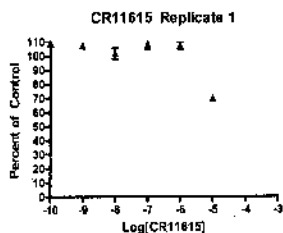
	CR11613-R1	CR11613-R2	CR11613-R3
Comparison of Fits			
Null hypothesis	Equation 2	Equation 2	Equation 2
Alternative hypothesis	Equation 1	Equation 1	Equation 1
P value	0.2151	P<0.0001	0.1205
Conclusion (alpha = 0.05)	Do not reject null hypothesis	Reject null hypothesis	Do not reject null hypothesis
Preferred model	Equation 2	Equation 1	Equation 2
F (Dfn, Dfd)	1.718 (2,14)	37.08 (2,20)	2.360 (2,19)
Equation 1			
Best-fit values			
BOTTOM	81.59	87.51	81.73
TOP	95.04	108.4	103.0
LOGEC50	-5.972	-6.049	-5.933
HILLSLOPE	-2.104	-1.014	-7.743
EC50	1.067e-006	8.836e-007	1.167e-008
Std. Error			
BOTTOM	2.948	4.820	47.76
TOP	0.8839	1.267	1.493
LOGEC50	0.1659	0.3148	268053
HILLSLOPE	6.808	0.6987	2.930e+007
Lower 95% conf. limit			
BOTTOM	75.48	77.46	-8.226
TOP	97.05	105.7	99.82
LOGEC50	-6.306	-6.705	-533800
HILLSLOPE	-16.27	-2.258	-61330000
EC50	4.942e-007	1.570e-007	
Upper 95% conf. limit			
BOTTOM	87.70	97.57	191.7
TOP	100.5	111.0	106.2
LOGEC50	-5.637	-5.392	533883
HILLSLOPE	12.06	0.2310	6.133e+007
EC50	2.304e-006	4.053e-006	
Goodness of Fit			
Degrees of Freedom	14	20	19
R ²	0.8865	0.7792	0.3413
Absolute Sum of Squares	97.39	301.3	653.0
Syx	2.638	3.891	5.776
Equation 2			
Best-fit values			
BOTTOM	0.0	0.0	0.0
TOP	100.0	100.0	100.0
LOGEC50	-0.537	-4.215	-4.847
HILLSLOPE	-0.4385	-1.158	-6.860
EC50	0.0002805	6.092e-005	1.421e-005
Std. Error			
LOGEC50	0.3164	1.830	26478
HILLSLOPE	0.07767	2.368	1.165e+006
Lower 95% conf. limit			
LOGEC50	-4.208	-7.595	-59080
HILLSLOPE	-0.8032	-6.089	-2488000
EC50	6.199e-005	2.541e-008	
Upper 95% conf. limit			
LOGEC50	-2.866	-0.8355	55069
HILLSLOPE	-0.2739	3.763	2.466e+006
EC50	0.001361	0.1461	
Goodness of Fit			
Degrees of Freedom	16	22	21
R ²	0.8586	0.03852	0.1771
Absolute Sum of Squares	121.3	1415	792.0
Syx	2.753	8.030	6.141
Constraints			
BOTTOM	BOTTOM = 0.0	BOTTOM = 0.0	BOTTOM = 0.0
TOP	TOP = 100.0	TOP = 100.0	TOP = 100.0
Data			
Number of X values	10	10	10
Number of Y replicates	3	3	3
Total number of values	18	24	23
Number of missing values	12	6	7

CR11614 Results



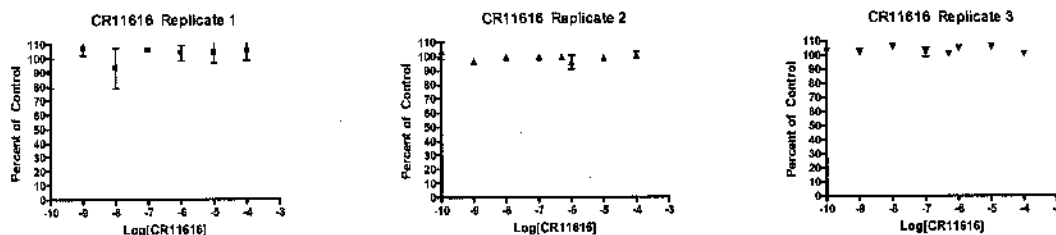
	CR11614.R1	CR11614.R2	CR11614.R3
Comparison of Fit	Does not converge.	Does not converge.	Does not converge.
Null hypothesis			
Alternative hypothesis			
P value			
Conclusion (alpha = 0.05)			
Preferred model			
F (DFn, DFd)			
Equation 1			
Best-fit values			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Std. Error			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
Lower 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Upper 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Goodness of Fit			
Degrees of Freedom			
R ²			
Absolute Sum of Squares			
Syx			
Equation 2			
Best-fit values			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Std. Error			
LOGEC50			
HILLSLOPE			
Lower 95% conf. limit			
LOGEC50			
HILLSLOPE			
EC50			
Upper 95% conf. limit			
LOGEC50			
HILLSLOPE			
EC50			
Goodness of Fit			
Degrees of Freedom			
R ²			
Absolute Sum of Squares			
Syx			
Constraints			
BOTTOM			
TOP			
Data			
Number of X values			
Number of Y replicates			
Total number of values			
Number of missing values			

CR11615 Results



	CR11615-R1	CR11615-R2	CR11615-R3
Comparison of Fits	Does not converge.	Equation 2	Equation 2
Null hypothesis		Equation 1	Equation 1
Alternative hypothesis		0.0004	0.0002
P value		Reject null hypothesis	Reject null hypothesis
Conclusion (alpha = 0.05)		Equation 1	Equation 1
Preferred model		12.95 (2,17)	15.17 (2,17)
F (DFn, Dfd)			
Equation 1			
Best-fit values			
BOTTOM		39.26	31.56
TOP		100.0	97.44
LOGEC50		-5.285	-5.171
HILLSLOPE		-1.498	-1.161
EC50		5.431e-008	6.739e-008
Std. Error			
BOTTOM		3.432	2.692
TOP		1.081	0.5429
LOGEC50		0.04847	0.04600
HILLSLOPE		0.1842	0.07892
Lower 95% conf. limit			
BOTTOM		32.02	25.48
TOP		98.61	96.39
LOGEC50		-5.381	-5.250
HILLSLOPE		-1.886	-1.327
EC50		4.355e-008	5.546e-008
Upper 95% conf. limit			
BOTTOM		48.50	37.70
TOP		103.2	98.59
LOGEC50		-5.169	-5.087
HILLSLOPE		-1.108	-0.9647
EC50		5.774e-008	8.184e-008
Goodness of Fit			
Degrees of Freedom		17	17
R ²		0.9865	0.9865
Absolute Sum of Squares		122.7	26.56
Syx		2.895	1.250
Equation 2			
Best-fit values			
BOTTOM		0.0	0.0
TOP		100.0	100.0
LOGEC50		-4.776	-4.768
HILLSLOPE		-0.8813	-0.7552
EC50		1.676e-008	1.631e-008
Std. Error			
LOGEC50		0.03280	0.01811
HILLSLOPE		0.08408	0.02751
Lower 95% conf. limit			
LOGEC50		-4.844	-4.825
HILLSLOPE		-1.017	-0.8128
EC50		1.431e-008	1.495e-008
Upper 95% conf. limit			
LOGEC50		-4.707	-4.750
HILLSLOPE		-0.7459	-0.6678
EC50		1.863e-008	1.783e-008
Goodness of Fit			
Degrees of Freedom		19	19
R ²		0.9861	0.9906
Absolute Sum of Squares		309.6	73.98
Syx		4.036	1.573
Constraints			
BOTTOM		BOTTOM = 0.0	BOTTOM = 0.0
TOP		TOP = 100.0	TOP = 100.0
Data			
Number of X values		10	10
Number of Y replicates		3	3
Total number of values		21	21
Number of missing values		0	0

CR11616 Results



	CR11616-R1	CR11616-R2	CR11616-R3
Comparison of Fits	Does not converge.	Does not converge.	Does not converge.
Null hypothesis			
Alternative hypothesis			
P value			
Conclusion (alpha = 0.05)			
Preferred model			
F (DfA, DfB)			
Equation 1			
Best-fit values			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Std. Error			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
Lower 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Upper 95% conf. limit			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Goodness of Fit			
Degrees of Freedom			
R ²			
Absolute Sum of Squares			
Syx			
Equation 2			
Best-fit values			
BOTTOM			
TOP			
LOGEC50			
HILLSLOPE			
EC50			
Std. Error			
LOGEC50			
HILLSLOPE			
Lower 95% conf. limit			
LOGEC50			
HILLSLOPE			
EC50			
Upper 95% conf. limit			
LOGEC50			
HILLSLOPE			
EC50			
Goodness of Fit			
Degrees of Freedom			
R ²			
Absolute Sum of Squares			
Syx			
Constraints			
BOTTOM			
TOP			
Data			
Number of X values			
Number of Y replicates			
Total number of values			
Number of missing values			