

Appendix 7

Final report from the second interlaboratory validation study

Estrogen Receptor Binding Assay using
Rat Uterine Cytosol
(ER-RUC assay)

Lab Y

Note: The analyses, summary, and conclusions in this report were prepared by the individual laboratory. For reasons described in the Integrated Summary Report (ISR) for the ER-RUC assay, data were normalized in a different way for the final analysis that is presented in the ISR. Thus the analyses, summary, and conclusions in this report may differ from those in the ISR.

***In Vitro* Estrogen Receptor Binding Assays**
Task Order 6
Task 7

Sub Contract No.: 5-340-0210114 (Mod. 1)

Final Report

Submitted to:

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***In Vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Uterine Cytosol**

Task Order 6 Task 7

Final Report

February 6, 2008 through July 10, 2008

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FOREWORD

This final report is submitted in compliance with the requirement of the subcontract # 5-340-0210114 (Modification # 1) and summarizes the work performed from 6 February 2008 through 10 July 2008 by The Hamner Institutes for Health Sciences as subcontractor to RTI International, Research Triangle Park, NC 27709. The information contained herein is submitted with the understanding that it is privileged or confidential in accordance with the Freedom of Information Act, 5 U.S.C., Section 552(b)(4). All of the assay procedures for ***In Vitro* Estrogen Receptor Binding Assays** were performed in accordance with the EPA Protocol for the ***In Vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays using Rat Uterine Cytosol** by the Hamner Institutes for Health Sciences.

SUMMARY

For the ER Binding Project under Task 7, we have performed 4 saturation binding assays with 3 cytosol preparations and over 170 competitive binding assays to obtain acceptable data. The protein value of cytosol preparation (Prep)-3, 4, and 5 from commercially obtained uteri was 8.09, 6.67, and 2.5 mg/ml, respectively. The saturation binding assay for cytosol prep-3 with 50 µg of protein, showed a K_d value of 0.061 nM which was within the range of the protocol criteria for K_d measurement (0.05 nM to 0.5 nM) and a linear Scatchard plot. The B_{max} value was 17.43 fmol ER/100 µg protein. The saturation binding assay for cytosol prep-4 with 50 µg of protein had a K_d value of 0.138 nM which was within the range of the protocol criteria for K_d measurement and a linear Scatchard plot. The B_{max} value was 15.17 fmol ER/100 µg protein. The saturation binding assay for cytosol prep-5 with 50 µg of protein, showed a K_d value of 0.235 nM which was within the range of the protocol criteria for K_d measurement and a linear Scatchard plot. The B_{max} value was 36.46 fmol ER/100 µg protein. The binding curves of specific [3 H]-17β-estradiol binding versus radioligand concentration for the saturation binding experiments of all the cytosol preparations reached a plateau for maximum specific binding indicative of saturation of the ER with the radioligand.

The competitive binding assays showed reproducible standard curves for estradiol, the weak positive, norethynodrel, and the negative control, R1881. The IC_{50} values for 17β-estradiol were reproducible for each experiment and within the expected values. A representative value for average IC_{50} value for 17β-estradiol (from 20 competitive binding assays), norethynodrel (from 19 competitive binding assays), and R1881 (from 10 competitive binding assays) was $-8.99 \pm 4.43E-02$, $-6.06 \pm 5.16E-02$, and $-4.77 \pm 1.7E-01$, respectively. Norethynodrel showed a low, but positive, relative binding affinity ($1.30E-03 \pm 9.59E-05$) when compared to 17β-estradiol.

Based on the criteria for determining the classification of a chemical from the Hill slope data of approximately -1.0, the test chemicals were determined to be positive, negative, or equivocal. There were 15 chemicals which were found to be positive. The positive chemicals were: code # 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 15, 17, and 23. There were 8 chemicals that were negative. The negative chemicals were: code # 8, 14, 16, 18, 19, 20, 21, and 22.

For the 20 optional chemicals, one within criteria run was performed for each of them. Based on the single run with the above classification criteria, 9 chemicals were classified as negative and 11 were positive. The negative chemicals were: code # 27, 28, 30, 31, 35, 36, 38, 45, and 46. The positive chemicals were: code # 29, 32, 33, 34, 37, 39, 40, 41, 42, 43, and 44.

SECTION I: BACKGROUND

The Food Quality Protection Act of 1996 requires that the Environmental Protection Agency (EPA) develop and implement a screening program using validated test systems to determine the potential estrogenic effects from pesticides in humans. One of the test systems being considered for inclusion in the screening program is an estrogen receptor (ER) binding assay. The ER binding assay protocol in this Task Order 6 uses rat uterine cytosol (RUC) as source of receptor. The purpose of Task 7 is to test a battery of chemicals and classify them as positive, negative, or equivocal in binding relative to 17 β - estradiol using the ER competitive binding assay. The Hamner was selected as one of the qualified laboratories to conduct Task 7. This report is a compilation of 23 chemicals with an additional 20 optional chemicals tested and submitted to RTI as a part of the subcontract requirement.

SECTION II: MATERIALS AND METHODS

II.1 Materials

- II.1.1 [³H]-17 β -estradiol, 2,4,6,7,16,17-³H-17 β -estradiol was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA). Catalog#: NET 51700, Batch#: 3589221 and 3589791, Specific Activity (SA): 110 Ci/mmol, Date SA certified: 8/8/07 and 3/10/08, Concentration: 1 mCi/ml. Both were stored at 4°C in their original containers as per the protocol. **Note: Batch#: 3589791 was used in all runs using cytosol prep-3. In mid-June, a sample of batch#: 3589791 was obtained from RTI which had been stored at -20°C in its original container. This batch of [³H]-17 β -estradiol was used in saturation assay of 061608 (cytosol prep-4 and 5) and all competitive binding assays using cytosol prep-5. For recording purposes, it was designated as 3589791-RTI. Batch#: 3589221 was not used in any run.**
- II.1.2 17 β -estradiol was purchased from SIGMA (St. Louis, MO). Catalog#: E8875, Lot#: 026K1806, CAS#: 50-28-2, 99% purity.
- II.1.3 Norethynodrel was purchased from US Pharmacopeial Convention, Inc. (Rockville, MD). Catalog#: 1471007, Lot#: G, CAS#: 68-23-5, 100% purity.
- II.1.4 Unknown test chemicals were provided by EPA via Battelle chemical repository and stored at ambient or appropriate temperature.

II.2 Methods

Detailed methodology can be found in The Hamner Protocol based on the original EPA protocol on “***In Vitro* ER Receptor Binding Assay**” included as Appendix A.

II.2.1 Ovariectomy and Uteri Collection

Ovariectomy and uteri collection was done at Harlan Sprague-Dawley, Inc. (Indianapolis, IN). Cytosol preparations were prepared from uteri collected from ovariectomized Sprague-Dawley rats that were 88–93 days old at time of surgery. Uteri were removed 8 days after ovariectomy, blotted weights were recorded, and the tissues were flash frozen.

II.2.2 Preparation of Cytosol

Cytosol preparation done on 2/29/08 was designated as cytosol prep-3. Cytosol preparation done on 4/20/08 was designated as cytosol prep-4. Cytosol preparation done on 6/12/08 was designated as cytosol prep-5. Uteri were placed in ice-cold TEDG + PMSF buffer (10 mM Tris (pH 7.4), 1.5 mM EDTA, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 1 mM DTT, made fresh) at a ratio of 0.1 g of tissue per 1.0 ml TEDG + PMSF buffer. The tissues were homogenized using a Polytron (PT 35/10) homogenizer for 5 bursts (~5 seconds per burst). The homogenate was transferred to pre-cooled centrifuge tubes and centrifuged for 10 min at $2,500 \times g$ at 4°C . The supernatant was then transferred to pre-cooled ultracentrifuge tubes and centrifuged at $105,000 \times g$ for 60 min at 4°C . The pellet was discarded, the cytosol supernatants combined, and aliquots of cytosol were stored at -80°C .

II.2.3 Determination of Protein Concentration

The protein concentration was determined using the Bio-Rad protein assay kit (Hercules, CA) using Hamner SOP. A 1:20 or 1:40 dilution of the cytosol was made in ddH₂O. Ten (10) μl of diluted cytosol or standard was assayed in a 96 well microtiter plate. Protein standard concentrations for standard curve were 0, 31.25, 62.5, 125, 250, 500, 1000 $\mu\text{g}/\text{ml}$. Bio-Rad dye reagent was diluted according to manufacturer's instructions and 200 μl was added to each well with mixing. The reaction was allowed to proceed at RT for 5 min. The absorbance was measured at a wavelength of 595 nm by using a SPECTRAMax 340 (00A ROM v2.04 Feb 1, 96) microplate reader (Molecular Devices, Sunnyvale, CA).

II.2.4 Estrogen Receptor Binding Assays

II.2.4.1 Saturation Binding Assay

A Saturation Binding assay was performed to determine the concentration of protein to use in the Competitive Binding assays for the unknown test chemicals. In the Saturation Binding assay, rat uterine cytosol was tested at a concentration of either 25 or 50 μg protein/tube. The concentration of [³H]-17 β -estradiol used was 0.03–3 nM. Before preparing the serial dilutions for the saturation binding assay, the specific activity (SA) was adjusted for decay over time. The calculation for SA was made

using the “QuickCalcs” (see Appendix E) webpage from GraphPad (<http://www.graphpad.com/quickcalcs/radcalcform.cfm>). The concentration of inert 17 β -estradiol used was 3–300 nM. The samples were incubated for 16–20 hr at 4° C. The bound [3 H]-17 β -estradiol was separated from the free [3 H]-17 β -estradiol by adding 250 μ l of hydrated hydroxyapatite (HAP; CAS# 1306-06-05) and vortexing. Ice cold buffer was added and the tubes were centrifuged to pellet the HAP which contains the estrogen-receptor-bound [3 H]-17 β -estradiol. The pellets were washed two more times by vortexing and centrifuging and suspended in 1.5 ml of 100% ethanol. One ml of ethanol sample was added to 14 ml of scintillation fluid in a 20 ml scintillation vial and counted in a Packard Tri-Carb 2200C liquid scintillation analyzer in a scintillation counter to determine DPMs/vial. The affinity of the radioligand for the receptor (K_d), maximum number of receptor bound (B_{max}) and 10% rule criteria (ratio of total binding in the absence of competitor to the total amount of [3 H]-17 β – estradiol added) were used for the evaluation of the saturation binding. All calculations were done using the worksheet and GraphPad Prism templates provided by EPA.

II.2.4.2 Competitive Binding Assay With Unknown Test Chemicals

The test and optional chemicals were tested in the order requested by EPA. Rat uterine cytosol was used at a concentration of 50 μ g protein/tube. **Note: Because of the progressive decrease in the specific total binding, the Sponsor (in a conference call with RTI and The Hamner) suggested that the concentration of cytosol should be increased to 100 μ g protein/tube. This was done for all competitive binding experiments between 4/17 and 6/18/2008. However, for all competitive binding runs using the [3 H]-17 β -estradiol received from RTI, 50 μ g protein of cytosol prep-5 was used.** The concentration of [3 H]-17 β -estradiol used was 1 nM. Before preparing the concentration of [3 H]-17 β -estradiol for the competitive binding assay, the specific activity (SA) was adjusted for decay over time. The calculation for SA was made using the “QuickCalcs” webpage from GraphPad (<http://www.graphpad.com/quickcalcs/radcalcform.cfm>) (see Appendix E). The final concentration range for generating the 17 β -estradiol standard curve was 1×10^{-7} to

1×10^{-11} M. The final concentration range of the weak positive control, norethynodrel, was 1×10^{-4} to $1 \times 10^{-8.5}$ M. The final concentration range of the negative control, R1881, was 1×10^{-3} to 1×10^{-10} M. Based on the solubility of each test chemical in ethanol, most test chemicals were diluted in ethanol and tested at a final concentration range of 1×10^{-15} to 1×10^{-3} M in log increments (see Table 6). The exceptions were chemical 5, 22, and 18 which were dissolved in DMSO at a final concentration range of 1×10^{-11} to 1×10^{-3} M in log increments.

The samples were incubated for 16–20 hr at 4°C. The bound [3 H]-17 β -estradiol was separated from the free [3 H]-17 β -estradiol by adding 250 μ l of hydrated hydroxyapatite (HAP) and vortexing. Ice cold buffer was added and the tubes were centrifuged to pellet the HAP which contains the estrogen-receptor-bound [3 H]-17 β -estradiol. The pellets were washed two more times by vortexing and centrifuging and suspended in 1.5 ml of 100% ethanol. One ml of the ethanol sample was added to 14 ml of scintillation fluid in a 20 ml scintillation vial and counted in a Packard Tri-Carb 2200C liquid scintillation analyzer to determine DPMs/vial. The log (IC₅₀) and relative binding affinity (RBA) were calculated according to the worksheet and GraphPad Prism templates provided by EPA and used for the evaluation of the competitive binding assay.

II.2.4.3 Preparation of Test Chemicals for Standard Curve Generation

1. Chemical Code # 7, 11, 12, 19, 20, and 23: For generating standard curve (final concentrations: 1×10^{-10} to 1×10^{-3} M), these chemicals were made as follows: a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a 1:2 dilution (5×10^{-2} M) was made in TEDG + PMSF buffer (hereafter referred to as TP buffer). The next 1:10 dilution (5×10^{-3} M) was cloudy when 100 μ l of 5×10^{-2} M was added to 900 μ l of TP buffer. Therefore, a combination of 700 μ l TP buffer and 200 μ l EtOH was used (which dissolved the chemicals very well) to make the 5×10^{-3} M concentration. Using this proportion of TP buffer and EtOH, the final EtOH concentration in sample tube was 1.6% which was within the criteria of a final concentration of EtOH in the sample tube of < 3%. For the

5×10^{-4} to 5×10^{-9} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).

2. Chemical Code # 10, 15, and 21: For generating standard curve (final concentrations: 1×10^{-12} to 1×10^{-3} M), these chemicals were made as follows: a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a 1:2 dilution (5×10^{-2} M) was made in TP buffer. For the 5×10^{-3} to 5×10^{-9} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-2} M concentration (100 μ l chemical + 900 μ l TP buffer).

Note: Other standard curves for Chemical Code # 10 were generated as follows: For final concentrations of 1×10^{-5} to 1×10^{-12} M, a 10 mM stock concentration in EtOH was made first. From the 10 mM stock, a 1:20 dilution (5×10^{-4} M) was made in 50% TP buffer. Therefore, using this proportion of TP buffer and EtOH, the final EtOH concentration in sample tube was within the criteria of a final concentration of EtOH in the sample tube of $< 3\%$. For the 5×10^{-4} to 5×10^{-11} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-4} M concentration (100 μ l chemical + 900 μ l TP buffer).

3. Chemical Code # 18 and 22: Since these two chemicals did not dissolve well in EtOH, DMSO was used as the solvent. For generating standard curve (final concentrations: 1×10^{-11} to 1×10^{-4} M), a 100 mM stock concentration in DMSO was made first. From the 100 mM stock, a dilution of 5×10^{-3} M was made using 500 μ l TP buffer, 450 μ l DMSO and 50 μ l of 100 mM stock. For the 5×10^{-4} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).
4. Chemical Code # 16: For generating standard curve (final concentrations: 1×10^{-10} to 1×10^{-3} M), a 100 mM stock concentration in EtOH was made. From the 100 mM stock, a 1:2 dilution (5×10^{-2} M) was made in TP buffer. The next 1:10 dilution (5×10^{-3} M) was cloudy when 100 μ l of 5×10^{-2} M was added to 900 μ l of TP buffer. Therefore, a combination of 600 μ l TP buffer and 300 μ l EtOH was used (which dissolved the chemical well) to make the 5×10^{-3} M concentration. The final EtOH concentration in sample tube was 1.8% which was within the

criteria of a final concentration of EtOH in the sample tube of < 3%. For the 5×10^{-4} to 5×10^{-9} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).

5. Chemical Code # 5: Since this chemical did not dissolve well in EtOH, DMSO was used as the solvent. For generating standard curve (final concentrations: 1×10^{-10} to 1×10^{-3} M), a 100 mM stock concentration in DMSO was made first. From the 100 mM stock, a dilution of 5×10^{-2} M was made using 500 μ l TP buffer, 500 μ l of 100 mM stock. The next 1:10 dilution (5×10^{-3} M) was cloudy when 100 μ l of 5×10^{-2} M was added to 900 μ l of TP buffer. Therefore, a combination of 700 μ l TP buffer and 200 μ l EtOH was used to make the 5×10^{-3} M concentration. For the 5×10^{-4} to 5×10^{-9} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).
6. Chemical Code # 13, 14, and 17: For generating standard curve (final concentrations: 1×10^{-11} to 1×10^{-4} M), a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a dilution of 5×10^{-3} M was made using 500 μ l TP buffer, 450 μ l EtOH and 50 μ l of 100 mM stock. A combination of 700 μ l TP buffer and 200 μ l EtOH was used to make the 5×10^{-4} M concentration. For the 5×10^{-5} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-4} M concentration (100 μ l chemical + 900 μ l TP buffer).
7. Chemical Code # 9: For generating standard curve (final concentrations: 1×10^{-13} to 1×10^{-5} M), a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a dilution of 5×10^{-4} M was made using 500 μ l TP buffer, 495 μ l EtOH and 5.0 μ l of 100 mM stock. For the 5×10^{-5} to 5×10^{-12} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-4} M concentration (100 μ l chemical + 900 μ l TP buffer).
8. Chemical Code # 4: For generating standard curve (final concentrations: 1×10^{-15} to 1×10^{-8} M), a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a dilution of 5×10^{-4} M was made using 700 μ l TP buffer, 290 μ l EtOH and 10 μ l of 100 mM stock. From the 5×10^{-4} M stock, a dilution of 5×10^{-6} M was made using 990 μ l TP buffer and 10 μ l of 5×10^{-4} M stock. For the

5×10^{-7} to 5×10^{-14} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-6} M concentration (100 μ l chemical + 900 μ l TP buffer).

9. Chemical Code # 1 and 3: For generating standard curve (final concentrations: 1×10^{-14} to 1×10^{-6} M), a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a dilution of 5×10^{-2} M was made using 500 μ l TP buffer and 500 μ l of 100 mM stock. From the 5×10^{-2} M stock, a dilution of 5×10^{-4} M was made using 990 μ l TP buffer and 10 μ l of 5×10^{-2} M stock. For the 5×10^{-5} to 5×10^{-12} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-4} M concentration (100 μ l chemical + 900 μ l TP buffer).
10. Chemical Code # 6: For generating standard curve (final concentrations: 1×10^{-11} to 1×10^{-4} M), a 100 mM stock concentration in EtOH was made. From the 100 mM stock, a dilution of 5×10^{-3} M was made using 500 μ l TP buffer, 450 μ l EtOH and 50 μ l of 100 mM stock. For the 5×10^{-4} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).
11. Chemical Code # 2: For generating standard curve (final concentrations: 1×10^{-12} to 1×10^{-5} M), a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a dilution of 5×10^{-2} M was made using 500 μ l TP buffer and 500 μ l of 100 mM stock. From the 5×10^{-2} M stock, a dilution of 5×10^{-4} M was made using 500 μ l TP buffer, 490 μ l EtOH and 10 μ l of 5×10^{-2} M stock. For the 5×10^{-5} to 5×10^{-11} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-4} M concentration (100 μ l chemical + 900 μ l TP buffer).
12. Chemical Code # 8: For generating standard curve (final concentrations: 1×10^{-10} to 1×10^{-3} M), a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a concentration of 5×10^{-2} M was made using 500 μ l TP buffer and 500 μ l of 100 mM stock. The 5×10^{-3} M concentration was made with 600 μ l TP buffer, 300 μ l EtOH, and 100 μ l of 5×10^{-2} M concentration. The 5×10^{-4} M concentration was made with 700 μ l TP buffer, 200 μ l EtOH, and 100 μ l of 5×10^{-2} M concentration. For the 5×10^{-4} to 5×10^{-9} M concentrations, 1:10 serial

dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).

13. Chemical Code # 27, 30, 36: For generating standard curve (final concentrations: 1×10^{-12} to 1×10^{-5} M), these chemicals were made as follows: a 5 mM stock concentration was made instead of 100 mM as the chemical would not dissolve at this concentration even after warming, vortexing, or sonication using EtOH. For the 5×10^{-4} to 5×10^{-11} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).
14. Chemical Code # 28, 29: For generating standard curve (final concentrations: 1×10^{-11} to 1×10^{-4} M), these chemicals were made as follows: a 10 mM stock concentration was made instead of 100 mM as the chemical would not dissolve at this concentration even after warming, vortexing, or sonication using EtOH. From the 10 mM stock, a concentration of 5×10^{-3} M was made using 500 μ l TP buffer and 500 μ l of 100 mM stock. For the 5×10^{-4} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l chemical + 900 μ l TP buffer).
15. Chemical Code # 32: For generating standard curve (final concentrations: 1×10^{-10} to 1×10^{-3} M), this chemical was made as follows: a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a 1:2 dilution (5×10^{-2} M) was made in TP buffer. For the 5×10^{-3} to 5×10^{-9} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-2} M concentration (100 μ l chemical + 900 μ l TP buffer).
16. Chemical Code # 34, 37, 39, 40: For generating standard curve (final concentrations: 1×10^{-11} to 1×10^{-4} M), these chemicals were made as follows: a 50 mM stock concentration in EtOH was made using sonication to dissolve the chemical. The next 1:10 dilution (5×10^{-3} M) was cloudy when 100 μ l of 5×10^{-2} M was added to 900 μ l of TP buffer. Therefore, a combination of 700 μ l TP buffer and 200 μ l EtOH was used to make the 5×10^{-3} M concentration. For the 5×10^{-4} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M concentration (100 μ l + 900 μ l TP buffer).

17. Chemical Code # 31, 38: For generating standard curve (final concentrations: 1×10^{-11} to 1×10^{-4} M), these chemicals were made as follows: a 50 mM stock concentration in EtOH was made using sonication to dissolve the chemical. For the 5×10^{-3} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-2} M chemical concentration (100 μ l chemical + 900 μ l TP buffer).
18. Chemical Code # 33, 42, 43: For generating standard curve (final concentrations: 1×10^{-10} to 1×10^{-3} M), these chemicals were made as follows: a 100 mM stock concentration in EtOH was made first. From the 100 mM stock, a 1:2 dilution (5×10^{-2} M) was made in TP buffer. The next 1:10 dilution (5×10^{-3} M) was cloudy when 100 μ l of 5×10^{-2} M was added to 900 μ l of TP buffer. Therefore, a combination of 700 μ l TP buffer and 200 μ l EtOH was used to make the 5×10^{-3} M. For the 5×10^{-4} to 5×10^{-9} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M chemical concentration (100 μ l chemical + 900 μ l TP buffer).
19. Chemical Code # 35, 42: For generating standard curve (final concentrations: 1×10^{-11} to 1×10^{-4} M), these chemicals were made as follows: a 50 mM stock concentration in EtOH was made using sonication to dissolve the chemical. The next 1:10 dilution (5×10^{-3} M) was cloudy when 100 μ l of 5×10^{-2} M was added to 900 μ l of TP buffer. Therefore, a combination of 600 μ l TP buffer and 300 μ l EtOH was used to make the 5×10^{-3} M. For the 5×10^{-4} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M chemical concentration (100 μ l chemical + 900 μ l TP buffer).
20. Chemical Code # 41, 45, and 46: For generating standard curve (final concentrations: 1×10^{-4} to 1×10^{-11} M), these chemicals were made as follows: a 10 mM stock concentration in EtOH was made first. From the 10 mM stock, a 1:2 dilution (5×10^{-3} M) was made in TP buffer. Therefore, using this proportion of TP buffer and EtOH, the final EtOH concentration in sample tube was within the criteria of a final concentration of EtOH in the sample tube of < 3%. For the

5×10^{-3} to 5×10^{-10} M concentrations, 1:10 serial dilutions were made in TP buffer from the 5×10^{-3} M chemical concentration (100 μ l chemical + 900 μ l TP buffer).

SECTION III: RESULTS

For the ER Binding Project, we have performed 4 saturation binding assays for 3 cytosol preparations and over 170 competitive binding assays. The summary results for the protein determination of the cytosol preparations are given in Table 1 and Table 2. The summary results for saturation binding assays are given in Table 3 and Figure 1. The summary results for the competitive binding assays are given in Figures 2–24 and Tables 4–8. A sponsor approved Hamner protocol with amendments and a QA Statement is included in Appendix A. The raw data and prism files for the saturation binding assays are included in Appendix B. The raw data and prism files for the competitive binding assays including the within run SD for controls are in Appendix C. Appendix E shows a sample calculation for correcting the specific activity of [³H]-17β –estradiol using GraphPad “Quick Calcs”.

III.1 Determination of Cytosol Protein Concentration

A standard curve was generated for determining the cytosol protein concentrations of cytosol prep-3, 4, and 5 (Table 1). The protein concentrations were determined to be 8.09 mg/ml for cytosol prep-3, 6.67 mg/ml for cytosol prep-4, and 2.5 mg/ml for cytosol prep-5 (Table 2).

Table 1. Data from Standard Protein Assay for Cytosol Preparations

Protein Conc. (ug/ml)	BSA	BSA	BSA
	Mean OD₅₉₅ Value- Prep3	Mean OD₅₉₅ Value- Prep4	Mean OD₅₉₅ Value- Prep5
0	-0.008	-0.001	-0.002
31.25	0.047	0.047	0.054
62.5	0.114	0.102	0.110
125	0.217	0.213	0.219
250	0.353	0.378	0.393
500	0.665	0.617	0.700
1000	1.045	0.941	0.990
R² value	0.999	0.998	1.000

Table 2. Data on Protein Concentrations for Cytosol Preparations

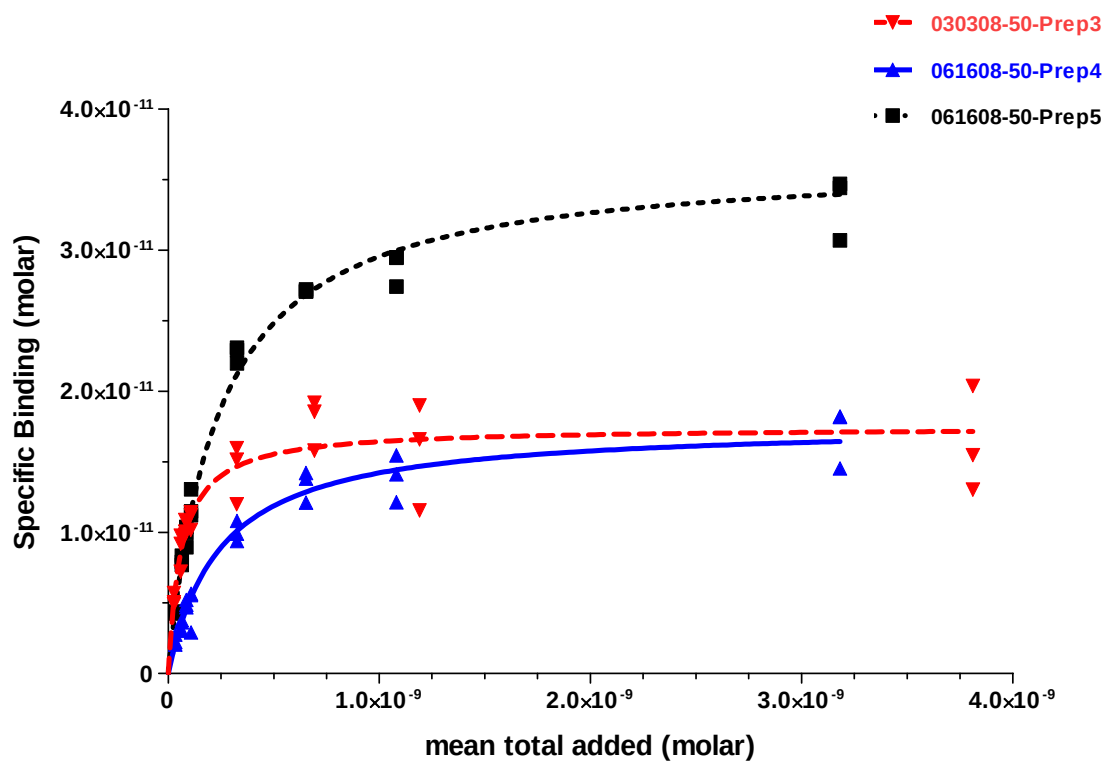
Replicate	Cytosol Prep-3	Cytosol Prep-4	Cytosol Prep-5
	Protein Conc. (mg/ml)	Protein Conc. (mg/ml)	Protein Conc. (mg/ml)
1	7.82	6.67	2.52
2	8.36		2.46
3			2.52
AVG±SE	8.09±0.27	6.67	2.50±0.02

III.2 Data on Saturation Binding Assay for Cytosol Preparations

The K_d and B_{max} values obtained by using either 25 or 50 μ g protein/tube in the saturation binding assay are presented in Table 3. All values using 25 μ g protein/tube were not within the expected criteria specifications. For cytosol prep-3, the K_d for 50 μ g of protein/ tube was 0.061 nM and was within the criteria of 0.05 to 0.5 nM. The B_{max} for 50 μ g of protein/tube was 17.43, which was slightly lower than the expected criteria of 36 to 44 fmol ER/100 μ g protein. For cytosol prep-4, the K_d for 50 μ g of protein/ tube was 0.138 nM and was within the criteria. The B_{max} for 50 μ g of protein/tube was 15.17, which was slightly lower than the expected criteria. For cytosol prep-5, the K_d for 50 μ g of protein/ tube was 0.235 nM and the B_{max} was 36.46. Both the K_d and the B_{max} values were within the expected criteria for cytosol prep-5. Therefore, 50 μ g of protein/ tube was selected to use in all competitive assays.

Table 3. Data on K_d and B_{max} Values for Cytosol Preparations

Cytosol Prep	μ g Protein/tube	K_d (nM)	B_{max} (fmol ER/100 μ g protein)
3	25	0.042	10.99
3	50	0.061	17.43
4	50	0.138	15.17
5	50	0.235	36.46



	061608-50-Prep5	061608-50-Prep4	030308-50-Prep3
Bmax	3.646e-011	1.770e-011	1.743e-011
Kd	2.345e-010	2.453e-010	6.140e-011
Std. Error			
Bmax	8.674e-013	1.066e-012	7.720e-013
Kd	9.677e-012	2.531e-011	7.368e-012
95% Confidence Intervals			
Bmax	3.466e-011 to 3.826e-011	1.549e-011 to 1.991e-011	1.583e-011 to 1.903e-011
Kd	2.145e-010 to 2.546e-010	1.928e-010 to 2.978e-010	4.612e-011 to 7.668e-011
Goodness of Fit			
Degrees of Freedom	22	22	22
R ² (unweighted)	0.9885	0.9580	0.8030
Weighted Sum of Squares (1/Y ²)	0.07400	0.4647	0.4062
Absolute Sum of Squares	2.950e-023	2.831e-023	9.291e-023
Sy.x	0.05800	0.1453	0.1359
Number of points			
Analyzed	24	24	24

Figure 1. Summary of Saturation Curves.

III.3 Representative Data on Competitive Binding Assay for Controls (Estradiol, Norethynodrel, and R1881) from 23 Test Chemicals

The mean IC_{50} values for 17β -estradiol (20 competitive binding assays), norethynodrel (19 competitive binding assays), and R1881 (10 competitive binding assays) for test agents was calculated to be $-8.99 \pm 4.43E-02$, $-6.06 \pm 5.16E-02$, and $-4.77 \pm 1.66E-01$ respectively. Norethynodrel showed a low, but positive relative binding affinity of $1.30E-03 \pm 9.59E-05$ when compared to 17β -estradiol. A summary of representative values for IC_{50} , average within run SD (WRSD), and average Relative Binding Affinity (RBA) for the controls are presented in Table 4. See Appendix D for tables of representative runs.

Table 4. Data on IC_{50} , Average RBA and Average WRSD of Controls for Test Chemicals

IC_{50} (M)	Estradiol		Norethynodrel		R1881	
	Mean	SE	Mean	SE	Mean	SE
	-8.99	4.43E-02	-6.06	5.16E-02	-4.77	1.66E-01
Average RBA	1.00	-	1.30E-03	9.59E-05	N/A	N/A
Average Within Run SD$\pm$SE	4.82 $\pm$ 4.54E-01		5.03 $\pm$ 5.59E-01		6.20 $\pm$ 6.85E-01	

III.4 Data on Competitive Binding Assay for Controls (Estradiol and Norethynodrel) from Optional Chemicals

The mean IC_{50} values for 17β -estradiol and norethynodrel from 6 competitive binding assays for optional chemicals was calculated to be $-9.12 \pm 1.40E-01$ and $-5.99 \pm 5.76E-02$, respectively. Norethynodrel showed a low, but positive relative binding affinity of $8.91E-04 \pm 1.53E-04$ when compared to 17β -estradiol. The IC_{50} , average WRSD, and average RBA for the controls are presented in Table 5. See Appendix D for tables of competitive runs.

Table 5. Data on IC₅₀, Average RBA and Average WRSD of Controls from Optional Chemicals

IC ₅₀ (M)	Estradiol		Norethynodrel	
	Mean	SE	Mean	SE
	-9.12	1.40E-01	-5.99	5.76E-02
Average RBA	1.00	-	8.91E-04	1.53E-04
Average WRSD±SE	2.98±3.05E-01		3.79±4.35E-01	

III.5 Data on Competitive Binding Assay for Test Chemicals and Optional Chemicals

The mean IC₅₀, final concentration range (M), average RBA, average Hill Slope and classification for the test chemicals are presented in Table 6. Table 7 gives Log (IC₅₀), RBA, Hill slope, and classification for each test chemical. Table 8 gives Log (IC₅₀), RBA, Hill slope, and classification for each optional chemical. A summary of the estradiol and test chemical graphs are shown in Figures 2 through 24.

Table 6. Data on IC₅₀, Concentration Range (M), Average RBA, Average Hill Slope, and Classification of Test Chemicals

Test Chemical	Chem 12	Chem 19	Chem 11	Chem 21	Chem 15
Average IC₅₀ ±SE	-7.63±7.80E-02	-3.88±6.51E-02	-5.77±7.17E-02	Not converged	-5.51±2.17E-02
Final Concentration Range (M)	1×10 ⁻¹⁰ -1×10 ⁻³	1×10 ⁻¹⁰ - 1×10 ⁻³	1×10 ⁻¹⁰ - 1×10 ⁻³	1×10 ⁻¹⁰ -1×10 ⁻³	1×10 ⁻¹⁰ -1×10 ⁻³
Average RBA ±SE	4.06E-02±4.23E-03	6.98E-06±1.69E-06	5.42E-04±3.41E-05	NA	3.25E-04±2.75E-05
Average Hill Slope ±SE	-1.02±6.74E-02	-1.95±1.09E+00	-0.91±9.53E-02	NA	-0.81±3.50E-02
Classification	Positive	Negative	Positive	Negative	Positive

Test Chemical	Chem 22	Chem 16	Chem 5	Chem 17	Chem 9
Average IC₅₀ ±SE	Not Converged	-5.32±1.56E-01	-7.47±2.64E-01	-5.63±6.00E-02	-10.96±6.99E-01
Final Concentration Range (M)	1×10 ⁻¹¹ - 1×10 ⁻⁴	1×10 ⁻¹⁰ -1×10 ⁻³	1×10 ⁻¹⁰ -1×10 ⁻³	1×10 ⁻¹¹ - 1×10 ⁻⁴	1×10 ⁻¹³ -1×10 ⁻⁵
Average RBA ±SE	NA	2.24E-04±1.04E-04	4.81E-02±1.89E-02	5.05E-04±1.67E-04	1.44E+02±7.20E+01
Average Hill Slope ±SE	NA	-1.29±7.79E-02	-0.74±8.55E-02	-0.91±2.34E-01	-1.21±2.51E-01
Classification	Negative	Negative	Positive	Positive	Positive

Table 6. Data on IC₅₀, Final Concentration Range (M), Average Relative Binding Affinity, Average Hill Slope, and Classification of Test Chemicals (Cont.)

Test Chemical	Chem 4	Chem 14	Chem 20	Chem 3	Chem 23
Average IC ₅₀ ±SE	-10.22±2.15E-01	Not converged	Not converged	-9.73±2.31E-01	-5.24±2.10E-01
Final Concentration Range (M)	1×10 ⁻¹⁵ - 1×10 ⁻⁸	1×10 ⁻¹¹ - 1×10 ⁻⁴	1×10 ⁻¹⁰ - 1×10 ⁻³	1×10 ⁻¹⁴ - 1×10 ⁻⁷	1×10 ⁻¹⁰ - 1×10 ⁻³
Average RBA ±SE	2.09E+01±5.04E+00	NA	NA	2.84E+00±9.22E-01	1.38E-04±2.59E-05
Average Hill Slope ±SE	-0.65±2.03E-02	NA	NA	-0.80±4.37E-02	-0.85±2.52E-01
Classification	Positive	Negative	Negative	Positive	Positive

Test Chemical	Chem 1	Chem 8	Chem 2	Chem 10
Average IC ₅₀ ±SE	-8.23±2.55E-01	-5.49±1.99E-01	-9.20±1.25E-01	-6.01±1.11E+00
Final Concentration Range (M)	1×10 ⁻¹³ - 1×10 ⁻⁶	1×10 ⁻¹⁰ - 1×10 ⁻³	1×10 ⁻¹² - 1×10 ⁻⁵	1×10 ⁻¹² - 1×10 ⁻⁴
Average RBA ±SE	1.44E+00±1.08E+00	1.01E-03±2.28E-04	2.68E+00±2.19E+00	2.04E-02±2.01E-02
Average Hill Slope ±SE	-0.71±1.56E-01	-1.66±7.45E-01	-0.73±2.71E-02	-0.84±3.24E-01
Classification	Positive	Negative	Positive	Positive

Test Chemical	Chem 18	Chem 13	Chem 7	Chem 6
Average IC ₅₀ ±SE	-5.24±4.05E-01	-7.43±1.30E-01	-5.04±5.22E-02	-6.19±8.82E-03
Final Concentration Range (M)	1×10 ⁻¹¹ - 1×10 ⁻⁴	1×10 ⁻¹¹ - 1×10 ⁻⁴	1×10 ⁻¹⁰ - 1×10 ⁻³	1×10 ⁻¹¹ - 1×10 ⁻⁴
Average RBA ±SE	7.80E-05±6.39E-05	8.54E-02±6.82E-02	1.05E-04±1.04E-05	1.47E-03±8.18E-05
Average Hill Slope ±SE	-0.42±8.07E-02	-1.36±1.39E-01	-1.12±1.34E-01	-1.02±3.33E-02
Classification	Negative	Positive	Positive	Positive

Table 7. Summary of Competition Binding Data

Experiment	Log (IC₅₀)	Relative Binding Affinity	Hill Slope	Classification
CHEM 12				POSITIVE
030508	-7.47	3.99E-02	-1.11	
030608	-7.69	3.37E-02	-0.89	
032408	-7.72	4.83E-02	-1.06	
AVG±SE	-7.63±7.80E-02	4.06E-02±4.23E-03	-1.02±6.74E-02	
CHEM 19				NEGATIVE
030608	-3.95	6.05E-06	-4.12	
031008	-3.75	4.62E-06	-0.98	
031108	-3.94	1.03E-05	-0.75	
AVG±SE	-3.88±6.51E-02	6.98E-06±1.69E-06	-1.95±1.09E+00	
CHEM 11				POSITIVE
030508	-5.63	5.72E-04	-1.07	
030608	-5.84	4.74E-04	-0.93	
031008	-5.85	5.81E-04	-0.74	
AVG±SE	-5.77±7.17E-02	5.42E-04±3.41E-05	-0.91±9.53E-02	
CHEM 21				NEGATIVE
031108	Not converged			
031208	Not converged			
031708	Not converged			
AVG±SE				
CHEM 15				POSITIVE
031108	-5.49	3.66E-04	-0.78	
031208	-5.49	2.72E-04	-0.77	
031708	-5.56	3.357E-04	-0.88	
AVG±SE	-5.51±2.17E-02	3.25E-04±2.75E-05	-0.81±3.50E-02	

Table 7. Summary of Competition Binding Data (Cont.)

Experiment	Log (IC₅₀)	Relative Binding Affinity	Hill Slope	Classification
CHEM 22 032608 032708 033108 AVG±SE	Not Converged Not Converged Not Converged			NEGATIVE
CHEM 16 031808 032608 061108 AVG±SE	-5.56 -5.37 -5.03 -5.32±1.56E-01	2.86E-04 3.66E-04 2.08E-05 2.24E-04±1.04E-04	-1.39 -1.35 -1.14 -1.29±7.79E-02	NEGATIVE
CHEM 5 032608 032708 033108 AVG±SE	-7.61 -7.84 -6.96 -7.47±2.64E-01	4.96E-02 8.02E-02 1.46E-02 4.81E-02±1.89E-02	-0.58 -0.87 -0.79 -0.74±8.55E-02	POSITIVE
CHEM 17 040308 040808 041008 AVG±SE	-5.53 -5.62 -5.74 -5.63±6.00E-02	8.38E-04 3.21E-04 3.56E-04 5.05E-04±1.67E-04	-0.49 -0.93 -1.30 -0.91±2.34E-01	POSITIVE
CHEM 9 062508 070108 070208 AVG±SE	-11.50 -9.57 -11.80 -10.96±6.99E-01	1.98E+02 1.56E+00 2.33E+02 1.44E+02±7.20E+01	-1.23 -1.63 -0.76 -1.21±2.51E-01	POSITIVE

Table 7. Summary of Competition Binding Data (Cont.)

Experiment	Log (IC₅₀)	Relative Binding Affinity	Hill Slope	Classification
CHEM 4				POSITIVE
040708	-10.23	2.20E+01	-0.66	
040808	-10.58	2.90E+01	-0.61	
040908	-9.84	1.17E+01	-0.68	
AVG±SE	-10.22±2.15E-01	2.09E+01±5.04E+00	-0.65±2.03E-02	
CHEM 14				NEGATIVE
041009	Not Converged			
041608	Not Converged			
041708	Not Converged			
AVG±SE				
CHEM 20				NEGATIVE
041009	Not Converged			
041608	Not Converged			
041708	Not Converged			
AVG±SE				
CHEM 3				POSITIVE
062308	-9.83	2.91E+00	-0.83	
062408	-10.07	4.40E+00	-0.85	
062508	-9.29	1.21E+00	-0.71	
AVG±SE	-9.73±2.31E-01	2.84E+00±9.22E-01	-0.80±4.37E-02	
CHEM 23				POSITIVE
041508	-5.09	1.64E-04	-0.82	
052708	-4.97	1.64E-04	-0.87	
061108	-5.65	8.63E-05	-1.60	
AVG±SE	-5.24±2.10E-01	1.38E-04±2.59E-05	-0.85±2.52E-01	

Table 7. Summary of Competition Binding Data (Cont.)

Experiment	Log (IC ₅₀)	Relative Binding Affinity	Hill Slope	Classification
CHEM 1				POSITIVE
050708	-7.96	1.45E-01	-0.44	
051808	-7.99	5.96E-01	-0.98	
052208	-8.74	3.58E+00	-0.72	
AVG±SE	-8.23±2.55E-01	1.44E+00±1.08E+00	-0.71±1.56E-01	
CHEM 8				NEGATIVE
051909	-5.74	1.47E-03	-2.95	
052208	-5.10	8.13E-04	-0.37	
052708	-5.63	7.53E-04	-1.65	
AVG±SE	-5.49±1.99E-01	1.01E-03±2.28E-04	-1.66±7.45E-01	
CHEM 2				POSITIVE
051909	-9.43	7.05E+00	-0.69	
062308	-9.16	6.12E-01	-0.73	
062408	-9.00	3.71E-01	-0.78	
AVG±SE	-9.20±1.25E-01	2.68E+00±2.19E+00	-0.73±2.71E-02	
CHEM 10				POSITIVE
052108	-4.68	5.75E-04	-1.35	
062308	-5.13	5.73E-05	-0.92	
062408	-8.21	6.07E-02	-0.24	
AVG±SE	-6.01±1.11E+00	2.04E-02±2.01E-02	-0.84±3.24E-01	
CHEM 18				NEGATIVE
060408	Not Converged			
060508	-5.64	1.42E-04	-0.33	
060608	-4.83	1.42E-05	-0.50	
AVG±SE	-5.24±4.05E-01	7.81E-05±6.39E-05	-0.42±8.07E-02	

Table 7. Summary of Competition Binding Data (Cont.)

Experiment	Log (IC₅₀)	Relative Binding Affinity	Hill Slope	Classification
CHEM 13				POSITIVE
052108	-7.27	2.22E-01	-1.31	
053008	-7.69	1.54E-02	-1.14	
060608	-7.33	1.90E-02	-1.62	
AVG±SE	-7.43±1.30E-01	8.54E-02±6.82E-02	-1.36±1.39E-01	
CHEM 7				POSITIVE
060308	-5.11	1.23E-04	-0.87	
060508	-4.94	8.67E-05	-1.16	
060608	-5.08	1.05E-04	-1.33	
AVG±SE	-5.04±5.22E-02	1.05E-04±1.04E-05	-1.12±1.34E-01	
CHEM 6				POSITIVE
060308	-6.19	1.48E-03	-1.06	
060508	-6.21	1.61E-03	-1.05	
060608	-6.18	1.32E-03	-0.96	
AVG±SE	-6.19±8.82E-03	1.47E-03±8.18E-05	-1.02±3.33E-02	

III.6 Summary Data on Competitive Binding for Test Chemicals and Respective Estradiol Controls

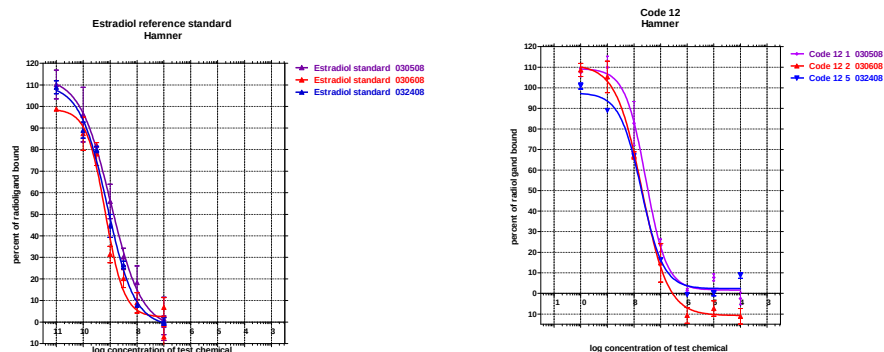


Figure 2. Summary of 17 β -estradiol Standard Curves and Chemical 12 curves

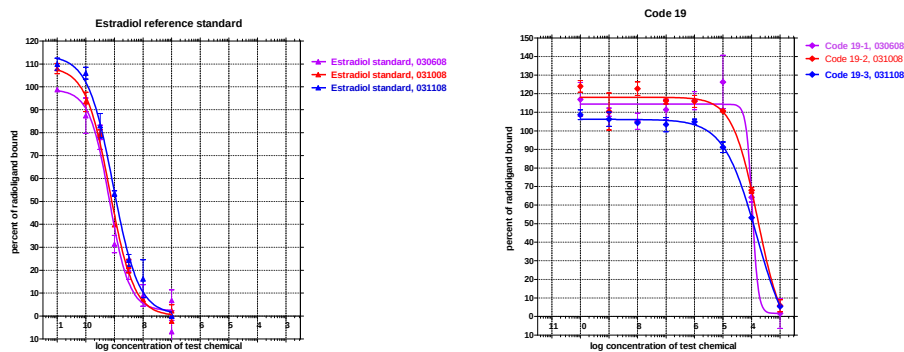


Figure 3. Summary of 17 β -estradiol Standard Curves and Chemical 19 curves

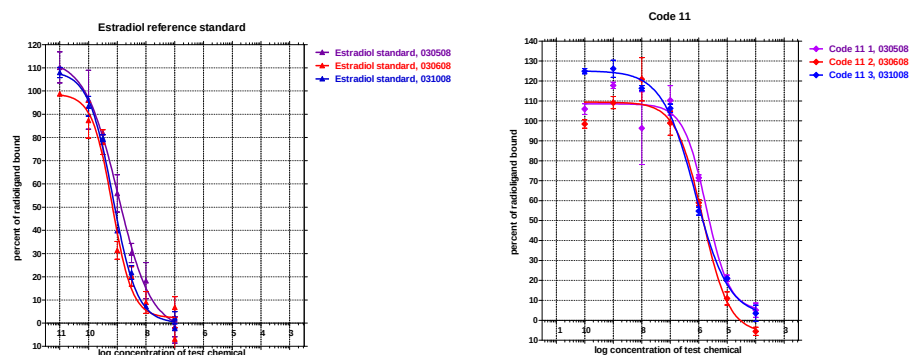


Figure 4. Summary of 17 β -estradiol Standard Curves and Chemical 11 curves

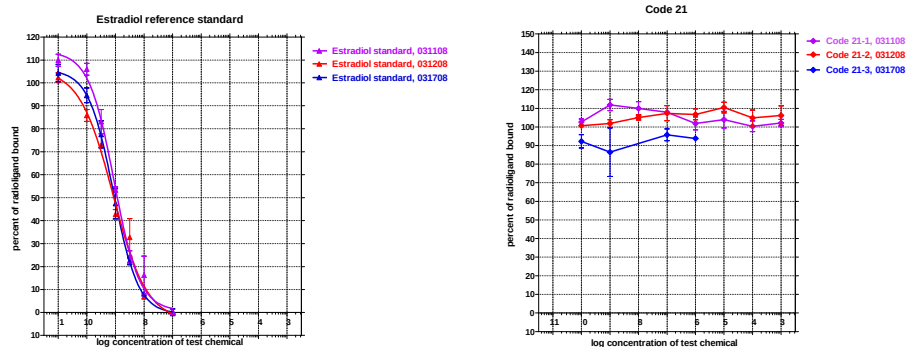


Figure 5. Summary of 17 β -estradiol Standard Curves and Chemical 21 curves

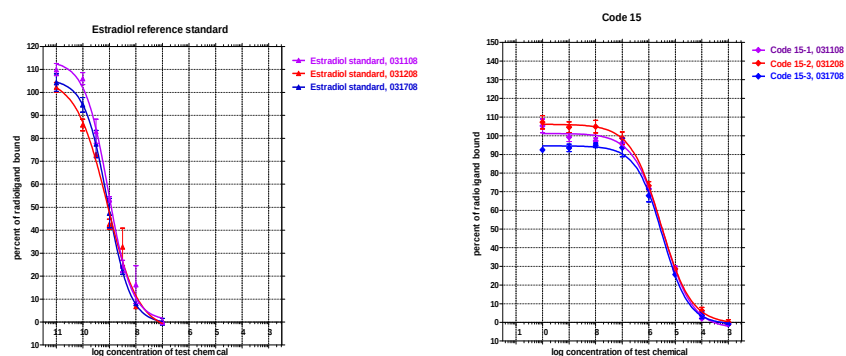


Figure 6. Summary of 17 β -estradiol Standard Curves and Chemical 15 curves

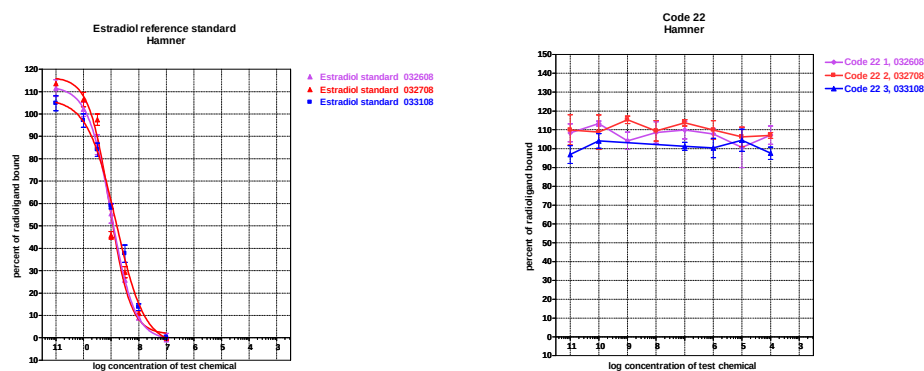


Figure 7. Summary of 17 β -estradiol Standard Curves and Chemical 22 curves

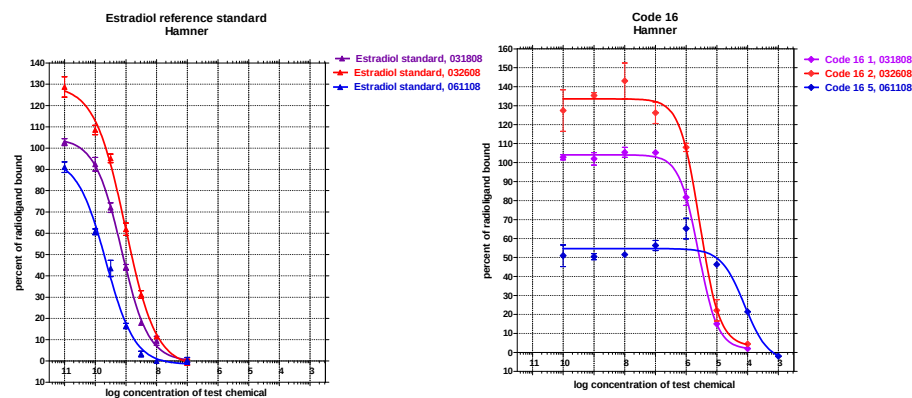


Figure 8. Summary of 17 β -estradiol Standard Curves and Chemical 16 curves

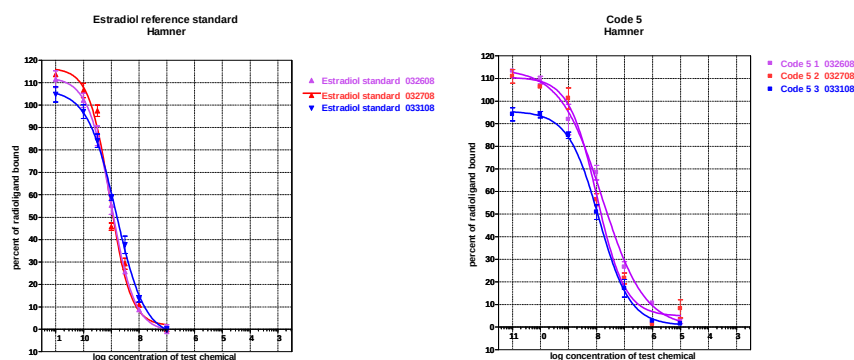


Figure 9. Summary of 17 β -estradiol Standard Curves and Chemical 5 curves

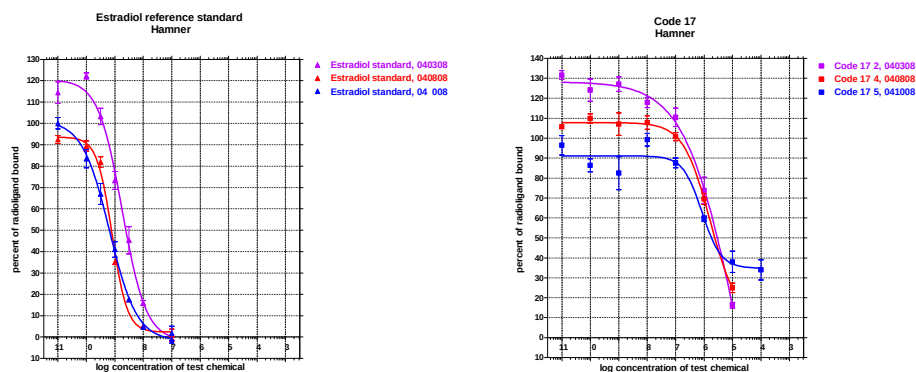


Figure 10. Summary of 17 β -estradiol Standard Curves and Chemical 17 curves

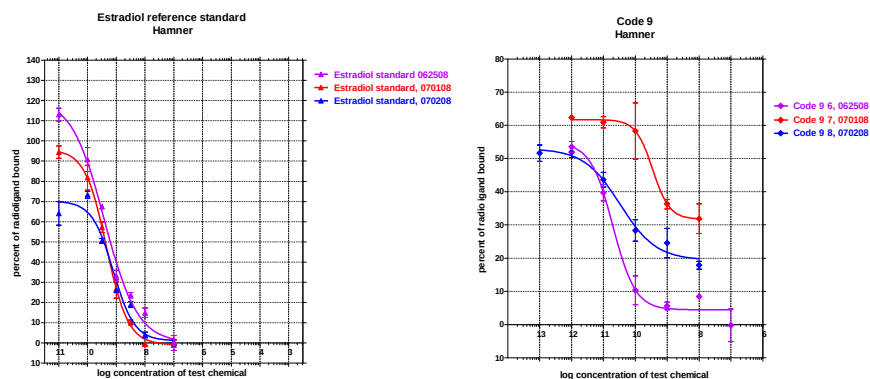


Figure 11. Summary of 17 β -estradiol Standard Curves and Chemical 9 curves

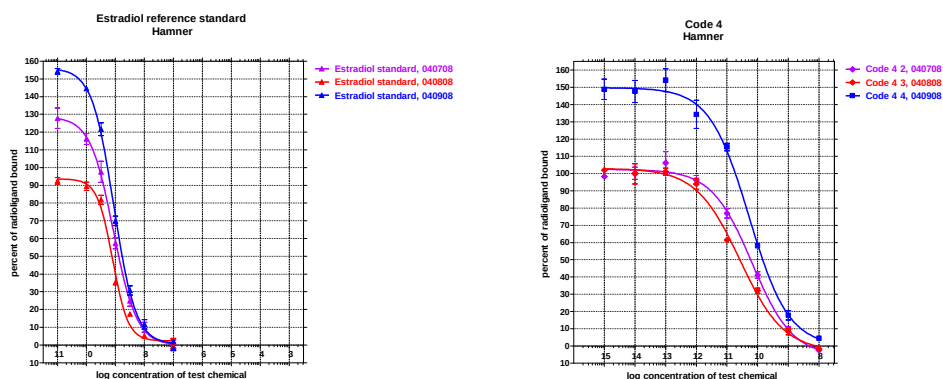


Figure 12. Summary of 17 β -estradiol Standard Curves and Chemical 4 curves

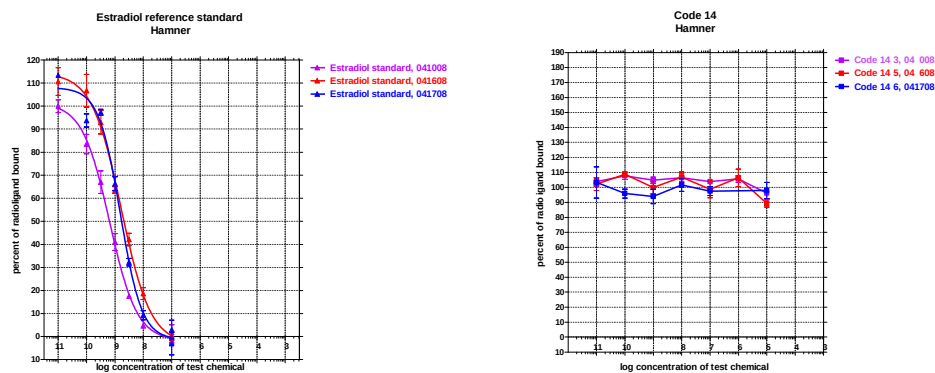


Figure 13. Summary of 17 β -estradiol Standard Curves and Chemical 14 curves

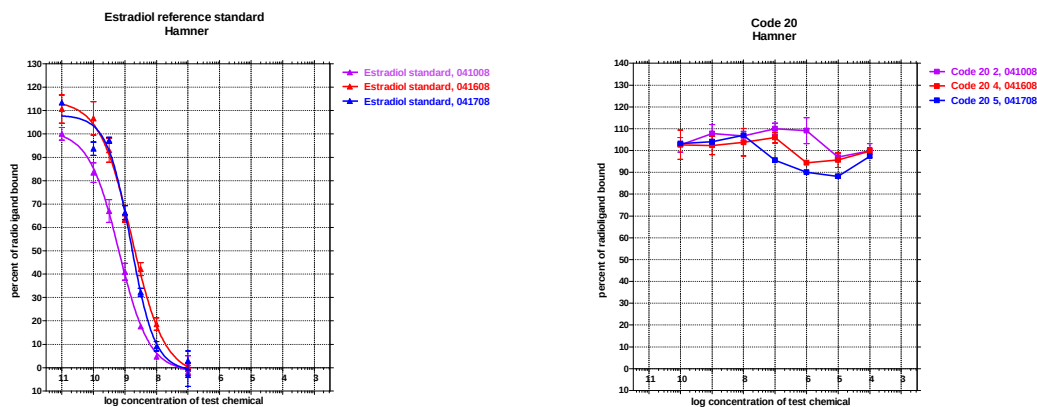


Figure 14. Summary of 17 β -estradiol Standard Curves and Chemical 20 curves

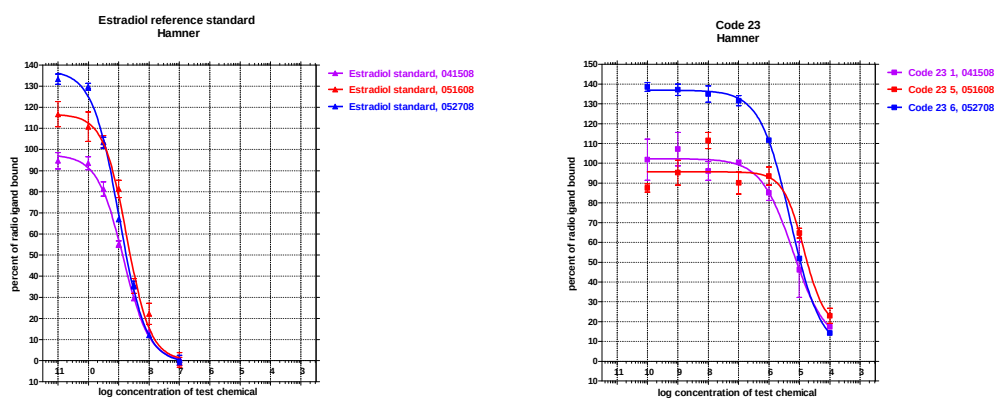


Figure 15. Summary of 17 β -estradiol Standard Curves and Chemical 23 curves

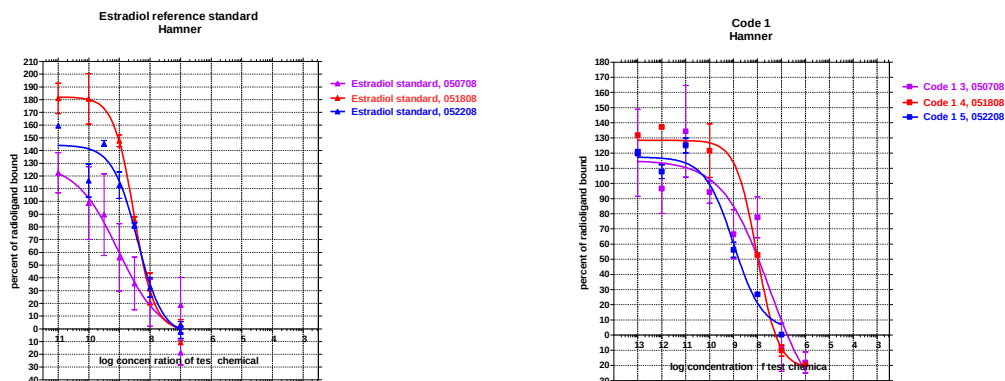


Figure 16. Summary of 17 β -estradiol Standard Curves and Chemical 1 curves

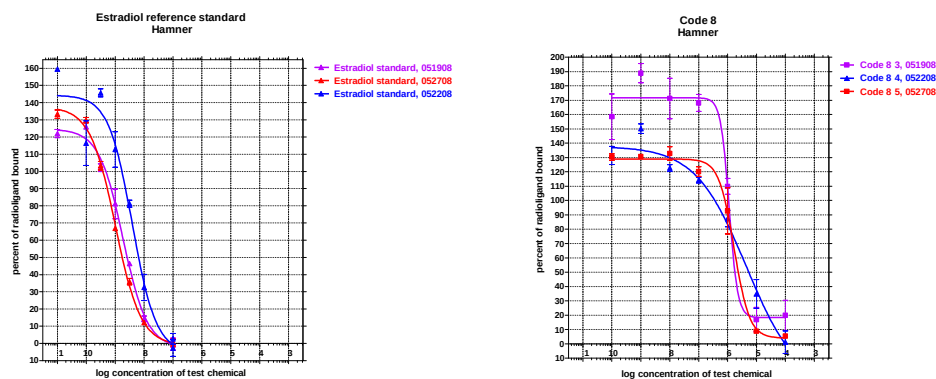


Figure 17. Summary of 17 β -estradiol Standard Curves and Chemical 8 curves

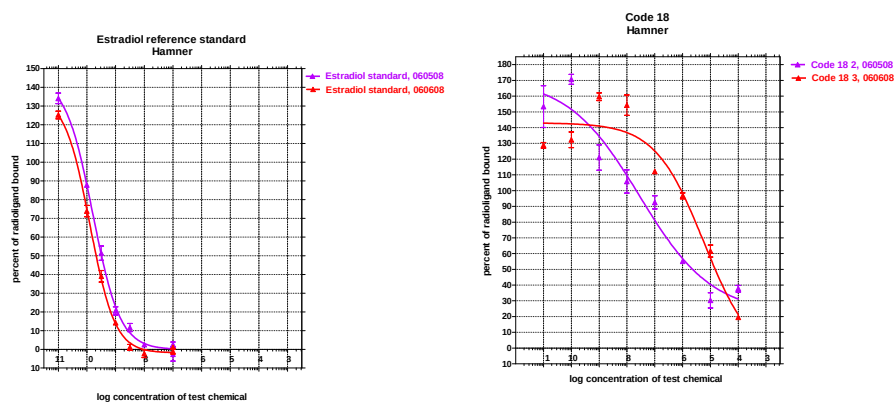


Figure 18. Summary of 17 β -estradiol Standard Curves and Chemical 18 curves

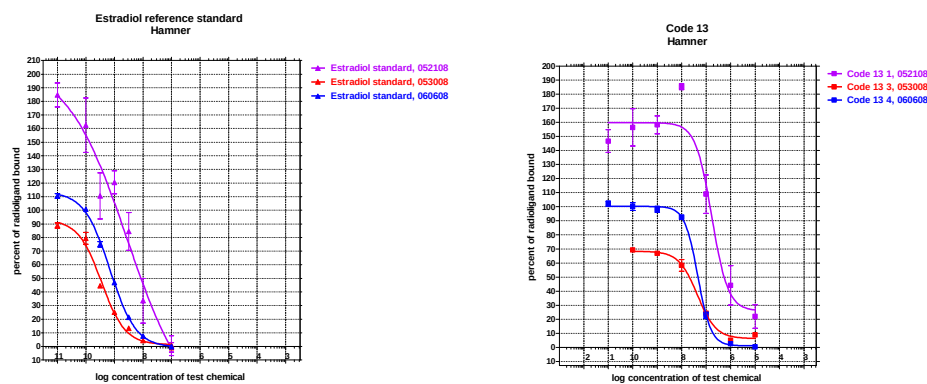


Figure 19. Summary of 17 β -estradiol Standard Curves and Chemical 13 curves

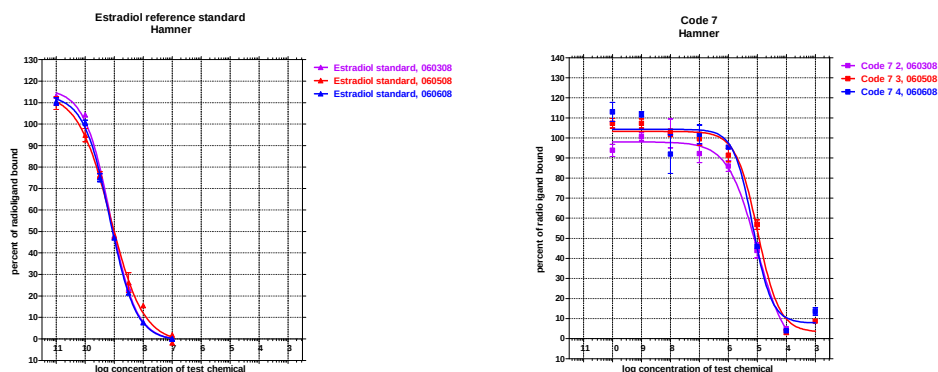


Figure 20. Summary of 17 β -estradiol Standard Curves and Chemical 7 curves

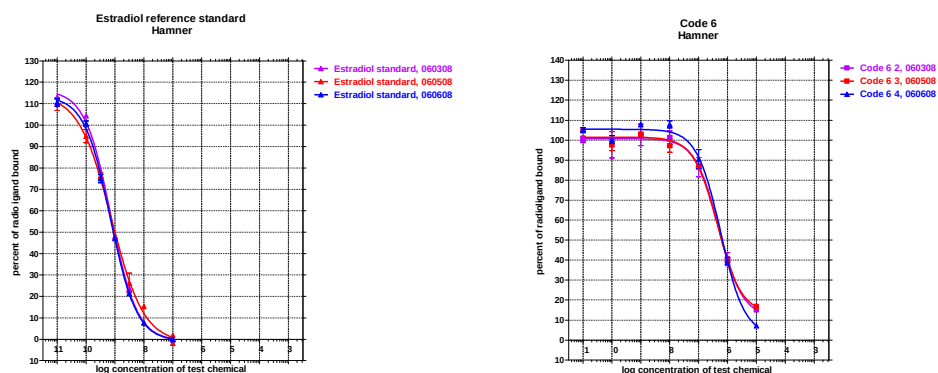


Figure 21. Summary of 17 β -estradiol Standard Curves and Chemical 6 curves

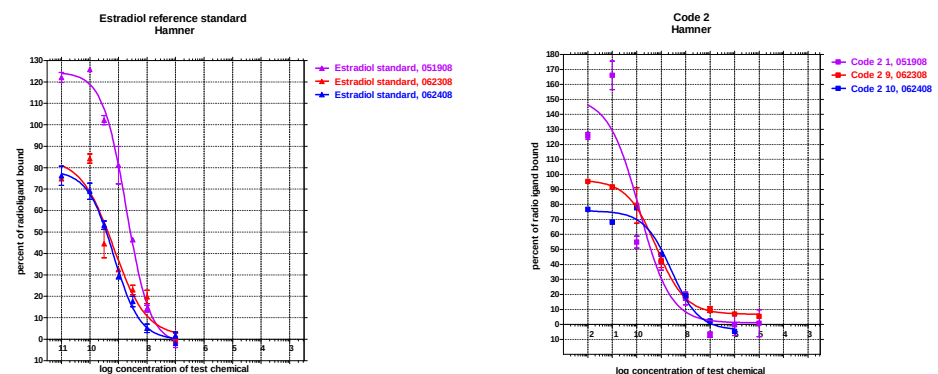


Figure 22. Summary of 17 β -estradiol Standard Curves and Chemical 2 curves

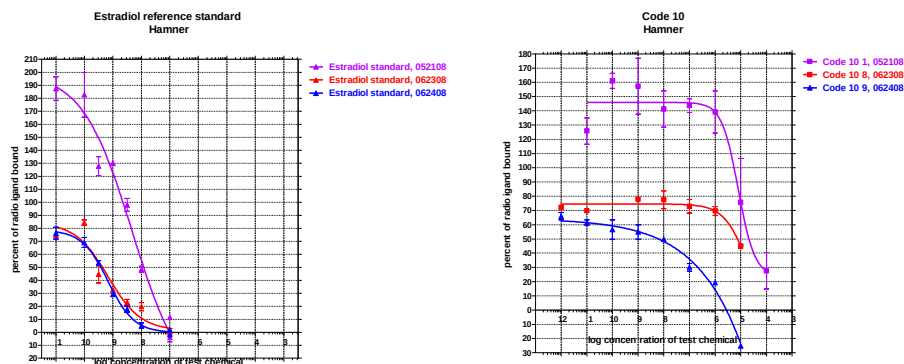


Figure 23. Summary of 17 β -estradiol Standard Curves and Chemical 10 Curves

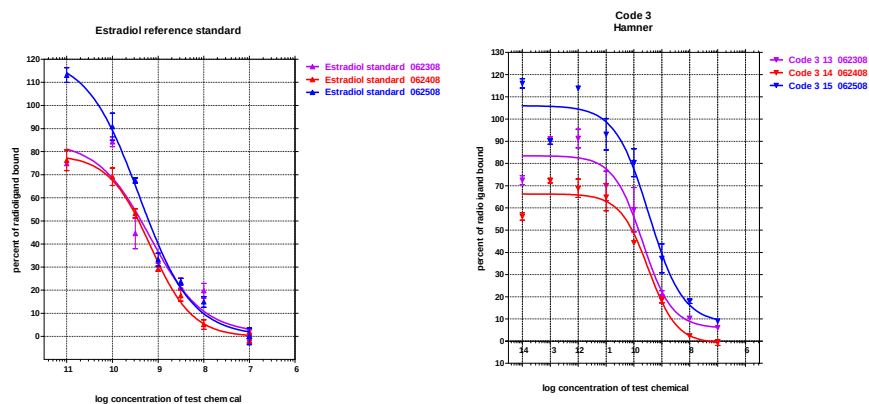


Figure 24. Summary of 17 β -estradiol Standard Curves and Chemical 3 curves

Table 8. Summary of Competition Binding Data for Optional Chemicals

	Log (IC ₅₀)	Relative Binding Affinity	Hill Slope	Classification
CHEM 27 061808	Not Converged	ND	ND	NEGATIVE
CHEM 28 061808	Not Converged	ND	ND	NEGATIVE
CHEM 29 061808	-5.47	2.43E-04	-1.17	POSITIVE
CHEM 32 061908	-7.22	1.46E-02	-0.88	POSITIVE
CHEM 34 061908	-6.84	6.03E-03	-0.82	POSITIVE
CHEM 31 061908	Not Converged	ND	ND	NEGATIVE
CHEM 33 062308	-6.86	7.80E-03	-1.09	POSITIVE
CHEM 30 062308	Not Converged	ND	ND	NEGATIVE
CHEM 36 062308	-5.18	1.61E-04	-0.46	NEGATIVE
CHEM 37 062408	-6.78	6.27E-03	-1.01	POSITIVE
CHEM 38 062408	Not Converged	ND	ND	NEGATIVE
CHEM 39 062408	-5.43	2.84E-04	-1.03	POSITIVE
CHEM 40 062408	-6.92	8.77E-03	-0.96	POSITIVE
CHEM 42 062608	-7.14	2.14E-02	-0.70	POSITIVE
CHEM 35 062608	Not Converged	ND	ND	NEGATIVE
CHEM 43 062608	-7.03	1.65E-02	-0.89	POSITIVE
CHEM 44 062608	-5.30	3.09E-04	-1.02	POSITIVE
CHEM 45 062608	-4.29	3.13E-06	-0.45	NEGATIVE
CHEM 46 062608	Not Converged	ND	ND	NEGATIVE
CHEM 41 062608	-6.68	7.76E-04	-1.13	POSITIVE

ND-Not Determined

SECTION IV: DISCUSSION

All three reference controls (17 β -estradiol, norethynodrel, and R1881) showed reproducible standard curves for the competitive binding assays with the 23 test agents. In addition, the IC₅₀ values (average IC₅₀ values for estradiol: $-8.99 \pm 4.43E-02$ and norethynodrel: $-6.06 \pm 5.16E-02$) were reproducible for each experiment and within the expected values for these compounds. Norethynodrel showed a low, but positive, relative binding affinity ($1.30E-03 \pm 9.59E-05$) when compared to 17 β -estradiol. The average within run SD for 17 β -estradiol ($4.82 \pm 4.54E-01$) was within expected criteria ($< 5\%$). The average within run SD for norethynodrel ($5.03 \pm 5.59E-01$) was within expected criteria ($< 5.7\%$). The average within run SD for R1881 ($6.20 \pm 6.85E-01$) was within the expected criteria ($< 10\%$). The representative average values for Bottom Plateau level ($-0.93 \pm 3.78E-01$), Top Plateau level ($107.80 \pm 1.83E+00$), and Hill slope ($-0.99 \pm 4.44E-02$) for 17 β -estradiol was within criteria. The representative average values for Bottom Plateau level ($4.55 \pm 1.76E+00$), Top Plateau level ($106.08 \pm 1.70E+00$), and Hill slope ($-1.04 \pm 4.37E-02$) for norethynodrel were within expected criteria. Among the twenty-three (23) test agents that were assayed for their relative binding affinity when compared to 17 β -estradiol, fifteen were found to be positive and eight were negative based on the criteria in section 9.7.4 of the EPA Protocol (page 44).

For the 20 optional chemicals 9 were negative and 11 were positive, based on the same criteria in section 9.7.4 of the EPA Protocol. The mean IC₅₀ values for 17 β -estradiol and norethynodrel from 6 competitive binding assays for optional chemicals was calculated to be $-9.12 \pm 1.40E-01$ and $-5.99 \pm 5.76E-02$, respectively. Norethynodrel showed a low, but positive relative binding affinity of $8.91E-04 \pm 1.53E-04$ when compared to 17 β -estradiol. The average within run SD for 17 β -estradiol ($2.98 \pm 3.05E-01$) was within expected criteria ($< 5\%$). The average within run SD for norethynodrel ($3.79 \pm 4.35E-01$) was within expected criteria ($< 5.7\%$). The value for Bottom Plateau level ($-0.90 \pm 5.08E-01$), Top Plateau level ($110.50 \pm 1.14E+01$), and Hill slope ($-0.89 \pm 2.78E-02$) for 17 β -estradiol was within criteria. The representative average values for Bottom Plateau level ($11.2 \pm 3.68E+00$), Top Plateau level ($108.24 \pm 1.10E+01$), and Hill slope ($-0.91 \pm 9.34E-02$) for norethynodrel were within expected criteria.

It is worth mentioning that many of the chemicals had to be rerun (shaded in red) for more than three/five runs as noted in the table below.

Chemical Code	Number of runs performed	Chemical Code	Number of runs performed	Chemical Code	Number of runs performed
1	5	9	8	17	5
2	10	10	9	18	3
3	15	11	3	19	4
4	8	12	5	20	5
5	3	13	4	21	3
6	4	14	6	22	3
7	4	15	3	23	8
8	5	16	5		

Some of the issues are addressed below:

1. For example, a significant number of runs (15 runs) were performed for Chemical Code # 3. The first run using the protocol criteria for making a standard curve yielded data which showed that the chemical had to be diluted extensively in order to attain a reasonable curve. The chemical was diluted to ten thousand-fold (top dose was 5×10^{-6} M) in order to achieve acceptable results. However, some of the variability in the data was also due to the difference in the ratio of ethanol/NSB in the later runs as there was a significant decrease in the percent specific activity in the ethanol samples when compared to the earlier runs. This observation is also relevant for a number of other chemical runs that were performed during the same period. However, when a different batch of [3 H]-17 β -estradiol provided by RTI was used, the variability in the ratio of ethanol/NSB was resolved.
2. It has been observed consistently that the “Bottom Plateau” values for norethynodrel did not meet acceptable criteria objectives. It may be necessary to reexamine the doses selected for norethynodrel and/or change the criteria for acceptance.

3. Due to time constraints, many of the chemical runs were done back to back before one can analyze and examine the data which has resulted in performing many unanticipated runs.

**APPENDIX A: Hamner Protocol, Amendments and
QA Statement**

Protocol for the *In Vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol

The Hamner Institutes for Health Sciences
P.O. Box 12137
Research Triangle Park, NC 27709

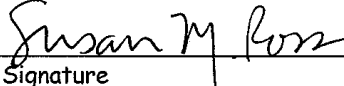
Protocol Number
07024
Page 1 of 6

TITLE: Protocol for the *In Vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol

PRINCIPAL INVESTIGATOR:
Sheela Sharma

 7/16/07
Signature Date

CO-INVESTIGATOR:
Susan Ross

 7/16/07
Signature Date

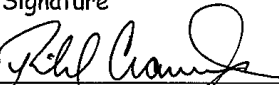
CO-INVESTIGATOR:

N/A
Signature Date

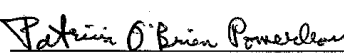
CO-INVESTIGATOR:

N/A
Signature Date

APPROVED BY:

 07 AUG 07
Health, Safety and Environment Date

 7/16/07
Management for Funding Date

 08/07/2007
Quality Assurance (Reviewed Only)

 8-10-07
RTI International (Sponsor) Date
Sponsor's Representative

Issue Date: 08/20/2007

N/A = Not Applicable

1. **BACKGROUND**

The Food Quality Protection Act of 1996 requires the Environmental Protection Agency (EPA) to develop and implement a screening program using validated test systems for determining the potential in humans for estrogenic effects from pesticides. One of the test systems being considered for inclusion in the screening program is an estrogen receptor (ER) binding assay. The ER binding assay protocol uses rat uterine cytosol (RUC) as source of receptor. For further details, see attached protocol dated April 2007 entitled "Protocol for the *In Vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol," hereafter referred to as "the EPA protocol."

2. **OBJECTIVE**

The two components of this project are: (a) generate data for the reference chemical (17 β -estradiol) and weak positive control (norethynodrel) within criteria for acceptable performance by using the EPA protocol and (b) generate data for up to 23 test chemicals using the same EPA protocol.

3. **IDENTIFICATION, HANDLING AND STORAGE OF TEST AGENTS**

Test chemicals as well as any pertinent information regarding test chemical composition and storage requirements will be provided by the Sponsor. The test chemicals are anticipated to be soluble in ethanol or DMSO. The primary routes of exposure include skin absorption, ingestion and inhalation. Personal protective equipment including safety glasses, gloves, and protective clothing will be employed.

Reagents and solutions will be labeled with identity, concentration, expiration date, storage condition, and initials of preparer.

4. **EXPERIMENTAL DESIGN/METHODS**

For details of design/methods, critical materials needed, how assays with test chemicals are run, record keeping, and what should be included in the test reports refer to section(s) 4.0–9.9 of the EPA protocol.

4.1 **Preparation of RUC.**

Rat uteri will be obtained from a reputable supplier. Uteri will be homogenized and ER isolated from the supernatant. Protein concentration of the purified ER will be determined. No IACUC statement will be required; and no animal care or animal health issues will need to be included.

4.2 **Demonstration of acceptable performance of RUC preparation and laboratory technique.**

Prior to routinely conducting the ER competitive binding assays, the RUC must be shown to be performing correctly in the laboratory in which it will be used. This can be accomplished in two steps as follows:

1) Conduct a saturation radioligand binding assay to demonstrate ER specificity and saturation. Nonlinear regression analysis of these data and the subsequent Scatchard plot should document ER binding affinity of the radioligand (K_d) and the number of receptors (B_{max}) for a particular batch of uterine cytosol.

2) Conduct a competitive binding assay using 17 β -estradiol and norethynodrel, which have known affinities for the ER. Comparison of IC₅₀ values (i.e., the concentration of a substance that inhibits [³H]-17 β -estradiol binding by 50%) from these assays with expected values will assist in documenting that the laboratory is performing the assay correctly.

5. DATA COLLECTION

Each assay (saturation and competitive binding) consists of at least three non-consecutive runs and each run contains three replicates. The data generated will be exported to an Excel spreadsheet and formatted for reporting purposes.

6. DATA ANALYSIS AND INTREPRETATION

Considerations for evaluating saturation binding assays are given in Section 8.6 of the EPA protocol but there are no specific performance criteria. Criteria for acceptable performance of known standards in the competitive binding assay are discussed in Section 9.7.3. Before running unknown test chemicals, a lab must meet the performance criteria for each of the standards (17 β -estradiol and norethynodrel) in order to indicate that a technician is capable of performing the assay correctly and consistently.

At least one successful Saturation Binding Assay must be performed each time a new batch of RUC is used in Competitive Binding Assays.

GraphPad Prism, version 4.03 and Microsoft Office XP Excel 2002 will be used for data analysis.

6.1 Criteria for acceptability of saturation binding assay.

ER saturation binding experiments measure total, non-specific, and specific binding of increasing concentrations of [³H]-17 β -estradiol under conditions of equilibrium. A graph of specific [³H]-17 β -estradiol binding versus radioligand concentration should reach a plateau for maximum specific binding indicative of saturation of the ER with the radioligand. In addition, analysis of the data should document the binding of the [³H]-17 β -estradiol to a single, high-affinity binding site (i.e., K_d = 0.05 to 0.5 nM and a linear Scatchard plot).

6.2 Criteria for acceptability of competitive binding assay.

The ER competitive binding assay measures the binding of a single concentration of [³H]-17 β -estradiol in the presence of increasing concentrations of a test substance. The competitive binding curve is plotted as specific [³H]-17 β -estradiol binding versus the concentration (log₁₀ units) of the competitor. The concentration of the test substance that inhibits 50% of the maximum specific [³H]-17 β -estradiol binding is estimated and recorded as the IC₅₀ value.

7. RECORDS

Experimental notes and data will be entered into The Hamner's laboratory notebook(s) and 3 ring binder(s) along with descriptions of procedures used, according to SOP QUA-007 and stored in Lab 210 and/or office 137D/E. After completion of the study, notebooks and other records will be archived at The Hamner.

8. **QUALITY ASSURANCE**

Both quality assurance (QA) and quality control (QC) procedures are integral parts of our research program. Research described in this protocol will be conducted under The Hamner's Research Quality Standards. These standards include: (1) scientifically reviewed protocols that are administratively approved for meeting requirements in data quality and safety regulations; (2) standardized laboratory notebooks and data recording procedures; (3) documented methods and/or SOPs for all experimental procedures including calibration of instruments; (4) all generated data reviewed by a member of the scientific staff for accuracy; and (5) a Quality Assessment audit of the in life phase and/or the draft report conducted by The Hamner's independent Quality Assurance group (SOP QUA-021). The Hamner's QA and QC processes assessing overall study performance and records ensure that conduct of the proposed research satisfies the intended protocol's objectives.

All assays will be conducted in the "spirit" of the Good Laboratory Practice (GLP) Standards as defined in the Federal Register (40 CFR, Part 160). All procedures will be performed in accordance with the EPA Protocol and with the Standard Operating Procedures (SOPs) of the Preclinical Prevention Research Program of The Hamner Institutes for Health Sciences.

9. **HEALTH AND SAFETY**

This study will be conducted in accordance with procedures set forth by The Hamner's Health, Safety, and Environment Office. All chemical and radioactive wastes will be handled and stored according to SOP HSE-034 and RAD-004. Study personnel will review the material safety data sheets (MSDS) for hazardous chemicals used in this study according to SOP HSE-003, controlled substances will be handled according to SOP HSE-024, and radioactive substances will be handled according to SOP RAD-005. A copy of the MSDS will be included in the study manual.

All chemical and their MSDSs along with other pertinent information will be sent to The Hamner from the Sponsor and received by the Health & Safety Department. Susan Ross will have custodial responsibility of all chemicals in use at The Hamner.

Upon completion of the study, all remaining chemicals (radioactive or non-radioactive) including all waste generated by this study will be returned to the Sponsor.

10. **PERSONNEL ASSIGNMENTS**

Principal Investigator	S. Sharma
Senior Research Associate	S. Ross
Research Investigators	B. Wetmore/P. Gao

11. REPORTS

A weekly status report and a monthly progress report will be submitted to the Sponsor, indicating the stage of completion of the requested assays. The final report will be submitted approximately 15 days before completion of the contract.

12. TIMELINE

Project will begin in June 2007 and be completed by November 2007. This timeline may be modified by consensus of the Sponsor and The Hamner.

13. REFERENCES

Cheng, Y and Prusoff, W.H. (1973) Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 percent inhibition (IC_{50}) of an enzymatic reaction. *Biochem. Pharmacol.* 22(23):3099–108.

Hulme, E.C. and Birdsall, N.J.M. (1992) Strategy and tactics in receptor-binding studies. In: *Receptor ligand interactions: a practical approach*. Ed., E.C. Hulme. IRL Press, New York. pp. 63–76.

Kuiper, G., Carlsson, B., Grandien, K., Enmark, E., Haggblad, J., Nilsson, S., Gustafsson, J. (1997) Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors α and β . *Endocrinology* 138(3):863–870.

Kuiper, G., Lemmen, J., Carlsson, B., Corton, J.C., Safe, S., Van Der Saag, P. Van Der Burg, B., Gustafsson, J. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor β . *Endocrinology* 139(10):4252–4263.

14. ATTACHMENTS

- a. Protocol dated April 2007 entitled “Protocol for the *In Vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol.”
- b. Radioactive Materials Use Authorization form will be kept on file at The Hamner.

Amendment

The Hamner Institutes for Health Sciences

Page 1 of 1

PROTOCOL AMENDMENT NO.: 07024A1 *sup 90/12/07*
RE

Protocol Number: 07024

Protocol Title: Protocol for the *In Vitro* Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol

Text/Attachment to be Amended:

The EPA Protocol, page 16 and 17 of 61:

2) Prepare a primary stock in absolute ethanol. For example, since the highest concentration in Column E is 30 nM, a stock concentration that is 300 nM in absolute ethanol would be appropriate. (Other concentrations could be used, but note that if the stock concentration in absolute ethanol is too low, the concentration of ethanol in the final assay tube could exceed the 3% limit for ethanol. See Section 9.2.1. Remember that there is ethanol in the buffer solution that contributes to the concentration of ethanol in the final assay tube.)

Amended to Read:

The EPA Protocol, page 16 of 61:

2) Prepare a primary stock in TEDG + PMSF buffer. For example, since the highest concentration in Column E is 30 nM, a stock concentration that is 300 nM would be appropriate.

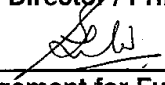
Reason for Amendment: Discrepancy noted by RTI and confirmed as an inconsistency by EPA (see attached email communication dated 9-10-07).

Accompanying Amendments: Email communication from Carol Sloan.

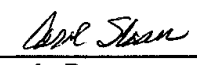

Study Director / Principal Investigator

9/27/07

Date


Management for Funding

Date


Sponsor's Representative

Date

9-28-07

The Hamner Institutes for Health Sciences

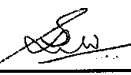
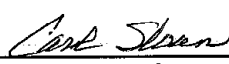
Page 1 of 1

PROTOCOL AMENDMENT NO.: 07024-2
Protocol Number: 07024
Protocol Title: Protocol for the <i>In Vitro</i> Estrogen Receptor Saturation Binding and Competitive Binding Assays Using Rat Uterine Cytosol
Text/Attachment to be Amended:

6. DATA ANALYSIS AND INTREPRETATION

GraphPad Prism, version 4.03 and Microsoft Office XP Excel 2002 will be used for data analysis.

Amended to Read:
GraphPad Prism, version 4.03 or newer version, and Microsoft Office XP Excel 2002, or newer version, will be used for data analysis.
Reason for Amendment:
Newer versions of software made available.

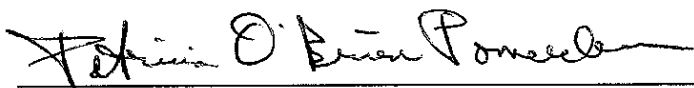
 / Susan M. Rose	3/6/08
Study Director / Principal Investigator	Date
	3/6/08
Management for Funding	Date
	3-24-08
Sponsor's Representative	Date

Statement of Quality Assurance

EPA contract EP-W-06-026, Endocrine Disruptor Screening Program: Laboratory Assay Validation Services, Task Order 6, Second Inter-Laboratory Validation of the Estrogen Receptor (ER) Binding Assay (Rat Uterine Cytosol) during Task 7, Test Coded Chemicals

The above mentioned study was conducted under the Research Quality Standards of The Hamner (The Hamner Institutes) Institutes for Health Sciences. These standards are designed to help assure the quality and integrity of the studies. Quality control procedures are integral parts of our research program. The study was subjected to quality assessments conducted by The Hamner Institutes' independent Quality Assurance personnel (see below).

Quality Assessment Date	Phase Reviewed	Quality Assurance Personnel Performing Quality Assessment
September 04-05, 2007	ER Saturation Binding Assay	Ann Matrone
October 09, 2007	Competitive Binding Assay	Ann Matrone
October 24-25, 2007	Report and Data	Ann Matrone and Patricia O'Brien Pomerleau
April 02, 08 and 11, 2008	ER Competitive Binding Assay And Data	Ann Matrone



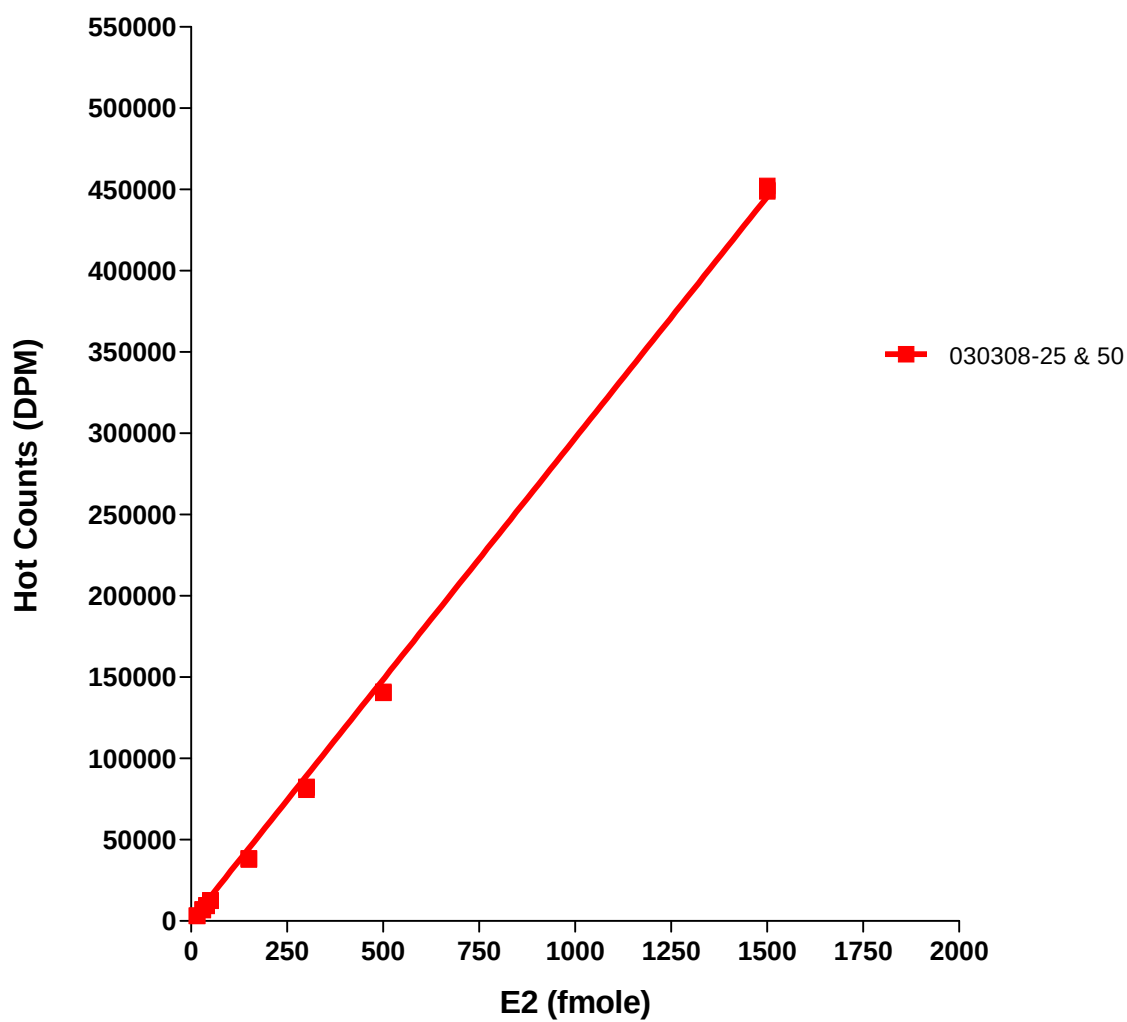
Patricia O'Brien Pomerleau, MS
Quality Assurance Director, CIIT

June 09, 2008

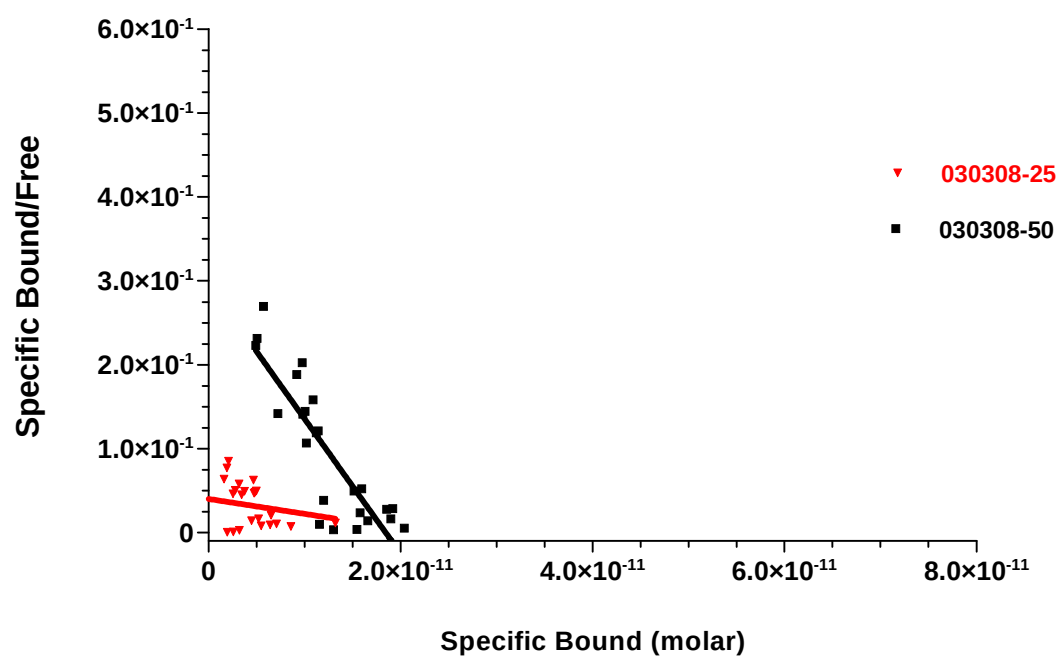
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APPENDIX B: Raw Data and Prism Files for Saturation Binding Assay

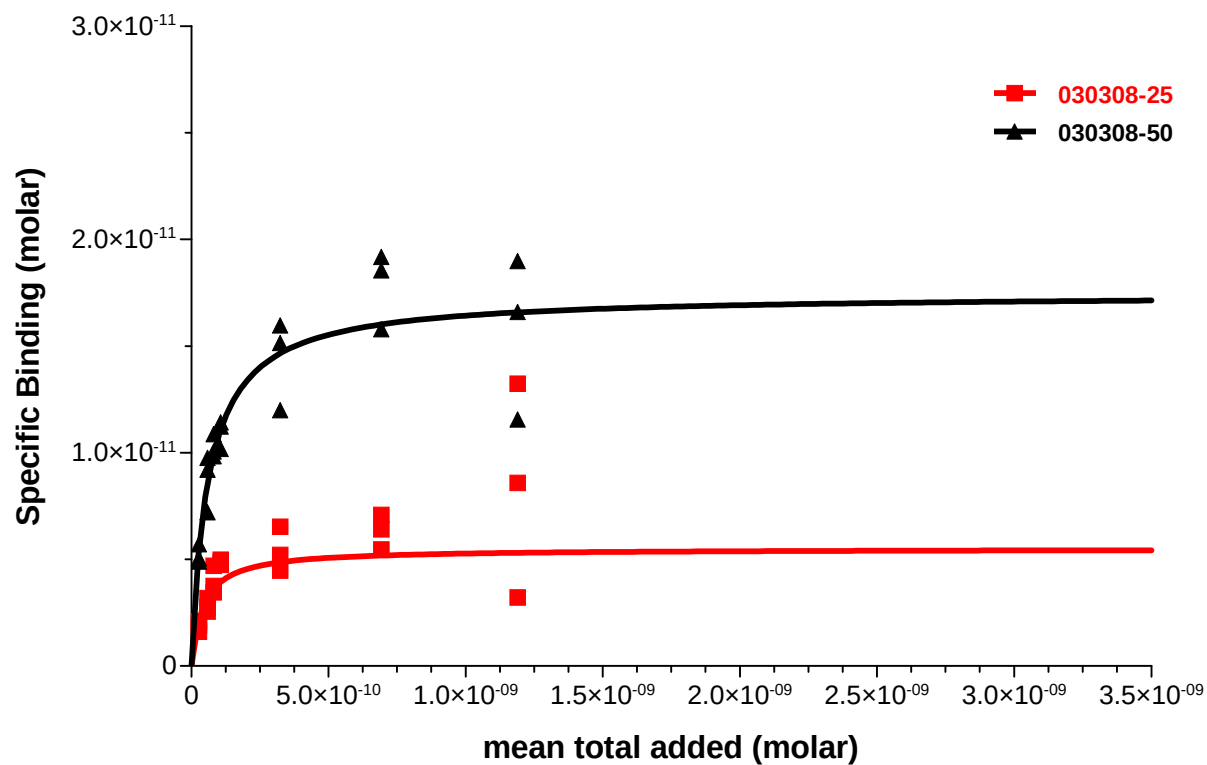
Hot Tubes Lab Hamner



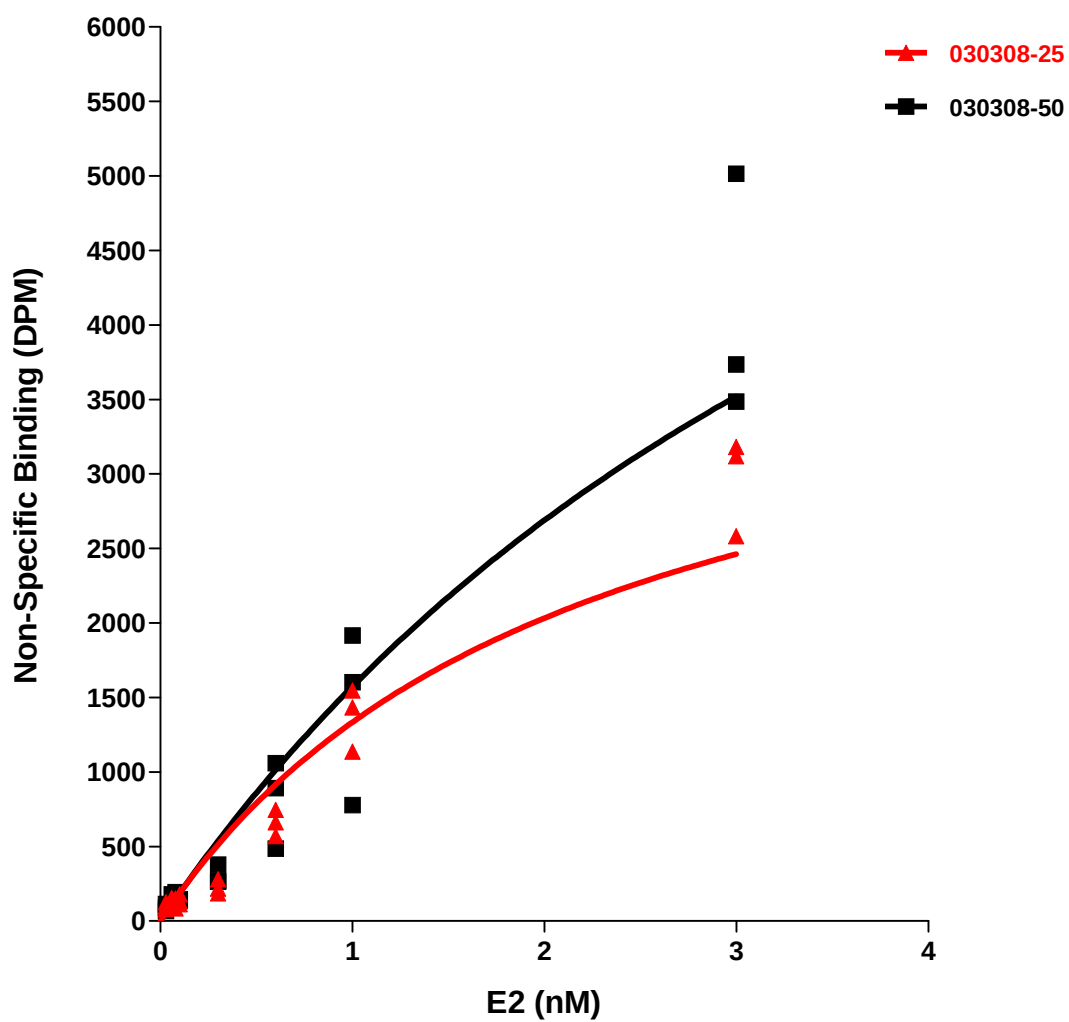
Scatchard Display Lab Hamner

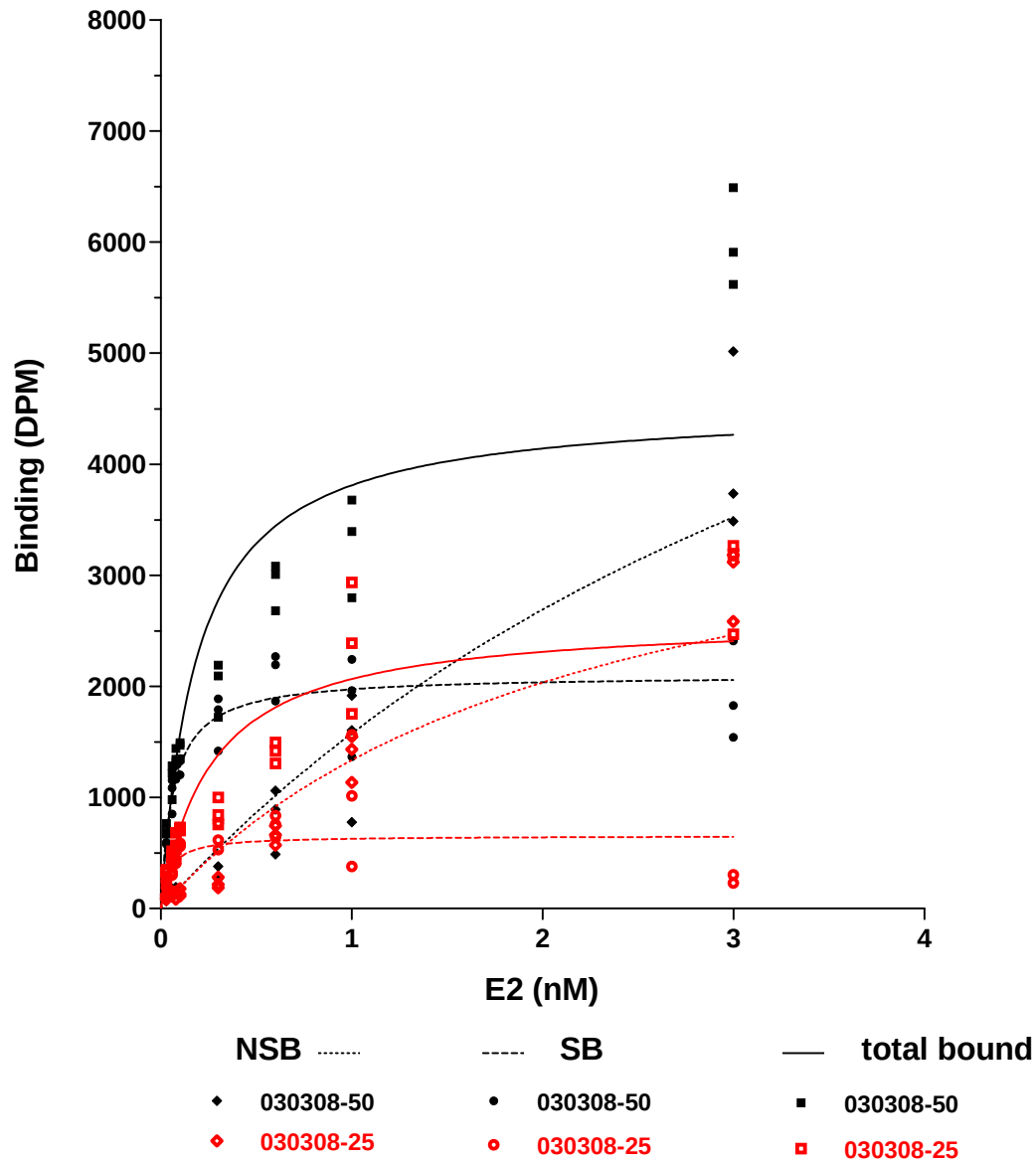


Lab Hamner

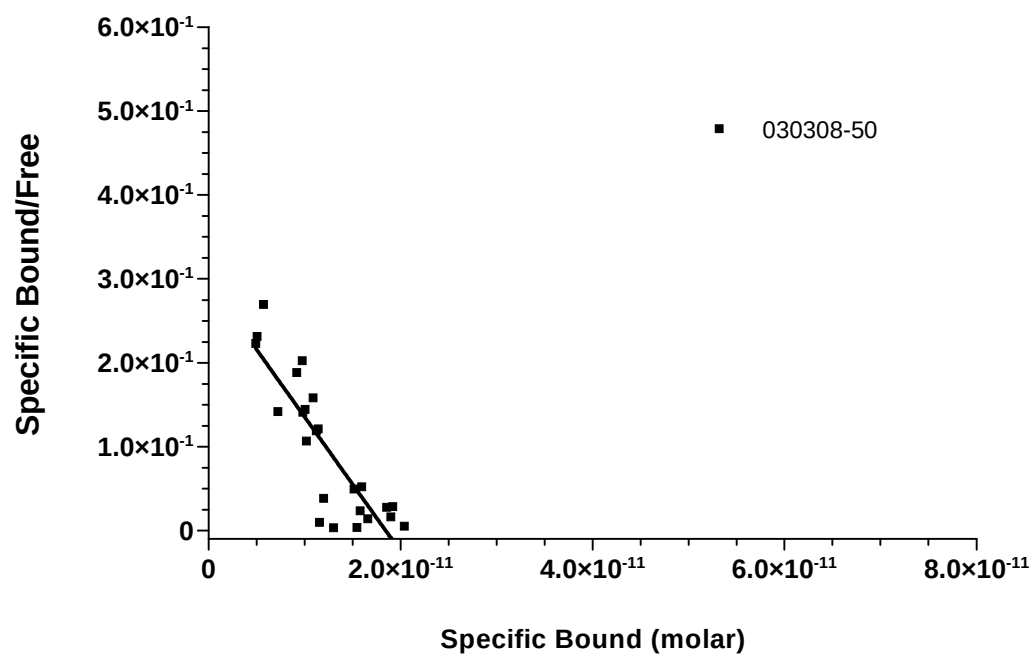


	030308-25	030308-50
BMAX	5.494e-012	2.851e-011
KD	4.197e-011	3.083e-010
Std. Error		
BMAX	9.911e-013	6.650e-013
KD	2.473e-011	1.115e-011
95% Confidence Intervals		
BMAX	3.438e-012 to 7.550e-012	2.713e-011 to 2.989e-011
KD	-9.312e-012 to 9.325e-011	2.852e-010 to 3.314e-010
Goodness of Fit		
Degrees of Freedom	22	22
R ² (unweighted)	0.09708	0.9899
Weighted Sum of Squares (1/Y ²)	7.291	0.05817
Absolute Sum of Squares	2.032e-022	1.632e-023
Sy.x	0.5757	0.05142
Number of points		
Analyzed	24	24

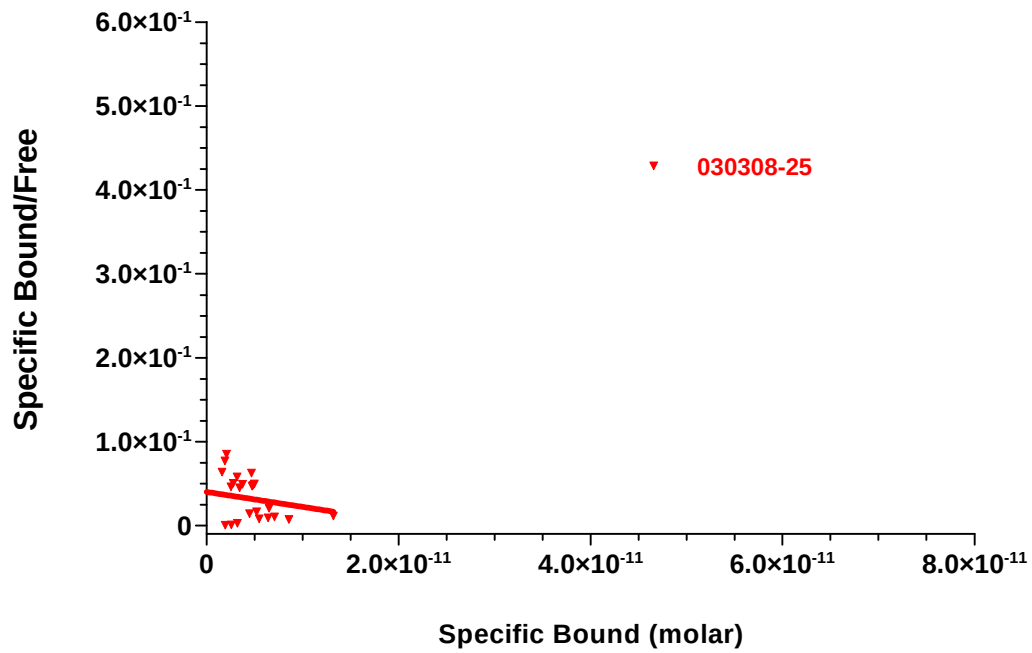
**NSB Tubes
Lab Hamner**

bound counts
Lab Hamner

Scatchard Display
Lab Hamner
030308
0.05 mg/tube



Scatchard Display
Lab Hamner
030308
0.025 mg/tube



Laboratory Code Hammer

ER Saturation Assay

72 assay tubes

Laboratory Code:	Hammer
Run identification:	030308
Assay start date:	3/3/2008

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable,

include the DPM value in column N, and

provide a reason in column Q;

the "TRUE" in column P will automatically change to "FALSE".

For your convenience, data reduction is performed in columns

V through BY, and the values needed for analysis are presented

in columns S, T and U; Prism-ready data in CF - CM.

Cells in column R are presented with a grey background

if the total binding exceeds 10% of the hot added at that concentration,

the cytosol concentration is probably too high for good competitive assays

Tracer lot number:	3589221
Specific activity on day of assay:	106.54 C/mmmole

Cytosol lot or vial number:	PREP-3
protein (cytosol) per tube:	25 ug
protein (cytosol) per tube:	0.025 mg
KD	4.20E-02 nM
Bmax	10.988 fmole/100 ug

volume of ethanol counted	1 mL
multiply DPM in sample by	1.5
total volume in tubes	500 uL

SAT8 030308, Lab Hammer
Sat-template

Saturation Assay Tube Layout										raw dpm		Use this value?	Notes to explain why "Use this value" is set to "FALSE"		Use Ten Percent Rule
Position	Replicate	Tube Type Code	Hot E2 Initial Concentration (nM)	Hot E2 Volume (uL)	Hot E2 Final Concentration (nM)	Cold E2 Initial Concentration (nM)	Cold E2 Volume (uL)	Cold E2 Final Concentration (nM)	Buffer Volume (uL)	Receptor Volume (uL)					
1	1	H	0.3	50	0.03	—	—	—	350	100	218.67	328.005	TRUE		10.0%
2	2	H	0.3	50	0.03	—	—	—	350	100	194.41	291.615	TRUE		8.9%
3	3	H	0.3	50	0.03	—	—	—	350	100	233.06	349.59	TRUE		10.7%
4	1	H	0.6	50	0.06	—	—	—	350	100	344.38	516.57	TRUE		7.4%
5	2	H	0.6	50	0.06	—	—	—	350	100	350	442.665	TRUE		6.3%
6	3	H	0.6	50	0.06	—	—	—	350	100	295.11	468.12	TRUE		6.7%
7	1	H	0.8	50	0.08	—	—	—	350	100	356.25	534.375	TRUE		5.6%
8	2	H	0.8	50	0.08	—	—	—	350	100	380.1	570.15	TRUE		6.0%
9	3	H	0.8	50	0.08	—	—	—	350	100	454.46	681.69	TRUE		7.1%
10	1	H	1.0	50	0.10	—	—	—	350	100	467.83	701.745	TRUE		5.6%
11	2	H	1.0	50	0.10	—	—	—	350	100	476.62	714.93	TRUE		5.7%
12	3	H	1.0	50	0.10	—	—	—	350	100	486.87	730.305	TRUE		5.8%
13	1	H	3.0	50	0.30	—	—	—	350	100	562.88	844.32	TRUE		2.2%
14	2	H	3.0	50	0.30	—	—	—	350	100	505.33	757.995	TRUE		2.0%
15	3	H	3.0	50	0.30	—	—	—	350	100	666.75	1000.125	TRUE		2.6%
16	1	H	6.0	50	0.60	—	—	—	350	100	998.16	1497.24	TRUE		1.8%
17	2	H	6.0	50	0.60	—	—	—	350	100	944.91	1417.365	TRUE		1.7%
18	3	H	6.0	50	0.60	—	—	—	350	100	870.91	1306.365	TRUE		1.6%
19	1	H	10.0	50	1.00	—	—	—	350	100	1592.28	2388.42	TRUE		1.7%
20	2	H	10.0	50	1.00	—	—	—	350	100	1168.33	1752.495	TRUE		1.2%
21	3	H	10.0	50	1.00	—	—	—	350	100	1958.03	2937.045	TRUE		2.1%
22	1	H	30.0	50	3.00	—	—	—	350	100	1648.22	2469.33	TRUE		0.5%
23	2	H	30.0	50	3.00	—	—	—	350	100	2127.72	3191.58	TRUE		0.7%
24	3	H	30.0	50	3.00	—	—	—	350	100	2177.82	3266.73	TRUE		0.7%
25	1	HC	0.3	50	0.03	30	50	3	300	100	53.69	80.535	TRUE		
26	2	HC	0.3	50	0.03	30	50	3	300	100	83.59	125.385	TRUE		
27	3	HC	0.3	50	0.03	30	50	3	300	100	65.46	98.19	TRUE		
28	1	HC	0.6	50	0.06	60	50	6	300	100	76.02	114.03	TRUE		
29	2	HC	0.6	50	0.06	60	50	6	300	100	101.38	152.07	TRUE		
30	3	HC	0.6	50	0.06	60	50	6	300	100	102.57	153.855	TRUE		
31	1	HC	0.8	50	0.08	80	50	8	300	100	95.83	143.745	TRUE		
32	2	HC	0.8	50	0.08	80	50	8	300	100	100.25	150.375	TRUE		
33	3	HC	0.8	50	0.08	80	50	8	300	100	55.97	83.955	TRUE		
34	1	HC	1.0	50	0.10	100	50	10	300	100	118.89	178.335	TRUE		
35	2	HC	1.0	50	0.10	100	50	10	300	100	87.56	131.34	TRUE		
36	3	HC	1.0	50	0.10	100	50	10	300	100	75.35	113.025	TRUE		
37	1	HC	3.0	50	0.30	300	50	30	300	100	187.28	280.92	TRUE		
38	2	HC	3.0	50	0.30	300	50	30	300	100	144.62	216.93	TRUE		
39	3	HC	3.0	50	0.30	300	50	30	300	100	124.36	186.54	TRUE		
40	1	HC	6.0	50	0.60	600	50	60	300	100	497.43	746.145	TRUE		
41	2	HC	6.0	50	0.60	600	50	60	300	100	441.8	662.7	TRUE		
42	3	HC	6.0	50	0.60	600	50	60	300	100	380.47	570.705	TRUE		
43	1	HC	10.0	50	1.00	1000	50	100	300	100	955.4	1433.1	TRUE		
44	2	HC	10.0	50	1.00	1000	50	100	300	100	757.63	1136.445	TRUE		
45	3	HC	10.0	50	1.00	1000	50	100	300	100	1031.39	1547.085	TRUE		
46	1	HC	30.0	50	3.00	3000	50	300	300	100	2078.83	3118.245	TRUE		
47	2	HC	30.0	50	3.00	3000	50	300	300	100	1722.12	2583.18	TRUE		
48	3	HC	30.0	50	3.00	3000	50	300	300	100	2120.82	3181.23	TRUE		
49	1	Hot	0.3	50	0.03	—	—	—	—	—	3249.64	3249.64	TRUE		

50	2	Hot	0.3	50	0.03	—	—	—	—	—	—	3315.85	3315.85	TRUE
51	3	Hot	0.3	50	0.03	—	—	—	—	—	—	3241.3	3241.3	TRUE
52	1	Hot	0.6	50	0.06	—	—	—	—	—	6810.14	6810.14	TRUE	
53	2	Hot	0.6	50	0.06	—	—	—	—	—	6912.33	6912.33	TRUE	
54	3	Hot	0.6	50	0.06	—	—	—	—	—	7234.69	7234.69	TRUE	
55	1	Hot	0.8	50	0.08	—	—	—	—	—	9508.31	9508.31	TRUE	
56	2	Hot	0.8	50	0.08	—	—	—	—	—	9773.82	9773.82	TRUE	
57	3	Hot	0.8	50	0.08	—	—	—	—	—	9406.59	9406.59	TRUE	
58	1	Hot	1.0	50	0.10	—	—	—	—	—	12620	12620	TRUE	
59	2	Hot	1.0	50	0.10	—	—	—	—	—	12627.1	12627.1	TRUE	
60	3	Hot	1.0	50	0.10	—	—	—	—	—	12566.5	12566.5	TRUE	
61	1	Hot	3.0	50	0.30	—	—	—	—	—	38501.3	38501.3	TRUE	
62	2	Hot	3.0	50	0.30	—	—	—	—	—	38391	38391	TRUE	
63	3	Hot	3.0	50	0.30	—	—	—	—	—	37954.3	37954.3	TRUE	
64	1	Hot	6.0	50	0.60	—	—	—	—	—	80799.9	80799.9	TRUE	
65	2	Hot	6.0	50	0.60	—	—	—	—	—	82559.9	82559.9	TRUE	
66	3	Hot	6.0	50	0.60	—	—	—	—	—	82251.9	82251.9	TRUE	
67	1	Hot	10.0	50	1.00	—	—	—	—	—	140382	140382	TRUE	
68	2	Hot	10.0	50	1.00	—	—	—	—	—	141043	141043	TRUE	
69	3	Hot	10.0	50	1.00	—	—	—	—	—	140374	140374	TRUE	
70	1	Hot	30.0	50	3.00	—	—	—	—	—	452306	452306	TRUE	
71	2	Hot	30.0	50	3.00	—	—	—	—	—	449005	449005	TRUE	
72	3	Hot	30.0	50	3.00	—	—	—	—	—	449645	449645	TRUE	

Output to Curve Fitting Software		
Saturation X values	Bound Y values	NSBV values
0.03	328.0	101.4
0.03	291.6	101.4
0.03	349.6	101.4
0.06	516.6	140.0
0.06	442.7	140.0
0.06	468.1	140.0
0.08	534.4	126.0
0.08	570.2	126.0
0.08	681.7	126.0
0.1	701.7	140.9
0.1	714.9	140.9
0.1	730.3	140.9
0.3	844.3	228.1
0.3	758.0	228.1
0.3	1000.1	228.1
0.6	1497.2	659.9
0.6	1417.4	659.9
0.6	1306.4	659.9
1	2388.4	1372.2
1	1752.5	1372.2
1	2937.0	1372.2
3	2469.3	2960.9
3	3191.6	2960.9
3	3266.7	2960.9

SAT8 030308, Lab Hammer
Sat-template

Total Binding – Positions 1-24 radiolabeled E2 plus cytosol (Panel A)													
Tube Identification					Assay tube contents								
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial (nM)	Hot E2 (u)	Cold Conc. Initial (nM)	Cold (u)	Buffer (u)	Cytosol (u)	Hot Conc. Final (nM)	Cold Conc. Final (nM)	Total Volume (u)
030308	1	1	H	c1	0.3	50	0	0	350	100	0.03	0	500
030308	2	2	H	c1	0.3	50	0	0	350	100	0.03	0	500
030308	3	3	H	c1	0.3	50	0	0	350	100	0.03	0	500
030308	4	1	H	c2	0.6	50	0	0	350	100	0.06	0	500
030308	5	2	H	c2	0.6	50	0	0	350	100	0.06	0	500
030308	6	3	H	c2	0.6	50	0	0	350	100	0.06	0	500
030308	7	1	H	c3	0.8	50	0	0	350	100	0.08	0	500
030308	8	2	H	c3	0.8	50	0	0	350	100	0.08	0	500
030308	9	3	H	c3	0.8	50	0	0	350	100	0.08	0	500
030308	10	1	H	c4	1	50	0	0	350	100	0.1	0	500
030308	11	2	H	c4	1	50	0	0	350	100	0.1	0	500
030308	12	3	H	c4	1	50	0	0	350	100	0.1	0	500
030308	13	1	H	c5	3	50	0	0	350	100	0.3	0	500
030308	14	2	H	c5	3	50	0	0	350	100	0.3	0	500
030308	15	3	H	c5	3	50	0	0	350	100	0.3	0	500
030308	16	1	H	c6	6	50	0	0	350	100	0.6	0	500
030308	17	2	H	c6	6	50	0	0	350	100	0.6	0	500
030308	18	3	H	c6	6	50	0	0	350	100	0.6	0	500
030308	19	1	H	c7	10	50	0	0	350	100	1	0	500
030308	20	2	H	c7	10	50	0	0	350	100	1	0	500
030308	21	3	H	c7	10	50	0	0	350	100	1	0	500
030308	22	1	H	c8	30	50	0	0	350	100	3	0	500
030308	23	2	H	c8	30	50	0	0	350	100	3	0	500
030308	24	3	H	c8	30	50	0	0	350	100	3	0	500

SAT8 030308, Lab Hammer
Sat-template

Total Binding -- Positions 1-24 radiolabeled E2 plus cytosol (Panel B)																		
Run	Position	Scintillation Results						Number of molecules						Ratio				
		Non Specific Binding (Mean of reps in pos. 25-48)		Specific Binding (Total - Non Specific)		Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)		Free (mean total added - total bound)		Total Binding molecules (fmole)	Non Specific Binding molecules (fmole)		Specific Binding molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (fmole)	Free (mean total added - total bound) (fmole)	Specific Bound / Free
		(dpm)	(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(dpm)	(dpm)							
030308	1	328.0	101.4	226.6	30.9%	10.0%	3268.9	2940.9	1	0	1	11	10	0.08				
030308	2	291.6	101.4	190.2	34.8%	8.9%	3268.9	2977.3	1	0	1	11	10	0.06				
030308	3	349.6	101.4	248.2	29.0%	10.7%	3268.9	2919.3	1	0	1	11	10	0.09				
030308	4	516.6	140.0	376.6	27.1%	7.4%	6985.7	6469.2	2	0	1	24	22	0.06				
030308	5	442.7	140.0	302.7	31.6%	6.3%	6985.7	6543.1	1	0	1	24	22	0.05				
030308	6	468.1	140.0	328.1	29.9%	6.7%	6985.7	6517.6	2	0	1	24	22	0.05				
030308	7	534.4	126.0	408.4	23.6%	5.6%	9562.9	9028.5	2	0	1	32	30	0.05				
030308	8	570.2	126.0	444.1	22.1%	6.0%	9562.9	8992.8	2	0	1	32	30	0.05				
030308	9	681.7	126.0	555.7	18.5%	7.1%	9562.9	8881.2	2	0	2	32	30	0.06				
030308	10	701.7	140.9	560.8	20.1%	5.6%	12604.5	11902.8	2	0	2	42	40	0.05				
030308	11	714.9	140.9	574.0	19.7%	5.7%	12604.5	11889.6	2	0	2	42	40	0.05				
030308	12	730.3	140.9	589.4	19.3%	5.8%	12604.5	11874.2	2	0	2	42	40	0.05				
030308	13	844.3	228.1	616.2	27.0%	2.2%	37437.9	37437.9	3	1	2	129	126	0.02				
030308	14	758.0	228.1	529.9	30.1%	2.0%	38282.2	37524.2	3	1	2	129	126	0.01				
030308	15	1000.1	228.1	772.0	22.8%	2.6%	38282.2	37282.1	3	1	3	129	126	0.02				
030308	16	1497.2	659.9	837.4	44.1%	1.8%	81870.6	80373.3	5	2	3	276	271	0.01				
030308	17	1417.4	659.9	757.5	46.6%	1.7%	81870.6	80453.2	5	2	3	276	271	0.01				
030308	18	1306.4	659.9	646.5	50.5%	1.6%	81870.6	80564.2	4	2	2	276	271	0.01				
030308	19	2388.4	1372.2	1016.2	57.5%	1.7%	140599.7	138211.2	8	5	3	473	465	0.01				
030308	20	1752.5	1372.2	380.3	78.3%	1.2%	140599.7	138847.2	6	5	1	473	468	0.00				
030308	21	2937.0	1372.2	1564.8	46.7%	2.1%	140599.7	137662.6	10	5	5	473	464	0.01				
030308	22	2469.3	2960.9	-491.6	119.9%	0.5%	450318.7	447849.3	8	10	-2	1516	1508	0.00				
030308	23	3191.6	2960.9	230.7	92.8%	0.7%	450318.7	447127.1	11	10	1	1516	1506	0.00				
030308	24	3266.7	2960.9	305.8	90.6%	0.7%	450318.7	447051.9	11	10	1	1516	1505	0.00				

SAT8 030308, Lab Hammer
Sat-template

Non Specific Binding -- Positions 25-48 radiolabeled E2 plus 100 X inert E2 plus cytosol															
Run	Position	Tube Identification			Assay tube contents								Scintillation Results		
		Rep	Tube Type Code	Conc. Code	Hot Conc. Initial (nM)	Hot (ul)	Cold Conc. Initial (nM)	Cold (ul)	Buffer (ul)	Cytosol (ul)	Hot Conc. Final (nM)	Cold Conc. Final (nM)	Total Binding (dpm)	Non Specific Binding (Mean of reps in pos. 25-48) (dpm)	
030308	25	1	HC		0.3	50	30	50	300	100	0.03	0.03	3	80.5	101.4
030308	26	2	HC		0.3	50	30	50	300	100	0.03	0.03	3	125.4	101.4
030308	27	3	HC		0.3	50	30	50	300	100	0.03	0.03	3	98.2	101.4
030308	28	1	HC		0.6	50	60	50	300	100	0.06	0.06	6	114.0	140.0
030308	29	2	HC		0.6	50	60	50	300	100	0.06	0.06	6	152.1	140.0
030308	30	3	HC		0.6	50	60	50	300	100	0.06	0.06	6	153.9	140.0
030308	31	1	HC		0.8	50	80	50	300	100	0.08	0.08	8	143.7	126.0
030308	32	2	HC		0.8	50	80	50	300	100	0.08	0.08	8	150.4	126.0
030308	33	3	HC		0.8	50	80	50	300	100	0.08	0.08	8	84.0	126.0
030308	34	1	HC		1	50	100	50	300	100	0.1	0.1	10	178.3	140.9
030308	35	2	HC		1	50	100	50	300	100	0.1	0.1	10	131.3	140.9
030308	36	3	HC		1	50	100	50	300	100	0.1	0.1	10	113.0	140.9
030308	37	1	HC		3	50	300	50	300	100	0.3	0.3	30	280.9	228.1
030308	38	2	HC		3	50	300	50	300	100	0.3	0.3	30	216.9	228.1
030308	39	3	HC		3	50	300	50	300	100	0.3	0.3	30	186.5	228.1
030308	40	1	HC		6	50	600	50	300	100	0.6	0.6	60	746.1	659.9
030308	41	2	HC		6	50	600	50	300	100	0.6	0.6	60	662.7	659.9
030308	42	3	HC		6	50	600	50	300	100	0.6	0.6	60	570.7	659.9
030308	43	1	HC		10	50	1000	50	300	100	1	1	100	1433.1	1372.2
030308	44	2	HC		10	50	1000	50	300	100	1	1	100	1136.4	1372.2
030308	45	3	HC		10	50	1000	50	300	100	1	1	100	1547.1	1372.2
030308	46	1	HC		30	50	3000	50	300	100	3	3	300	3118.2	2960.9
030308	47	2	HC		30	50	3000	50	300	100	3	3	300	2583.2	2960.9
030308	48	3	HC		30	50	3000	50	300	100	3	3	300	3181.2	2960.9

Free -- Positions 49-72, radiolabeled E2 without cytosol													
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc.		Counts per Scintillation			Experimental number of molecules		Total Added (Mean of reps in pos. 49-72)	predicted dpm
					Initial	Hot	Molecules of E2	Vial	(uM)	(fmole)	(dpm)		
030308	49	1	Hot	c1	0.3	50	15	3249.6			11	3268.9	3548
030308	50	2	Hot	c1	0.3	50	15	3315.9			11	3268.9	3548
030308	51	3	Hot	c1	0.3	50	15	3241.3			11	3268.9	3548
030308	52	1	Hot	c2	0.6	50	30	6810.1			23	6985.7	7096
030308	53	2	Hot	c2	0.6	50	30	6912.3			23	6985.7	7096
030308	54	3	Hot	c2	0.6	50	30	7234.7			24	6985.7	7096
030308	55	1	Hot	c3	0.8	50	40	9508.3			32	9562.9	9461
030308	56	2	Hot	c3	0.8	50	40	9773.8			33	9562.9	9461
030308	57	3	Hot	c3	0.8	50	40	9406.6			32	9562.9	9461
030308	58	1	Hot	c4	1	50	50	12620.0			42	12604.5	11826
030308	59	2	Hot	c4	1	50	50	12627.1			43	12604.5	11826
030308	60	3	Hot	c4	1	50	50	12566.5			42	12604.5	11826
030308	61	1	Hot	c5	3	50	150	38501.3			130	38282.2	35478
030308	62	2	Hot	c5	3	50	150	38391.0			129	38282.2	35478
030308	63	3	Hot	c5	3	50	150	37954.3			128	38282.2	35478
030308	64	1	Hot	c6	6	50	300	80799.9			272	81870.6	70955
030308	65	2	Hot	c6	6	50	300	82559.9			278	81870.6	70955
030308	66	3	Hot	c6	6	50	300	82251.9			277	81870.6	70955
030308	67	1	Hot	c7	10	50	500	140382.0			473	140599.7	118259
030308	68	2	Hot	c7	10	50	500	141043.0			475	140599.7	118259
030308	69	3	Hot	c7	10	50	500	140374.0			473	140599.7	118259
030308	70	1	Hot	c8	30	50	1500	452306.0			1523	450318.7	354777
030308	71	2	Hot	c8	30	50	1500	449005.0			1512	450318.7	354777
030308	72	3	Hot	c8	30	50	1500	449645.0			1514	450318.7	354777

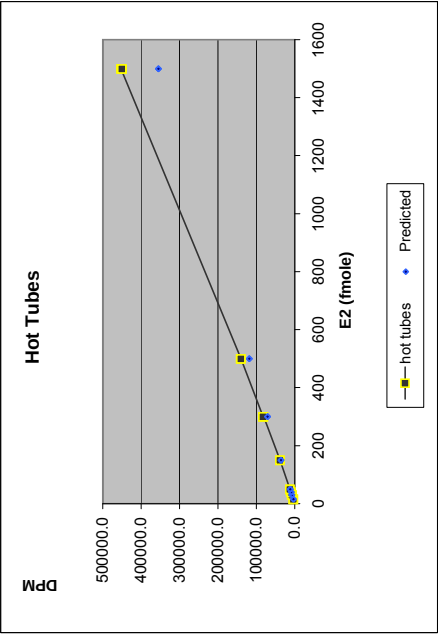
Linear regression results (LINEST function)
(Regression line forced through 0.0)

Slope	296.9573511 dpm/fmole
1/slope	0.003367487 fmole/dpm
origin	x y
end point	0 452306

SLOPE function, used if missing HOT tubes

Slope	301.3 dpm/fmole
1/slope	0.003319 fmole/dpm
origin	x y
end point	0 452306

Computation Check	
3/3/08	specific activity date
106.54	Ci/mMole Estradiol
2.22E+12	DPM/Ci (definition)
2.3652E+14	DPM/nmole
2.3652E+11	DPM/nmole
236.5	DPM/fmole
0.004228	fmole/DPM



Prism input for bound/free	Prism input for specific bound		HOT tubes NSB Tubes Specific bound Total Bound			
	specific bound/molar	bound/free %	average total added molar	specific bound/molar	total added dpm	total bound dpm
1.91643E-12	0.0771		2.76E-11	1.9164E-12	3249.64	328.01
1.60871E-12	0.0639		2.76E-11	1.6087E-12	3315.85	226.64
2.09895E-12	0.0850		2.76E-11	2.0989E-12	3241.30	190.25
3.1844E-12	0.0582		5.91E-11	3.1844E-12	6810.14	248.22
2.55946E-12	0.0463		5.91E-11	2.5595E-12	6912.33	114.03
2.77471E-12	0.0503		5.91E-11	2.7747E-12	7234.69	152.07
3.45301E-12	0.0452		8.09E-11	3.4530E-12	9508.31	153.86
3.75552E-12	0.0494		8.09E-11	3.7555E-12	9773.82	143.75
4.69871E-12	0.0626		8.09E-11	4.6987E-12	9406.59	150.38
4.74251E-12	0.0471		1.07E-10	4.7425E-12	12620.00	83.96
4.854E-12	0.0483		1.07E-10	4.8540E-12	12627.10	178.34
4.98401E-12	0.0496		1.07E-10	4.9840E-12	12566.50	131.34
5.21051E-12	0.0165		3.24E-10	5.2105E-12	38501.30	113.03
4.48054E-12	0.0141		3.24E-10	4.4805E-12	38391.00	280.92
6.52799E-12	0.0207		3.24E-10	6.5280E-12	37954.30	216.93
7.08097E-12	0.0104		6.92E-10	7.0810E-12	80799.90	186.54
6.40555E-12	0.0094		6.92E-10	6.4056E-12	82559.90	746.15
5.46693E-12	0.0080		6.92E-10	5.4669E-12	82251.90	662.70
8.59308E-12	0.0074		1.19E-09	8.5931E-12	140382.00	570.71
3.21569E-12	0.0027		1.19E-09	3.2157E-12	141043.00	1433.10
1.32323E-11	0.0114		1.19E-09	1.3232E-11	140374.00	1136.45
-4.15665E-12	-0.0011		3.81E-09	-4.1566E-12	452306.00	1547.09
1.95076E-12	0.0005		3.81E-09	1.9508E-12	449005.00	3118.25
2.58623E-12	0.0007		3.81E-09	2.5862E-12	449645.00	2583.18
						3181.23
						305.85
						3266.73

(total volume in tube uL/1000)	mole to molar conversion value	Free (total added - bound)/ dpm	Free (total added - bound)/ molar	specific bound/dpm	mean specific bound/dpm	mean specific bound/molar	sp/free %	use for bound/free	
0.5	0.0005	2940.9	2.48685E-11	226.6	221.7	1.8747E-12	7.71%	specific bound/molar	bound/free %
		2977.3	2.51762E-11	190.2		0	6.39%	1.91643E-12	7.71%
		2919.3	2.46860E-11	248.2		0	8.50%	1.60871E-12	6.39%
		6469.2	5.47032E-11	376.6	335.8	2.83953E-12	5.82%	3.1844E-12	8.50%
		6543.1	5.53281E-11	302.7		0	4.63%	2.55946E-12	5.82%
		6517.6	5.51129E-11	328.1		0	5.03%	2.77471E-12	4.63%
		9028.5	7.63453E-11	408.4	469.4	3.96908E-12	4.52%	3.45301E-12	5.03%
		8992.8	7.60428E-11	444.1		0	4.94%	3.75552E-12	4.52%
		8881.2	7.50996E-11	555.7		0	6.26%	4.69871E-12	4.94%
		11902.8	1.00650E-10	560.8	574.8	4.86017E-12	4.71%	4.74251E-12	6.26%
		11889.6	1.00539E-10	574.0		0	4.83%	4.854E-12	4.71%
		11874.2	1.00409E-10	589.4		0	4.96%	4.98401E-12	4.83%
		37437.9	3.16575E-10	616.2	639.4	5.40635E-12	1.65%	5.21051E-12	4.96%
		37524.2	3.17305E-10	529.9		0	1.41%	4.48054E-12	1.65%
		37282.1	3.15257E-10	772.0		0	2.07%	6.52799E-12	1.41%
		80373.3	6.79637E-10	837.4	747.1	6.31782E-12	1.04%	7.08097E-12	2.07%
		80453.2	6.80313E-10	757.5		0	0.94%	6.40555E-12	1.04%
		80564.2	6.81251E-10	646.5		0	0.80%	5.46693E-12	0.94%
		138211.2	1.16872E-09	1016.2	987.1	8.34701E-12	0.74%	8.59308E-12	0.80%
		138847.2	1.17409E-09	380.3		0	0.27%	3.21569E-12	0.74%
		137662.6	1.16408E-09	1564.8		0	1.14%	1.32323E-11	0.27%
		447649.3	3.78702E-09	-491.6	15.0	1.26798E-13	-0.11%	-4.15659E-12	1.14%
		447127.1	3.78091E-09	230.7		0	0.05%	1.95076E-12	-0.11%
		447051.9	3.78027E-09	305.8		0	0.07%	2.58623E-12	0.05%

Molecules of E2 (fmole)	total added dpm		mean total added dpm	total added molar		use for specific bound	
						average total added molar	specific bound/molar
15	3249.64		3288.93	2.7479E-11		2.76421E-11	1.91643E-12
15	3315.85			2.80388E-11		2.76421E-11	1.60871E-12
15	3241.30			2.74084E-11		2.76421E-11	2.09895E-12
30	6810.14		6985.72	5.75866E-11		5.90713E-11	3.1844E-12
30	6912.33			5.84507E-11		5.90713E-11	2.55946E-12
30	7234.69			6.11766E-11		5.90713E-11	2.77471E-12
40	9508.31		9562.91	8.04023E-11		8.0864E-11	3.45301E-12
40	9773.82			8.26475E-11		8.0864E-11	3.75552E-12
40	9406.59			7.95422E-11		8.0864E-11	4.69871E-12
50	12620.00		12604.53	1.06715E-10		1.06584E-10	4.74251E-12
50	12627.10			1.06775E-10		1.06584E-10	4.854E-12
50	12866.50			1.06262E-10		1.06584E-10	4.98401E-12
150	38501.30		38282.20	3.25567E-10		3.23714E-10	5.21051E-12
150	38391.00			3.24634E-10		3.23714E-10	4.48054E-12
150	37954.30			3.20942E-10		3.23714E-10	6.52799E-12
300	80799.90		81870.57	6.83244E-10		6.92298E-10	7.08097E-12
300	82559.90			6.98127E-10		6.92298E-10	6.40555E-12
300	82251.90			6.95522E-10		6.92298E-10	5.46693E-12
500	140382.00		140599.67	1.18707E-09		1.18891E-09	8.59308E-12
500	141043.00			1.19266E-09		1.18891E-09	3.21569E-12
500	140374.00			1.187E-09		1.18891E-09	1.32323E-11
1500	452306.00		450318.67	3.8247E-09		3.8079E-09	4.15659E-12
1500	449005.00			3.79679E-09		3.8079E-09	1.95076E-12
1500	449645.00			3.8022E-09		3.8079E-09	2.58623E-12

total bound dpm	total bound/molar	average bound/dpm	average bound/molar	NSB dpm	NSB molar	Hot Final Concentration (nM)
328.0	2.77361E-12	323.07	2.73188E-12	80.5	6.81004E-13	0.03
291.6	2.4659E-12		0	125.4	1.06028E-12	0.03
349.6	2.95613E-12		0	98.2	8.30296E-13	0.03
516.6	4.36812E-12	475.79	4.02324E-12	114.0	9.64238E-13	0.06
442.7	3.74318E-12		0	152.1	1.2859E-12	0.06
468.1	3.95843E-12		0	153.9	1.301E-12	0.06
534.4	4.51868E-12	595.41	5.03475E-12	143.7	1.21551E-12	0.08
570.2	4.82119E-12		0	150.4	1.27157E-12	0.08
681.7	5.76437E-12		0	84.0	7.09924E-13	0.08
701.7	5.93396E-12	715.66	6.05162E-12	178.3	1.508E-12	0.10
714.9	6.04545E-12		0	131.3	1.11061E-12	0.10
730.3	6.17546E-12		0	113.0	9.5574E-13	0.10
844.3	7.13957E-12	867.48	7.33542E-12	280.9	2.37546E-12	0.30
758.0	6.40961E-12		0	216.9	1.83438E-12	0.30
1000.1	8.45706E-12		0	186.5	1.57738E-12	0.30
1497.2	1.26607E-11	1406.99	1.18978E-11	746.1	6.30941E-12	0.60
1417.4	1.19852E-11		0	662.7	5.60379E-12	0.60
1306.4	1.10466E-11		0	570.7	4.82588E-12	0.60
2388.4	2.01965E-11	2359.32	1.99504E-11	1433.1	1.21183E-11	1.00
1752.5	1.48191E-11		0	1136.4	9.60978E-12	1.00
2937.0	2.48357E-11		0	1547.1	1.30822E-11	1.00
2469.3	2.08807E-11	2975.88	2.51641E-11	3118.2	2.63679E-11	3.00
3191.6	2.6988E-11		0	2583.2	2.18434E-11	3.00
3266.7	2.76235E-11		0	3181.2	2.69006E-11	3.00

Bmax molar	5.49E-12	4.20E-11
mole to molar conversion value	0.0005	1.00E+09
DPM/mole = (DPM/nmole)*1000	2.37E+17	4.20E-02
Bmax molar to Bmax moles	2.747E+15	
= DPM/((DPM/nmole)*1000)	2.747E-15	
=Bmax DPM	6.50E+02	
assay date	3/3/2008	
Bmax(dpm)	649.715783	
DPM/Ci (definition)	2.22E+12	
Ci/nmole	106.54	
DPM/nmole	2.37E+14	
DPM/pmol	2.37E+05	
1/(DPM/nmole)	4.23E-15	
1/(DPM/pmol)	4.23E-06	
SA(dpm/pmol)	2.37E+05	
protein/tube (ug)	25	
protein /tube(mg)	0.025	
bmax pmole	0.002747	
bmax pmole/mg	0.10988	
Bmax fmole/mg	109.88	
Bmax (fmole/100 ug)	10.988	
Bmax(fmole/100 ug)/Bmax molar	2.00E+12	

Laboratory Code Hammer

ER Saturation Assay
72 assay tubes

Provide information in all blue cells in columns N and DM

If the DPM value for a tube was judged unreliable, include the DPM value in column Q; provide a reason in column Q; the "TRUE" in column P will automatically change to "FALSE". For your convenience, data reduction is performed in columns V through BY, and the values needed for analysis are presented in columns S, T and U; Phen-ready data in CF - CM.

Cells in column R are presented with a grey background. If the total binding exceeds 10% of the total added at that concentration, the cytosol concentration is probably too high for good competitive assays

Laboratory Code: Hammer
Run Identification: 030308
Assay start date: 3/3/2008

Tracer lot number: 3589221
Specific activity on day of assay: 106.54 Ci/mmol

Cytosol lot or vial number: PREP-3
protein (cytosol) per tube: 50 ug
protein (cytosol) per tube: 0.05 mg
KD: 3.08E-07 nM
Bmax: 28.5 fmole/100 ug

volume of ethanol counted: 1 mL
multiply DPM in sample by: 1.5
total volume in tubes 500 uL

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot E2 Initial Concentration (nM)	Hot E2 Volume (uL)	Hot E2 Final Concentration (nM)	Cold E2 Initial Concentration (nM)	Cold E2 Volume (uL)	Cold E2 Final Concentration (nM)	Buffer Volume (uL)	Receptor Volume (uL)
1	1	H	0.3	50	0.03	—	—	—	350	100
2	2	H	0.3	50	0.03	—	—	—	350	100
3	3	H	0.3	50	0.03	—	—	—	350	100
4	1	H	0.6	50	0.06	—	—	—	350	100
5	2	H	0.6	50	0.06	—	—	—	350	100
6	3	H	0.6	50	0.06	—	—	—	350	100
7	1	H	0.8	50	0.08	—	—	—	350	100
8	2	H	0.8	50	0.08	—	—	—	350	100
9	3	H	0.8	50	0.08	—	—	—	350	100
10	1	H	1.0	50	0.10	—	—	—	350	100
11	2	H	1.0	50	0.10	—	—	—	350	100
12	3	H	1.0	50	0.10	—	—	—	350	100
13	1	H	3.0	50	0.30	—	—	—	350	100
14	2	H	3.0	50	0.30	—	—	—	350	100
15	3	H	3.0	50	0.30	—	—	—	350	100
16	1	H	6.0	50	0.60	—	—	—	350	100
17	2	H	6.0	50	0.60	—	—	—	350	100
18	3	H	6.0	50	0.60	—	—	—	350	100
19	1	H	10.0	50	1.00	—	—	—	350	100
20	2	H	10.0	50	1.00	—	—	—	350	100
21	3	H	10.0	50	1.00	—	—	—	350	100
22	1	H	30.0	50	3.00	—	—	—	350	100
23	2	H	30.0	50	3.00	—	—	—	350	100
24	3	H	30.0	50	3.00	—	—	—	350	100
25	1	HC	0.3	50	0.03	30	50	3	300	100
26	2	HC	0.3	50	0.03	30	50	3	300	100
27	3	HC	0.3	50	0.03	30	50	3	300	100
28	1	HC	0.6	50	0.06	60	50	6	300	100
29	2	HC	0.6	50	0.06	60	50	6	300	100
30	3	HC	0.6	50	0.06	60	50	6	300	100
31	1	HC	0.8	50	0.08	80	50	8	300	100
32	2	HC	0.8	50	0.08	80	50	8	300	100
33	3	HC	0.8	50	0.08	80	50	8	300	100
34	1	HC	1.0	50	0.10	100	50	10	300	100
35	2	HC	1.0	50	0.10	100	50	10	300	100
36	3	HC	1.0	50	0.10	100	50	10	300	100
37	1	HC	3.0	50	0.30	300	50	30	300	100
38	2	HC	3.0	50	0.30	300	50	30	300	100
39	3	HC	3.0	50	0.30	300	50	30	300	100
40	1	HC	6.0	50	0.60	600	50	60	300	100
41	2	HC	6.0	50	0.60	600	50	60	300	100
42	3	HC	6.0	50	0.60	600	50	60	300	100
43	1	HC	10.0	50	1.00	1000	50	100	300	100
44	2	HC	10.0	50	1.00	1000	50	100	300	100
45	3	HC	10.0	50	1.00	1000	50	100	300	100
46	1	HC	30.0	50	3.00	3000	50	300	300	100
47	2	HC	30.0	50	3.00	3000	50	300	300	100
48	3	HC	30.0	50	3.00	3000	50	300	300	100
49	1	Hdt	0.3	50	0.03	—	—	—	—	—
50	2	Hdt	0.3	50	0.03	—	—	—	—	—
51	3	Hdt	0.3	50	0.03	—	—	—	—	—
52	1	Hdt	0.6	50	0.06	—	—	—	—	—
53	2	Hdt	0.6	50	0.06	—	—	—	—	—
54	3	Hdt	0.6	50	0.06	—	—	—	—	—
55	1	Hdt	0.8	50	0.08	—	—	—	—	—
56	2	Hdt	0.8	50	0.08	—	—	—	—	—
57	3	Hdt	0.8	50	0.08	—	—	—	—	—
58	1	Hdt	1.0	50	0.10	—	—	—	—	—
59	2	Hdt	1.0	50	0.10	—	—	—	—	—
60	3	Hdt	1.0	50	0.10	—	—	—	—	—
61	1	Hdt	3.0	50	0.30	—	—	—	—	—
62	2	Hdt	3.0	50	0.30	—	—	—	—	—
63	3	Hdt	3.0	50	0.30	—	—	—	—	—
64	1	Hdt	6.0	50	0.60	—	—	—	—	—
65	2	Hdt	6.0	50	0.60	—	—	—	—	—
66	3	Hdt	6.0	50	0.60	—	—	—	—	—
67	1	Hdt	10.0	50	1.00	—	—	—	—	—
68	2	Hdt	10.0	50	1.00	—	—	—	—	—
69	3	Hdt	10.0	50	1.00	—	—	—	—	—
70	1	Hdt	30.0	50	3.00	—	—	—	—	—
71	2	Hdt	30.0	50	3.00	—	—	—	—	—
72	3	Hdt	30.0	50	3.00	—	—	—	—	—

raw dpm	dpm for 1.5 mL	Use this value? Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule
459	688.5	TRUE	21.1%
510.73	766.095	TRUE	23.4%
448.85	670.275	TRUE	20.5%
810.56	1215.84	TRUE	17.4%
654.13	981.195	TRUE	14.0%
855.22	1282.83	TRUE	18.4%
986.91	1345.355	TRUE	14.1%
1379.48	1591.97	TRUE	13.5%
1591.97	1591.97	TRUE	15.1%
951.48	1432.22	TRUE	11.7%
979.07	1469.955	TRUE	10.7%
897.26	1345.89	TRUE	11.8%
995.33	1492.995	TRUE	4.5%
1148.33	1722.495	TRUE	5.5%
1396.89	2095.335	TRUE	5.7%
1461.4	2192.1	TRUE	3.3%
1787.14	2680.71	TRUE	3.8%
2054.51	3081.765	TRUE	3.7%
1858.68	2568.68	TRUE	2.6%
1688.03	2399.045	TRUE	2.6%
2451.78	3677.67	TRUE	2.4%
2263.78	3395.67	TRUE	1.3%
3938.87	5908.305	TRUE	1.2%
3746.59	5619.885	TRUE	1.4%
4325.5	6488.25	TRUE	
44.92	67.38	TRUE	
76.78	115.17	TRUE	
59.85	89.775	TRUE	
119.07	178.605	TRUE	
120.07	180.105	TRUE	
65.07	97.695	TRUE	
84.13	126.195	TRUE	
99.29	148.935	TRUE	
129.7	194.55	TRUE	
97	145.5	TRUE	
97.15	145.725	TRUE	
90.85	136.275	TRUE	
178.76	269.64	TRUE	
178.15	264.225	TRUE	
378.45	567.675	TRUE	
594.62	891.93	TRUE	
324.73	487.095	TRUE	
706.75	1060.125	TRUE	
1277.88	1916.82	TRUE	
1068.97	1603.455	TRUE	
518.9	778.35	TRUE	
2324.6	3486.9	TRUE	
2490.04	3735.06	TRUE	
3343.95	5015.925	TRUE	
3349.64	5024.46	TRUE	
3315.86	3315.86	TRUE	
3241.3	3241.3	TRUE	
6810.14	6810.14	TRUE	
6912.33	6912.33	TRUE	
7234.69	7234.69	TRUE	
9508.31	9508.31	TRUE	
9773.82	9773.82	TRUE	
9406.59	9406.59	TRUE	
12520	12520	TRUE	
12567	12567	TRUE	
12566.5	12566.5	TRUE	
38501.3	38501.3	TRUE	
38391	38391	TRUE	
37954.3	37954.3	TRUE	
80799.9	80799.9	TRUE	
82559.9	82559.9	TRUE	
82251.9	82251.9	TRUE	
140382	140382	TRUE	
141043	141043	TRUE	
452306	452306	TRUE	
452306	452306	TRUE	
449205	449205	TRUE	
449645	449645	TRUE	

Output to Curve Fitting Software		
Saturation X values	Round y values	ASP y values
0.03	688.5	90.8
0.03	766.1	90.8
0.03	670.3	90.8
0.06	1215.2	128.1
0.06	1215.2	128.1
0.06	1282.8	128.1
0.08	1345.4	156.6
0.08	1319.1	156.6
0.08	1442.2	156.6
0.1	1470.0	142.5
0.1	1345.9	142.5
0.1	1493.0	142.5
0.3	1722.5	304.0
0.3	1595.3	304.0
0.3	2067.1	304.0
0.6	2860.7	813.1
0.6	3081.8	813.1
0.6	3006.7	813.1
1	2799.0	1432.9
1	3677.7	1432.9
1	3395.7	1432.9
3	5908.3	4079.3
3	5619.9	4079.3
3	6466.3	4079.3

Total Binding -- Positions 1-24 radiolabeled E2 plus cytozol (Panel A)													
Tube Identification				Assay tube contents									
Run	Position	Rep	Tube Type Code	Hot Conc. Initial	Hot E2	Cold Conc. Initial	Cold	Buffer	Cytozol	Hot Conc. Final	Cold Conc. Final	Total Volume	
				(nM)	(uM)	(nM)	(uM)	(uM)	(uM)	(nM)	(nM)	(uL)	
030308	1	1	H	c1	0.3	50	0	0	350	100	0.03	0	500
030308	2	2	H	c1	0.3	50	0	0	350	100	0.03	0	500
030308	3	3	H	c1	0.3	50	0	0	350	100	0.03	0	500
030308	4	1	H	c2	0.6	50	0	0	350	100	0.06	0	500
030308	5	2	H	c2	0.6	50	0	0	350	100	0.06	0	500
030308	6	3	H	c2	0.6	50	0	0	350	100	0.06	0	500
030308	7	1	H	c3	0.8	50	0	0	350	100	0.08	0	500
030308	8	2	H	c3	0.8	50	0	0	350	100	0.08	0	500
030308	9	3	H	c3	0.8	50	0	0	350	100	0.08	0	500
030308	10	1	H	c4	1	50	0	0	350	100	0.1	0	500
030308	11	2	H	c4	1	50	0	0	350	100	0.1	0	500
030308	12	3	H	c4	1	50	0	0	350	100	0.1	0	500
030308	13	1	H	c5	3	50	0	0	350	100	0.3	0	500
030308	14	2	H	c5	3	50	0	0	350	100	0.3	0	500
030308	15	3	H	c5	3	50	0	0	350	100	0.3	0	500
030308	16	1	H	c6	6	50	0	0	350	100	0.6	0	500
030308	17	2	H	c6	6	50	0	0	350	100	0.6	0	500
030308	18	3	H	c6	6	50	0	0	350	100	0.6	0	500
030308	19	1	H	c7	10	50	0	0	350	100	1	0	500
030308	20	2	H	c7	10	50	0	0	350	100	1	0	500
030308	21	3	H	c7	10	50	0	0	350	100	1	0	500
030308	22	1	H	c8	30	50	0	0	350	100	3	0	500
030308	23	2	H	c8	30	50	0	0	350	100	3	0	500
030308	24	3	H	c8	30	50	0	0	350	100	3	0	500

Total Binding -- Positions 1-24 radiolabeled E2 plus cytozol (Panel B)																		
Run	Position	Scintillation Results					Number of molecules							Ratio				
		Counts per Scintillation Val	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSBP total binding	Ratio Total binding/Hot	Total Added (Mean of reps in pos. 49-72)	Free (mean total added - total bound)	Total Binding molecules		Non Specific Binding molecules		Specific Binding molecules		Total Added reps in pos. 49-72	Free (mean total added - total bound)	Specific Bound Free	
									(dpm)	(dpm)	(fmole)	(fmole)	(fmole)					(fmole)
030308	1	688.5	90.8	597.7	13.2%	21.1%	3268.9	2580.4	2	0	2	11	9	0.23				
030308	2	766.1	90.8	675.3	11.8%	23.4%	3268.9	2580.6	3	0	2	11	8	0.27				
030308	3	670.3	90.8	579.5	13.5%	20.5%	3268.9	2580.7	2	0	2	11	9	0.22				
030308	4	1215.8	128.1	1087.8	10.5%	17.4%	6985.7	5769.9	4	0	4	24	19	0.19				
030308	5	1215.8	128.1	1087.8	10.5%	17.4%	6985.7	5769.5	3	0	3	24	19	0.16				
030308	6	1282.8	128.1	1154.8	10.0%	18.4%	6985.7	5769.5	4	0	4	24	19	0.20				
030308	7	1345.4	156.6	1188.8	11.6%	14.1%	9562.9	8217.5	5	1	4	32	28	0.14				
030308	8	1319.1	156.6	1162.5	11.9%	13.8%	9562.9	8243.8	4	1	4	32	28	0.14				
030308	9	1442.2	156.6	1285.7	10.9%	15.1%	9562.9	8120.7	5	1	4	32	27	0.16				
030308	10	1470.0	142.5	1327.5	9.7%	11.7%	12804.5	11134.6	5	0	4	42	37	0.12				
030308	11	1345.9	142.5	1203.4	10.6%	10.7%	12804.5	11258.6	5	0	4	42	38	0.11				
030308	12	1493.0	142.5	1350.5	9.5%	11.8%	12804.5	11111.5	5	0	5	42	37	0.12				
030308	13	1722.5	304.0	1418.5	17.6%	4.5%	38282.2	36593.7	6	1	6	129	123	0.04				
030308	14	2095.3	304.0	1791.4	14.5%	5.5%	38282.2	36166.9	7	1	6	129	122	0.05				
030308	15	2552.1	304.0	2248.1	15.5%	4.5%	38282.2	36166.9	7	1	6	129	122	0.05				
030308	16	2860.7	813.1	1987.7	39.3%	3.3%	81870.6	79159.9	9	3	8	276	267	0.02				
030308	17	3081.8	813.1	2268.7	28.4%	3.8%	81870.6	78788.8	10	3	9	276	265	0.03				
030308	18	3006.7	813.1	2193.6	27.0%	3.7%	81870.6	78653.9	10	3	8	276	266	0.03				
030308	19	2799.0	1432.9	1366.2	51.2%	2.0%	146596.7	137800.6	9	5	5	473	464	0.01				
030308	20	3877.7	1432.9	2244.8	39.0%	2.6%	146596.7	136922.0	12	5	8	473	461	0.02				
030308	21	3395.7	1432.9	1962.8	42.2%	2.4%	146596.7	137204.0	11	5	7	473	462	0.01				
030308	22	5908.3	4079.3	1829.0	69.0%	1.3%	450316.7	444110.4	20	14	6	1516	1497	0.00				
030308	23	5619.9	4079.3	1540.6	72.6%	1.2%	450316.7	444698.6	19	14	5	1516	1498	0.00				
030308	24	6488.3	4079.3	2409.0	62.9%	1.4%	450316.7	443830.4	22	14	8	1516	1495	0.01				

Non Specific Binding -- Positions 25-48 radiolabeled E2 plus 100 X inert E2 plus cytosol											
Run	Position	Tube Identification		Assay tube contents							
		Rep	Tube Type Code	Hot Conc. Initial	Hot Conc. Final	Cold Conc. Initial	Cold Conc. Final	Buffer	Cytosol	Hot Conc. Final	Cold Conc. Final
				(nM)	(nM)	(nM)	(nM)	(uL)	(uL)	(nM)	(nM)
030308 25	1	1	HC	0.3	50	30	50	300	100	0.03	3
030308 26	2	1	HC	0.3	50	30	50	300	100	0.03	3
030308 27	3	1	HC	0.3	50	30	50	300	100	0.03	3
030308 28	1	1	HC	0.6	50	60	50	300	100	0.06	6
030308 29	2	1	HC	0.6	50	60	50	300	100	0.06	6
030308 30	3	1	HC	0.6	50	60	50	300	100	0.06	6
030308 31	1	1	HC	0.8	50	80	60	300	100	0.08	8
030308 32	2	1	HC	0.8	50	80	60	300	100	0.08	8
030308 33	3	1	HC	0.8	50	80	60	300	100	0.08	8
030308 34	1	1	HC	1	50	100	50	300	100	0.1	10
030308 35	2	1	HC	1	50	100	50	300	100	0.1	10
030308 36	3	1	HC	1	50	100	50	300	100	0.1	10
030308 37	1	1	HC	3	50	300	50	300	100	0.3	30
030308 38	2	1	HC	3	50	300	50	300	100	0.3	30
030308 39	3	1	HC	3	50	300	50	300	100	0.3	30
030308 40	1	1	HC	6	50	600	60	300	100	0.6	60
030308 41	2	1	HC	6	50	600	60	300	100	0.6	60
030308 42	3	1	HC	6	50	600	50	300	100	0.6	60
030308 43	1	1	HC	10	50	1000	50	300	100	1	100
030308 44	2	1	HC	10	50	1000	50	300	100	1	100
030308 45	3	1	HC	10	50	1000	50	300	100	1	100
030308 46	1	1	HC	30	50	3000	50	300	100	3	300
030308 47	2	1	HC	30	50	3000	50	300	100	3	300
030308 48	3	1	HC	30	50	3000	50	300	100	3	300

Free Positions 49-72, radiolabeled E2 without cytosol												
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc.		Hot	Molecules of E2	Counts per Scintillation Vial		Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)
					Initial	Final			Val	Val		
030308 49		1	Hot	c1	0.3	50	15	3249.6	(dpm)	(fmole)	11	3268.9
030308 50		2	Hot	c1	0.3	50	15	3315.9			11	3268.9
030308 51		3	Hot	c1	0.3	50	15	3241.3			11	3268.9
030308 52		1	Hot	c2	0.6	50	30	6810.1			23	6855.7
030308 53		2	Hot	c2	0.6	50	30	6912.3			23	6855.7
030308 54		3	Hot	c2	0.6	50	30	7234.7			24	6855.7
030308 55		1	Hot	c3	0.8	50	40	9508.3			32	9562.9
030308 56		2	Hot	c3	0.8	50	40	9773.8			33	9562.9
030308 57		3	Hot	c3	0.8	50	40	9408.6			32	9562.9
030308 58		1	Hot	c4	1	50	50	12620.0			42	12604.5
030308 59		2	Hot	c4	1	50	50	12627.1			43	12604.5
030308 60		3	Hot	c4	1	50	50	12566.5			42	12604.5
030308 61		1	Hot	c5	3	50	150	38591.3			130	38262.2
030308 62		2	Hot	c5	3	50	150	38391.0			129	38262.2
030308 63		3	Hot	c5	3	50	150	37954.3			128	38262.2
030308 64		1	Hot	c6	6	50	300	80769.9			272	81970.6
030308 65		2	Hot	c6	6	50	300	82559.9			278	81970.6
030308 66		3	Hot	c6	6	50	300	82251.9			277	81970.6
030308 67		1	Hot	c7	10	50	500	140382.0			473	140599.7
030308 68		2	Hot	c7	10	50	500	141043.0			475	140599.7
030308 69		3	Hot	c7	10	50	500	140374.0			473	140599.7
030308 70		1	Hot	c8	30	50	1500	452006.0			1523	450318.7
030308 71		2	Hot	c8	30	50	1500	448005.0			1512	450318.7
030308 72		3	Hot	c8	30	50	1500	448645.0			1514	450318.7

Linear regression results (LINEST function)

Slope: 266.5673511 dpm/fmole

Intercept: 0.003397487 fmole/dpm

Slope: 0.003397487 fmole/dpm

Intercept: 266.5673511 dpm/fmole

SLOPE function, used if missing HOT tubes

Slope: 301.3 dpm/fmole

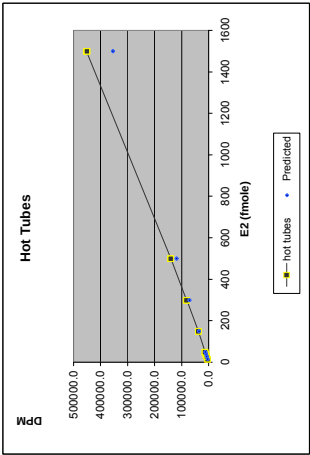
Intercept: 0.003319 fmole/dpm

SLOPE function, used if missing HOT tubes

Slope: 301.3 dpm/fmole

Intercept: 0.003319 fmole/dpm

Computation Check	
3/3/08	Specific activity calc
10634	Count/Estimate
2.22E+12	DPM/CI (definition)
2.3652E+14	DPM/Imole
2.3652E+11	DPM/Imole
236.5	DPM/Imole
0.004428	Imole/DPM



Prism input for bound/free		Prism input for specific bound		HOT tubes		NSB Tubes		Specific bound		Total Bound	
specific bound/molar	bound/free %	average total added molar	specific bound/molar	total added dpm	NSB dpm	specific bound/dpm	total bound dpm				
5.05437E-12	0.2316	2.78E-11	5.0544E-12	3249.64	67.38	597.73	688.50				
5.71051E-12	0.2688	2.78E-11	5.7105E-12	3315.85	115.17	675.52	766.10				
4.90025E-12	0.2230	2.78E-11	4.9003E-12	3241.30	88.76	579.50	670.28				
9.18619E-12	0.1895	5.91E-11	9.1862E-12	6610.14	173.81	1087.77	1211.64				
7.54453E-12	0.1410	5.91E-11	7.5445E-12	5842.53	119.45	854.51	954.51				
9.76468E-12	0.2025	5.91E-11	9.7647E-12	7234.69	97.61	1154.76	1232.83				
1.06295E-11	0.1447	8.09E-11	1.063E-11	9508.31	120.20	1188.81	1345.37				
9.83044E-12	0.1410	8.09E-11	9.8304E-12	9773.82	143.04	1182.54	1319.10				
1.08718E-11	0.1583	8.09E-11	1.0872E-11	9406.59	194.55	1285.66	1442.22				
1.1225E-11	0.1192	1.07E-10	1.1225E-11	12620.00	144.50	1327.46	1469.96				
1.07759E-11	0.1069	1.07E-10	1.0776E-11	12627.10	145.73	1203.39	1345.89				
1.14198E-11	0.1215	1.07E-10	1.1420E-11	12566.50	136.28	1350.50	1483.00				
1.19951E-11	0.0388	3.24E-10	1.1995E-11	38501.30	269.84	1418.53	1722.90				
1.51478E-11	0.0495	3.24E-10	1.5148E-11	38391.00	284.23	1791.37	2035.34				
1.57455E-11	0.0388	3.24E-10	1.5745E-11	38456.50	284.23	1791.37	2035.34				
1.57825E-11	0.0236	6.92E-10	1.5783E-11	80769.90	89.93	1067.66	2981.71				
1.81843E-11	0.0288	6.92E-10	1.8184E-11	82569.90	487.10	2268.72	3081.77				
1.85492E-11	0.0278	6.92E-10	1.8549E-11	82251.90	1050.13	2193.61	3066.66				
1.15523E-11	0.0099	1.19E-09	1.1552E-11	140382.00	1919.82	1386.17	2790.05				
1.8892E-11	0.0164	1.19E-09	1.8892E-11	141043.00	1603.46	2244.80	3877.67				
1.68974E-11	0.0143	1.19E-09	1.6897E-11	140374.00	776.35	1962.80	3395.67				
1.54661E-11	0.0041	3.81E-09	1.5466E-11	452200.00	3466.90	1829.01	5908.31				
1.30272E-11	0.0035	3.81E-09	1.3027E-11	449005.00	3735.00	1540.59	5918.89				
2.03701E-11	0.0054	3.81E-09	2.0370E-11	448645.00	5915.93	2405.96	6483.25				

(total volume in tube uL/1000)	made to molar conversion value	Free (total added bound) dpm	Free (total added bound) molar	specific bound/dpm	mean specific bound/dpm	mean specific bound molar	spfree %	use for bound/free	
0.5	0.0005							specific bound/molar	bound/free %
2580.4	2.16201E-11	2580.4	2.16201E-11	597.7	617.5	5.22171E-12	23.16%	5.05437E-12	23.16%
2502.8	2.11640E-11	2502.8	2.11640E-11	675.3		0	25.98%	5.71051E-12	25.98%
2599.7	2.19742E-11	2599.7	2.19742E-11	579.5	1031.9	8.72552E-12	22.30%	4.90025E-12	22.30%
5769.9	4.67901E-11	5769.9	4.67901E-11	1097.8		0	18.85%	9.19819E-12	18.85%
5769.9	4.67901E-11	5769.9	4.67901E-11	1097.8		0	18.85%	9.19819E-12	18.85%
5702.9	4.62297E-11	5702.9	4.62297E-11	1154.8		0	20.25%	9.76966E-12	20.25%
8217.5	6.94879E-11	8217.5	6.94879E-11	1189.8	1212.3	1.02519E-11	14.47%	1.00525E-11	14.47%
8243.8	6.97097E-11	8243.8	6.97097E-11	1162.5		0	14.10%	9.83044E-12	14.10%
8120.7	6.86686E-11	8120.7	6.86686E-11	1285.7		0	15.83%	1.08715E-11	15.83%
11134.6	9.41541E-11	11134.6	9.41541E-11	1327.5	1293.8	1.09402E-11	11.92%	1.1225E-11	11.92%
11258.6	9.52091E-11	11258.6	9.52091E-11	1203.4		0	10.69%	1.01759E-11	10.69%
11111.5	9.39592E-11	11111.5	9.39592E-11	1350.5		0	12.15%	1.14198E-11	12.15%
3659.7	3.09149E-10	3659.7	3.09149E-10	1419.5	1699.3	1.43619E-11	3.88%	1.19951E-11	3.88%
36168.9	3.05989E-10	36168.9	3.05989E-10	1791.4		0	4.95%	1.9178E-11	4.95%
36168.9	3.05989E-10	36168.9	3.05989E-10	1791.4		0	4.95%	1.9178E-11	4.95%
70789.9	6.66509E-10	70789.9	6.66509E-10	1887.7	2110.0	1.78421E-11	2.36%	1.67929E-11	2.36%
78788.8	6.66229E-10	78788.8	6.66229E-10	2268.7		0	2.88%	1.91843E-11	2.88%
78863.9	6.66897E-10	78863.9	6.66897E-10	2193.6		0	2.79%	1.85492E-11	2.79%
137800.6	1.16524E-09	137800.6	1.16524E-09	1365.2	1857.9	1.57108E-11	0.99%	1.15523E-11	0.99%
138622.0	1.15781E-09	138622.0	1.15781E-09	2244.8		0	1.64%	1.8982E-11	1.64%
137204.0	1.16000E-09	137204.0	1.16000E-09	1962.8		0	1.43%	1.65974E-11	1.43%
444410.4	3.75794E-09	444410.4	3.75794E-09	1820.0	1926.2	1.62878E-11	0.41%	1.54661E-11	0.41%
444698.8	3.76008E-09	444698.8	3.76008E-09	1540.6		0	0.35%	1.30272E-11	0.35%
443830.4	3.75303E-09	443830.4	3.75303E-09	2469.0		0	0.54%	2.03701E-11	0.54%

SAT8 030308: Lab Hammer

Sat-template

Molecules of E2 (fmole)	total added dpm	mean total added		use for specific bound	
		total added molar	dpm	average total added molar	specific bound/molar
15	3249.64	2.7479E-11	3268.93	2.7642E-11	5.05437E-12
15	3315.85	2.80388E-11		2.7642E-11	5.71105E-12
15	3241.30	2.74084E-11		2.7642E-11	4.90025E-12
30	6670.14	5.75986E-11	6985.72	5.90713E-11	9.18619E-12
30	6670.14	5.75986E-11		5.90713E-11	9.18619E-12
30	7234.69	6.11708E-11		5.90713E-11	9.76469E-12
40	9508.31	8.94023E-11	9562.91	8.0864E-11	1.00625E-11
40	9773.82	8.26475E-11		8.0864E-11	9.83044E-12
40	9406.59	7.95422E-11		8.0864E-11	1.08715E-11
50	12620.00	1.08715E-10	12604.53	1.06584E-10	1.1225E-11
50	12627.10	1.08775E-10		1.06584E-10	1.01759E-11
50	12566.50	1.06262E-10		1.06584E-10	1.14198E-11
150	38501.30	3.25607E-10	38262.20	3.23714E-10	1.19961E-11
150	38391.00	3.24634E-10		3.23714E-10	1.51478E-11
150	37942.60	3.19565E-10		3.23714E-10	1.50565E-11
300	80759.90	6.83244E-10	81670.57	6.92298E-10	1.57809E-11
300	82559.90	6.88127E-10		6.92298E-10	1.91843E-11
300	82251.90	6.95522E-10		6.92298E-10	1.85492E-11
500	140382.00	1.18707E-09	140599.67	1.18891E-09	1.15523E-11
500	141043.00	1.19208E-09		1.18891E-09	1.8892E-11
500	140374.00	1.1187E-09		1.18891E-09	1.65974E-11
1500	462306.00	3.8247E-09	460318.67	3.80779E-09	1.54468E-11
1500	449005.00	3.76079E-09		3.80779E-09	1.30272E-11
1500	449845.00	3.8022E-09		3.80779E-09	2.03701E-11

SAT8 030308, Lab Hammer

Sat-template

total bound $\mu\text{g/m}$	total bound/molar	average bound/ $\mu\text{g/m}$	average bound/molar	NSB $\mu\text{g/m}$	NSB molar	Hot Final Concentration (mM)
688.5	5.82198E-12	708.29	5.9893E-12	67.4	5.69768E-13	0.03
786.1	6.4787E-12	0	0	115.2	9.73978E-13	0.03
670.3	5.66786E-12	0	0	89.8	7.59738E-13	0.03
1215.8	1.02871E-11	1159.96	9.80859E-12	178.6	1.51028E-12	0.06
1362.6	1.15697E-11	0	0	178.6	1.51028E-12	0.06
1292.6	1.08478E-11	0	0	97.6	8.25348E-13	0.06
1346.4	1.13794E-11	1368.90	1.15794E-11	126.2	1.06711E-12	0.08
1319.1	1.11643E-11	0	0	148.9	1.25944E-12	0.08
1442.2	1.21954E-11	0	0	194.6	1.64512E-12	0.08
1470.0	1.24208E-11	1436.28	1.21462E-11	145.5	1.23035E-12	0.10
1345.9	1.13809E-11	0	0	145.7	1.23225E-12	0.10
1493.0	1.26248E-11	0	0	136.3	1.12324E-12	0.10
1722.5	1.45934E-11	2003.31	1.894E-11	269.6	2.28008E-12	0.30
2056.3	1.77182E-11	0	0	269.2	2.23428E-12	0.30
1716.3	1.45875E-11	0	0	167.6	1.40678E-12	0.30
2680.7	2.26891E-11	2923.05	2.47173E-11	891.9	7.55218E-12	0.60
3081.8	2.65984E-11	0	0	487.1	4.11889E-12	0.60
3008.7	2.54243E-11	0	0	1050.1	8.96442E-12	0.60
2799.0	2.36887E-11	3290.80	2.7827E-11	1916.8	1.62086E-11	1.00
3677.7	3.10884E-11	0	0	1603.5	1.35988E-11	1.00
3395.7	2.87138E-11	0	0	778.4	6.56173E-12	1.00
5908.3	4.99007E-11	6005.48	5.07924E-11	3486.9	2.94852E-11	3.00
5619.9	4.75218E-11	0	0	3735.1	3.1937E-11	3.00
6488.3	5.48847E-11	0	0	5015.9	4.24147E-11	3.00

SAT8 030308; Lab Hammer

Sat-template

One site binding (hyperbola)	
Best-fit values	
BMAX	
KD	2.85E-11
Std. Error	3.08E-10
BMAX	
KD	
95% Confidence Intervals	
BMAX	
KD	
Goodness of Fit	
Degrees of Freedom	
R ² (Unweighted)	
Weighted Sum of Squares (1/Y ²)	
Absolute Sum of Squares	
Sig. X	
Data	
Number of X values	
Number of Y replicates	
Total number of values	
Number of missing values	
Bmax(mole/100 ug)/Bmax molar	
Bmax molar	2.85E-11
mole to molar conversion value	0.0005
DPM/mole (DPM/mole)*1000	2.37E+17
Bmax molar to Bmax molar	1.45E-14
DPM(DPM/mole)	4.23E-08
Bmax DPM	3.37E+03
assay date	
Bmax(dpm)	3371.56843
DPM/C (definition)	2.22E+12
C/mole	106.54
DPM/mole	2.37E+14
1/(DPM/mole)	4.23E-08
1/(DPM/mole)	4.23E-08
SA(dpm/pmol)	2.37E+05
protein/tube (ug)	50
protein/tube(mg)	0.05
bmax pmole	0.014255
bmax pmole/mg	0.2851
Bmax fmole/mg	285.1
Bmax (mole/100 ug)	28.51
Bmax(mole/100 ug)/Bmax molar	1.00E+12

APPENDIX C: Raw Data, Prism and Within Run SD Files for Competitive Binding Assays with Test Chemicals

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.13

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 032608
Assay start date: 3/26/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-0.338591221
1	1	2	-7.0	-1.13245405
1	1	3	-7.0	-1.372357653
1	2	1	-8.0	8.803916972
1	2	2	-8.0	9.730817253
1	2	3	-8.0	8.012235084
1	3	1	-8.5	25.49248393
1	3	2	-8.5	26.57859297
1	3	3	-8.5	25.25148986
1	4	1	-9.0	56.22740681
1	4	2	-9.0	62.28933465
1	4	3	-9.0	47.83187119
1	5	1	-9.5	77.51667057
1	5	2	-9.5	89.93277247
1	5	3	-9.5	91.3263943
1	6	1	-10.0	95.75479671
1	6	2	-10.0	105.8296575
1	6	3	-10.0	104.8384195
1	7	1	-11.0	104.16
1	7	2	-11.0	115.08
1	7	3	-11.0	115.48
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.45

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 032608
Assay start date: 3/26/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-4.0	81.25153347
1	1	2	-4.0	84.76175937
1	1	3	-4.0	74.64218923
1	2	1	-4.5	10.87472125
1	2	2	-4.5	12.37520923
1	2	3	-4.5	13.00877284
1	3	1	-5.5	23.88731074
1	3	2	-5.5	21.55152202
1	3	3	-5.5	29.45852666
1	4	1	-6.0	43.76441466
1	4	2	-6.0	41.87789997
1	4	3	-6.0	47.8242379
1	5	1	-6.5	76.71844586
1	5	2	-6.5	91.59792156
1	5	3	-6.5	79.04223939
1	6	1	-7.0	81.36385198
1	6	2	-7.0	86.73224032
1	6	3	-7.0	96.07430469
1	7	1	-7.5	94.25
1	7	2	-7.5	108.52
1	7	3	-7.5	103.97
1	8	1	-8.5	107.10
1	8	2	-8.5	110.71
1	8	3	-8.5	114.07
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.94

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 032608
Assay start date: 3/26/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-4.0	14.13086742
1	1	2	-4.0	15.76003097
1	1	3	-4.0	11.22803383
1	2	1	-5.0	63.23586341
1	2	2	-5.0	60.73650406
1	2	3	-5.0	57.87837978
1	3	1	-6.0	101.9246812
1	3	2	-6.0	111.6713103
1	3	3	-6.0	100.7306155
1	4	1	-7.0	103.5920112
1	4	2	-7.0	115.1477315
1	4	3	-7.0	111.5557203
1	5	1	-8.0	109.3835023
1	5	2	-8.0	107.4860829
1	5	3	-8.0	112.4150114
1	6	1	-9.0	105.7784054
1	6	2	-9.0	119.3754873
1	6	3	-9.0	111.7759955
1	7	1	-10.0	119.81
1	7	2	-10.0	110.78
1	7	3	-10.0	115.04
1	8	1	-11.0	106.30
1	8	2	-11.0	117.47
1	8	3	-11.0	116.61
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.44

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 032708	1	1	1	-7.0	-0.402945318
Assay start date: 3/27/2008	1	1	2	-7.0	1.979097742
Technician ID: SMR	1	1	3	-7.0	-1.056989193
	1	2	1	-8.0	10.10374782
	1	2	2	-8.0	9.71692031
	1	2	3	-8.0	13.05712492
	1	3	1	-8.5	26.41794337
	1	3	2	-8.5	34.17612528
	1	3	3	-8.5	27.22468231
	1	4	1	-9.0	45.84839076
	1	4	2	-9.0	48.5994469
	1	4	3	-9.0	42.93191497
	1	5	1	-9.5	92.38475764
	1	5	2	-9.5	100.220595
	1	5	3	-9.5	99.81210024
	1	6	1	-10.0	109.031065
	1	6	2	-10.0	110.3175209
	1	6	3	-10.0	100.0385979
	1	7	1	-11.0	
	1	7	2	-11.0	113.72
	1	7	3	-11.0	113.50
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.23

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 032708
Assay start date: 3/26/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	13.97584025
1	2	2	-4.5	15.01925654
1	2	3	-4.5	12.30891909
1	3	1	-5.5	32.31579037
1	3	2	-5.5	34.50823705
1	3	3	-5.5	43.85699259
1	4	1	-6.0	53.11540354
1	4	2	-6.0	53.86360937
1	4	3	-6.0	58.25359258
1	5	1	-6.5	90.30683226
1	5	2	-6.5	89.70877657
1	5	3	-6.5	89.26977825
1	6	1	-7.0	105.3880151
1	6	2	-7.0	112.3992637
1	6	3	-7.0	108.0767208
1	7	1	-7.5	116.57
1	7	2	-7.5	128.12
1	7	3	-7.5	133.53
1	8	1	-8.5	132.95
1	8	2	-8.5	115.11
1	8	3	-8.5	127.63
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.34

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 032708
Assay start date: 3/27/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-4.0	10.82777693
1	1	2	-4.0	9.962504878
1	1	3	-4.0	10.61654875
1	2	1	-5.0	43.65467162
1	2	2	-5.0	45.68297111
1	2	3	-5.0	48.44420692
1	3	1	-6.0	100.3376258
1	3	2	-6.0	104.2886106
1	3	3	-6.0	104.3471438
1	4	1	-7.0	93.81754636
1	4	2	-7.0	107.0969274
1	4	3	-7.0	113.6628154
1	5	1	-8.0	123.1184574
1	5	2	-8.0	125.6811897
1	5	3	-8.0	123.100643
1	6	1	-9.0	119.5059466
1	6	2	-9.0	118.2423949
1	6	3	-9.0	114.6731477
1	7	1	-10.0	112.34
1	7	2	-10.0	116.91
1	7	3	-10.0	116.93
1	8	1	-11.0	125.05
1	8	2	-11.0	112.10
1	8	3	-11.0	131.67
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.58

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 033108
Assay start date: 3/31/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-1.614850828
1	1	2	-7.0	-0.397009815
1	1	3	-7.0	1.135420266
1	2	1	-8.0	10.83524986
1	2	2	-8.0	14.41250889
1	2	3	-8.0	15.71853017
1	3	1	-8.5	34.9215712
1	3	2	-8.5	32.73851373
1	3	3	-8.5	45.30420207
1	4	1	-9.0	60.2614823
1	4	2	-9.0	59.9218691
1	4	3	-9.0	56.24093868
1	5	1	-9.5	87.91076316
1	5	2	-9.5	86.07208538
1	5	3	-9.5	78.24549069
1	6	1	-10.0	94.49568433
1	6	2	-10.0	103.1611832
1	6	3	-10.0	93.65201365
1	7	1	-11.0	105.93
1	7	2	-11.0	109.98
1	7	3	-11.0	98.44
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.63

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 033108
Assay start date: 3/31/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	12.18774453
1	2	2	-4.5	10.23824561
1	2	3	-4.5	12.01734212
1	3	1	-5.5	33.68347255
1	3	2	-5.5	39.56056833
1	3	3	-5.5	30.74254142
1	4	1	-6.0	64.43336233
1	4	2	-6.0	61.01935597
1	4	3	-6.0	54.46660867
1	5	1	-6.5	79.23811453
1	5	2	-6.5	87.35427416
1	5	3	-6.5	90.29163042
1	6	1	-7.0	90.88744305
1	6	2	-7.0	97.9192237
1	6	3	-7.0	78.26217345
1	7	1	-7.5	97.12
1	7	2	-7.5	102.46
1	7	3	-7.5	99.65
1	8	1	-8.5	91.47
1	8	2	-8.5	107.78
1	8	3	-8.5	97.61
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.88

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 033108
Assay start date: 3/31/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-4.0	17.73833498
1	1	2	-4.0	16.95067069
1	1	3	-4.0	18.87752873
1	2	1	-5.0	69.44414654
1	2	2	-5.0	62.52557029
1	2	3	-5.0	55.1756257
1	3	1	-6.0	86.3795247
1	3	2	-6.0	96.85271909
1	3	3	-6.0	99.42067152
1	4	1	-7.0	91.37362616
1	4	2	-7.0	96.89919248
1	4	3	-7.0	107.7358326
1	5	1	-8.0	108.5640121
1	5	2	-8.0	114.0919617
1	5	3	-8.0	109.1383755
1	6	1	-9.0	93.05620102
1	6	2	-9.0	103.2863039
1	6	3	-9.0	99.12634008
1	7	1	-10.0	94.54
1	7	2	-10.0	110.50
1	7	3	-10.0	98.77
1	8	1	-11.0	106.76
1	8	2	-11.0	109.81
1	8	3	-11.0	110.26
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.67

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 060408
Assay start date: 6/4/2008
Technician ID: JZ

run	xgroup	rep	x	y
1	1	1	-7.0	0.742681904
1	1	2	-7.0	0.937902368
1	1	3	-7.0	2.085503785
1	2	1	-8.0	-0.921019592
1	2	2	-8.0	-1.511168812
1	2	3	-8.0	-0.410689576
1	3	1	-8.5	5.810079445
1	3	2	-8.5	4.081304462
1	3	3	-8.5	3.323823415
1	4	1	-9.0	7.933142066
1	4	2	-9.0	5.811361681
1	4	3	-9.0	5.892463122
1	5	1	-9.5	16.68280147
1	5	2	-9.5	14.18019693
1	5	3	-9.5	13.81508017
1	6	1	-10.0	22.72469854
1	6	2	-10.0	22.3461183
1	6	3	-10.0	22.62917194
1	7	1	-11.0	70.40
1	7	2	-11.0	67.22
1	7	3	-11.0	63.18
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.72

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060408
Assay start date: 6/4/2008
Technician ID: JZ

run	xgroup	rep	X	Y
1	1	1	-4.0	3.9392968
1	1	2	-4.0	4.243186784
1	1	3	-4.0	4.559578572
1	2	1	-4.5	8.46462898
1	2	2	-4.5	6.167182232
1	2	3	-4.5	6.701233618
1	3	1	-5.5	26.64481522
1	3	2	-5.5	24.52047037
1	3	3	-5.5	23.9110876
1	4	1	-6.0	35.08417347
1	4	2	-6.0	33.96317845
1	4	3	-6.0	33.50862571
1	5	1	-6.5	44.16689373
1	5	2	-6.5	39.63130366
1	5	3	-6.5	43.12187121
1	6	1	-7.0	55.61822485
1	6	2	-7.0	51.27817581
1	6	3	-7.0	55.15341422
1	7	1	-7.5	60.69
1	7	2	-7.5	56.21
1	7	3	-7.5	55.68
1	8	1	-8.5	62.18
1	8	2	-8.5	60.62
1	8	3	-8.5	61.73
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.82

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 060508
Assay start date: 6/5/2008
Technician ID: JZ

run	xgroup	rep	x	y
1	1	1	-7.0	4.943761444
1	1	2	-7.0	2.252088547
1	1	3	-7.0	0.668751548
1	2	1	-8.0	2.486811312
1	2	2	-8.0	2.818756525
1	2	3	-8.0	2.052088084
1	3	1	-8.5	9.83960611
1	3	2	-8.5	16.02712043
1	3	3	-8.5	9.184049037
1	4	1	-9.0	22.6118579
1	4	2	-9.0	22.84380288
1	4	3	-9.0	16.22434311
1	5	1	-9.5	50.18414394
1	5	2	-9.5	45.52718872
1	5	3	-9.5	58.57166336
1	6	1	-10.0	86.43561675
1	6	2	-10.0	88.87590018
1	6	3	-10.0	88.02450932
1	7	1	-11.0	139.09
1	7	2	-11.0	133.79
1	7	3	-11.0	129.39
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 9.28

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060508
Assay start date: 6/5/2008
Technician ID: JZ

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	17.76323556
1	2	2	-4.5	14.43128341
1	2	3	-4.5	13.54378135
1	3	1	-5.5	30.36048695
1	3	2	-5.5	52.32581557
1	3	3	-5.5	48.58691803
1	4	1	-6.0	67.70362894
1	4	2	-6.0	60.60639029
1	4	3	-6.0	62.73556189
1	5	1	-6.5	121.3662532
1	5	2	-6.5	110.0328936
1	5	3	-6.5	113.4676238
1	6	1	-7.0	104.5134364
1	6	2	-7.0	113.6759576
1	6	3	-7.0	121.625976
1	7	1	-7.5	179.77
1	7	2	-7.5	144.11
1	7	3	-7.5	145.68
1	8	1	-8.5	128.18
1	8	2	-8.5	136.86
1	8	3	-8.5	130.92
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.65

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 060608
Assay start date: 6/6/2008
Technician ID: JZ

run	xgroup	rep	x	y
1	1	1	-7.0	3.188299128
1	1	2	-7.0	0.396045677
1	1	3	-7.0	-0.317625128
1	2	1	-8.0	1.686041799
1	2	2	-8.0	-5.108945419
1	2	3	-8.0	-3.427284656
1	3	1	-8.5	1.203689709
1	3	2	-8.5	-1.676622572
1	3	3	-8.5	3.772510312
1	4	1	-9.0	13.22897705
1	4	2	-9.0	14.23902496
1	4	3	-9.0	15.084784
1	5	1	-9.5	44.37223036
1	5	2	-9.5	39.02692804
1	5	3	-9.5	33.68162571
1	6	1	-10.0	79.98282634
1	6	2	-10.0	70.49153036
1	6	3	-10.0	71.19008659
1	7	1	-11.0	126.17
1	7	2	-11.0	121.36
1	7	3	-11.0	128.21
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.35

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060608
Assay start date: 6/6/2008
Technician ID: JZ

run	xgroup	rep	X	Y
1	1	1	-4.0	8.835892954
1	1	2	-4.0	9.665880271
1	1	3	-4.0	6.690937607
1	2	1	-4.5	53.08151137
1	2	2	-4.5	48.27639081
1	2	3	-4.5	47.68626523
1	3	1	-5.5	46.5585865
1	3	2	-5.5	30.63899604
1	3	3	-5.5	42.19901733
1	4	1	-6.0	45.778543
1	4	2	-6.0	40.26632319
1	4	3	-6.0	49.73330442
1	5	1	-6.5	111.161566
1	5	2	-6.5	102.2472525
1	5	3	-6.5	108.4107134
1	6	1	-7.0	101.8023583
1	6	2	-7.0	100.4827902
1	6	3	-7.0	
1	7	1	-7.5	113.64
1	7	2	-7.5	119.41
1	7	3	-7.5	115.21
1	8	1	-8.5	88.25
1	8	2	-8.5	82.53
1	8	3	-8.5	91.33
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.99

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 030508	1	1	1	-7.0	11.92324155
Assay start date: 3/5/2008	1	1	2	-7.0	-8.09556443
Technician ID: SMR	1	1	3	-7.0	0.6922246745
	1	2	1	-8.0	20.45901617
	1	2	2	-8.0	9.670622146
	1	2	3	-8.0	24.72519591
	1	3	1	-8.5	27.84867334
	1	3	2	-8.5	34.96443712
	1	3	3	-8.5	27.77763864
	1	4	1	-9.0	54.55498586
	1	4	2	-9.0	48.71921261
	1	4	3	-9.0	64.51486947
	1	5	1	-9.5	81.87943682
	1	5	2	-9.5	81.32618585
	1	5	3	-9.5	82.57134205
	1	6	1	-10.0	107.3440083
	1	6	2	-10.0	99.0944215
	1	6	3	-10.0	82.47435238
	1	7	1	-11.0	112.26
	1	7	2	-11.0	102.73
	1	7	3	-11.0	115.59
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 11.19

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 030508
Assay start date: 3/5/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	-4.335506874
1	2	2	-4.5	-2.1443598
1	2	3	-4.5	0.171781013
1	3	1	-5.5	10.81059237
1	3	2	-5.5	14.53171745
1	3	3	-5.5	24.18902059
1	4	1	-6.0	31.72416227
1	4	2	-6.0	54.85756633
1	4	3	-6.0	42.88685448
1	5	1	-6.5	70.75567708
1	5	2	-6.5	81.50650468
1	5	3	-6.5	86.34369408
1	6	1	-7.0	107.7729485
1	6	2	-7.0	99.05890415
1	6	3	-7.0	95.09462185
1	7	1	-7.5	109.72
1	7	2	-7.5	103.96
1	7	3	-7.5	100.04
1	8	1	-8.5	114.37
1	8	2	-8.5	115.83
1	8	3	-8.5	69.74
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.63

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: R1881	run	xgroup	rep	x	y
Run ID: 030508	1	1	1	-3.0	
Assay start date: 3/5/2008	1	1	2	-3.0	
Technician ID: SMR	1	1	3	-3.0	
	1	2	1	-4.0	25.64728087
	1	2	2	-4.0	15.26597028
	1	2	3	-4.0	16.25977296
	1	3	1	-5.0	62.16935855
	1	3	2	-5.0	68.47232151
	1	3	3	-5.0	55.58977009
	1	4	1	-6.0	86.15791103
	1	4	2	-6.0	104.461639
	1	4	3	-6.0	95.24830268
	1	5	1	-7.0	107.0707979
	1	5	2	-7.0	119.574953
	1	5	3	-7.0	110.5603772
	1	6	1	-8.0	93.08652572
	1	6	2	-8.0	114.3047252
	1	6	3	-8.0	102.3934366
	1	7	1	-9.0	109.75
	1	7	2	-9.0	117.30
	1	7	3	-9.0	113.18
	1	8	1	-10.0	109.88
	1	8	2	-10.0	117.76
	1	8	3	-10.0	108.59
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 8.42

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 030608
Assay start date: 3/6/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-9.65172333
1	1	2	-7.0	-13.614004
1	1	3	-7.0	2.758025934
1	2	1	-8.0	0.542780671
1	2	2	-8.0	16.80979157
1	2	3	-8.0	9.591920827
1	3	1	-8.5	23.46865474
1	3	2	-8.5	25.20146881
1	3	3	-8.5	11.84326638
1	4	1	-9.0	30.23253694
1	4	2	-9.0	25.46401639
1	4	3	-9.0	38.47215537
1	5	1	-9.5	78.94386587
1	5	2	-9.5	86.6080675
1	5	3	-9.5	68.38070129
1	6	1	-10.0	90.36796774
1	6	2	-10.0	98.95764962
1	6	3	-10.0	72.84291632
1	7	1	-11.0	98.19
1	7	2	-11.0	99.37
1	7	3	-11.0	
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 9.00

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 030608
Assay start date: 3/6/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	3.966838781
1	2	2	-4.5	7.273844426
1	2	3	-4.5	2.060086932
1	3	1	-5.5	16.78244286
1	3	2	-5.5	17.05046019
1	3	3	-5.5	23.60430432
1	4	1	-6.0	43.59949095
1	4	2	-6.0	46.3628043
1	4	3	-6.0	52.93962135
1	5	1	-6.5	53.53801106
1	5	2	-6.5	76.28775945
1	5	3	-6.5	62.99081813
1	6	1	-7.0	112.6691973
1	6	2	-7.0	88.16584985
1	6	3	-7.0	94.31383918
1	7	1	-7.5	106.77
1	7	2	-7.5	86.83
1	7	3	-7.5	103.58
1	8	1	-8.5	101.08
1	8	2	-8.5	128.02
1	8	3	-8.5	121.03
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 11.38

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 030608
Assay start date: 3/6/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	
1	2	1	-4.0	10.30408116
1	2	2	-4.0	
1	2	3	-4.0	8.094305635
1	3	1	-5.0	49.54291194
1	3	2	-5.0	73.61852565
1	3	3	-5.0	58.47499964
1	4	1	-6.0	92.51429426
1	4	2	-6.0	83.1161846
1	4	3	-6.0	70.68346242
1	5	1	-7.0	100.134738
1	5	2	-7.0	111.6321344
1	5	3	-7.0	117.5263277
1	6	1	-8.0	105.1723698
1	6	2	-8.0	107.9717834
1	6	3	-8.0	138.4196459
1	7	1	-9.0	139.99
1	7	2	-9.0	107.69
1	7	3	-9.0	122.13
1	8	1	-10.0	119.16
1	8	2	-10.0	101.90
1	8	3	-10.0	104.68
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.67

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 031008	1	1	1	-7.0	-2.595547061
Assay start date: 3/10/2008	1	1	2	-7.0	-2.599826219
Technician ID: SMR	1	1	3	-7.0	-1.74684739
	1	2	1	-8.0	8.13087567
	1	2	2	-8.0	6.859965743
	1	2	3	-8.0	6.594657947
	1	3	1	-8.5	26.81439271
	1	3	2	-8.5	16.55083224
	1	3	3	-8.5	21.99891357
	1	4	1	-9.0	40.04697565
	1	4	2	-9.0	40.97697932
	1	4	3	-9.0	38.44514417
	1	5	1	-9.5	78.74625425
	1	5	2	-9.5	82.66311021
	1	5	3	-9.5	77.7121244
	1	6	1	-10.0	96.07541778
	1	6	2	-10.0	85.17782873
	1	6	3	-10.0	99.1200387
	1	7	1	-11.0	
	1	7	2	-11.0	110.19
	1	7	3	-11.0	105.74
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.96

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 031008
Assay start date: 3/10/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	4.591298808
1	2	2	-4.5	5.209637139
1	2	3	-4.5	9.438871634
1	3	1	-5.5	15.93392029
1	3	2	-5.5	12.70529558
1	3	3	-5.5	18.63906134
1	4	1	-6.0	48.86346752
1	4	2	-6.0	46.80162656
1	4	3	-6.0	42.08742082
1	5	1	-6.5	73.14055727
1	5	2	-6.5	74.35726452
1	5	3	-6.5	72.78182119
1	6	1	-7.0	102.4841701
1	6	2	-7.0	94.5556035
1	6	3	-7.0	103.0818258
1	7	1	-7.5	98.48
1	7	2	-7.5	104.64
1	7	3	-7.5	104.05
1	8	1	-8.5	109.55
1	8	2	-8.5	104.11
1	8	3	-8.5	106.29
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.30

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 031008
Assay start date: 3/10/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	
1	2	1	-4.0	5.163279594
1	2	2	-4.0	5.1932337
1	2	3	-4.0	7.700107098
1	3	1	-5.0	33.63465738
1	3	2	-5.0	34.3756649
1	3	3	-5.0	38.52145582
1	4	1	-6.0	76.00830632
1	4	2	-6.0	91.75917374
1	4	3	-6.0	85.71914222
1	5	1	-7.0	105.5409153
1	5	2	-7.0	104.94112
1	5	3	-7.0	108.0855879
1	6	1	-8.0	102.4820305
1	6	2	-8.0	123.2159774
1	6	3	-8.0	123.990505
1	7	1	-9.0	116.19
1	7	2	-9.0	
1	7	3	-9.0	116.45
1	8	1	-10.0	112.33
1	8	2	-10.0	111.26
1	8	3	-10.0	110.98
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.13

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 031108
Assay start date: 3/11/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	0.887399353
1	1	2	-7.0	1.042897448
1	1	3	-7.0	-1.556747969
1	2	1	-8.0	7.353999658
1	2	2	-8.0	32.76662917
1	2	3	-8.0	8.659476843
1	3	1	-8.5	20.75157412
1	3	2	-8.5	23.06566849
1	3	3	-8.5	29.19582747
1	4	1	-9.0	51.50202913
1	4	2	-9.0	55.53579107
1	4	3	-9.0	53.33407941
1	5	1	-9.5	79.14817673
1	5	2	-9.5	93.53175047
1	5	3	-9.5	76.48067759
1	6	1	-10.0	102.1018159
1	6	2	-10.0	104.8930067
1	6	3	-10.0	110.9121966
1	7	1	-11.0	106.58
1	7	2	-11.0	115.13
1	7	3	-11.0	108.01
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.07

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 031108
Assay start date: 3/11/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	1.404783922
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	5.756610142
1	2	2	-4.5	5.821636617
1	2	3	-4.5	7.783739847
1	3	1	-5.5	15.42859162
1	3	2	-5.5	16.44639733
1	3	3	-5.5	15.40526691
1	4	1	-6.0	39.11095142
1	4	2	-6.0	35.15493854
1	4	3	-6.0	39.01765257
1	5	1	-6.5	66.28141621
1	5	2	-6.5	71.388115
1	5	3	-6.5	64.85154055
1	6	1	-7.0	82.06942048
1	6	2	-7.0	92.56766229
1	6	3	-7.0	94.17706757
1	7	1	-7.5	95.87
1	7	2	-7.5	106.25
1	7	3	-7.5	92.90
1	8	1	-8.5	92.62
1	8	2	-8.5	104.72
1	8	3	-8.5	111.88
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.88

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 031208
Assay start date: 3/12/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	3.890950034
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	8.387323034
1	2	2	-4.5	8.11631591
1	2	3	-4.5	6.605581487
1	3	1	-5.5	15.11233875
1	3	2	-5.5	18.38657386
1	3	3	-5.5	20.52401225
1	4	1	-6.0	36.57922995
1	4	2	-6.0	34.60074766
1	4	3	-6.0	37.93621995
1	5	1	-6.5	68.08445954
1	5	2	-6.5	60.73469423
1	5	3	-6.5	70.54111306
1	6	1	-7.0	83.0452254
1	6	2	-7.0	85.03152521
1	6	3	-7.0	83.16183664
1	7	1	-7.5	98.26
1	7	2	-7.5	104.04
1	7	3	-7.5	95.10
1	8	1	-8.5	98.94
1	8	2	-8.5	102.19
1	8	3	-8.5	104.36
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.17

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 031108
Assay start date: 3/11/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	
1	2	1	-4.0	7.819080323
1	2	2	-4.0	12.16242483
1	2	3	-4.0	10.75304664
1	3	1	-5.0	50.73443399
1	3	2	-5.0	47.55167072
1	3	3	-5.0	31.05332289
1	4	1	-6.0	82.13868781
1	4	2	-6.0	77.65468821
1	4	3	-6.0	87.62140926
1	5	1	-7.0	91.53501358
1	5	2	-7.0	96.42754908
1	5	3	-7.0	105.1573535
1	6	1	-8.0	102.4481526
1	6	2	-8.0	119.8879707
1	6	3	-8.0	109.6653846
1	7	1	-9.0	104.92
1	7	2	-9.0	108.33
1	7	3	-9.0	108.71
1	8	1	-10.0	106.28
1	8	2	-10.0	107.77
1	8	3	-10.0	96.89
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.96

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 031208
Assay start date: 3/12/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	1.023877074
1	1	2	-7.0	-0.431483297
1	1	3	-7.0	-1.245807587
1	2	1	-8.0	6.851833153
1	2	2	-8.0	6.042069079
1	2	3	-8.0	7.447918533
1	3	1	-8.5	16.47388896
1	3	2	-8.5	39.46063502
1	3	3	-8.5	42.26907663
1	4	1	-9.0	38.4332835
1	4	2	-9.0	44.07752802
1	4	3	-9.0	45.54852341
1	5	1	-9.5	72.27920683
1	5	2	-9.5	73.02968809
1	5	3	-9.5	73.35867511
1	6	1	-10.0	89.93831765
1	6	2	-10.0	81.18139996
1	6	3	-10.0	86.37288018
1	7	1	-11.0	102.12
1	7	2	-11.0	105.91
1	7	3	-11.0	98.79
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.39

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 031208
Assay start date: 3/12/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	
1	2	1	-4.0	10.53713916
1	2	2	-4.0	13.09542035
1	2	3	-4.0	14.74230979
1	3	1	-5.0	44.72116993
1	3	2	-5.0	33.63202748
1	3	3	-5.0	31.65680249
1	4	1	-6.0	79.87978402
1	4	2	-6.0	79.2569888
1	4	3	-6.0	78.08761912
1	5	1	-7.0	94.79494771
1	5	2	-7.0	98.1610386
1	5	3	-7.0	107.3850527
1	6	1	-8.0	100.6176921
1	6	2	-8.0	106.0241539
1	6	3	-8.0	101.4000949
1	7	1	-9.0	99.53
1	7	2	-9.0	103.78
1	7	3	-9.0	110.96
1	8	1	-10.0	99.96
1	8	2	-10.0	103.80
1	8	3	-10.0	107.64
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.65

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 031708	1	1	1	-7.0	1.157228561
Assay start date: 3/17/2008	1	1	2	-7.0	-0.984782614
Technician ID: SMR	1	1	3	-7.0	-1.361887024
	1	2	1	-8.0	7.453201028
	1	2	2	-8.0	8.877333288
	1	2	3	-8.0	7.907324835
	1	3	1	-8.5	
	1	3	2	-8.5	24.02690351
	1	3	3	-8.5	20.77392385
	1	4	1	-9.0	37.93253779
	1	4	2	-9.0	44.61070085
	1	4	3	-9.0	59.7871552
	1	5	1	-9.5	69.50685787
	1	5	2	-9.5	89.09521584
	1	5	3	-9.5	73.98197552
	1	6	1	-10.0	88.86219583
	1	6	2	-10.0	99.85032079
	1	6	3	-10.0	94.91308676
	1	7	1	-11.0	110.28
	1	7	2	-11.0	97.19
	1	7	3	-11.0	105.46
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.78

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 031708
Assay start date: 3/17/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	3.244744879
1	1	2	-4.0	2.127237014
1	1	3	-4.0	3.629841868
1	2	1	-4.5	4.570059799
1	2	2	-4.5	9.304572966
1	2	3	-4.5	8.527839606
1	3	1	-5.5	27.29950131
1	3	2	-5.5	23.13173466
1	3	3	-5.5	24.80145707
1	4	1	-6.0	41.16008651
1	4	2	-6.0	44.9747265
1	4	3	-6.0	37.82718107
1	5	1	-6.5	72.38273312
1	5	2	-6.5	65.4618863
1	5	3	-6.5	66.46967785
1	6	1	-7.0	80.6565056
1	6	2	-7.0	84.46024661
1	6	3	-7.0	89.65396977
1	7	1	-7.5	95.52
1	7	2	-7.5	100.82
1	7	3	-7.5	93.81
1	8	1	-8.5	93.30
1	8	2	-8.5	101.80
1	8	3	-8.5	106.11
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.11

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 031708
Assay start date: 3/17/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-3.0	26.1631019
1	1	2	-3.0	31.95990147
1	1	3	-3.0	51.104308
1	2	1	-4.0	12.3766297
1	2	2	-4.0	11.76919369
1	2	3	-4.0	11.41824682
1	3	1	-5.0	37.51765028
1	3	2	-5.0	39.83985778
1	3	3	-5.0	39.29054962
1	4	1	-6.0	76.5425072
1	4	2	-6.0	89.85015125
1	4	3	-6.0	72.05867037
1	5	1	-7.0	86.22951296
1	5	2	-7.0	98.13191631
1	5	3	-7.0	92.42448829
1	6	1	-8.0	94.94215068
1	6	2	-8.0	103.3634226
1	6	3	-8.0	100.6590245
1	7	1	-9.0	103.92
1	7	2	-9.0	112.33
1	7	3	-9.0	101.45
1	8	1	-10.0	109.71
1	8	2	-10.0	95.27
1	8	3	-10.0	97.63
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.25

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 031808
Assay start date: 3/18/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-0.673195914
1	1	2	-7.0	0.789328765
1	1	3	-7.0	0.617751843
1	2	1	-8.0	6.377941252
1	2	2	-8.0	13.51224452
1	2	3	-8.0	8.5807192
1	3	1	-8.5	18.37334089
1	3	2	-8.5	18.3988152
1	3	3	-8.5	17.06066505
1	4	1	-9.0	45.76645809
1	4	2	-9.0	41.46429789
1	4	3	-9.0	44.97150999
1	5	1	-9.5	69.74302173
1	5	2	-9.5	76.59186296
1	5	3	-9.5	69.81494917
1	6	1	-10.0	95.69908854
1	6	2	-10.0	95.65713087
1	6	3	-10.0	86.05556645
1	7	1	-11.0	105.80
1	7	2	-11.0	100.20
1	7	3	-11.0	102.42
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.33

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 031808
Assay start date: 3/18/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	-0.228144859
1	1	2	-4.0	
1	1	3	-4.0	2.149956232
1	2	1	-4.5	5.072757856
1	2	2	-4.5	3.269327066
1	2	3	-4.5	4.80602692
1	3	1	-5.5	17.7462235
1	3	2	-5.5	11.03224622
1	3	3	-5.5	12.93457725
1	4	1	-6.0	35.68163115
1	4	2	-6.0	37.04675409
1	4	3	-6.0	
1	5	1	-6.5	66.58870365
1	5	2	-6.5	68.54872647
1	5	3	-6.5	67.2270597
1	6	1	-7.0	90.93089846
1	6	2	-7.0	84.80582712
1	6	3	-7.0	90.2857992
1	7	1	-7.5	83.13
1	7	2	-7.5	104.40
1	7	3	-7.5	97.72
1	8	1	-8.5	105.42
1	8	2	-8.5	105.64
1	8	3	-8.5	101.70
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.42

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: R1881	run	xgroup	rep	x	y
Run ID: 031808	1	1	1	-3.0	5.017313785
Assay start date: 3/18/2008	1	1	2	-3.0	4.239598305
Technician ID: SMR	1	1	3	-3.0	3.990099987
	1	2	1	-4.0	8.507293268
	1	2	2	-4.0	12.67084329
	1	2	3	-4.0	7.801505232
	1	3	1	-5.0	47.08138166
	1	3	2	-5.0	44.76471859
	1	3	3	-5.0	42.22852697
	1	4	1	-6.0	88.90868836
	1	4	2	-6.0	81.81259654
	1	4	3	-6.0	71.46703263
	1	5	1	-7.0	89.92766047
	1	5	2	-7.0	95.672865
	1	5	3	-7.0	107.6667661
	1	6	1	-8.0	102.1763047
	1	6	2	-8.0	108.813859
	1	6	3	-8.0	111.9914037
	1	7	1	-9.0	100.71
	1	7	2	-9.0	105.04
	1	7	3	-9.0	95.51
	1	8	1	-10.0	108.73
	1	8	2	-10.0	102.17
	1	8	3	-10.0	108.95
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.02

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 032408
Assay start date: 3/24/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-2.692379462
1	1	2	-7.0	3.289501243
1	1	3	-7.0	0.272665303
1	2	1	-8.0	7.291892777
1	2	2	-8.0	7.975079305
1	2	3	-8.0	8.332286085
1	3	1	-8.5	23.93818571
1	3	2	-8.5	29.8652287
1	3	3	-8.5	25.54142723
1	4	1	-9.0	34.16465785
1	4	2	-9.0	51.67464479
1	4	3	-9.0	47.99442483
1	5	1	-9.5	77.37144555
1	5	2	-9.5	80.39437456
1	5	3	-9.5	82.39823605
1	6	1	-10.0	89.42507988
1	6	2	-10.0	82.53152217
1	6	3	-10.0	95.14952798
1	7	1	-11.0	103.44
1	7	2	-11.0	109.66
1	7	3	-11.0	113.63
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.80

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 032408
Assay start date: 3/24/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-4.0	2.518726699
1	1	2	-4.0	4.308568774
1	1	3	-4.0	2.024425632
1	2	1	-4.5	5.554603512
1	2	2	-4.5	9.227968758
1	2	3	-4.5	7.026082188
1	3	1	-5.5	15.41625253
1	3	2	-5.5	16.96846451
1	3	3	-5.5	18.2244005
1	4	1	-6.0	38.1411538
1	4	2	-6.0	43.94176539
1	4	3	-6.0	40.70100879
1	5	1	-6.5	65.16472259
1	5	2	-6.5	71.42155351
1	5	3	-6.5	69.58068189
1	6	1	-7.0	79.0866474
1	6	2	-7.0	79.9785219
1	6	3	-7.0	100.5129611
1	7	1	-7.5	101.46
1	7	2	-7.5	99.56
1	7	3	-7.5	
1	8	1	-8.5	103.72
1	8	2	-8.5	106.00
1	8	3	-8.5	110.27
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.79

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: R1881
Run ID: 032408
Assay start date: 3/24/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-3.0	
1	1	2	-3.0	
1	1	3	-3.0	
1	2	1	-4.0	10.78398891
1	2	2	-4.0	9.339167457
1	2	3	-4.0	9.374202663
1	3	1	-5.0	55.16978747
1	3	2	-5.0	55.33506224
1	3	3	-5.0	51.10113369
1	4	1	-6.0	87.41893348
1	4	2	-6.0	91.91943426
1	4	3	-6.0	93.11596273
1	5	1	-7.0	88.29252874
1	5	2	-7.0	103.2906437
1	5	3	-7.0	103.2914053
1	6	1	-8.0	106.027198
1	6	2	-8.0	108.5436398
1	6	3	-8.0	108.3585625
1	7	1	-9.0	105.66
1	7	2	-9.0	107.98
1	7	3	-9.0	105.55
1	8	1	-10.0	107.59
1	8	2	-10.0	111.00
1	8	3	-10.0	116.63
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.17

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 032608
Assay start date: 3/26/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	2.014472735
1	1	2	-7.0	-0.658981935
1	1	3	-7.0	-2.985199469
1	2	1	-8.0	11.74118174
1	2	2	-8.0	9.900553635
1	2	3	-8.0	13.00534194
1	3	1	-8.5	33.55201436
1	3	2	-8.5	27.67611094
1	3	3	-8.5	32.43841401
1	4	1	-9.0	62.6941623
1	4	2	-9.0	56.61479963
1	4	3	-9.0	66.4310307
1	5	1	-9.5	98.37142166
1	5	2	-9.5	91.34258244
1	5	3	-9.5	95.7237385
1	6	1	-10.0	110.5586313
1	6	2	-10.0	110.9682626
1	6	3	-10.0	104.0954088
1	7	1	-11.0	119.75
1	7	2	-11.0	
1	7	3	-11.0	
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.86

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 032608
Assay start date: 3/26/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	0.563130017
1	1	2	-4.0	
1	1	3	-4.0	1.831359402
1	2	1	-4.5	9.108418918
1	2	2	-4.5	9.693025191
1	2	3	-4.5	10.66420406
1	3	1	-5.5	37.54795422
1	3	2	-5.5	29.71884191
1	3	3	-5.5	29.59134077
1	4	1	-6.0	63.47544586
1	4	2	-6.0	63.97188645
1	4	3	-6.0	61.2658783
1	5	1	-6.5	89.21846777
1	5	2	-6.5	83.41038411
1	5	3	-6.5	95.15676536
1	6	1	-7.0	89.63216827
1	6	2	-7.0	85.97125799
1	6	3	-7.0	97.82072526
1	7	1	-7.5	108.37
1	7	2	-7.5	106.06
1	7	3	-7.5	115.44
1	8	1	-8.5	121.23
1	8	2	-8.5	
1	8	3	-8.5	
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.54

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: R1881	run	xgroup	rep	x	y
Run ID: 032608	1	1	1	-3.0	
Assay start date: 3/26/2008	1	1	2	-3.0	
Technician ID: SMR	1	1	3	-3.0	
	1	2	1	-4.0	16.64454989
	1	2	2	-4.0	18.63709424
	1	2	3	-4.0	18.97212382
	1	3	1	-5.0	59.87150417
	1	3	2	-5.0	62.16109904
	1	3	3	-5.0	59.53783099
	1	4	1	-6.0	112.5050582
	1	4	2	-6.0	109.3446577
	1	4	3	-6.0	103.8838112
	1	5	1	-7.0	111.6600241
	1	5	2	-7.0	106.4338339
	1	5	3	-7.0	117.8628187
	1	6	1	-8.0	108.6501835
	1	6	2	-8.0	127.8933602
	1	6	3	-8.0	123.0510298
	1	7	1	-9.0	113.86
	1	7	2	-9.0	116.30
	1	7	3	-9.0	114.45
	1	8	1	-10.0	112.52
	1	8	2	-10.0	117.75
	1	8	3	-10.0	116.81
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 9.08

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 040208
Assay start date: 4/2/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-1.560595942
1	1	2	-7.0	-1.711371507
1	1	3	-7.0	0.640107677
1	2	1	-8.0	12.45740074
1	2	2	-8.0	31.26097254
1	2	3	-8.0	14.55483342
1	3	1	-8.5	33.25410158
1	3	2	-8.5	33.49782098
1	3	3	-8.5	35.84103849
1	4	1	-9.0	65.22368484
1	4	2	-9.0	63.9926953
1	4	3	-9.0	74.44991015
1	5	1	-9.5	97.35953432
1	5	2	-9.5	102.4032868
1	5	3	-9.5	120.5263031
1	6	1	-10.0	115.1624108
1	6	2	-10.0	93.65107505
1	6	3	-10.0	116.1465828
1	7	1	-11.0	116.97
1	7	2	-11.0	138.55
1	7	3	-11.0	132.59
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.99

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 040208
Assay start date: 4/2/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	8.196441972
1	2	2	-4.5	8.778890044
1	2	3	-4.5	15.391328
1	3	1	-5.5	17.11078906
1	3	2	-5.5	22.64817658
1	3	3	-5.5	23.37004041
1	4	1	-6.0	47.15850023
1	4	2	-6.0	42.81389201
1	4	3	-6.0	44.88240883
1	5	1	-6.5	72.87399569
1	5	2	-6.5	73.6041212
1	5	3	-6.5	85.35222273
1	6	1	-7.0	123.5986134
1	6	2	-7.0	106.2583908
1	6	3	-7.0	106.2439328
1	7	1	-7.5	120.82
1	7	2	-7.5	119.54
1	7	3	-7.5	114.54
1	8	1	-8.5	123.81
1	8	2	-8.5	124.78
1	8	3	-8.5	119.96
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.27

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: R1881	run	xgroup	rep	x	y
Run ID: 040208	1	1	1	-3.0	
Assay start date: 4/2/2008	1	1	2	-3.0	
Technician ID: SMR	1	1	3	-3.0	
	1	2	1	-4.0	14.60337076
	1	2	2	-4.0	9.410908165
	1	2	3	-4.0	15.78375755
	1	3	1	-5.0	48.13647598
	1	3	2	-5.0	55.34995077
	1	3	3	-5.0	49.6297737
	1	4	1	-6.0	102.3557821
	1	4	2	-6.0	91.60631054
	1	4	3	-6.0	93.28859407
	1	5	1	-7.0	117.265007
	1	5	2	-7.0	117.4157826
	1	5	3	-7.0	118.4856694
	1	6	1	-8.0	118.9947951
	1	6	2	-8.0	125.8984571
	1	6	3	-8.0	133.7356884
	1	7	1	-9.0	134.45
	1	7	2	-9.0	126.68
	1	7	3	-9.0	101.87
	1	8	1	-10.0	130.94
	1	8	2	-10.0	123.82
	1	8	3	-10.0	123.25
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.05

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 040308
Assay start date: 4/3/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	0.693754273
1	1	2	-7.0	0.399829425
1	1	3	-7.0	1.252000028
1	2	1	-8.0	13.82838877
1	2	2	-8.0	10.63539222
1	2	3	-8.0	12.09127177
1	3	1	-8.5	44.38811472
1	3	2	-8.5	28.49925638
1	3	3	-8.5	31.27145405
1	4	1	-9.0	56.55322013
1	4	2	-9.0	61.49179195
1	4	3	-9.0	50.40300128
1	5	1	-9.5	78.85027454
1	5	2	-9.5	84.19590056
1	5	3	-9.5	74.17073721
1	6	1	-10.0	92.5040353
1	6	2	-10.0	95.70654741
1	6	3	-10.0	92.58861799
1	7	1	-11.0	81.54
1	7	2	-11.0	94.75
1	7	3	-11.0	86.53
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.87

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 040308
Assay start date: 4/3/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	-0.626792977
1	1	2	-4.0	2.26699231
1	1	3	-4.0	-0.090750178
1	2	1	-4.5	5.577347346
1	2	2	-4.5	7.041685169
1	2	3	-4.5	6.782650681
1	3	1	-5.5	19.24485279
1	3	2	-5.5	19.15709825
1	3	3	-5.5	18.88114722
1	4	1	-6.0	46.03324805
1	4	2	-6.0	44.93790221
1	4	3	-6.0	39.90734671
1	5	1	-6.5	63.81675865
1	5	2	-6.5	67.59231848
1	5	3	-6.5	68.65806038
1	6	1	-7.0	87.8287271
1	6	2	-7.0	85.65706653
1	6	3	-7.0	87.95031472
1	7	1	-7.5	85.74
1	7	2	-7.5	94.88
1	7	3	-7.5	89.86
1	8	1	-8.5	99.18
1	8	2	-8.5	89.54
1	8	3	-8.5	94.21
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.72

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 040708
Assay start date: 4/7/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-0.431014543
1	1	2	-7.0	4.008656494
1	1	3	-7.0	1.138980569
1	2	1	-8.0	14.90208146
1	2	2	-8.0	9.593543028
1	2	3	-8.0	5.540035278
1	3	1	-8.5	30.86269281
1	3	2	-8.5	21.32240813
1	3	3	-8.5	22.66999733
1	4	1	-9.0	62.69099495
1	4	2	-9.0	52.00875303
1	4	3	-9.0	57.31125767
1	5	1	-9.5	103.3322674
1	5	2	-9.5	103.7039495
1	5	3	-9.5	85.7714919
1	6	1	-10.0	114.2389667
1	6	2	-10.0	112.0161143
1	6	3	-10.0	121.9972285
1	7	1	-11.0	128.90
1	7	2	-11.0	137.25
1	7	3	-11.0	117.18
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.63

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 040708
Assay start date: 4/7/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	10.67962727
1	1	2	-4.0	10.67962727
1	1	3	-4.0	1.210179425
1	2	1	-4.5	6.716626542
1	2	2	-4.5	11.35661978
1	2	3	-4.5	8.315583901
1	3	1	-5.5	26.09840326
1	3	2	-5.5	32.43992848
1	3	3	-5.5	26.56421272
1	4	1	-6.0	42.79996541
1	4	2	-6.0	40.02200326
1	4	3	-6.0	36.57308241
1	5	1	-6.5	92.83586652
1	5	2	-6.5	
1	5	3	-6.5	94.97665925
1	6	1	-7.0	124.8994869
1	6	2	-7.0	121.0511284
1	6	3	-7.0	122.0563597
1	7	1	-7.5	142.89
1	7	2	-7.5	148.81
1	7	3	-7.5	144.34
1	8	1	-8.5	143.98
1	8	2	-8.5	145.02
1	8	3	-8.5	133.47
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.26

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 040808	1	1	1	-7.0	-1.511929334
Assay start date: 4/8/2008	1	1	2	-7.0	6.381655304
Technician ID: SMR	1	1	3	-7.0	-1.839264989
	1	2	1	-8.0	7.086365184
	1	2	2	-8.0	4.567757091
	1	2	3	-8.0	3.547178728
	1	3	1	-8.5	17.86863485
	1	3	2	-8.5	18.63589295
	1	3	3	-8.5	16.05994899
	1	4	1	-9.0	36.66228829
	1	4	2	-9.0	34.52209689
	1	4	3	-9.0	34.00398919
	1	5	1	-9.5	83.69437553
	1	5	2	-9.5	85.05688413
	1	5	3	-9.5	76.98920696
	1	6	1	-10.0	93.57178103
	1	6	2	-10.0	85.45927764
	1	6	3	-10.0	89.13502769
	1	7	1	-11.0	92.35
	1	7	2	-11.0	95.75
	1	7	3	-11.0	89.32
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.06

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 040808
Assay start date: 4/8/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	-0.785327579
1	2	2	-4.5	-1.213782846
1	2	3	-4.5	2.365017479
1	3	1	-5.5	13.95520158
1	3	2	-5.5	10.11369875
1	3	3	-5.5	11.53875557
1	4	1	-6.0	42.38128001
1	4	2	-6.0	38.90881165
1	4	3	-6.0	37.58174704
1	5	1	-6.5	47.87718311
1	5	2	-6.5	58.48014789
1	5	3	-6.5	50.49691081
1	6	1	-7.0	93.86158775
1	6	2	-7.0	102.5411949
1	6	3	-7.0	101.437219
1	7	1	-7.5	93.83
1	7	2	-7.5	94.46
1	7	3	-7.5	92.90
1	8	1	-8.5	108.57
1	8	2	-8.5	109.39
1	8	3	-8.5	104.27
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.99

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 040908	1	1	1	-7.0	-2.489233521
Assay start date: 4/9/2008	1	1	2	-7.0	-2.164474149
Technician ID: SMR	1	1	3	-7.0	-1.376109006
	1	2	1	-8.0	7.025274751
	1	2	2	-8.0	11.57661262
	1	2	3	-8.0	16.38563999
	1	3	1	-8.5	35.34052667
	1	3	2	-8.5	30.58327254
	1	3	3	-8.5	26.444494387
	1	4	1	-9.0	74.30106135
	1	4	2	-9.0	65.02423929
	1	4	3	-9.0	70.57103523
	1	5	1	-9.5	127.4533453
	1	5	2	-9.5	115.0571859
	1	5	3	-9.5	122.2077754
	1	6	1	-10.0	143.3406679
	1	6	2	-10.0	146.1340691
	1	6	3	-10.0	144.2384628
	1	7	1	-11.0	154.33
	1	7	2	-11.0	151.60
	1	7	3	-11.0	156.85
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 8.62

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 040908
Assay start date: 4/9/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	-0.959569811
1	1	2	-4.0	2.018567764
1	1	3	-4.0	2.881062763
1	2	1	-4.5	10.19167863
1	2	2	-4.5	9.59981644
1	2	3	-4.5	14.92069282
1	3	1	-5.5	37.87035512
1	3	2	-5.5	35.62527946
1	3	3	-5.5	34.11326571
1	4	1	-6.0	65.91732756
1	4	2	-6.0	68.0541501
1	4	3	-6.0	34.41802179
1	5	1	-6.5	57.07234133
1	5	2	-6.5	62.95330996
1	5	3	-6.5	59.23740381
1	6	1	-7.0	
1	6	2	-7.0	149.9582284
1	6	3	-7.0	141.7262843
1	7	1	-7.5	138.05
1	7	2	-7.5	156.77
1	7	3	-7.5	136.68
1	8	1	-8.5	150.08
1	8	2	-8.5	149.81
1	8	3	-8.5	164.62
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.49

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 041008
Assay start date: 4/10/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-1.309879549
1	1	2	-7.0	-1.68254839
1	1	3	-7.0	-2.405731663
1	2	1	-8.0	5.29213492
1	2	2	-8.0	3.507913608
1	2	3	-8.0	5.385499938
1	3	1	-8.5	16.48723352
1	3	2	-8.5	17.3362222
1	3	3	-8.5	18.93846085
1	4	1	-9.0	45.41456442
1	4	2	-9.0	43.90727324
1	4	3	-9.0	33.86420401
1	5	1	-9.5	
1	5	2	-9.5	71.90649256
1	5	3	-9.5	62.11740783
1	6	1	-10.0	87.93125275
1	6	2	-10.0	87.50794526
1	6	3	-10.0	75.08723299
1	7	1	-11.0	101.34
1	7	2	-11.0	103.89
1	7	3	-11.0	94.85
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.30

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 041008
Assay start date: 4/10/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	8.433313904
1	2	2	-4.5	9.27597275
1	2	3	-4.5	7.905564186
1	3	1	-5.5	15.58365004
1	3	2	-5.5	15.81548013
1	3	3	-5.5	13.46078274
1	4	1	-6.0	32.03171773
1	4	2	-6.0	37.40890977
1	4	3	-6.0	40.75501705
1	5	1	-6.5	60.73038346
1	5	2	-6.5	73.49369786
1	5	3	-6.5	61.69489156
1	6	1	-7.0	79.58774334
1	6	2	-7.0	101.7476929
1	6	3	-7.0	93.06158135
1	7	1	-7.5	98.80
1	7	2	-7.5	95.99
1	7	3	-7.5	98.57
1	8	1	-8.5	102.79
1	8	2	-8.5	95.94
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.12

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 041508
Assay start date: 4/15/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	0.809879889
1	1	2	-7.0	-1.995660205
1	1	3	-7.0	-2.2650824
1	2	1	-8.0	14.45845332
1	2	2	-8.0	14.0341537
1	2	3	-8.0	13.93735531
1	3	1	-8.5	29.68806718
1	3	2	-8.5	28.65716429
1	3	3	-8.5	30.02040833
1	4	1	-9.0	56.15920109
1	4	2	-9.0	52.21466657
1	4	3	-9.0	57.08523905
1	5	1	-9.5	87.38958933
1	5	2	-9.5	81.04606797
1	5	3	-9.5	75.62535795
1	6	1	-10.0	89.8692415
1	6	2	-10.0	99.69911833
1	6	3	-10.0	91.03082222
1	7	1	-11.0	91.56
1	7	2	-11.0	102.41
1	7	3	-11.0	90.18
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.35

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 041508
Assay start date: 4/15/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	4.373674064
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	9.442683251
1	2	2	-4.5	19.15317539
1	2	3	-4.5	23.28324016
1	3	1	-5.5	29.22343489
1	3	2	-5.5	27.53914285
1	3	3	-5.5	25.23050117
1	4	1	-6.0	46.22768595
1	4	2	-6.0	44.60953948
1	4	3	-6.0	56.6528729
1	5	1	-6.5	61.90579903
1	5	2	-6.5	70.97419516
1	5	3	-6.5	85.36811622
1	6	1	-7.0	85.71820374
1	6	2	-7.0	95.76587695
1	6	3	-7.0	89.83536207
1	7	1	-7.5	88.38
1	7	2	-7.5	102.87
1	7	3	-7.5	114.00
1	8	1	-8.5	95.09
1	8	2	-8.5	99.00
1	8	3	-8.5	
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run.

It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.28

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 041708
Assay start date: 4/17/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	11.01214083
1	1	2	-7.0	-2.623269666
1	1	3	-7.0	0.82478613
1	2	1	-8.0	5.377824376
1	2	2	-8.0	9.992858337
1	2	3	-8.0	12.20829345
1	3	1	-8.5	31.94845846
1	3	2	-8.5	29.56527024
1	3	3	-8.5	35.40563128
1	4	1	-9.0	69.4339852
1	4	2	-9.0	69.15682789
1	4	3	-9.0	60.48107459
1	5	1	-9.5	98.60661592
1	5	2	-9.5	94.79570285
1	5	3	-9.5	98.51726915
1	6	1	-10.0	88.29162298
1	6	2	-10.0	95.25884731
1	6	3	-10.0	97.78
1	7	1	-11.0	112.55
1	7	2	-11.0	112.55
1	7	3	-11.0	115.20
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.61

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 041608
Assay start date: 4/16/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-0.873078111
1	1	2	-7.0	4.274137972
1	1	3	-7.0	-5.005109194
1	2	1	-8.0	21.05035527
1	2	2	-8.0	21.44673368
1	2	3	-8.0	13.46213255
1	3	1	-8.5	37.43019415
1	3	2	-8.5	42.12970224
1	3	3	-8.5	46.8862432
1	4	1	-9.0	62.61590742
1	4	2	-9.0	66.34870844
1	4	3	-9.0	61.69767829
1	5	1	-9.5	82.8853877
1	5	2	-9.5	98.84888667
1	5	3	-9.5	97.2091918
1	6	1	-10.0	93.30529217
1	6	2	-10.0	117.5043369
1	6	3	-10.0	109.27
1	7	1	-11.0	112.30
1	7	2	-11.0	99.67
1	7	3	-11.0	120.11
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.96

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 041608
Assay start date: 4/16/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	20.21482379
1	2	2	-4.5	35.21446734
1	2	3	-4.5	28.96651696
1	3	1	-5.5	37.60414439
1	3	2	-5.5	41.55652195
1	3	3	-5.5	33.76012927
1	4	1	-6.0	61.23000879
1	4	2	-6.0	61.79178251
1	4	3	-6.0	52.6608208
1	5	1	-6.5	84.99275207
1	5	2	-6.5	
1	5	3	-6.5	87.33965448
1	6	1	-7.0	94.96209691
1	6	2	-7.0	111.4075236
1	6	3	-7.0	100.1064613
1	7	1	-7.5	97.67
1	7	2	-7.5	90.80
1	7	3	-7.5	99.00
1	8	1	-8.5	107.15
1	8	2	-8.5	108.02
1	8	3	-8.5	112.16
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 9.26

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 041708
Assay start date: 4/17/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	1.384570968
1	2	2	-4.5	6.871191746
1	2	3	-4.5	18.0067162
1	3	1	-5.5	47.04076826
1	3	2	-5.5	33.07349835
1	3	3	-5.5	31.96304569
1	4	1	-6.0	68.42746653
1	4	2	-6.0	67.2258437
1	4	3	-6.0	53.55031834
1	5	1	-6.5	66.64417802
1	5	2	-6.5	74.5541019
1	5	3	-6.5	81.64349425
1	6	1	-7.0	73.53664281
1	6	2	-7.0	69.94453815
1	6	3	-7.0	96.80874018
1	7	1	-7.5	106.24
1	7	2	-7.5	99.49
1	7	3	-7.5	99.82
1	8	1	-8.5	99.19
1	8	2	-8.5	98.15
1	8	3	-8.5	74.72
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.93

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol-sialinized
Run ID: 050508
Assay start date: 5/5/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-7.0	-2.24695264
1	1	2	-7.0	1.743325324
1	1	3	-7.0	-2.194376162
1	2	1	-8.0	5.393239772
1	2	2	-8.0	8.940768447
1	2	3	-8.0	9.153841542
1	3	1	-8.5	24.04820433
1	3	2	-8.5	27.5141011
1	3	3	-8.5	33.00419228
1	4	1	-9.0	59.28412974
1	4	2	-9.0	60.74105162
1	4	3	-9.0	59.48613411
1	5	1	-9.5	80.89859658
1	5	2	-9.5	82.52570021
1	5	3	-9.5	85.07012503
1	6	1	-10.0	92.03650837
1	6	2	-10.0	84.35895899
1	6	3	-10.0	101.42
1	7	1	-11.0	99.33
1	7	2	-11.0	100.20
1	7	3	-11.0	
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.41

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol-uncoated

Run ID: 050508

Assay start date: 5/5/2008

Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	1.567090867
1	1	2	-7.0	5.523507484
1	1	3	-7.0	-2.693458455
1	2	1	-8.0	10.61974889
1	2	2	-8.0	10.14740119
1	2	3	-8.0	11.80532815
1	3	1	-8.5	28.41689507
1	3	2	-8.5	30.91185983
1	3	3	-8.5	31.82156651
1	4	1	-9.0	58.00523934
1	4	2	-9.0	61.3574277
1	4	3	-9.0	62.09353651
1	5	1	-9.5	88.41985565
1	5	2	-9.5	91.13148132
1	5	3	-9.5	98.96491708
1	6	1	-10.0	95.05560066
1	6	2	-10.0	96.94364573
1	6	3	-10.0	99.83559788
1	7	1	-11.0	100.12
1	7	2	-11.0	96.64
1	7	3	-11.0	83.71
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 36.13

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 050708
Assay start date: 5/7/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-5.609689741
1	1	2	-7.0	-12.7569476
1	1	3	-7.0	-37.63692994
1	2	1	-8.0	52.56169854
1	2	2	-8.0	20.74600663
1	2	3	-8.0	
1	3	1	-8.5	39.4237444
1	3	2	-8.5	69.45701385
1	3	3	-8.5	
1	4	1	-9.0	107.234854
1	4	2	-9.0	18.12983502
1	4	3	-9.0	42.90221321
1	5	1	-9.5	72.71807835
1	5	2	-9.5	151.5077391
1	5	3	-9.5	44.8515319
1	6	1	-10.0	153.3611656
1	6	2	-10.0	86.50824538
1	6	3	-10.0	56.49107996
1	7	1	-11.0	119.85
1	7	2	-11.0	151.10
1	7	3	-11.0	96.90
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 50.10

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 050708
Assay start date: 5/7/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	-9.514915131
1	2	2	-4.5	68.93216913
1	2	3	-4.5	-44.91155598
1	3	1	-5.5	87.1018982
1	3	2	-5.5	117.0941756
1	3	3	-5.5	14.52326604
1	4	1	-6.0	32.4624148
1	4	2	-6.0	98.93542653
1	4	3	-6.0	120.4818763
1	5	1	-6.5	114.0907754
1	5	2	-6.5	155.4100365
1	5	3	-6.5	-15.3606752
1	6	1	-7.0	121.6794299
1	6	2	-7.0	96.29363489
1	6	3	-7.0	61.61874703
1	7	1	-7.5	34.90
1	7	2	-7.5	56.94
1	7	3	-7.5	50.69
1	8	1	-8.5	138.31
1	8	2	-8.5	75.99
1	8	3	-8.5	32.65
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 19.57

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 051508
Assay start date: 5/15/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-3.405938917
1	1	2	-7.0	13.02343373
1	1	3	-7.0	-7.926761441
1	2	1	-8.0	2.876449941
1	2	2	-8.0	53.35516453
1	2	3	-8.0	31.11447454
1	3	1	-8.5	32.84159323
1	3	2	-8.5	16.86779066
1	3	3	-8.5	22.00651778
1	4	1	-9.0	88.1537427
1	4	2	-9.0	82.80273199
1	4	3	-9.0	59.07519562
1	5	1	-9.5	71.2188509
1	5	2	-9.5	131.8441187
1	5	3	-9.5	107.2932836
1	6	1	-10.0	109.8949419
1	6	2	-10.0	104.5193872
1	6	3	-10.0	120.300283
1	7	1	-11.0	140.75
1	7	2	-11.0	124.52
1	7	3	-11.0	91.95
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 20.18

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 051508
Assay start date: 5/15/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	38.68563584
1	2	2	-4.5	39.76858361
1	2	3	-4.5	37.57943591
1	3	1	-5.5	19.38763574
1	3	2	-5.5	52.53229646
1	3	3	-5.5	33.44356579
1	4	1	-6.0	43.84632332
1	4	2	-6.0	39.21182358
1	4	3	-6.0	77.9985776
1	5	1	-6.5	97.73190978
1	5	2	-6.5	74.65629506
1	5	3	-6.5	75.09291893
1	6	1	-7.0	81.15699585
1	6	2	-7.0	154.8835634
1	6	3	-7.0	156.1206644
1	7	1	-7.5	103.40
1	7	2	-7.5	131.26
1	7	3	-7.5	142.92
1	8	1	-8.5	135.55
1	8	2	-8.5	127.74
1	8	3	-8.5	115.47
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.88

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 051608
Assay start date: 5/16/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	3.313010535
1	1	2	-7.0	-2.929290117
1	1	3	-7.0	-1.487206613
1	2	1	-8.0	12.45409391
1	2	2	-8.0	25.37995049
1	2	3	-8.0	28.70189285
1	3	1	-8.5	38.11371487
1	3	2	-8.5	39.68455583
1	3	3	-8.5	28.41514652
1	4	1	-9.0	87.91918672
1	4	2	-9.0	82.41115156
1	4	3	-9.0	73.78370603
1	5	1	-9.5	106.5382108
1	5	2	-9.5	
1	5	3	-9.5	100.8172039
1	6	1	-10.0	105.2193169
1	6	2	-10.0	102.6427758
1	6	3	-10.0	124.8273374
1	7	1	-11.0	107.71
1	7	2	-11.0	114.53
1	7	3	-11.0	128.00
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run.

It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 12.37

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 051608
Assay start date: 5/16/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	4.130794376
1	2	2	-4.5	12.88212545
1	2	3	-4.5	9.469008892
1	3	1	-5.5	30.34650835
1	3	2	-5.5	14.86986217
1	3	3	-5.5	16.18388421
1	4	1	-6.0	24.11047158
1	4	2	-6.0	57.61385755
1	4	3	-6.0	61.95750772
1	5	1	-6.5	104.6736636
1	5	2	-6.5	73.46912025
1	5	3	-6.5	92.94351144
1	6	1	-7.0	104.2212726
1	6	2	-7.0	107.7617546
1	6	3	-7.0	96.81597892
1	7	1	-7.5	111.22
1	7	2	-7.5	128.98
1	7	3	-7.5	91.82
1	8	1	-8.5	92.75
1	8	2	-8.5	107.49
1	8	3	-8.5	108.55
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 13.05

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 051808
Assay start date: 5/18/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	
1	1	2	-7.0	
1	1	3	-7.0	-10.53
1	2	1	-8.0	43.99
1	2	2	-8.0	
1	2	3	-8.0	18.96
1	3	1	-8.5	49.81
1	3	2	-8.5	83.22
1	3	3	-8.5	87.97
1	4	1	-9.0	154.75
1	4	2	-9.0	138.93
1	4	3	-9.0	149.42
1	5	1	-9.5	
1	5	2	-9.5	
1	5	3	-9.5	
1	6	1	-10.0	160.77
1	6	2	-10.0	
1	6	3	-10.0	200.63
1	7	1	-11.0	192.87
1	7	2	-11.0	169.11
1	7	3	-11.0	
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.26

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 051808
Assay start date: 5/18/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	
1	2	2	-4.5	25.50
1	2	3	-4.5	24.27
1	3	1	-5.5	104.38
1	3	2	-5.5	
1	3	3	-5.5	107.97
1	4	1	-6.0	131.27
1	4	2	-6.0	
1	4	3	-6.0	125.48
1	5	1	-6.5	
1	5	2	-6.5	
1	5	3	-6.5	129.36
1	6	1	-7.0	
1	6	2	-7.0	178.01
1	6	3	-7.0	156.65
1	7	1	-7.5	
1	7	2	-7.5	101.54
1	7	3	-7.5	121.04
1	8	1	-8.5	
1	8	2	-8.5	
1	8	3	-8.5	
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.30

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 051908
Assay start date: 5/19/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	5.788712012
1	1	2	-7.0	-2.173060472
1	1	3	-7.0	-2.138658459
1	2	1	-8.0	11.62902704
1	2	2	-8.0	14.33302524
1	2	3	-8.0	17.0966536
1	3	1	-8.5	45.92668702
1	3	2	-8.5	44.93132212
1	3	3	-8.5	48.4024852
1	4	1	-9.0	89.85461709
1	4	2	-9.0	72.48160066
1	4	3	-9.0	
1	5	1	-9.5	100.0272923
1	5	2	-9.5	104.3481851
1	5	3	-9.5	
1	6	1	-10.0	126.8780632
1	6	2	-10.0	124.9182952
1	6	3	-10.0	
1	7	1	-11.0	123.30
1	7	2	-11.0	117.58
1	7	3	-11.0	125.49
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.61

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 051908
Assay start date: 5/19/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	13.06
1	2	2	-4.5	24.54
1	2	3	-4.5	9.85
1	3	1	-5.5	52.05
1	3	2	-5.5	47.22
1	3	3	-5.5	41.48
1	4	1	-6.0	78.56
1	4	2	-6.0	91.59
1	4	3	-6.0	83.20
1	5	1	-6.5	117.57
1	5	2	-6.5	133.03
1	5	3	-6.5	
1	6	1	-7.0	171.01
1	6	2	-7.0	176.68
1	6	3	-7.0	173.62
1	7	1	-7.5	163.25
1	7	2	-7.5	
1	7	3	-7.5	176.30
1	8	1	-8.5	158.45
1	8	2	-8.5	164.92
1	8	3	-8.5	
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 9.73

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 052108
Assay start date: 5/21/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	0.383236941
1	1	2	-7.0	-1.034621822
1	1	3	-7.0	-10.95302969
1	2	1	-8.0	
1	2	2	-8.0	46.03394968
1	2	3	-8.0	50.62713286
1	3	1	-8.5	102.9181036
1	3	2	-8.5	
1	3	3	-8.5	93.15534893
1	4	1	-9.0	129.1989601
1	4	2	-9.0	130.7102108
1	4	3	-9.0	
1	5	1	-9.5	135.1571743
1	5	2	-9.5	120.5653983
1	5	3	-9.5	
1	6	1	-10.0	165.4472788
1	6	2	-10.0	
1	6	3	-10.0	199.8013457
1	7	1	-11.0	189.29
1	7	2	-11.0	170.90
1	7	3	-11.0	202.13
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 14.79

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 052108
Assay start date: 5/21/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	86.86130124
1	2	2	-4.5	60.89835456
1	2	3	-4.5	53.66472782
1	3	1	-5.5	44.58118688
1	3	2	-5.5	45.25002378
1	3	3	-5.5	47.36313331
1	4	1	-6.0	126.9764217
1	4	2	-6.0	70.97052888
1	4	3	-6.0	114.8354754
1	5	1	-6.5	101.7332529
1	5	2	-6.5	116.4995492
1	5	3	-6.5	127.3622529
1	6	1	-7.0	186.8330793
1	6	2	-7.0	165.4463354
1	6	3	-7.0	154.1817636
1	7	1	-7.5	
1	7	2	-7.5	152.64
1	7	3	-7.5	175.51
1	8	1	-8.5	135.55
1	8	2	-8.5	
1	8	3	-8.5	131.66
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 7.09

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 052208	1	1	1	-7.0	
Assay start date: 5/22/2008	1	1	2	-7.0	-0.422458223
Technician ID: SMR	1	1	3	-7.0	5.753553022
	1	2	1	-8.0	24.97735437
	1	2	2	-8.0	
	1	2	3	-8.0	40.14055911
	1	3	1	-8.5	
	1	3	2	-8.5	83.24457286
	1	3	3	-8.5	79.26909261
	1	4	1	-9.0	102.5261596
	1	4	2	-9.0	123.1532094
	1	4	3	-9.0	
	1	5	1	-9.5	148.0064032
	1	5	2	-9.5	
	1	5	3	-9.5	143.4171482
	1	6	1	-10.0	
	1	6	2	-10.0	103.5280337
	1	6	3	-10.0	129.4697798
	1	7	1	-11.0	158.55
	1	7	2	-11.0	
	1	7	3	-11.0	160.52
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 8.27

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 052208
Assay start date: 5/22/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	
1	2	2	-4.5	3.267218491
1	2	3	-4.5	0.561455568
1	3	1	-5.5	43.9192566
1	3	2	-5.5	33.16882711
1	3	3	-5.5	42.61596127
1	4	1	-6.0	
1	4	2	-6.0	77.20287365
1	4	3	-6.0	100.7754178
1	5	1	-6.5	
1	5	2	-6.5	121.9904732
1	5	3	-6.5	102.0841793
1	6	1	-7.0	
1	6	2	-7.0	145.9745432
1	6	3	-7.0	125.9206622
1	7	1	-7.5	152.78
1	7	2	-7.5	
1	7	3	-7.5	148.43
1	8	1	-8.5	161.31
1	8	2	-8.5	
1	8	3	-8.5	187.20
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.90

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 052708
Assay start date: 5/27/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-1.274727861
1	1	2	-7.0	-0.195080511
1	1	3	-7.0	-1.010525697
1	2	1	-8.0	12.67089009
1	2	2	-8.0	12.99322712
1	2	3	-8.0	10.43996495
1	3	1	-8.5	32.66097603
1	3	2	-8.5	39.00857576
1	3	3	-8.5	35.93106175
1	4	1	-9.0	67.74328616
1	4	2	-9.0	67.56628628
1	4	3	-9.0	65.59852517
1	5	1	-9.5	99.64219379
1	5	2	-9.5	102.1913035
1	5	3	-9.5	108.1734841
1	6	1	-10.0	127.654371
1	6	2	-10.0	128.9738246
1	6	3	-10.0	132.8465404
1	7	1	-11.0	131.64
1	7	2	-11.0	138.26
1	7	3	-11.0	130.19
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.76

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 052708
Assay start date: 5/27/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	8.864614126
1	2	2	-4.5	13.79621483
1	2	3	-4.5	12.83906593
1	3	1	-5.5	23.90389408
1	3	2	-5.5	32.77205513
1	3	3	-5.5	36.70861546
1	4	1	-6.0	58.78169409
1	4	2	-6.0	63.54823333
1	4	3	-6.0	55.7950158
1	5	1	-6.5	95.30543725
1	5	2	-6.5	96.22106126
1	5	3	-6.5	102.2520336
1	6	1	-7.0	118.3071164
1	6	2	-7.0	
1	6	3	-7.0	119.6639425
1	7	1	-7.5	131.04
1	7	2	-7.5	139.62
1	7	3	-7.5	130.50
1	8	1	-8.5	139.36
1	8	2	-8.5	137.89
1	8	3	-8.5	142.93
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.09

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 052808
Assay start date: 5/28/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-6.012923075
1	1	2	-7.0	-3.231913945
1	1	3	-7.0	7.392834955
1	2	1	-8.0	11.69117458
1	2	2	-8.0	2.308346367
1	2	3	-8.0	7.459253951
1	3	1	-8.5	9.109422431
1	3	2	-8.5	0.758951534
1	3	3	-8.5	1.372182083
1	4	1	-9.0	5.97456038
1	4	2	-9.0	2.070154109
1	4	3	-9.0	3.838273564
1	5	1	-9.5	2.207000142
1	5	2	-9.5	10.62789808
1	5	3	-9.5	3.203285068
1	6	1	-10.0	3.864612131
1	6	2	-10.0	13.10257822
1	6	3	-10.0	1.902961466
1	7	1	-11.0	5.35
1	7	2	-11.0	13.82
1	7	3	-11.0	4.52
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 6.14

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 052808
Assay start date: 5/28/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	-2.030073681
1	2	2	-4.5	13.69233309
1	2	3	-4.5	7.852042146
1	3	1	-5.5	13.94025917
1	3	2	-5.5	10.89987241
1	3	3	-5.5	8.893560698
1	4	1	-6.0	21.24348574
1	4	2	-6.0	19.44616483
1	4	3	-6.0	11.19417727
1	5	1	-6.5	15.79884587
1	5	2	-6.5	16.41894735
1	5	3	-6.5	13.92365442
1	6	1	-7.0	18.22142146
1	6	2	-7.0	29.56017456
1	6	3	-7.0	17.98952756
1	7	1	-7.5	5.97
1	7	2	-7.5	28.46
1	7	3	-7.5	13.23
1	8	1	-8.5	25.61
1	8	2	-8.5	35.71
1	8	3	-8.5	32.46
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.34

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 053008
Assay start date: 5/30/2008
Technician ID: JZ

run	xgroup	rep	x	y
1	1	1	-7.0	1.962854796
1	1	2	-7.0	0.03741791
1	1	3	-7.0	0.654813434
1	2	1	-8.0	6.729833173
1	2	2	-8.0	3.802674429
1	2	3	-8.0	2.589763348
1	3	1	-8.5	15.64258919
1	3	2	-8.5	10.78904226
1	3	3	-8.5	13.83321125
1	4	1	-9.0	25.73115548
1	4	2	-9.0	25.31733876
1	4	3	-9.0	24.26614916
1	5	1	-9.5	45.52966955
1	5	2	-9.5	43.9210165
1	5	3	-9.5	44.24921597
1	6	1	-10.0	88.00819389
1	6	2	-10.0	74.83550389
1	6	3	-10.0	75.54517578
1	7	1	-11.0	90.41
1	7	2	-11.0	85.15
1	7	3	-11.0	91.08
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 2.39

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 053008
Assay start date: 5/30/2008
Technician ID: JZ

run	xgroup	rep	X	Y
1	1	1	-4.0	4.4362421
1	1	2	-4.0	-0.798777259
1	1	3	-4.0	1.428222618
1	2	1	-4.5	2.464191377
1	2	2	-4.5	2.958868838
1	2	3	-4.5	3.186230209
1	3	1	-5.5	66.88356371
1	3	2	-5.5	58.60057015
1	3	3	-5.5	58.92972092
1	4	1	-6.0	78.71681935
1	4	2	-6.0	73.95364619
1	4	3	-6.0	76.92932137
1	5	1	-6.5	105.0916897
1	5	2	-6.5	106.6470698
1	5	3	-6.5	107.4946806
1	6	1	-7.0	99.6616533
1	6	2	-7.0	98.68466532
1	6	3	-7.0	96.6460234
1	7	1	-7.5	109.99
1	7	2	-7.5	105.69
1	7	3	-7.5	105.99
1	8	1	-8.5	110.54
1	8	2	-8.5	108.44
1	8	3	-8.5	110.47
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.88

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol	run	xgroup	rep	x	y
Run ID: 060208	1	1	1	-7.0	-1.6333228
Assay start date: 6/2/2008	1	1	2	-7.0	-0.617976999
Technician ID: SMR	1	1	3	-7.0	3.728072993
	1	2	1	-8.0	9.200458002
	1	2	2	-8.0	18.88735023
	1	2	3	-8.0	10.06370747
	1	3	1	-8.5	37.21958309
	1	3	2	-8.5	29.17800324
	1	3	3	-8.5	31.04580789
	1	4	1	-9.0	50.29061876
	1	4	2	-9.0	56.55637113
	1	4	3	-9.0	47.04829487
	1	5	1	-9.5	74.6486412
	1	5	2	-9.5	77.89404812
	1	5	3	-9.5	79.65446042
	1	6	1	-10.0	
	1	6	2	-10.0	87.77054692
	1	6	3	-10.0	96.67537624
	1	7	1	-11.0	97.12
	1	7	2	-11.0	99.45
	1	7	3	-11.0	
	1	8	1		
	1	8	2		
	1	8	3		
	1	9	1		
	1	9	2		
	1	9	3		
	1	10	1		
	1	10	2		
	1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run.

It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.77

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060208
Assay start date: 6/2/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	5.416547841
1	2	2	-4.5	5.707894536
1	2	3	-4.5	7.594711227
1	3	1	-5.5	24.84890993
1	3	2	-5.5	20.74744729
1	3	3	-5.5	19.78245771
1	4	1	-6.0	23.64704059
1	4	2	-6.0	34.1473399
1	4	3	-6.0	35.07584691
1	5	1	-6.5	25.7912906
1	5	2	-6.5	32.81752465
1	5	3	-6.5	27.43454762
1	6	1	-7.0	26.98134165
1	6	2	-7.0	25.18752981
1	6	3	-7.0	22.15690758
1	7	1	-7.5	31.49
1	7	2	-7.5	21.40
1	7	3	-7.5	29.35
1	8	1	-8.5	32.93
1	8	2	-8.5	32.74
1	8	3	-8.5	25.63
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.32

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 060308
Assay start date: 6/3/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	0.794244482
1	1	2	-7.0	0.07739337
1	1	3	-7.0	-0.571755137
1	2	1	-8.0	8.069088517
1	2	2	-8.0	7.314403596
1	2	3	-8.0	8.053556743
1	3	1	-8.5	22.11498956
1	3	2	-8.5	22.29460058
1	3	3	-8.5	26.57260872
1	4	1	-9.0	48.90889287
1	4	2	-9.0	47.09725081
1	4	3	-9.0	48.20876828
1	5	1	-9.5	77.58532685
1	5	2	-9.5	78.11380542
1	5	3	-9.5	78.36549981
1	6	1	-10.0	105.4393733
1	6	2	-10.0	104.8475729
1	6	3	-10.0	102.6790983
1	7	1	-11.0	112.61
1	7	2	-11.0	111.91
1	7	3	-11.0	114.66
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.58

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060308
Assay start date: 6/3/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	5.166239764
1	2	2	-4.5	5.532232082
1	2	3	-4.5	6.758445734
1	3	1	-5.5	26.83545413
1	3	2	-5.5	25.64667603
1	3	3	-5.5	21.918652
1	4	1	-6.0	40.79772254
1	4	2	-6.0	36.585824
1	4	3	-6.0	41.19716791
1	5	1	-6.5	68.37896732
1	5	2	-6.5	63.44583692
1	5	3	-6.5	60.99579912
1	6	1	-7.0	90.78255583
1	6	2	-7.0	93.03904383
1	6	3	-7.0	93.75071768
1	7	1	-7.5	99.79
1	7	2	-7.5	93.90
1	7	3	-7.5	104.58
1	8	1	-8.5	107.92
1	8	2	-8.5	98.80
1	8	3	-8.5	87.11
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 4.28

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 060508
Assay start date: 6/5/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	2.303863409
1	1	2	-7.0	1.11632605
1	1	3	-7.0	2.075240797
1	2	1	-8.0	15.59070172
1	2	2	-8.0	14.79323137
1	2	3	-8.0	16.05047515
1	3	1	-8.5	35.48050779
1	3	2	-8.5	21.54789186
1	3	3	-8.5	21.74906531
1	4	1	-9.0	47.85646738
1	4	2	-9.0	45.6005798
1	4	3	-9.0	49.03136368
1	5	1	-9.5	75.61081906
1	5	2	-9.5	74.21235658
1	5	3	-9.5	79.19401801
1	6	1	-10.0	92.02938986
1	6	2	-10.0	100.9640916
1	6	3	-10.0	91.58153514
1	7	1	-11.0	108.60
1	7	2	-11.0	115.35
1	7	3	-11.0	105.35
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.40

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060508
Assay start date: 6/5/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	6.150718765
1	2	2	-4.5	6.49166623
1	2	3	-4.5	7.665479009
1	3	1	-5.5	18.7401317
1	3	2	-5.5	17.95385772
1	3	3	-5.5	19.71854979
1	4	1	-6.0	39.75390865
1	4	2	-6.0	38.63174363
1	4	3	-6.0	38.2189227
1	5	1	-6.5	76.49424862
1	5	2	-6.5	69.65326774
1	5	3	-6.5	68.5758882
1	6	1	-7.0	
1	6	2	-7.0	90.48248521
1	6	3	-7.0	92.60654458
1	7	1	-7.5	93.70
1	7	2	-7.5	96.28
1	7	3	-7.5	94.69
1	8	1	-8.5	84.18
1	8	2	-8.5	95.72
1	8	3	-8.5	100.34
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 1.86

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Estradiol
Run ID: 060608
Assay start date: 6/6/2008
Technician ID: SMR

run	xgroup	rep	x	y
1	1	1	-7.0	-0.236378295
1	1	2	-7.0	0.342116982
1	1	3	-7.0	0.254061557
1	2	1	-8.0	7.5774581
1	2	2	-8.0	7.633394948
1	2	3	-8.0	7.709902121
1	3	1	-8.5	21.54073899
1	3	2	-8.5	22.11959515
1	3	3	-8.5	20.53279307
1	4	1	-9.0	47.08980426
1	4	2	-9.0	47.11001371
1	4	3	-9.0	46.90431046
1	5	1	-9.5	74.85035991
1	5	2	-9.5	78.37510312
1	5	3	-9.5	71.74676703
1	6	1	-10.0	101.6365317
1	6	2	-10.0	102.1240845
1	6	3	-10.0	98.46725818
1	7	1	-11.0	112.68
1	7	2	-11.0	111.84
1	7	3	-11.0	107.27
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.60

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060608
Assay start date: 6/6/2008
Technician ID: SMR

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	10.29093657
1	2	2	-4.5	11.2458327
1	2	3	-4.5	10.33099457
1	3	1	-5.5	27.26506343
1	3	2	-5.5	22.16542727
1	3	3	-5.5	23.48950661
1	4	1	-6.0	41.46760968
1	4	2	-6.0	38.31818467
1	4	3	-6.0	37.84181925
1	5	1	-6.5	72.4963208
1	5	2	-6.5	60.09421931
1	5	3	-6.5	75.00590044
1	6	1	-7.0	93.2604398
1	6	2	-7.0	96.99521686
1	6	3	-7.0	91.61228763
1	7	1	-7.5	107.79
1	7	2	-7.5	112.80
1	7	3	-7.5	105.58
1	8	1	-8.5	115.32
1	8	2	-8.5	115.64
1	8	3	-8.5	110.77
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".

Use the lab and chemical code assigned by the study coordinator.

2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).

A template for data entry titled "ER-RUC data entry templates.xls" is provided.

x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.

y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.

3. x values representing a triplicate have to be exactly the same.

4. Up to 30 (x, y) pairs can be input.

5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.

SDwithin-run (estradiol) should be < 5.0%.

SDwithin-run (norethynodrel) should be < 5.7%.

SDwithin-run (R1881) should be < 10%.

2. These upper limits are a part of provisional performance criteria.

3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 3.59

Provide information in all blue cells.

Laboratory Code: HAMNER

Compound ID: Estradiol
Run ID: 060708
Assay start date: 6/7/2008
Technician ID: JZ

run	xgroup	rep	X	Y
1	1	1	-7.0	0.991812505
1	1	2	-7.0	-1.033079935
1	1	3	-7.0	1.036983611
1	2	1	-8.0	-2.596780955
1	2	2	-8.0	-0.802763059
1	2	3	-8.0	-3.373054784
1	3	1	-8.5	7.176908084
1	3	2	-8.5	5.285298292
1	3	3	-8.5	6.353232476
1	4	1	-9.0	11.14583111
1	4	2	-9.0	9.472269495
1	4	3	-9.0	10.70806174
1	5	1	-9.5	54.57589815
1	5	2	-9.5	50.0325771
1	5	3	-9.5	45.50877444
1	6	1	-10.0	69.05072827
1	6	2	-10.0	54.04388289
1	6	3	-10.0	58.51303409
1	7	1	-11.0	87.70
1	7	2	-11.0	85.80
1	7	3	-11.0	82.98
1	8	1		
1	8	2		
1	8	3		
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

SDwithin-run calculation for the ER Competitive Binding Assay

Data & results worksheet

Instruction:

This spreadsheet is used to estimate within-run standard deviation for data from a single run.

Within-run standard deviation (**SDwithin-run**) is a measure of variation of y at each concentration in a single run. It is SD around a mean specific to each run-concentration combination.

How to use this spreadsheet:

1. Save the file under a new name defined by the naming convention, e.g., "lab A run 1 chem B YYYYMMDD SDw.xls".
Use the lab and chemical code assigned by the study coordinator.
2. Enter the values of x and y from a single run (cut-and-paste-special, values-only) from the data entry Excel file).
A template for data entry titled "ER-RUC data entry templates.xls" is provided.
x: log10(concentration) in units of log10(M), from column T of the "compet-#unk-template" worksheet.
y: % radioligand bound (%), from column U of the "compet-#unk-template" worksheet.
3. x values representing a triplicate have to be exactly the same.
4. Up to 30 (x, y) pairs can be input.
5. The first three columns (run, xgroup, replicate) are guides for data entry and are not used in the calculation.

The use of estimated SDwithin-run:

1. SDwithin-run is estimated separately for each run.
SDwithin-run (estradiol) should be < 5.0%.
SDwithin-run (norethynodrel) should be < 5.7%.
SDwithin-run (R1881) should be < 10%.
2. These upper limits are a part of provisional performance criteria.
3. If a run does not satisfy **all** of the criteria, the entire run including the test chemicals shall be flagged as unacceptable by adding "UN" before the file name.

Result:
SDwithin-run = 5.50

Provide information in all blue cells.

Laboratory Code: HAMNER
Compound ID: Norethynodrel
Run ID: 060708
Assay start date: 6/7/2008
Technician ID: JZ

run	xgroup	rep	X	Y
1	1	1	-4.0	
1	1	2	-4.0	
1	1	3	-4.0	
1	2	1	-4.5	24.25270167
1	2	2	-4.5	24.55997673
1	2	3	-4.5	22.3153631
1	3	1	-5.5	43.84190485
1	3	2	-5.5	44.32596065
1	3	3	-5.5	60.88925736
1	4	1	-6.0	64.21407387
1	4	2	-6.0	50.12403465
1	4	3	-6.0	58.39090481
1	5	1	-6.5	83.75308692
1	5	2	-6.5	94.81721967
1	5	3	-6.5	84.01686388
1	6	1	-7.0	99.36054071
1	6	2	-7.0	96.04743524
1	6	3	-7.0	96.50026164
1	7	1	-7.5	89.43
1	7	2	-7.5	76.52
1	7	3	-7.5	87.48
1	8	1	-8.5	83.31
1	8	2	-8.5	80.82
1	8	3	-8.5	85.30
1	9	1		
1	9	2		
1	9	3		
1	10	1		
1	10	2		
1	10	3		

Although all of the cells in columns x and y have been shaded blue, fill in only the cells for which there are data. For example, if there were only 6 concentrations tested, the x and y cells for xgroups 7, 8, 9, and 10 should be left blank.

APPENDIX D: Representative Runs for 23 Chemicals (Estradiol, Norethynodrel, and R1881) and Optional Chemicals (Estradiol and Norethynodrel)

Representative Runs of Competition Binding Data for Estradiol-23 Chemicals

Estradiol	EtOH/Hot Tube Ratio (>10%)	NSB/EtOH (around 0.25)	R1881/EtOH (close to 1.0)	Within Run SD (< 5.0%)	Log (IC₅₀)	Bottom Plateau Level (-5.0 to 1)	Top Plateau Level (90 to 110)	Hill Slope (-1.1 to -0.7)
030508	2.12	0.26	0.91	7.99	-8.87	-3.1	114	-0.75
030608	1.48	0.32	0.9	8.42	-9.17	2.3	99	-1.24
031008	2.05	0.2	0.89	3.67	-9.09	0.0	109	-1.03
031108	1.83	0.11	0.84	7.13	-8.93	0.6	114	-0.98
031208	2.05	0.11	0.84	5.96	-9.05	-3.8	106	-0.74
031708	1.89	0.1	0.82	6.65	-9.03	-0.7	106	-1.00
031808	1.82	0.11	0.85	3.25	-9.11	-0.1	105	-0.99
032408	1.82	0.13	0.95	5.02	-9.03	-2.1	110	-0.85
032608	0.77	0.17	1.12	3.17	-8.81	-1.2	120	-1.00
040308	1.04	0.17		6.6	-8.61	-1.6	121	-1.03
040808	1.39	0.13		3.26	-9.12	2.4	94	-1.63
041008	1.6	0.12		4.49	-9.19	-1.7	101	-0.90
041508	0.89	0.19		4.12	-8.87	-0.5	98	-1.01
041608	0.55	0.32		7.61	-8.68	-3.3	114	-0.84
041708	0.81	0.31		4.28	-8.76	-1.3	108	-1.14
053008	1.27	0.17		3.34	-9.50	1.3	93	-1.05
060208	1.98	0.08		3.88	-8.92	-2.5	102	-0.83
060308	2.89	0.04		1.32	-9.02	-0.7	116	-0.96
060508	3.26	0.06		4.28	-9.00	-1.7	114	-0.77
060608	3.15	0.05		1.86	-9.06	-0.8	114	-0.96
AVG	1.73	0.16	0.90	4.82	-8.99	-0.93	107.80	-0.99
SE	1.70E-01	1.95E-02	3.05E-02	4.54E-01	4.43E-02	3.78E-01	1.83E+00	4.44E-02

Representative Runs of Competition Binding Data for Norethynodrel-23 Chemicals

Norethynodrel	Within Run SD (< 5.7%)	Log (IC ₅₀)	Relative Binding Affinity	Bottom Plateau Level (-5.0 to 1)	Top Plateau Level (90 to 110)	Hill Slope (-1.1 to -0.7)
030508	11.19	-6.07	1.58E-03	-1.7	104	-1.30
030608	9	-6.13	9.25E-04	-0.9	117	-0.79
031008	2.96	-6.10	1.04E-03	4.6	107	-1.20
031108	5.07	-6.22	1.93E-03	5.0	103	-1.12
031208	2.88	-6.24	1.55E-03	6.1	103	-1.02
031708	3.78	-6.14	1.28E-03	3.0	103	-0.85
031808	4.33	-6.23	1.32E-03	1.6	103	-1.07
032408	4.8	-6.19	1.44E-03	3.0	107	-0.94
032608	3.86	-5.79	9.51E-04	-2.4	117	-0.72
040308	3.75	-5.92	2.03E-03	2.6	124	-1.03
040808	3.06	-6.287	1.48E-03	-1.2	108	-0.97
041008	5.3	-6.239	1.12E-03	7.7	101	-1.26
041508	7.35	-6.043	1.49E-03	16.1	101	-1.11
041608	4.96	-5.834	1.42E-03	27.7	104	-1.34
041708	9.26	-5.766	1.01E-03	-2.9	96	-0.75
053008	2.39	-5.392	7.76E-05	-6.2	108	-0.97
060308	4.58	-6.173	1.42E-03	6.7	102	-1.03
060508	3.4	-6.154	1.42E-03	7.4	95	-1.35
060608	3.6	-6.178	1.33E-03	10.2	116	-0.99
AVG	5.03	-6.06	1.30E-03	4.55	106.08	-1.04
SE	5.59E-01	5.16E-02	9.59E-05	1.76E+00	1.70E+00	4.37E-02

Representative Runs of Competition Binding Data for R1881-23 Chemicals

R1881	Within Run SD (< 10%)	Hill Slope (-1.1 to -0.7)	Log (IC ₅₀)
030508	6.63	-1.30	-4.75
030608	11.38	-0.79	-4.84
031008	5.3	-1.20	-3.75
031108	6.17	-1.12	-3.94
031208	4.39	-1.02	-5.31
031708	7.11	-0.67	-5.25
031808	5.42	-0.71	-5.17
032408	3.79	-0.53	-4.89
032608	4.54	-1.15	-4.84
040208	7.27	-0.67	-4.99
AVG	6.20	-0.92	-4.77
SE	6.85E-01	8.53E-02	1.66E-01

Runs of Competition Binding Data for Estradiol-Optional Chemicals

Estradiol	EtOH/Hot Tube Ratio (>10%)	NSB/EtOH (around 0.25)	Within Run SD (< 5.0%)	Log (IC₅₀)	Bottom Plateau Level (-5.0 to 1)	Top Plateau Level (90 to 110)	Hill Slope (-1.1 to -0.7)
061808	2.99	0.07	3.1	-9.08	-0.5	105	-0.98
061908	2.97	0.07	3.56	-9.06	-2.0	104	-0.81
062308	2.93	0.08	3.29	-8.97	-0.4	109	-0.96
062408	2.72	0.06	3.55	-8.98	-2.7	96	-0.83
062608	1.9	0.1	2.82	-8.81	-0.5	165	-0.90
062608-JZ	2.95	0.17	1.57	-9.79	0.7	84	-0.88
AVG	2.74	0.09	2.98	-9.12	-0.90	110.5	-0.89
SE	1.73E-01	1.66E-02	3.05E-01	1.40E-01	5.08E-01	1.14E+01	2.78E-02

Runs of Competition Binding Data for Norethynodrel-Optional Chemicals

Norethynodrel	Within Run SD (< 5.7%)	Log (IC₅₀)	Relative Binding Affinity	Bottom Plateau Level (-5.0 to 1)	Top Plateau Level (90 to 110)	Hill Slope (-1.1 to -0.7)
061808	2.82	-6.13	1.11E-03	15.2	98	-1.06
061908	5.54	-6.10	1.09E-03	17.0	101	-1.06
062308	3.24	-6.02	1.12E-03	19.3	106	-1.12
062408	4.27	-5.99	1.03E-03	1.9	97	-0.72
062608	4.09	-5.73	8.40E-04	-2.3	162	-0.55
062608-JZ	2.77	-5.99	1.57E-04	15.9	87	-0.98
AVG	3.79	-5.99	8.91E-04	11.20	108.24	-0.91
SE	4.35E-01	5.76E-02	1.53E-04	3.68E+00	1.10E+01	9.34E-02

**APPENDIX E: Calculation of Specific Activity Using the “QuickCalcs”
Webpage from GraphPad-Example from Run 061808**

Try our
free demos

QuickCalcs

Online Calculators for Scientists

1. Select category

2. Choose calculator

3. Choices and results

Radioactivity calculator

Radioactively labeled compounds are commonly used in biological and chemical research. Use these calculators to perform the radioactivity calculations commonly used in setting up and analyzing biochemical and pharmacological experiments. Note that this page has seven independent calculators, and that the results will appear right on this page. [How do these calculations work?](#)

Isotope decay

Isotope: ☐ ^{125}I ☐ ^{32}P ☐ ^{33}P ☐ ^{35}S ☒ ^3H ☐ OtherHalf life (days): Number of days: Percent Remaining =

Concentration of stock

% remaining isotope: Original dilution: Original activity: Stock Concentration =

Assumes compound was 100% labeled originally (carrier free)

Dilution of stock

Concentration of stock solution (micromolar): Dilute to this concentration in nM: Final dilution volume in ml: Stock volume needed =

Specific activity (cpm/fmol)

Specific radioactivity: Counter efficiency (%): Specific Activity =

Cpm to fmol/mg

cpm/fmol: cpm: mg protein: fmol/mg =

Cpm to sites/cell

cpm/fmol: cpm: cell count: sites/cell =

Cpm to nMcpm/fmol: cpm: microliters:

concentration =

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