DRAFT REPORT

Validation of an Androgen Receptor Binding Assay–Task 5

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EPA Contract Number:

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Participation

The following principal staff participated in the conduct of this study:

Study Director:	James E. Morris, Ph.D.
Scientists:	Lyle Sasser, Ph.D.
	Lucie Fritz, Ph.D..
Technical Assistant:	Jeff Creim
Data Facilitator:	Nancy Holter
QA :	Mary Lynn
	Janet Cloutier

Study Dates and Data Retention

Study initiation date:	June 26, 2005
Experimental start date:	June 29, 2005
Experimental end date:	August 3, 2005

Richland, Battelle will retain all supporting documentation, including raw data and written records, for a period of up to five years following submission of the final report to Battelle Memorial Institute. Also, electronic data files will be provided to Data Co-ordination Center at Battelle, Columbus. At the end of this period, Battelle will be notified to determine whether the data (excluding proprietary information) will be transferred, retained, or destroyed.

Statement of Compliance

This study was conducted in compliance with U.S. EPA Good Laboratory Practice Regulations as set forth in Part 160 Title 40 of the Code of Federal Regulations. This study was conducted under my scientific guidance and management.

Exception: The dose solutions used in the study were not analyzed; however, the stability of the stock concentrations of methyltrienolone and dexamethasone in ethanol were evaluated and are reported separately by Battelle.

James E. Morris, Ph.D.		
Study Director	Signature	Date

Quality Assurance Statement

This study was inspected in accordance with Battelle, Richland standard operating procedures. Based on audits conducted, the results reported herein accurately reflect the methods used and the data collected for this study.

All findings were reported to the Study Director and Battelle Management.

Inspection/Audit Dates:	Study Phase Audited:	Date(s) reported to Study Director and Management:
June 23,2005	Technical System Audit, Cytosol Preparation	June 23, 2005
February 8-10,13-15,2006	Audit of Data Quality, Data and Draft Report	February 15,2006

Mary Lynn

Janet Cloutier		
Quality Assurance	Signature	Date

Glossary of Abbreviations

B_{max} ..... binding maximum
EPA ..... Environmental Protection Agency
HAP ..... hydroxylapatite
IC_{50} ..... concentration at which 50% of specific activity is inhibited
K_d ..... dissociation constant
K_i ..... inhibitory constant
TEDG + PMSF buffer ..... buffer with Tris, ethylene diamine tetraacetic acid, dithiothreitol, glycerol, sodium molybdate, triamcinolone acetonide and phenylmethylsulfonyl fluoride

Summary

The objective of this study was to evaluate the inhibition of androgen receptor binding of R1881 in rat ventral prostate cytosol by known chemicals. This *in vitro* test method involved combining cytosol, substrate, and test or control substance in a common reaction vessel. The inhibitory effect of the test substance on androgen receptor binding of R1881 was evaluated by measuring the amount of bound ^{3}H-R1881 (substrate). Battelle, Richland conducted three separate experiments to evaluate the inhibition of androgen receptor binding of ^{3}H-R1881 by the test substance.

The cytosol used in these experiments was prepared and characterized at Battelle, Richland. A series of saturation and competitive binding experiments were conducted using the cytosol.

A total of three saturation experiments were conducted and the amount of cytosolic protein used for each incubation in the saturation binding experiments was 0.6 mg. The K_d observed across the three saturation experiments was 0.930, 0.834, and 0.926 nM and the B_{max} observed was 14.40, 12.26, and 15.58 fmoles/100ug of protein. A total of three competitive binding experiments were conducted and the amount of cytosolic protein used for each incubation in the competitive binding experiments was 1.0 mg per assay tube. The IC_{50} values for R1881 were determined to be 1.475, 1.546, and 1.636 nM. The IC_{50} values for dexamethasone were determined to be 48.91, 50.44, and 49.21 μM. The RBA values for dexamethasone in comparison to R1881 were, 0.0030, 0.0031, and 0.0033 %.

Introduction

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

One potential endocrine target for environmental chemicals is the androgen receptor. The objective of this study was to evaluate the inhibition of androgen receptor (AR) binding of R1881 in rat ventral prostate cytosol by known chemicals, thus validating androgen receptor binding as a potential tool for screening environmental chemicals. Rat ventral prostate cytosol was selected because it provided a biological source of the androgen receptor. Since the assay was evaluated for its potential to serve as a screening assay, the use of rat tissue enhanced its availability.

Experimental Methods

Test, Marker/Tracer and Reference Substance Information and Preparation

Chemical Repository, Battelle, Sequim provided the following test materials at the necessary dilutions:

- Marker/Tracer: ^{3}H-R1881 (CAS No. 965-93-5)(Lot # 3559-507)
- Reference Substance: Unlabelled R1881–Methyltrienolone (CAS No. 965-93-5) (Lot# 3411228)
- Test Substance: Dexamethasone (CAS No. 50-02-2)(Lot # P4311T)

These test and reference articles were used in the androgen receptor-binding assay to determine relative binding affinities (RBAs).

The following were prepared at Battelle, Richland or were supplied by Battelle, Richland:

- Buffer with Tris(Tris base; Lot# 120K5444 & Tris HCl; Lot# 072K5420), ethylene diamine tetraacetic acid(EDTA; Lot#99H0095), dithiothreitol (DTT) (Lot# 51K17412), glycerol (Lot# 10655KA), Sodium molybdate (Lot # 30K0210) and phenylmethylsulfonyl fluoride (Lot# 60K0701)- (TEDG + PMSF Buffer) (pH 7.4) (lot 0677-10-01)

- Triamcinolone acetonide (Sigma; Lot # 40K1266)
- Absolute ethanol (AAPER ; Lot# 03K10UA)
- Hydroxylapatite (HAP) (lot# 190682)

The following reagent was obtained from supplier as identified:
- Substrate: ^{3}H-R1881 (CAS No. 965-93-5) (Perkin-Elmer Cat # NET 590; Lot # 3559-507)

R1881 Preparation

Battelle, Richland provided the R1881 stock solutions prepared in absolute ethanol. Battelle was responsible for the preparation and analysis of the R1881 stocks.
Dilutions of the unlabeled R1881 stock were prepared in ethanol for the saturation assays at 1.0 x 10^{-6} and 1.0 x 10^{-5} M. In the assay, 7.5 ul, 15.0 ul, 21.0 ul, 30.0 and 45.0 ul of the 1.0 x 10^{-6} M stock was used to generate a final R1881 concentration in the assay tubes of 0.025, 0.05 0.07, 0.10, and 0.15 uM. To generate a final R1881 concentration in the assay tubes of 0.250, 0.050 and 0.100 uM, 7.5, 15 or 30 ul of 1.0 x 10^{-5} M stock were used.
Dilutions of the R1881 stock solutions were prepared in ethanol for competitive assays. The final target concentrations for R1881 in the assay were 1.0 × 10^{-6} (NSB), 1.0 x10^{-7}, 1.0 × 10^{-8}, 1.0 x10^{-9}, 1.0 x 10^{-10}, and 1.0 x 10^{-11} M. The total volume of solvent used in each assay was no more than 3.33% of the total assay volume.

Dexamethasone Preparation

Chemical Repository, Battelle, Sequim provided the dexamethasone stock solutions (30 mM) prepared in absolute ethanol. Chemical Repository, Battelle, Sequim was responsible for the preparation of the dexamethasone stock.

Fresh dilutions of the dexamethasone stock solutions were prepared in ethanol. The final target concentrations for dexamethasone in assay tubes were 1.0 x 10^{-3}, 1.0 x 10^{-4}, 1.0 x 10^{-5}, 1.0 x 10^{-6}, 1.0 x 10^{-7}, 1.0 x 10^{-8}, 1.0 x 10^{-9}, and 1.0 x 10^{-10} M. The total volume of solvent used in each assay was no more than 3.33% of the total assay volume.

Marker/ Tracer Preparation

The marker/tracer solutions were prepared from the radiolabeled R1881 received from vendor and stored at -20°C in the original container.
Dilutions of the ^{3}H R1881 stock were prepared in ethanol for the saturation assays at 1.0 x 10^{-7} and 1.0 x 10^{-8} M. In the saturation assay, 7.5 ul, 15.0 ul, 21.0 ul, 30.0 and 45.0 ul of the 1.0 x 10^{-8} M stock was used to generate a final ^{3}H R1881 concentration in the assay tubes of 0.25, 0.5 0.7, 1.0, and 1.5 nM. To generate a final^{3}H R1881 concentration in the assay tubes of 2.5, 5.0 and 10.0 nM, 7.5, 15 or 30 ul of 1.0 x 10^{-7} M stock were used.

The [^{3}H]-R1881 was diluted with ethanol to achieve a marker solution at a concentration of 10 nM for the competitive binding assays. The marker solution (30 µL) was added to the incubation mixtures to achieve a final concentration of 1 nM [^{3}H]-R1881 in the assay.

Preparation of Rat Ventral Prostate Cytosol

Rat ventral prostate cytosol was prepared by Battelle, Richland for saturation and competitive AR assays.

- The rat prostate cytosol was prepared by Battelle – Richland per Battelle SOPs # 331-ED-I-99-00 and 331-AR-I-01-01. Briefly, the ventral prostate tissues were collected from 143 Sprague-Dawley male rats (85 to 100 days of age) castrated 24 hours prior to being humanely killed. The supplier was Charles River. For the study, weighed and trimmed prostate tissues are placed in ice-cold buffer prepared with Tris, Ethylenediaminetetraacetic acid and Glycerol (TEDG) with phenylmethylsulfonyl fluoride (PMSF) with final extraction volume equaling a ratio of 0.1 g of tissue per 1.0 ml TEDG buffer with PMSF. The tissues are homogenized and the cytosol pooled, aliquoted and stored at –80 degrees centigrade. The appropriate cytosol was shipped to the participating laboratories on dry ice.

Assays

Protein Assay:

The protein concentration of the cytosol preparation was determined for each batch of the cytosol as described in Battelle, Richland SOP (331-ER-I-02-00). In brief, a six-point curve was prepared, ranging from 0.2 to 2.0 mg protein / ml. The protein standards were made from bovine serum albumin (BSA). Protein was determined using a BioRad Protein Assay Kit purchased from BioRad (Hercules, CA). To a 1-ul aliquot of standard or unknown, 200 uL of dye reagent was added and mixed. The samples were placed at room temperature for at least 5 minutes and up to an hour to allow for color development. Absorbance (600 nm) was measured using a plate reader. The protein concentration of the cytosol sample was determined by extrapolation of the absorbance value using the standard curve developed using the protein standard.

Evaluation of the Cytosol for Conducting Androgen Receptor Competitive Binding Assays

Prior to conducting the androgen receptor competitive binding assays with unknown chemicals, the cytosol was characterized at Battelle, Richland. This was accomplished in two steps as follows:

1) A series of saturation radioligand binding assays was conducted to demonstrate androgen receptor specificity and saturation. Nonlinear regression analysis of these data and subsequent

Scatchard plots document androgen receptor binding affinity (K_d) and the number of receptors (B_{max}).

2) A series of androgen receptor competitive binding assays were conducted using R1881 and dexamethasone, substances with known affinities for the androgen receptor. Comparison of IC_{50} values (i.e., the concentration of a substance that inhibits [^{3}H]-R1881 binding by 50%) from these assays with reported values in the literature assisted in documenting that the cytosol was appropriate for routine use in the laboratory.

Saturation Radioligand Binding Assay

Androgen receptor saturation binding experiments measured total, non-specific, and specific binding of increasing concentrations of ^{3}H-R1881 under conditions of equilibrium.

The experimental setup and details of the saturation binding assay are specified in the assay protocol attached to the study protocol for this task. The saturation binding experiments were conducted as three independent replicates. The same technician conducted all three replicate experiments.

Competitive Binding Assay (Inhibition of Androgen Receptor Binding of [^{3}H]-R1881 by Test substance)

An androgen receptor competitive binding assay measures the binding of a single concentration of [^{3}H]-R1881 in the presence of increasing concentrations of a test substance. The experimental setup and details of the competitive binding assay are specified in the assay protocol attached to the study protocol for this task.

These experiments tested the androgen receptor binding of [^{3}H]-R1881 in the presence of multiple concentrations of a test substance. The inhibition experiments were conducted as three independent replicates. All three replicate experiments for a given test substance were conducted by the same technician and there were three (triplicate) repetitions for each concentration within a given replicate.

Control samples were included for each replicate experiment. These included:

- Vehicle or ethanol control (substrate, buffer, vehicle [used for preparation of test substance solutions], and cytosol)
- Non-specific background control (substrate, buffer, R1881 (at 10 fold excess of the highest concentration used in standard curve), and cytosol).

Six repetitions of each type of control were included with each replicate experiment and were treated the same as the other samples. The control sets were split so that three tubes (of each control type) were run at the beginning and three at the end of each replicate set.

Description of Data Calculations

All raw data was used to populate a format using Microsoft Excel® spreadsheets and Prism templates for analysis.

IC_{50} Calculation

Data for the non-radiolabeled R1881 standard curve and each test substance were plotted as the percentage of [^{3}H]-R1881 bound versus the molar concentration (log) of competitor. Estimates of IC_{50} values were determined using appropriate nonlinear curve fitting software to fit a one-site competitive binding model. The model was constrained to fit the bottom of the curve to 0% and the top to 100%.

Relative Binding Affinity Values

The RBA value for each test substance was calculated by dividing the IC_{50} value for R1881 by the IC_{50} of the test substance and expressing the value as a percent (e.g., RBA for R1881 = 100%).

Criteria for Data Acceptance

All data obtained were reported.

Results

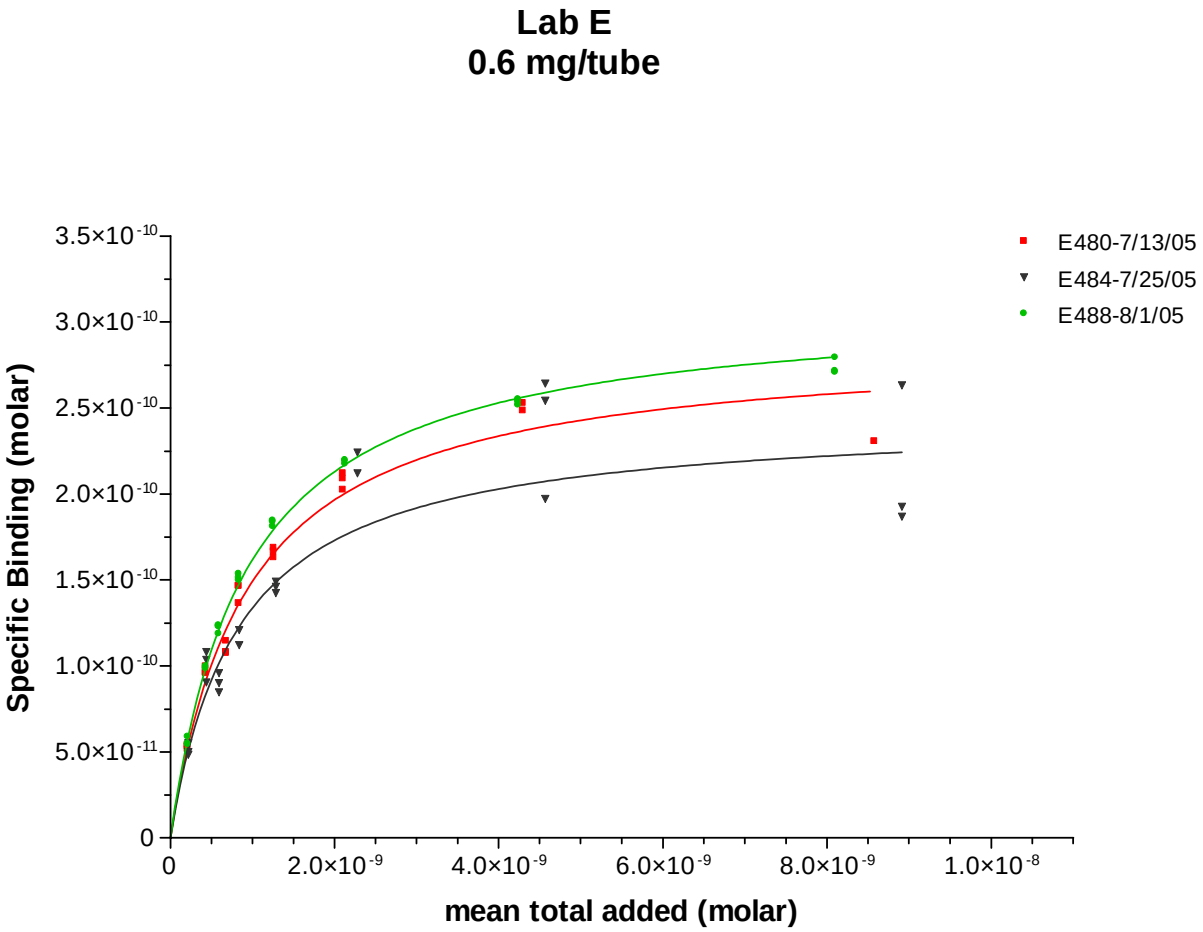
Rat ventral prostate cytosol was prepared by Battelle, Richland at a protein concentration of 4.28 mg/mL. A series of saturation and competitive binding experiments were conducted using the cytosol.

A total of three saturation assays were conducted and the data are presented in Table 1 and Figure 1. The raw data and all supporting calculations are presented in Appendices 2 and 3. The amount of cytosolic protein used in the saturation binding experiments was 0.6 mg. The K_d observed across the three saturation experiments was 0.93, 0.83, and 0.93 nM and the B_{max} observed was 14.40, 12.26, and 15.58 fmoles/100ug of protein.

A total of three competitive binding experiments were conducted and the data are presented in Table 2 and Figure 2. The raw data and all supporting calculations are presented in Appendices 4 and 5. The amount of cytosolic protein used for each incubation in the competitive binding experiments was 1.0 mg. The IC_{50} values for R1881 were determined to be 1.48, 1.55, and 1.64 nM. The IC_{50} values for dexamethasone were determined to be 48.9, 50.4, and 49.2 µM. The RBA values for dexamethasone in comparison to R1881 were, 0.0030, 0.0031, and 0.0033 %.

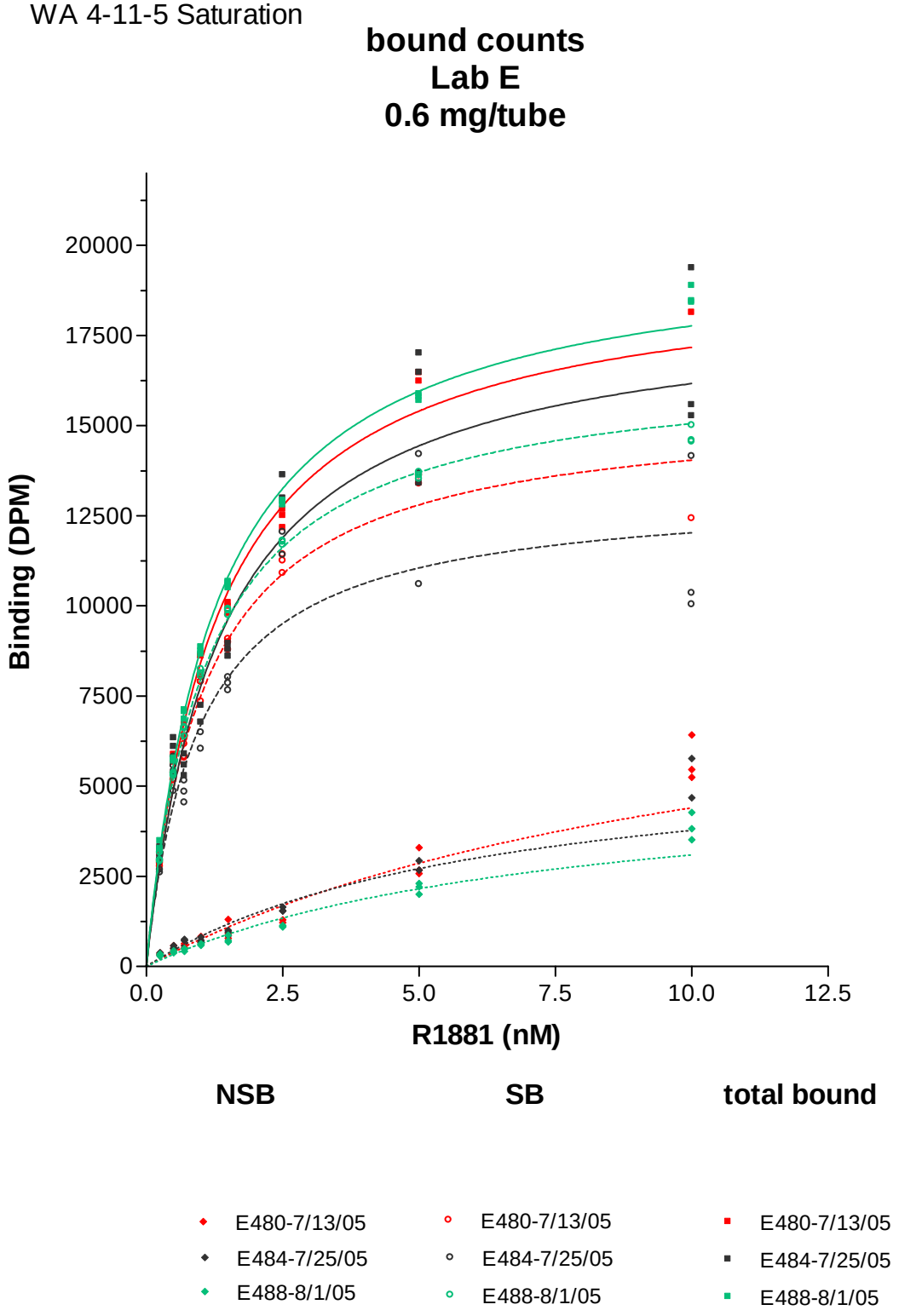
Figure 1: Saturation Binding and Scatchard Plots of ^{3}H-R1881 to the Androgen Receptor

WA 4-11-5 Saturation



	E480-7/13/05	E484-7/25/05	E488-8/1/05
BMAX	2.880e-010	2.452e-010	3.115e-010
KD	9.302e-010	8.337e-010	9.257e-010
Std. Error			
BMAX	1.109e-011	1.554e-011	3.465e-012
KD	6.621e-011	1.064e-010	2.006e-011
95% Confidence Intervals			
BMAX	2.648e-010 to 3.112e-010	2.128e-010 to 2.776e-010	3.043e-010 to 3.187e-010
KD	7.916e-010 to 1.069e-009	6.119e-010 to 1.056e-009	8.841e-010 to 9.673e-010
Goodness of Fit			
Degrees of Freedom	19	20	22
R^2 (unweighted)	0.9711	0.8601	0.9974
Weighted Sum of Squares ($1/Y^2$)	0.08935	0.4046	0.01389
Absolute Sum of Squares	2.279e-021	1.411e-020	3.164e-022
Sy.x	1.095e-011	2.656e-011	3.792e-012
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	21	22	24
Number of missing values	3	2	0

WA 4-11-5 Saturation



WA 4-11-5 Saturation

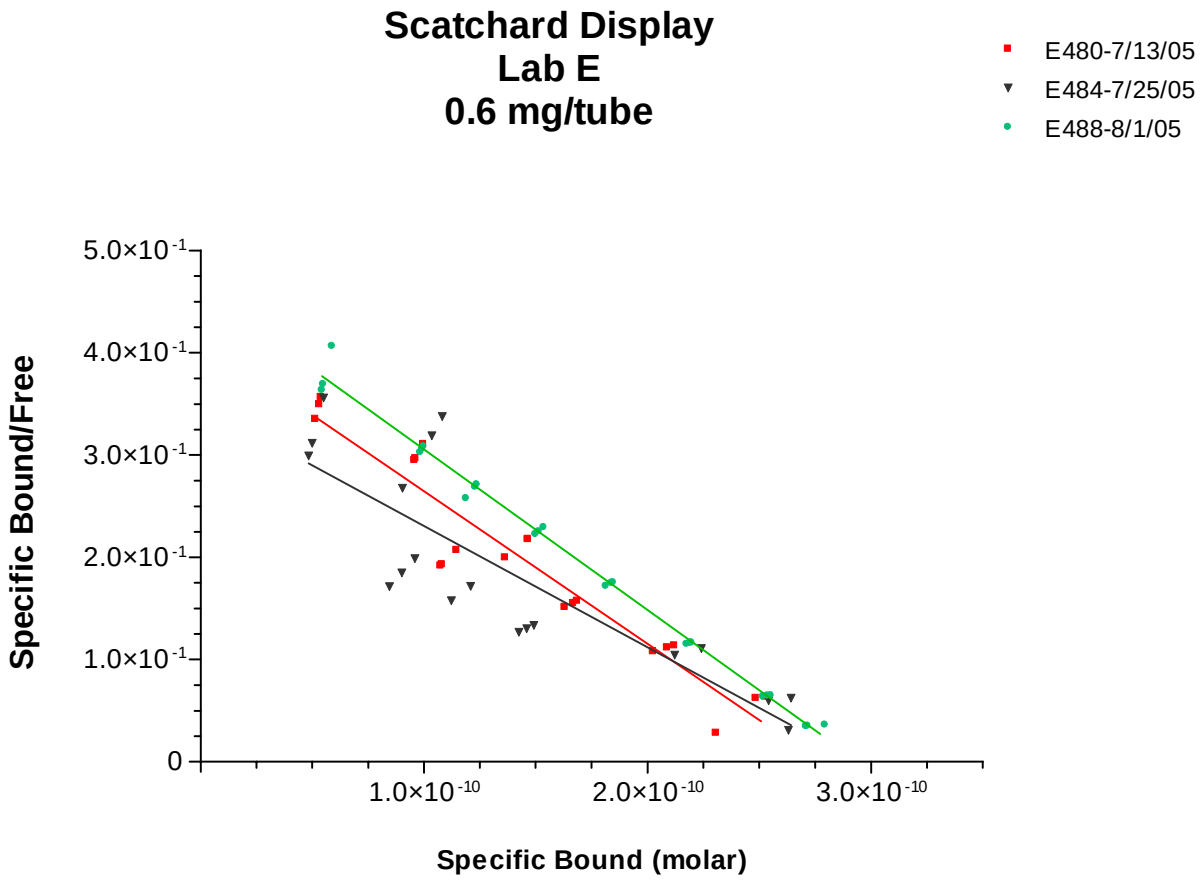


Figure 2: Competitive Binding of R1881 and Dexamethasone (Weak Positive) to the Androgen Receptor

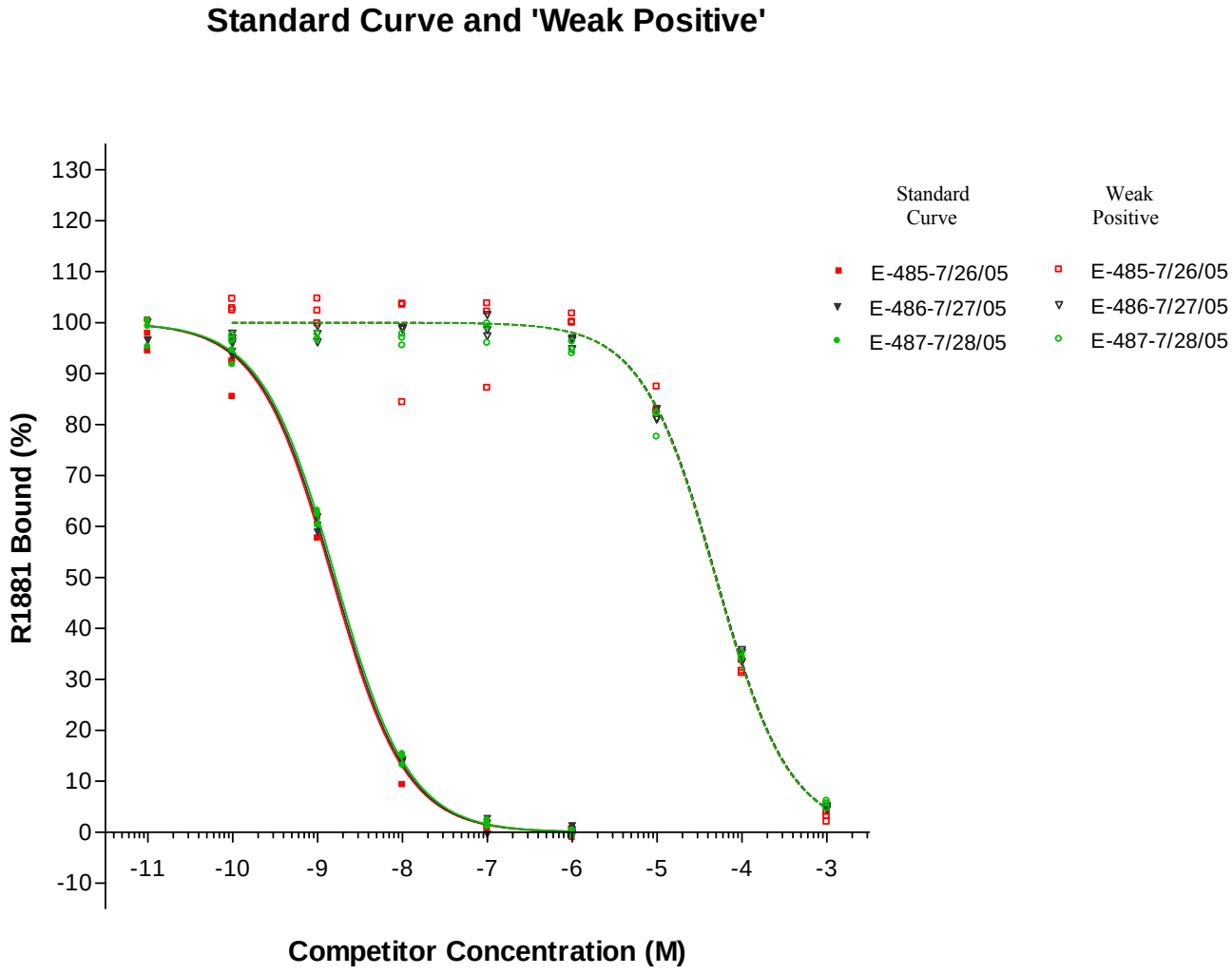


Table 1: Saturation Binding Experiments

Run number	Assay date	Technician	K_d (M)	B_{max} (M)	B_{max} (fmole/100 ug
480	13 July 2005	JEC	0.93×10^{-9}	2.88×10^{-10}	14.40
484	25 July 2005	JEC	0.83×10^{-9}	2.45×10^{-10}	12.26
488	01 August 2005	JEC	0.93×10^{-9}	3.12×10^{-10}	15.58
Mean			0.90×10^{-9}	2.82×10^{-10}	14.08

Table 2: Competitive Binding Experiments

Run number	Assay date	Technician	R1881 IC_{50} (M)	Dexamethasone IC_{50} (M)	Dexamethasone RBA (%)
485	26 July 2005	JEC	1.48×10^{-9}	4.89×10^{-5}	0.0030
486	27 July 2005	JEC	1.55×10^{-9}	5.04×10^{-5}	0.0031
487	28 July 2005	JEC	1.64×10^{-9}	4.92×10^{-5}	0.0033
			1.55 x 10-9	4.95×10^{-5}	0.0031

Abbreviations: IC_{50}, concentration at which 50% of activity is inhibited; RBA, relative binding affinity;
RBA of dexamethasone = (IC_{50} of R1881 / IC_{50} of dexamethasone) × 100

Appendix 1: Copy of Battelle Richland Task 5 Study Protocol SR-04-WA 4-11-01

Assay Protocol	Battelle Pacific Northwest Laboratories P.O. Box 999 Battelle Boulevard Richland, Washington 99352	SR-04-WA 4-11-01

PROTOCOL

1. **Title:** Validation of an Androgen Receptor Binding Assay – Task 5

2. **Sponsor:** Battelle Memorial Institute
505 King Avenue
Columbus, Ohio 43201-2693

3. **Testing Facility:** Battelle, Richland
331 Building, P7-51
Richland, WA 99352

4. **Objective:** To provide data to establish laboratory variability among five laboratories using the same assay and rat prostate cytosol prepared in each participating laboratory, radiolabeled R 1881, R1881 and dexamethasone (test substance). This protocol is specific to the study to be conducted at Battelle-Richland.

5. **Duration:** approximately 45 days

6. **Proposed Study Dates:**

   a. Initiation of *In Vitro* Studies: June 26, 2005
   b. Completion of *In Vitro* Studies: August 4, 2005

7. **Protocol Approval:**

   a. Study Director: James E. Morris Date: 6/10/05
   James E. Morris, Ph.D.

   b. WAL: Michael L. Blanton Date: 6/8/05
   Michael L.Blanton

   c. Sponsor: David P. Houchens Date: 6/6/05
   David P. Houchens, Ph.D.

Assay Protocol	Battelle Pacific Northwest Laboratories P.O. Box 999 Battelle Boulevard Richland, Washington 99352	SR-04-WA 4-11-01

Reviewed By:

a. MSL QA Representative: Mary E Lynn Date: 6/7/05
Mary E. Lynn

b. EDSP Battelle QAM: Terri L Pollock Date: 6-6-05
Terri L. Pollock

8. **Test, marker and reference substances:**

a.1 Test Substance: Dexamethasone (CAS # 50-02-2) will be prepared by and supplied by the Chemical Repository, Battelle, Sequim, Washington. Also, identified as weak positive.

a.2 Reference substance: Radioinert Methyltrienolone (CAS 965-93-5, Perkin-Elmer, NEN, catalog number NLP 005005MG) will be prepared by and supplied by the Chemical Repository, Battelle, Sequim, Washington.

a.3 Marker: Radiolabeled R 1881 Tracer (CAS # 965-93-5) Perkin-Elmer [17 α–methyl–^{3}H]-R 1881. Specific Activity: 82Ci (3.04TBq)/mmol.

b. Storage: ^{3}H-R 1881 will be stored at -20±-5 °C. Other substances will be stored according to conditions specified by the supplier.

c. Disposition: All quantities of the test and reference substances which are dispensed will be documented.

9. **Test System:**

a. Identification: The test system is rat prostate cytosol. Each tube within the assay will be uniquely labeled as defined in the assay. Cytosol will be stored at -80 + 10 °C.

b. Justification for selection of the test system: Rat prostate cytosol is used because of the extensive data that exist for comparison and the inability to identify appropriate recombinant system(s) without false negative and positive acting chemicals in the *in vitro* binding assay.

c. Source: The rat prostate cytosol will be prepared by Battelle – Richland per Battelle SOPs. The ventral prostate tissues are collected from 130 Sprague-Dawley male rats (60 to 90 days of age) castrated 24 hours prior to being humanely killed. Weighed and trimmed prostate tissues are placed in ice-cold buffer prepared with tris, ethylenediaminetetraacetic acid and glycerol (TEDG) with phenylmethylsulfonyl fluoride (PMSF) at a ratio of 0.1 g of tissue per 1.0 ml TEDG buffer with PMSF. Homogenize the tissue using an appropriate homogenizer. Cytosol pooled and stored at -80 + 10 °C.

10. **Experimental Design:**

a. Assay to be Performed: Competitive binding of dexamethasone to the androgen receptor using rat prostate cytosol, compared to R 1881 will be performed (Appendix 1). The radio tracer for the assay will be Radiolabeled R 1881 Tracer. Prior to conducting the competitive binding assay, a saturation assay will be performed to demonstrate that the assay is properly standardized (Appendix 1). Each assay (competitive and saturation binding assay) will be run in triplicate (one each day on three separate days by the same technician).

b. Frequency of Tests: Three tubes will be run per concentration and each assay will be run three times for the saturation and competitive binding assays. The assays are described in detail in Appendix 1.

c. Route of administration and Reason for its choice: The cytosol is added directly to the test and reference substances in assay tubes in the appropriate sequence with the other reagents in the assay. The direct application and sequence is required for this assay type.

d. Analysis of Data: Nonlinear curve fitting of unlabeled R 1881 (nM) (X axis) and total radiolabeled binding (DPM) (Y axis) to obtain K_d and B_{max} and then $IC_{50,}$ Relative Binding Activity ratio (RBA) for each test substance(s) using GraphPad program.

11. **Quality Assurance:** This study will be audited by the Quality Assurance Unit to assure adherence to Good Laboratory Practice Regulations, adherence to the study protocol and

compliance with Battelle Standard Operating Procedures. The Quality Assurance Unit will conduct a review of the raw data for accuracy and traceability and will audit the final report.

12. **Reports:** A Report will be prepared at the completion of the study. The report will include, but not be limited to, the following:

    a. Design of the study and the results obtained.
    b. Name and address of the facility performing the study.
    c. Copy of the approved protocol, including all changes and revisions.
    d. Date of the completed report.
    e. Instances of ambiguity or unclear direction.
    f. Prism or other data files.
    g. Description of all circumstances that may have affected the quality or integrity of data.
    h. Name, chemical structure, Chemical Abstract Service Registry Number (if known), physical nature and purity (if known) of the test and reference substances.
    i. Justification for choice of solvent/vehicle if other than water or ethanol, and information to demonstrate that the solvent/vehicle, if other than an established solvent does not bind to or otherwise affect the Androgen Receptor (AR).
    j. Type and source of AR, its isolation from tissues, protein concentration of AR preparation and method of storage.
    k. Test conditions.
    l. Results including extent of precipitation of test and reference substance, K_d and IC_{50} values with confidence limits for R 1881 and test substance, and RBA values for the test substance(s).

13. **Alteration of Design:** Alterations of the protocol may be made as the study progresses. Changes will be documented as required by Battelle SOPs.

14. **Data Notebooks:** All original data will be maintained in data notebooks. These will include, but not necessarily be limited to the following:

    a. The original signed protocol and all amendments.
    b. Test system records.
    c. Test and reference substances receipt and use records.
    d. Test and reference substances preparation data.
    e. Sample preparation data.
    f. Scintillation counting data.
    g. Calculations to determine final reported values.

15. **Records to be Maintained:** The protocol, any amendments, the final report and all raw data collected as a result of this study will be archived by Battelle-Richland. The associated facility records will also be archived as required by Battelle-Richland SOPs.

16. **Personnel:** *Curricula vitae* for all personnel involved in the execution of the study are on file at Battelle, Pacific Northwest Division.

17. **Compliance Statement:** This study will be conducted in compliance with the U.S. EPA Good Laboratory Practice Regulations as set forth in Part 160 Title 40 of the Code of Federal Regulations.

Appendix 1

Assay Protocol for the *In Vitro* Androgen Receptor (AR) Saturation and Competitive Binding Assay Using Rat Ventral Prostate Cytosol (RVPC)

OP No. NHEERL-H/RTD/EB/VW/2002-03-000

1.0 Purpose and Applicability

Determine ability of compound to compete with [^{3}H] ligand for binding in rat ventral prostate tissue homogenate.

2.0 Safety and Operating Precautions

All procedures with radioisotopes should follow the regulations and procedures as described in the Hazardous Agent Protocol and in the Radiation Safety Manual and Protocols for U.S. EPA.

3.0 Animal Use

Follow U.S. EPA approved animal use protocols

4.0 Equipment and Materials

4.1 Equipment

Corning Stir/hot Plates
Pipettes
Balance
Sample rotor
Polytron PT 35/10 Tissue Homogenizer
Vacuum Concentrator
Refrigerated General Laboratory Centrifuge
pH Meter with Tris Compatible Electrode
Scintillation Counter
Refrigerators and freezers (-20^o and -80^o)

4.2 Chemicals

Tris HCL & Tris Base
Phenylmethylsulfonyl Fluoride (PMSF)
Glycerol 99%+
Sodium Molybdate
Ethylenediaminetetraacetic acid (EDTA); Disodium salt
Dithiothreitol (DTT)
Hydroxylapatite (HAP; BIO-RAD)
Scintillation Cocktail (Optifluor)
Ethyl Alcohol, anhydrous
[^{3}H]-R1881 (NEN; Purity >97%)
Radioinert R1881 (NEN)

Triamcinolone Acetonide

4.3 Supplies
20 ml Polypropylene Scintillation Vials
12 x 75 mm Borosilicate Glass Test Tubes
1000 ml graduated cylinders
100 ml Erlenmeyer or plastic containers
pipette tips

5.0 Stock Preparations

5.1. Preparation of Stock Solutions for making TEDG Buffer

5.1.1. *EDTA Stock Solution*: Add 7.444g disodium EDTA to 100 ml ddH_2O = 200mM. Store at 4+ 2^oC. Use 750 ul/100ml TEDG buffer = 1.5 mM.

5.1.2. *PMSF Stock Solution*: Add 1.742 g PMSF to 100 ml ethanol = 100 mM. Store at 4+ 2 ^{o} C. Use 1.00 ml/100ml TEDG buffer = 1.0 mM.

5.1.3. *Sodium Molybdate Stock:* Add 2.419 g sodium molybdate to 8.0 ml ddH_2O in a 10 ml volumetric flask; bring the total volume to 10 mls = 1.0 M. Store at 4^oC. Use 100ul/100ml TEDG buffer = 1.0 mM.

5.1.4. *1 M Tris Buffer:* Add 147.24 g Tris-HCL + 8.0 g Tris base to 800mls ddH_2O in a volumetric flask; bring the final volume to 1.0 liter. Refrigerate to 4^oC and pH the cooled solution to 7.4. Store at 4+ 2 ^{o} C. Use 1.0 ml/100 ml TEDG buffer = 10mM. (50 mM Tris = 50 ml 1 M Tris/1 L ddH_2O)

5.1.5. Triamcinolone Acetonide (TRIAM) Stock: Add 0.0653grams to 250 ml ethanol = 0.6 mM. Store at 4+ 2^o C. Use 100ul/1.0ml TEDG buffer = 60 uM.

5.1.6. Add 15.4 mg DTT directly to 100 ml TEDG buffer the morning of the receptor isolation = 1.0 mM.

5.2. Preparation of Low-Salt TEDG Buffer (pH 7.4)

To make 100 ml of low-salt TEDG buffer add the following together in this order:

87.15 ml ddH_2O
1.0 ml 1M TRIS
10.0 ml glycerol
100 ul 1 M sodium molybdate
750 ul 200mM EDTA
1.0 ml 100mM PMSF
15.4 mg DTT (add immediately before use)

Check pH of the final solution to make sure it is 7.4 at 4+ 2 ^{o} C.

5.3 Preparation of 50 mM TRIS Buffer

Add 50.0 ml of 1.0 M TRIS to 950 ml ddH_2O. Store at 4+ 2 ^{o} C. Check pH of the final solution to make sure it is 7.4 at 4+ 2 ^{o} C.

5.4 Preparation of 60% Hydroxylapatite (HAP) Slurry

5.4.1. Shake BIO-RAD HT-GEL until all the HAP is in suspension (i.e., looks like milk). The evening before the receptor extraction, pour 100 ml (or an appropriate volume) into a 100 ml graduated cylinder, parafilm seal the top and place in the refrigerator for at least 2h.

5.4.2. Pour off the phosphate buffer supernatant, and bring the volume to 100 ml with 50 mM TRIS. Suspend the HAP by parafilm sealing the top of the graduated cylinder and inverting the cylinder several times. Decant supernatant and repeat wash step times 2 with fresh 50 mM TRIS buffer.

5.4.3. Place in the refrigerator overnight.

5.4.4. Decant supernatant. Add enough 50 mM TRIS to make the final solution a 60% slurry (i.e., if the volume of the settled HAP is 60 ml bring the final volume of the slurry to 100 mls with 50 mM TRIS).

5.4.5. Store at 4+ 2 ^{o} C until ready for use in the extraction.

5.5 Preparation of [^{3}H]-R1881 Stock Solutions

Dilute the original 1.0 mCi/ml stock of [^{3}H]-R1881 to 0.1 µM (i.e., 1 x 10^{-7} M). This is most easily accomplished by pipetting 1 ul of the stock solution for every specific activity unit (Ci/mmol) and diluting this to 10.0 mls with ethanol. Thus, if the specific activity of the stock vial is 86 Ci/mmol, then pipette 86.0 µl into an amber colored vial (i.e., R1881 is photosensitive) and add 10.0 ml ethanol to the vial; this solution is 1 x 10^{-7}M.

Note: [^{3}H]-R1881 stock solution and dilutions should be stored at -20 + 4^{0}C. Store stock solution in original protective vial and store dilutions in amber glass vials. This product is light-sensitive; care should be taken to minimize exposure to light.

5.6 Calculation Check and Dilutions

86 µl x 1.0 mCi/1000 µl = 86 x 10^{-3} mCi R1881 = 86 x 10^{-6} Ci R1881
86 x 10^{-6} Ci ÷ 86.0 Ci/mmol = 1 x 10^{-6} mmol R1881 = 1 x 10^{-9} moles R1881
1 x 10^{-9} moles R1881 ÷ 0.010 liters = 1 x 10^{-7} moles/liter = 0.1 µM

To prepare the 1 x 10^{-8} M stock simply make a 10-fold dilution of the 1 x 10^{-7} M stock (i.e., pipette 1.0 ml of the 1 x 10^{-7} M stock into a clean amber colored vial and add 9 ml ethanol = 0.01 uM).

To prepare the 1 x 10^{-9} M stock simply make a 10-fold dilution of the 1 x 10^{-8} M stock (i.e., pipette 1.0 ml of the 1 x 10^{-8} M stock into a clean amber colored vial and add 9 ml ethanol = 0.001 uM).

5.7 Preparation of 100X Radioinert R1881 Solutions

The R1881 comes as a 5.00 mg quantity. Dilute the original stock to 5.0 ml with ethanol = 3.52 mM. Take 56.82 µl and dilute to 20 ml in an amber vial with ethanol = 1 x 10^{-5} M R1881. This is the 10 µM radioinert R1881 stock.

To make the 1.0 µM radioinert R1881 stock, pipette 2 ml of the 10 µM stock into an amber vial and dilute to 20 ml with ethanol = 1 x 10^{-6}M = 1.0 µM radioinert R1881 stock. To make the 0.10 µM radioinert R1881 stock, pipette 2 ml of the 1 µM stock into an amber vial and dilute to 20 ml with ethanol = 1 x 10^{-7}M = 0.10 µM radioinert R1881 stock.

5.8 Compound Stock Preparations

5.8.1. Make stocks 30X above desired final concentration (this accounts for the use of 10 µl stock in 300 µl cytosol). Initial Stock of each test chemical solution will be diluted in 100% ethanol at a concentration of 3.0 x 10^{2} M (i.e., 30 mM).

EXAMPLE:

4 (t) octyl phenol FW 206.33
1M = 206.33 g/L
1mM= 0.20633 mg/ml x 30 (30 mM desired final stock conc.) = 6.1899 mg/ml

2 ml Stock = 6.1899 mg x 2 = 12.37mg/2ml

5.8.2. Prepare serial dilutions of R1881 for standard curve in ethanol (100%) to yield the Initial Concentrations as indicated in Table 1.

Table 1: Standard Curve

Standards	*Initial R1881 Concentration (Molar)*	**Final R1881 Concentration (Molar) in AR assay tube*
Negative Control	0	

0	0 (EtOH)	0
NSB	1×10^{-5}	1×10^{-6}
S1	3×10^{-6}	1×10^{-7}
S2	3×10^{-7}	1×10^{-8}
S3	3×10^{-8}	1×10^{-9}
S4	3×10^{-9}	1×10^{-10}
S5	3×10^{-10}	1×10^{-11}
** Final concentration = 10 ul of each standard is added to the assay tube, except for the NSB which is 30 ul.*		

5.8.3. Prepare serial dilutions of the test chemicals as indicated in Table 2.

Table 2: Test Chemical Concentrations (this subject to adjustment)

Serial Dilutions of the Test Chemical	*Initial Concentration (Molar)*	**Final Concentration (Molar) in AR assay tube*
Concentration 1	3×10^{-3}	1×10^{-4}
Concentration 2	3×10^{-4}	1×10^{-5}
Concentration 3	3×10^{-5}	1×10^{-6}
Concentration 4	3×10^{-6}	1×10^{-7}
Concentration 5	3×10^{-7}	1×10^{-8}
Concentration 6	3×10^{-8}	1×10^{-9}
Tube 7	0 (vehicle only)	0

** Final concentration = 10 ul of each Initial Concentration of test chemical is added to the assay tube along with 300 ul of ventral prostate cytosol.*

6.0 Tissue Homogenate Collection

6.1. Castrate 90 day old rats (60-90 day old acceptable; 90 day old preferred) as per laboratory animal protocols.

6.2. 24 hours after castration, make low salt TEDG buffer and place in an ice-water bucket.

6.3. Kill rat and excise ventral prostate. Tissue should be trimmed of fat, weighed and the weights recorded.

6.4. Add low-salt TEDG buffer at 10ml/g tissue.

6.5. Mince tissues with Metzenbaum scissors until all pieces are small 1-2 mm cubes. Then homogenize the tissues using an ice bath with a Polytron homgenizer using three to five 5-sec bursts of the Polytron. [Note: place probe of the Polytron in TEDG buffer in an ice-water bath to cool it down prior to its use for homogenization. Re-cool probe as needed.]

6.6. Transfer homogenates to pre-cooled centrifuge tubes, balance, and centrifuge at 30,000x g for 30 minutes (i.e., 15, 262 rpm using JA-17/JA-21 Beckman rotors).

6.7. The supernatant contains the low-salt cytosolic receptor. Pool the supernatant from all rats. Aliquot as needed for proposed studies and store -80 + 10 ^{o}C .until needed for assay. Discard after 6 months.

6.8. Determine the protein content for each batch of cytosol according to the method by Bradford (1976) using the commercially available BioRad Protein Assay Kit (BioRad Chemical Division, Richmond, CA). Protein concentrations usually range from 5.5 - 8 mg/ml in undiluted cytosol.

7.0. Assay procedure for chemicals: Day 1

7.1. Set up tubes: 12x75 mm glass tubes

7.1.1. Label sufficient glass tubes as needed for the assay.

7.1.2. Add 30ul of 0.01uM [3H] R1881 (1 x 10^{-8}M) and 50 ul triamcinolone acetonide (60 uM stock) to ALL tubes.

7.1.3. For 3 tubes (NSB) at beginning of assay and at end of assay, also add 100x inert R1881 (30ul of 1.0uM, ie 1 x 10^{-6}M). These tubes are for determining nonspecific binding.

7.1.4. Place tubes in speed-vac and dry the tubes according to instructions. Remove when dry.

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Position	*Replicate*	*Competitor*	*Competitor Code*	*Concentration Code*	*Labels on vials in set 1-1-E supplied by Battelle to laboratory "E"*	*Competitor Initial Concentration (M)*	*Cytosol (uL)*	*Tracer (Hot R1881) Volume (uL)*	*Competitor Volume (uL)*	*triamcelenone Volume (uL)*	*Competitor Final Concentration (M)*	*Aliquot (uL)*	*HAP (500 ul)*
1	1	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
2	2	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
3	3	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	1.0E-07	100	500
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	1.0E-07	100	500
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	1.0E-07	100	500
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	1.0E-08	100	500

11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	1.0E-08	100	500
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	1.0E-08	100	500
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	1.0E-09	100	500
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	1.0E-09	100	500
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	1.0E-09	100	500
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	1.0E-10	100	500
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	1.0E-10	100	500
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	1.0E-10	100	500
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	1.0E-11	100	500
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	1.0E-11	100	500
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	1.0E-11	100	500
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	1.E-03	100	500
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	1.E-03	100	500
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	1.E-03	100	500
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	1.E-04	100	500
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	1.E-04	100	500
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	1.E-04	100	500
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	1.E-05	100	500
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	1.E-05	100	500
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	1.E-05	100	500
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	1.E-06	100	500
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	1.E-06	100	500
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	1.E-06	100	500
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	1.E-07	100	500
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	1.0E-07	100	500
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	1.0E-07	100	500
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	1.0E-08	100	500
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	1.0E-08	100	500
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	1.0E-08	100	500
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	1.0E-09	100	500
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	1.0E-09	100	500
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	1.0E-09	100	500
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	1.0E-10	100	500
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	1.0E-10	100	500
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	1.0E-10	100	500
46	1	unknown 1	C	1	**E-1-C11**	3.00E-02	300	30	10	50	1.0E-03	100	500
47	2	unknown 1	C	1	**E-1-C11**	3.00E-02	300	30	10	50	1.0E-03	100	500
48	3	unknown 1	C	1	**E-1-C11**	3.00E-02	300	30	10	50	1.0E-03	100	500
49	1	unknown 1	C	2	**E-1-C12**	3.00E-03	300	30	10	50	1.0E-04	100	500
50	2	unknown 1	C	2	**E-1-C12**	3.00E-03	300	30	10	50	1.0E-04	100	500
51	3	unknown 1	C	2	**E-1-C12**	3.00E-03	300	30	10	50	1.0E-04	100	500
52	1	unknown 1	C	3	**E-1-C13**	3.00E-04	300	30	10	50	1.0E-05	100	500

53	2	unknown 1	C	3	**E-1-C13**	3.00E-04	300	30	10	50	1.0E-05	100	500
54	3	unknown 1	C	3	**E-1-C13**	3.00E-04	300	30	10	50	1.0E-05	100	500
55	1	unknown 1	C	4	**E-1-C14**	3.00E-05	300	30	10	50	1.0E-06	100	500
56	2	unknown 1	C	4	**E-1-C14**	3.00E-05	300	30	10	50	1.0E-06	100	500
57	3	unknown 1	C	4	**E-1-C14**	3.00E-05	300	30	10	50	1.0E-06	100	500
58	1	unknown 1	C	5	**E-1-C15**	3.00E-06	300	30	10	50	1.0E-07	100	500
59	2	unknown 1	C	5	**E-1-C15**	3.00E-06	300	30	10	50	1.0E-07	100	500
60	3	unknown 1	C	5	**E-1-C15**	3.00E-06	300	30	10	50	1.0E-07	100	500
61	1	unknown 1	C	6	**E-1-C16**	3.00E-07	300	30	10	50	1.0E-08	100	500
62	2	unknown 1	C	6	**E-1-C16**	3.00E-07	300	30	10	50	1.0E-08	100	500
63	3	unknown 1	C	6	**E-1-C16**	3.00E-07	300	30	10	50	1.0E-08	100	500
64	1	unknown 1	C	7	**E-1-C17**	3.00E-08	300	30	10	50	1.0E-09	100	500
65	2	unknown 1	C	7	**E-1-C17**	3.00E-08	300	30	10	50	1.0E-09	100	500
66	3	unknown 1	C	7	**E-1-C17**	3.00E-08	300	30	10	50	1.0E-09	100	500
67	1	unknown 1	C	8	**E-1-C18**	3.00E-09	300	30	10	50	1.0E-10	100	500
68	2	unknown 1	C	8	**E-1-C18**	3.00E-09	300	30	10	50	1.0E-10	100	500
69	3	unknown 1	C	8	**E-1-C18**	3.00E-09	300	30	10	50	1.0E-10	100	500
70	1	ethanol	EtOH	0	**—**	—	300	30	10	50	—	100	500
71	2	ethanol	EtOH	0	**—**	—	300	30	10	50	—	100	500
72	3	ethanol	EtOH	0	**—**	—	300	30	10	50	—	100	500
73	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
74	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
75	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
76	1	none	Hot		**—**	—	—	30	—	—	—	—	—
77	2	none	Hot		**—**	—	—	30	—	—	—	—	—
78	3	none	Hot		**—**	—	—	30	—	—	—	—	—
79	1	none	Hot		**—**	—	—	30	—	—	—	—	—
80	2	none	Hot		**—**	—	—	30	—	—	—	—	—
81	3	none	Hot		**—**	—	—	30	—	—	—	—	—

7.2. Add 10ul of compound stocks (see 5.8 for concentrations 1-7 in triplicate)

7.3. Remove aliquot of prostate cytosol and thaw on ice. Cytosol should be diluted with ice-cold low-salt TEDG buffer to give a protein concentration of ~1 mg per 300 ul assay tube. The amount of cytosol protein is subject to adjustment based on activity identified in saturation binding assays.

7.4. Add 300 ul of diluted cytosol to every tube ON ICE. Gently vortex and place tubes in refrigerator overnight in rotor (18-20hr).

Note: Make sure that all components are concentrated at the bottom of tube. If any of the liquid remains on the side of the tube, centrifuge assay tubes for 1 minute at 600 x g (4°C) to concentrate fluid at bottom of tube.

7.5. Before leaving for the day, prepare the washes of the HAP slurry as described in section 5.4 above.

7.6. Label the HAP tubes and the scintillation vials to be used the following day - see underlines below.

8.0 Assay procedure: Day 2

8.1. The following morning, wash the HAP as described in section 5.4 above, dilute with 50 mM TRIS to yield a 60% slurry, and transfer contents to a plastic or glass container. Place a stir bar in the container and place in a beaker containing ice-water; stir the HAP slurry by placing the beaker on a magnetic stir plate.

8.2. While the HAP slurry is constantly being stirred, pipette 500 µl of the HAP slurry into clean pre-labelled 12 x 75 mm glass test tubes. Place these tubes in a rack in an ice-water bath prior to pipetting the HAP slurry and keep them in the ice-water bath for the remainder of the assay.

8.3. One HAP tube should be prepared for each incubation tube.

8.4. Take the incubation tubes from the refrigerator and place them in an ice-water bath with the HAP tubes. Pipette 100 µl from each of the incubation tubes into the appropriate pre-labelled tubes containing HAP. Repeat for all tubes. Quickly take each rack from the ice-water bath and vortex each rack of tubes using the whole-rack vortex unit. Place racks back into the ice-water bath and vortex as above every 5 minutes for 20 minutes.

8.5. Centrifuge the HAP tubes for 10-11 minutes at 4 + 2^{o}C and 600 x *g* (1780 rpm in a Beckman GLC refrigerated centrifuge). Place the tubes back into the rack and into the ice-water bath.

8.6. While the tubes remain in the ice-water bath, aspirate or decant the supernatant from each tube. If aspirating, use a 9 inch pipette connected to an aspiration apparatus as per the radiation safety protocol.

8.7. Add 2 ml of 50 mM TRIS to each tube, vortex and centrifuge at 600 x *g* as above. Place the tubes into decanting racks in an ice-water bath and decant the supernatant TRIS wash into the radiation safety container. Gently tap the tube openings on a clean adsorbent diaper, place the rack back in the ice-water bath and add 2 mls of 50 mM TRIS.

8.8. Repeat the TRIS washing procedure 3 or 4 times (to be determined empirically) keeping the tubes on ice at all times.

8.9. Following the last wash and decanting, add 2 mls of ethanol to each tube, vortex 3 times at 5 minute intervals and centrifuge the tubes at 600 x *g* for 10 minutes. Decant the supernatants into pre-labelled 20 ml scintillation vials containing 14 ml of Optifluor scintillation cocktail. Count samples using the single label DPM program with quench correction.

9.0. Saturation Radioligand Binding Assay

Prior to routinely conducting the AR competitive binding assays, the methods should be standardized within each laboratory. A series of saturation radioligand binding assays should be conducted to demonstrate AR specificity and saturation. Nonlinear regression analysis of these data and subsequent Scatchard plots will document AR binding affinity (K_d) and maximum specific binding number (B_{max}). Scatchard assay is to be conducted as follows:

Day 1 9.1. Set up tubes: 12x75 glass tubes and label for 8 concentrations in triplicate each with and without 100X inert (48 tubes total 1 through 48 below).

9.2. Add [^{3}H] R1881 from the appropriate stock solutions to tubes as listed below:

9.3. Place 50 µl of 60 uM stock triamcinolone acetonide to ALL tubes.

9.4 An aliquot of each concentration of [^{3}H]R1881 should also be counted on scintillation counter to determine total counts added (tube # 49-72 below).

Saturation Assay Tube Layout

Position	*Replicate*	*Tube Type Code*	*Hot Initial Concentration (nM)*	*Hot R1881 Volume (uL)*	*Hot Final Concentration (nM)*	*Cold Initial Concentration (uM)*	*Cold Volume (uL)*	*Cold Final Concentration (nM)*	*Triamcelenone Acetate (uL)*	*Cytosol (ul)*
1	1	H	10.0	7.5	0.25	—	—	—	50	300
2	2	H	10.0	7.5	0.25	—	—	—	50	300
3	3	H	10.0	7.5	0.25	—	—	—	50	300
4	1	H	10.0	15	0.50	—	—	—	50	300
5	2	H	10.0	15	0.50	—	—	—	50	300
6	3	H	10.0	15	0.50	—	—	—	50	300
7	1	H	10.0	21	0.70	—	—	—	50	300
8	2	H	10.0	21	0.70	—	—	—	50	300
9	3	H	10.0	21	0.70	—	—	—	50	300
10	1	H	10.0	30	1.00	—	—	—	50	300
11	2	H	10.0	30	1.00	—	—	—	50	300
12	3	H	10.0	30	1.00	—	—	—	50	300
13	1	H	10.0	45	1.50	—	—	—	50	300
14	2	H	10.0	45	1.50	—	—	—	50	300
15	3	H	10.0	45	1.50	—	—	—	50	300
16	1	H	100.0	7.5	2.50	—	—	—	50	300
17	2	H	100.0	7.5	2.50	—	—	—	50	300
18	3	H	100.0	7.5	2.50	—	—	—	50	300
19	1	H	100.0	15	5.00	—	—	—	50	300
20	2	H	100.0	15	5.00	—	—	—	50	300
21	3	H	100.0	15	5.00	—	—	—	50	300
22	1	H	100.0	30	10.00	—	—	—	50	300
23	2	H	100.0	30	10.00	—	—	—	50	300
24	3	H	100.0	30	10.00	—	—	—	50	300
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
28	1	HC	10.0	15	0.5	1.00	15	50	50	300
29	2	HC	10.0	15	0.5	1.00	15	50	50	300
30	3	HC	10.0	15	0.5	1.00	15	50	50	300
31	1	HC	10.0	21	0.7	1.00	21	70	50	300
32	2	HC	10.0	21	0.7	1.00	21	70	50	300
33	3	HC	10.0	21	0.7	1.00	21	70	50	300
34	1	HC	10.0	30	1	1.00	30	100	50	300
35	2	HC	10.0	30	1	1.00	30	100	50	300

36	3	HC	10.0	30	1	1.00	30	100	50	300
37	1	HC	10.0	45	1.5	1.00	45	150	50	300
38	2	HC	10.0	45	1.5	1.00	45	150	50	300
39	3	HC	10.0	45	1.5	1.00	45	150	50	300
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300
43	1	HC	100.0	15	5	10.00	15	500	50	300
44	2	HC	100.0	15	5	10.00	15	500	50	300
45	3	HC	100.0	15	5	10.00	15	500	50	300
46	1	HC	100.0	30	10	10.00	30	1000	50	300
47	2	HC	100.0	30	10	10.00	30	1000	50	300
48	3	HC	100.0	30	10	10.00	30	1000	50	300
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—
52	1	Hot	10.0	15	0.06	—	—	—	—	—
53	2	Hot	10.0	15	0.06	—	—	—	—	—
54	3	Hot	10.0	15	0.06	—	—	—	—	—
55	1	Hot	10.0	21	0.08	—	—	—	—	—
56	2	Hot	10.0	21	0.08	—	—	—	—	—
57	3	Hot	10.0	21	0.08	—	—	—	—	—
58	1	Hot	10.0	30	0.10	—	—	—	—	—
59	2	Hot	10.0	30	0.10	—	—	—	—	—
60	3	Hot	10.0	30	0.10	—	—	—	—	—
61	1	Hot	10.0	45	0.30	—	—	—	—	—
62	2	Hot	10.0	45	0.30	—	—	—	—	—
63	3	Hot	10.0	45	0.30	—	—	—	—	—
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—
67	1	Hot	100.0	15	1.00	—	—	—	—	—
68	2	Hot	100.0	15	1.00	—	—	—	—	—
69	3	Hot	100.0	15	1.00	—	—	—	—	—
70	1	Hot	100.0	30	3.00	—	—	—	—	—
71	2	Hot	100.0	30	3.00	—	—	—	—	—
72	3	Hot	100.0	30	3.00	—	—	—	—	—

9.5. Place tubes in speed-vac (Tubes 1-48) and dry the tubes according to instructions. Remove when dry and place on ice.

9.6. Cytosol should be diluted with the low salt TEDG buffer to a protein concentration of ~ 0.6 mg per 300 ul assay (The 0.6 mg is for initial assessment, it may be necessary to adjust depending upon the linearity of the Scatchard plot). Add 300 ul of diluted prostate cytosol to all tubes (1-48). Keep tubes and cytosol on ice at all times during this procedure. Gently vortex and place tubes in refrigerator overnight in rotor (18-20hr).

9.7 Before leaving for the day, conduct washes of the HAP slurry as described in section 5.4 above. If desired, label the HAP tubes and the scintillation vials to be used the following day.

Day 2

9.8. Continue as with Day 2 protocol for competitive binding assay above in section 8.0.

10.0 Data Processing

10.1 Free Concentration of [^{3}H]-R1881

Multiply the DPM in the total counts tubes by 1.8047 x 10^{-5}. This value will yield the free concentration (i.e., nM) of [^{3}H]-R1881 initially present in each incubation tube.

Calculation Check -

$$\frac{\text{X DPM}}{2.22 \times 10^{12}\ \text{dpm/Ci}} = \frac{4.5045 \times 10^{-13}\ \text{Ci}}{*83.2\ \text{Ci/mmole}} = \frac{5.4141 \times 10^{-15}\ \text{mmole}}{1000\ \text{mmole/mole}} = \frac{5.4141 \times 10^{-18}\ \text{moles}}{0.0003\ \text{liters}}$$

$$= 1.8047 \times 10^{-14}\ \text{moles/liter} = \text{X}\ (1.8047 \times 10^{-5})\ \text{nM}$$
$$1 \times 10^{-9}\ \text{moles/nmole}$$

*Note this value will be the Specific activity of the radioligand ([^{3}H]R1881) used in the assay.

10.2 Calculation of Total, Nonspecific and Specific [^{3}H]-R1881 Binding

10.2.1. Total binding is calculated by multiplying the DPM from the tubes that contained only radiolabelled R1881 x (1.6242 x 10^{-2}). This value will be total binding in fmoles.

10.2.2. Nonspecific binding is calculated by multiplying the DPM from the tubes containing radiolabelled R1881 + 100-fold molar excess of radioinert R1881 x (1.6242 x 10^{-2}). This value will be nonspecific binding in fmoles.

10.2.3. Specific binding is calculated by subtracting nonspecific binding from total binding i.e., fmoles total binding - fmoles nonspecific binding = specific binding in fmoles.

10.3 Graphical Presentation of the Data

10.3.1. Standard Curve and Test Chemical Competitive Binding Curves: Data for the standard curve and each test chemical will be plotted as the percent 3H_R1881 bound versus the molar concentration. Estimates of the IC_{50}s will be determined using

appropriate non linear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA). A Scatchard Analysis may also be performed for the standard curve using R1881 to demonstrate that the assay meets acceptable QA standards.

10.3.2. Relative Binding Affinity: The RBA for each competitor should be calculated by dividing the IC_{50} for R1881 by the IC_{50} of the competitor and expressing as a percent (e.g., RBA for R1881 =100 %).

10.3.3. Maximal binding capacity (Bmax) and association/dissociation constants (Ka / Kd) can be estimated using a number of commercially available iterative nonlinear regression analysis programs. One of the better programs was developed by Munson and Rodbard and is called LIGAND.

10.4 References

1) Nonneman, D.J., Ganjam, V.K., Welshons, W.V., and Vom Saal, F.S. (1992) *Biol. Reprod.* **47**, 723-729

2) Segel, I.H. (1975) *Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems*. 1st Ed, John Wiley and Sons, Inc., New York, NY

3) Munson, P.J., and Rodbard, D. (1980) *Anal. Biochem.* **107**, 220-239.

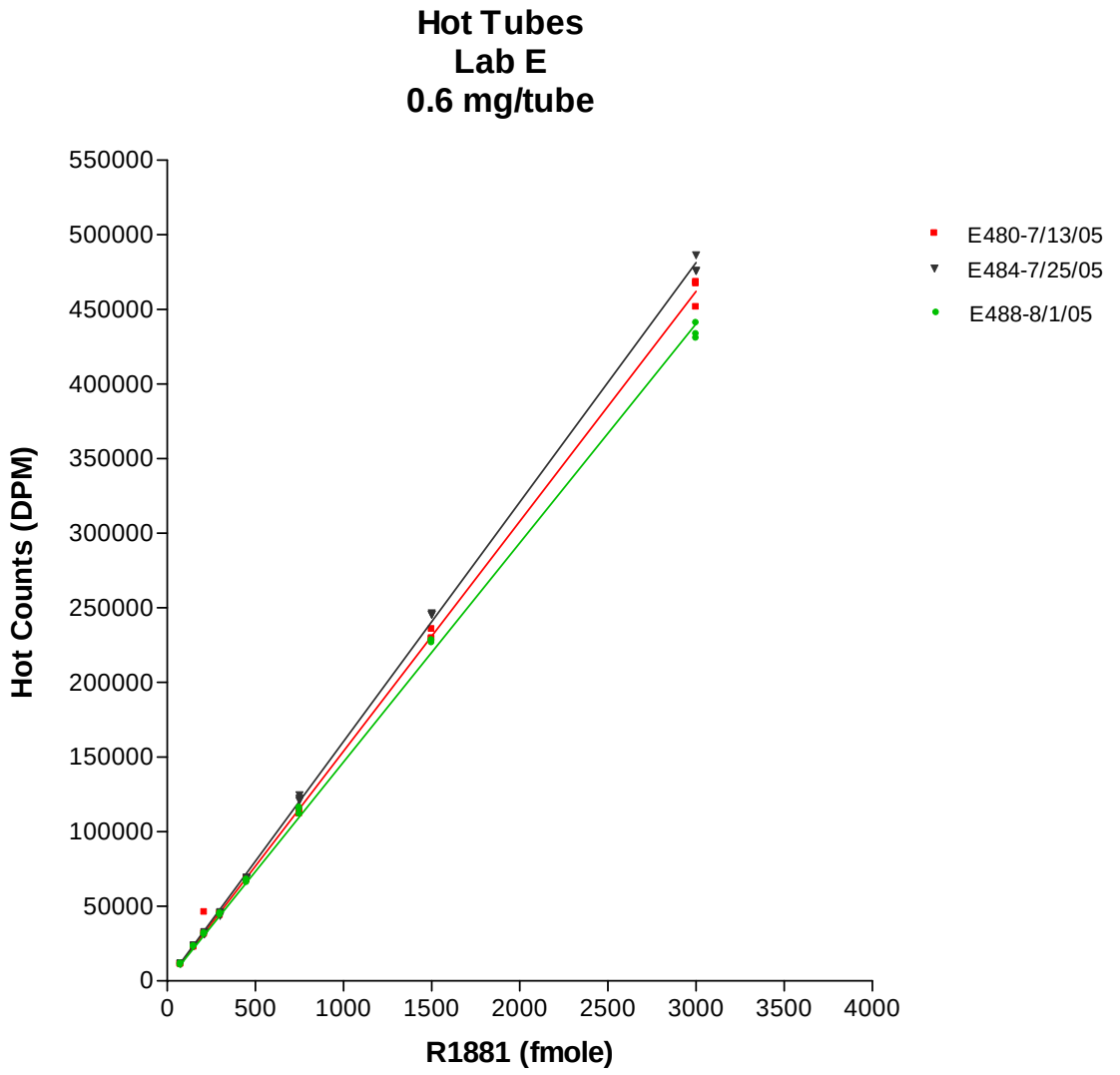
4) Tekpetey, F.R., and Amann, R.P. (1988) *Biol. Reprod.* **38**, 1051-1060.

5) Wilson, V.S., Lambright, C.S., Ostby, J. and Gray, Jr., L.E.. *In* vitro and in vivo effects of 17-beta trenbolone: A feedlot effluent contaminant. (2002). *Toxicol. Sci.**70**(2):202-211.*.

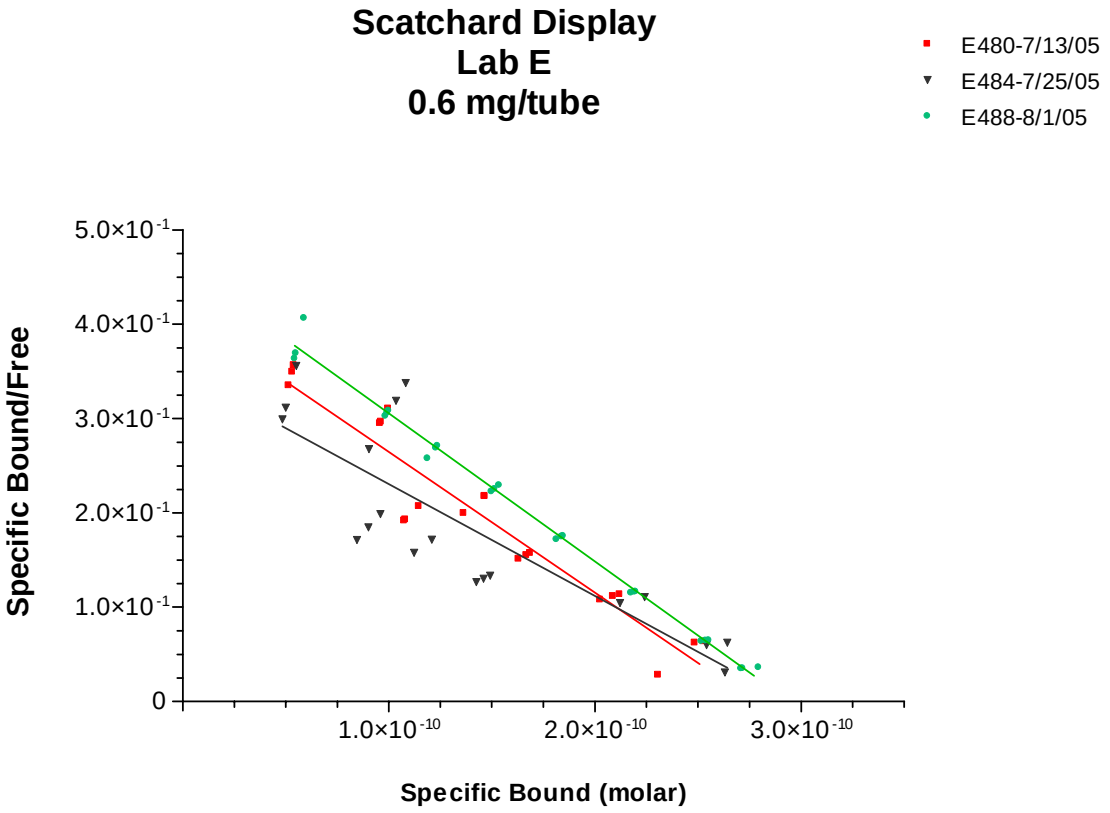
6) Bradford, M. (1976). *Anal. Biochem.* **72**, 248-254.

Appendix 2: Prism Files for Saturation Binding Experiments

WA 4-11-5 Saturation

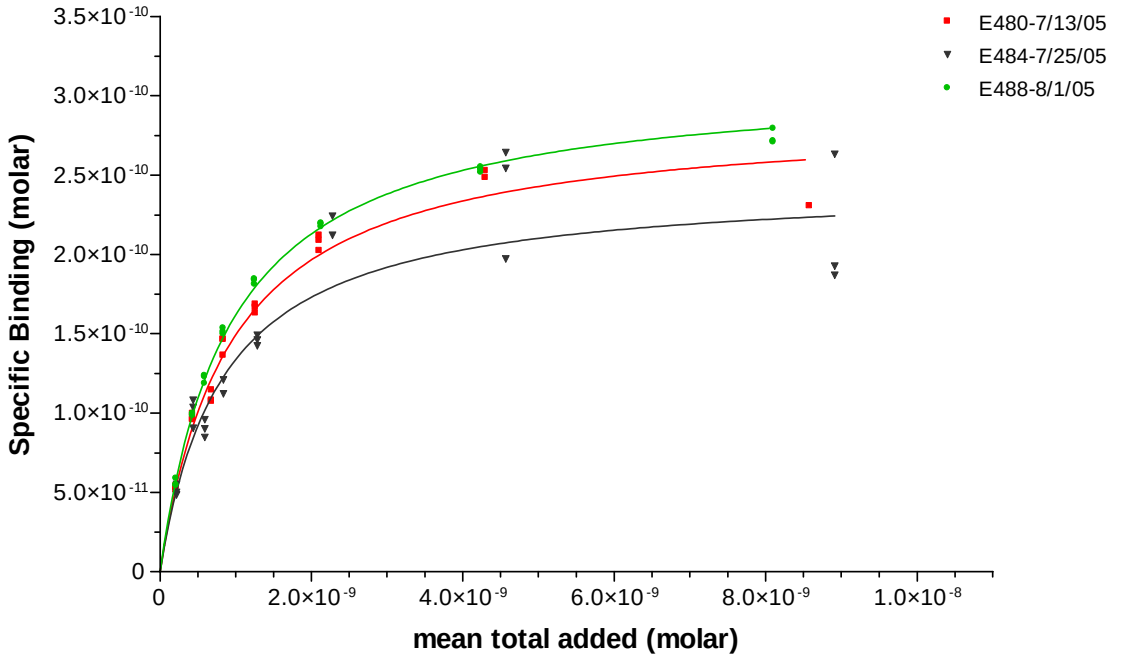


WA 4-11-5 Saturation



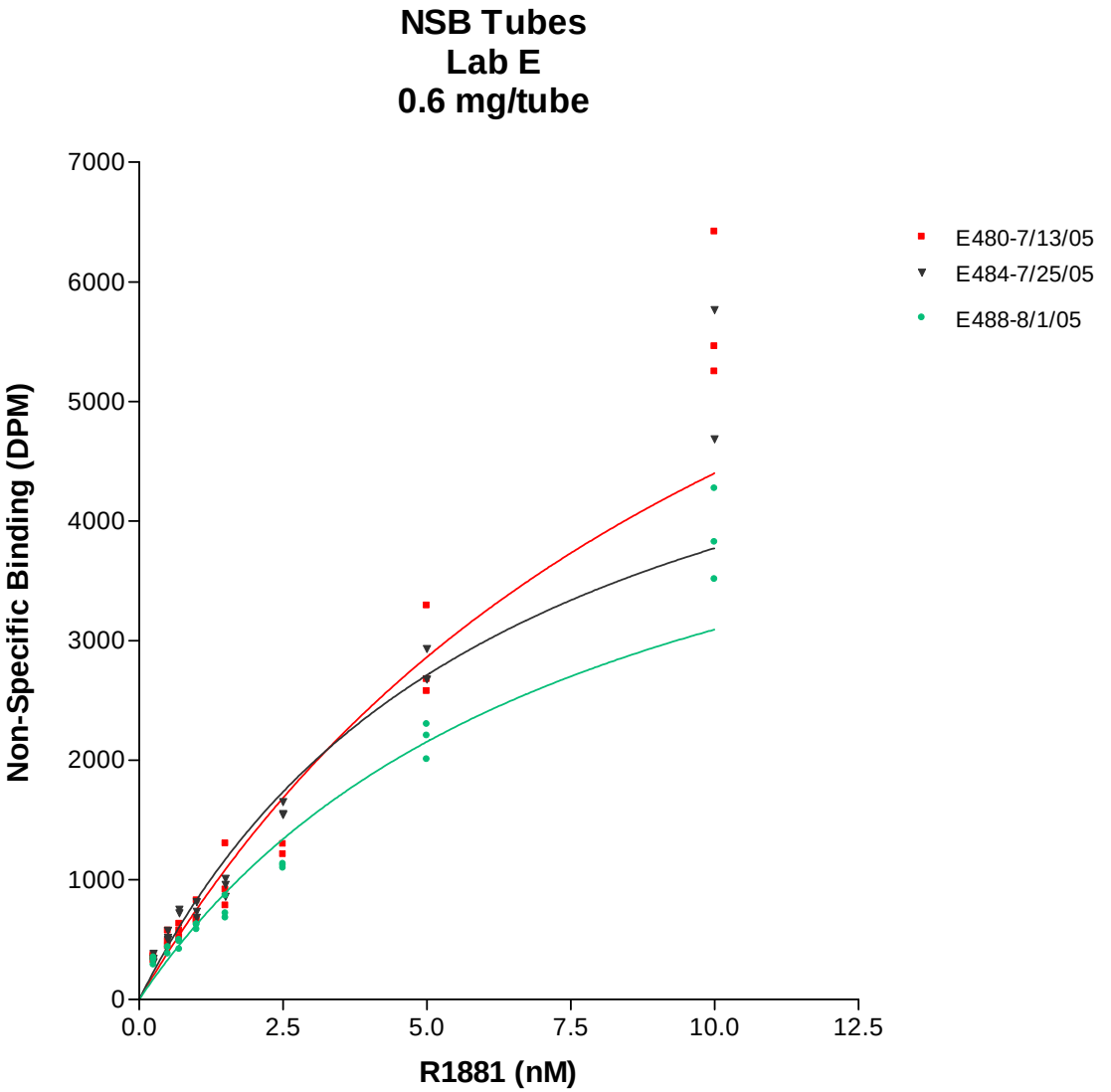
WA 4-11-5 Saturation

Lab E
0.6 mg/tube

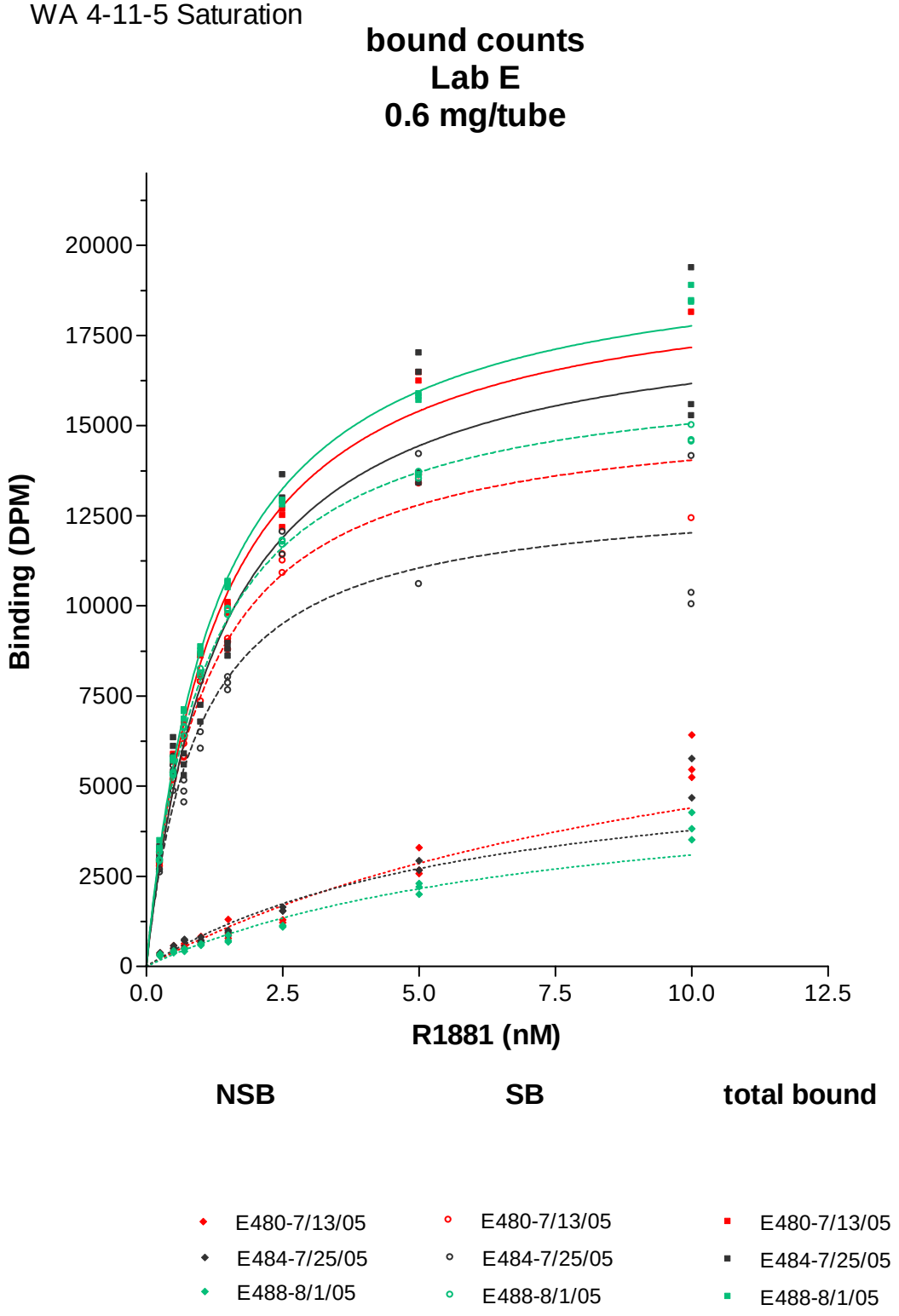


	E480-7/13/05	E484-7/25/05	E488-8/1/05
BMAX	2.880e-010	2.452e-010	3.115e-010
KD	9.302e-010	8.337e-010	9.257e-010
Std. Error			
BMAX	1.109e-011	1.554e-011	3.465e-012
KD	6.621e-011	1.064e-010	2.006e-011
95% Confidence Intervals			
BMAX	2.648e-010 to 3.112e-010	2.128e-010 to 2.776e-010	3.043e-010 to 3.187e-010
KD	7.916e-010 to 1.069e-009	6.119e-010 to 1.056e-009	8.841e-010 to 9.673e-010
Goodness of Fit			
Degrees of Freedom	19	20	22
R^2 (unweighted)	0.9711	0.8601	0.9974
Weighted Sum of Squares ($1/Y^2$)	0.08935	0.4046	0.01389
Absolute Sum of Squares	2.279e-021	1.411e-020	3.164e-022
Sy.x	1.095e-011	2.656e-011	3.792e-012
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	21	22	24
Number of missing values	3	2	0

WA 4-11-5 Saturation



WA 4-11-5 Saturation



Appendix 3: Excel Files for Saturation Binding Experiments

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

- Include the DPM value in column O
- Provide a reason in column R
- The value in column Q will automatically change to FALSE

For your convenience, data reduction is performed in columns U through BZ, and the values needed for analysis are presented in columns CF through CN

Cells in column S 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

Laboratory Code:	E	
Run identification:	480	
Assay start date:	7/13/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.92	**Ci/mmole**
Cytosol lot or vial number:	062305	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	9.30E-01	**nM**
Bmax	14.40	**fmole/100 ug**
total volume in tubes	300	**uL**
volume of ethanol counted:	2	**mL**
mulitply DPM in sample by :	3	

Receptor_Notes
diluted to 2 mg/ml for use (0.6 mg/300 ul)

protcol calls for counting decanted EtOH supe

reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	1071.22	3213.66	TRUE		28.2%	0.25	3213.7	351.8
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	1041.59	3124.77	TRUE		27.4%	0.25	3124.8	351.8
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	1085.65	3256.95	TRUE		28.6%	0.25	3257.0	351.8
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1959.20	5877.6	TRUE		25.4%	0.5	5877.6	504.1
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1896.40	5689.2	TRUE		24.5%	0.5	5689.2	504.1
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1889.00	5667	TRUE		24.4%	0.5	5667.0	504.1
7	1	H	10.0	21	0.70	—	—	—	50	300	—	2121.20	6363.6	TRUE		17.4%	0.7	6363.6	575.3
8	2	H	10.0	21	0.70	—	—	—	50	300	—	2132.30	6396.9	TRUE		17.5%	0.7	6396.9	575.3
9	3	H	10.0	21	0.70	—	—	—	50	300	—	2248.50	6745.5	TRUE		18.4%	0.7	6745.5	575.3
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2871.80	8615.4	TRUE		19.2%	1	8615.4	716.6
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2688.10	8064.3	TRUE		18.0%	1	8064.3	716.6
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2869.40	8608.2	TRUE		19.2%	1	8608.2	716.6
13	1	H	10.0	45	1.50	—	—	—	50	300	—	3261.20	9783.6	TRUE		14.4%	1.5	9783.6	1002.6
14	2	H	10.0	45	1.50	—	—	—	50	300	—	3362.70	10088.1	TRUE		14.9%	1.5	10088.1	1002.6
15	3	H	10.0	45	1.50	—	—	—	50	300	—	3329.10	9987.3	TRUE		14.7%	1.5	9987.3	1002.6
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	4227.50	12682.5	TRUE		11.2%	2.5	12682.5	1256.5
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	4169.70	12509.1	TRUE		11.0%	2.5	12509.1	1256.5
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	4055.70	12167.1	TRUE		10.7%	2.5	12167.1	1256.5
19	1	H	100.0	15	5.00	—	—	—	50	300	—	5412.00	16236	TRUE		7.0%	5	16236.0	2849.1
20	2	H	100.0	15	5.00	—	—	—	50	300	—	5489.80	16469.4	TRUE		7.1%	5	16469.4	2849.1
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4382.90	13148.7	FALSE	out?		5		2849.1
22	1	H	100.0	30	10.00	—	—	—	50	300	—	4799.30	14397.9	FALSE	out?		10		5711.3
23	2	H	100.0	30	10.00	—	—	—	50	300	—	5332.40	15997.2	FALSE	out?		10		5711.3
24	3	H	100.0	30	10.00	—	—	—	50	300	—	6047.10	18141.3	TRUE		3.9%	10	18141.3	5711.3

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	108.87	326.61	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	118.60	355.8	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	124.30	372.9	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	191.97	575.91	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	163.63	490.89	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	148.48	445.44	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	210.61	631.83	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	191.84	575.52	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	172.89	518.67	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	220.92	662.76	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	219.85	659.55	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	275.81	827.43	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	434.43	1303.29	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	262.03	786.09	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	306.11	918.33	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	432.72	1298.16	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	404.94	1214.82	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	851.03	2553.09	FALSE	out?				
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	859.12	2577.36	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	892.32	2676.96	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	1097.70	3293.1	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1820.50	5461.5	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1750.40	5251.2	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	2140.40	6421.2	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11394.9	11394.9	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11260.7	11260.7	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11559.3	11559.3	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	22662.0	22662	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	23488.0	23488	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	23386.0	23386	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	46082.0	46082	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	32236.0	32236	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	31423.0	31423	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	45249.0	45249	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	45394.0	45394	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	44047.0	44047	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	67668.0	67668	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	68304.0	68304	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	67786.0	67786	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	114301	114301	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	113641	113641	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	112144	112144	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	228978	228978	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	229587	229587	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	235659	235659	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	468373	468373	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	467237	467237	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	451629	451629	TRUE					

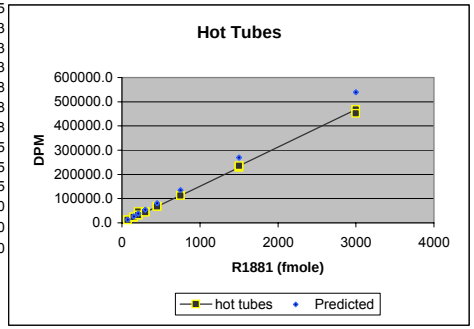
		Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)												
		Tube Identification			Assay tube contents									
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
480	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
480	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
480	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
480	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
480	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
480	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
480	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
480	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
480	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
480	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
480	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
480	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
480	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
480	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
480	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
480	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
480	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
480	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
480	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
480	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
480	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
480	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
480	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
480	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

										Number of molecules					Ratio
Run	Position	Total Counts	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 (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
		(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
480	1	3213.7	351.8	2861.9	10.9%	28.2%	11405.0	8191.3	21	2	19	74	53	0.35
480	2	3124.8	351.8	2773.0	11.3%	27.4%	11405.0	8280.2	20	2	18	74	54	0.33
480	3	3257.0	351.8	2905.2	10.8%	28.6%	11405.0	8148.0	21	2	19	74	53	0.36
480	4	5877.6	504.1	5373.5	8.6%	25.4%	23178.7	17301.1	38	3	35	151	112	0.31
480	5	5689.2	504.1	5185.1	8.9%	24.5%	23178.7	17489.5	37	3	34	151	114	0.30
480	6	5667.0	504.1	5162.9	8.9%	24.4%	23178.7	17511.7	37	3	34	151	114	0.29
480	7	6363.6	575.3	5788.3	9.0%	17.4%	36580.3	30216.7	41	4	38	238	196	0.19
480	8	6396.9	575.3	5821.6	9.0%	17.5%	36580.3	30183.4	42	4	38	238	196	0.19
480	9	6745.5	575.3	6170.2	8.5%	18.4%	36580.3	29834.8	44	4	40	238	194	0.21
480	10	8615.4	716.6	7898.8	8.3%	19.2%	44896.7	36281.3	56	5	51	292	236	0.22
480	11	8064.3	716.6	7347.7	8.9%	18.0%	44896.7	36832.4	52	5	48	292	239	0.20
480	12	8608.2	716.6	7891.6	8.3%	19.2%	44896.7	36288.5	56	5	51	292	236	0.22
480	13	9783.6	1002.6	8781.0	10.2%	14.4%	67919.3	58135.7	64	7	57	441	377	0.15
480	14	10088.1	1002.6	9085.5	9.9%	14.9%	67919.3	57831.2	66	7	59	441	376	0.16
480	15	9987.3	1002.6	8984.7	10.0%	14.7%	67919.3	57932.0	65	7	58	441	376	0.16
480	16	12682.5	1256.5	11426.0	9.9%	11.2%	113362.0	100679.5	82	8	74	736	654	0.11
480	17	12509.1	1256.5	11252.6	10.0%	11.0%	113362.0	100852.9	81	8	73	736	655	0.11
480	18	12167.1	1256.5	10910.6	10.3%	10.7%	113362.0	101194.9	79	8	71	736	657	0.11
480	19	16236.0	2849.1	13386.9	17.5%	7.0%	231408.0	215172.0	105	18	87	1503	1397	0.06
480	20	16469.4	2849.1	13620.3	17.3%	7.1%	231408.0	214938.6	107	18	88	1503	1396	0.06
480	21		2849.1				231408.0			18	-18	1503		
480	22		5711.3				462413.0			37	-37	3002		
480	23		5711.3				462413.0			37	-37	3002		
480	24	18141.3	5711.3	12430.0	31.5%	3.9%	462413.0	444271.7	118	37	81	3002	2885	0.03

		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
		Tube Identification			Assay tube contents								Scintillation Results	
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
480	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	326.6	351.8
480	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	355.8	351.8
480	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	372.9	351.8
480	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	575.9	504.1
480	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	490.9	504.1
480	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	445.4	504.1
480	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	631.8	575.3
480	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	575.5	575.3
480	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	518.7	575.3
480	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	662.8	716.6
480	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	659.6	716.6
480	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	827.4	716.6
480	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	1303.3	1002.6
480	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	786.1	1002.6
480	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	918.3	1002.6
480	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1298.2	1256.5
480	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1214.8	1256.5
480	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1256.5
480	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2577.4	2849.1
480	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	2677.0	2849.1
480	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	3293.1	2849.1
480	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	5461.5	5711.3
480	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	5251.2	5711.3
480	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	6421.2	5711.3

Free -- Positions 49-72, radiolabeled R1881 without cytosol												
Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial	Hot R1881 Volume	Molecules of R1881	Counts per Scintillation Vial	Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)	predicted dpm	
					(nM)	(ul)	(fmole)	(dpm)	(fmole)	(dpm)		
480	49	1	Hot	c1	10	7.5	75	11394.9	74	11405.0	13474	
480	50	2	Hot	c1	10	7.5	75	11260.7	73	11405.0	13474	
480	51	3	Hot	c1	10	7.5	75	11559.3	75	11405.0	13474	
480	52	1	Hot	c2	10	15	150	22662.0	147	23178.7	26948	
480	53	2	Hot	c2	10	15	150	23488.0	153	23178.7	26948	
480	54	3	Hot	c2	10	15	150	23386.0	152	23178.7	26948	
480	55	1	Hot	c3	10	21	210	46082.0	299	36580.3	37727	
480	56	2	Hot	c3	10	21	210	32236.0	209	36580.3	37727	
480	57	3	Hot	c3	10	21	210	31423.0	204	36580.3	37727	
480	58	1	Hot	c4	10	30	300	45249.0	294	44896.7	53895	
480	59	2	Hot	c4	10	30	300	45394.0	295	44896.7	53895	
480	60	3	Hot	c4	10	30	300	44047.0	286	44896.7	53895	
480	61	1	Hot	c5	10	45	450	67668.0	439	67919.3	80843	
480	62	2	Hot	c5	10	45	450	68304.0	444	67919.3	80843	
480	63	3	Hot	c5	10	45	450	67786.0	440	67919.3	80843	
480	64	1	Hot	c6	100	7.5	750	114301.0	742	113362.0	134738	
480	65	2	Hot	c6	100	7.5	750	113641.0	738	113362.0	134738	
480	66	3	Hot	c6	100	7.5	750	112144.0	728	113362.0	134738	
480	67	1	Hot	c7	100	15	1500	228978.0	1487	231408.0	269475	
480	68	2	Hot	c7	100	15	1500	229587.0	1491	231408.0	269475	
480	69	3	Hot	c7	100	15	1500	235659.0	1530	231408.0	269475	
480	70	1	Hot	c8	100	30	3000	468373.0	3041	462413.0	538950	
480	71	2	Hot	c8	100	30	3000	467237.0	3034	462413.0	538950	
480	72	3	Hot	c8	100	30	3000	451629.0	2932	462413.0	538950	

Computation Check	
7/13/05	specific activity date
80.92	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7965E+14	DPM/mmole
1.7965E+11	DPM/nmole
179.7	DPM/fmole
0.005566	fmole/DPM



Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	154.0103958 dpm/fmole	
1/slope	0.006493068 fmole/dpm	
	x	y
origin	0	0
end point	3041.2	468373
SLOPE function, used if missing HOT tubes		
Slope	154.0 dpm/fmole	
1/slope	0.006494 fmole/dpm	
	x	y
origin	0	0
end point	3041.4	468373

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
5.31012E-11	0.34938	2.11614E-10	5.31012E-11
5.14519E-11	0.33490	2.11614E-10	5.14519E-11
5.39044E-11	0.35655	2.11614E-10	5.39044E-11
9.97034E-11	0.31059	4.3007E-10	9.97034E-11
9.62077E-11	0.29647	4.3007E-10	9.62077E-11
9.57958E-11	0.29483	4.3007E-10	9.57958E-11
1.07399E-10	0.19156	6.78733E-10	1.07399E-10
1.08017E-10	0.19287	6.78733E-10	1.08017E-10
1.14485E-10	0.20681	6.78733E-10	1.14485E-10
1.46559E-10	0.21771	8.33039E-10	1.46559E-10
1.36334E-10	0.19949	8.33039E-10	1.36334E-10
1.46426E-10	0.21747	8.33039E-10	1.46426E-10
1.62928E-10	0.15104	1.26021E-09	1.62928E-10
1.68578E-10	0.15710	1.26021E-09	1.68578E-10
1.66708E-10	0.15509	1.26021E-09	1.66708E-10
2.12005E-10	0.11349	2.10338E-09	2.12005E-10
2.08787E-10	0.11157	2.10338E-09	2.08787E-10
2.02442E-10	0.10782	2.10338E-09	2.02442E-10
2.48388E-10	0.06221	4.29368E-09	2.48388E-10
2.52718E-10	0.06337	4.29368E-09	2.52718E-10
		4.29368E-09	
		8.57988E-09	
		8.57988E-09	
2.30633E-10	0.02798	8.57988E-09	2.30633E-10

Bmax molar	2.88E-10	KD molar	9.30E-10
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.80E+17	**kd nM =**	9.30E-01
Bmax molar to Bmax moles	8.64E-14		
= DPM/((DPM/mmole)*1000)	8.64E-14		
=Bmax DPM	15521.7738		
assay date	7/13/2005		
Bmax(dpm)	15521.7738		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.92		
DPM/mmole	1.80E+14		
DPM/pmole	1.80E+05		
1/(DPM/mmole)	5.57E-15		
1/(DPM/pmole)	5.57E-06		
SA(dpm/pmole)	1.80E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.086400		
bmax pmole/mg	0.144		
Bmax fmole/mg	144		
Bmax (fmole/100 ug)	14.4		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

- Include the DPM value in column O
- Provide a reason in column R
- The value in column Q will automatically change to FALSE

For your convenience, data reduction is performed in columns U through BZ, and the values needed for analysis are presented in columns CF through CN

Cells in column S 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

Laboratory Code:	E	
Run identification:	484	
Assay start date:	7/25/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.77	**Ci/mmole**
Cytosol lot or vial number:	062305	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	8.34E-01	**nM**
Bmax	12.26	**fmole/100 ug**
total volume in tubes	300	**uL**
volume of ethanol counted:	2	**mL**
mulitply DPM in sample by :	3	

Receptor_Notes
diluted to 2 mg/ml for use (0.6 mg/300 ul)

protcol calls for counting decanted EtOH supe

reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	1009.29	3027.87	TRUE		26.0%	0.25	3027.9	344.8
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	981.38	2944.14	TRUE		25.3%	0.25	2944.1	344.8
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	1102.70	3308.1	TRUE		28.4%	0.25	3308.1	344.8
4	1	H	10.0	15	0.50	—	—	—	50	300	—	2114.30	6342.9	TRUE		26.9%	0.5	6342.9	529.5
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1796.50	5389.5	TRUE		22.9%	0.5	5389.5	529.5
6	3	H	10.0	15	0.50	—	—	—	50	300	—	2031.90	6095.7	TRUE		25.9%	0.5	6095.7	529.5
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1963.50	5890.5	TRUE		18.5%	0.7	5890.5	735.7
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1861.10	5583.3	TRUE		17.5%	0.7	5583.3	735.7
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1761.20	5283.6	TRUE		16.6%	0.7	5283.6	735.7
10	1	H	10.0	30	1.00	—	—	—	50	300	—	3079.60	9238.8	FALSE	poor replicate		1		743.3
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2258.90	6776.7	TRUE		15.0%	1	6776.7	743.3
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2413.10	7239.3	TRUE		16.1%	1	7239.3	743.3
13	1	H	10.0	45	1.50	—	—	—	50	300	—	2866.40	8599.2	TRUE		12.4%	1.5	8599.2	941.4
14	2	H	10.0	45	1.50	—	—	—	50	300	—	2931.00	8793	TRUE		12.7%	1.5	8793.0	941.4
15	3	H	10.0	45	1.50	—	—	—	50	300	—	2986.80	8960.4	TRUE		13.0%	1.5	8960.4	941.4
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	4330.10	12990.3	TRUE		10.6%	2.5	12990.3	1580.5
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	3285.70	9857.1	FALSE	poor replicate		2.5		1580.5
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	4544.80	13634.4	TRUE		11.1%	2.5	13634.4	1580.5
19	1	H	100.0	15	5.00	—	—	—	50	300	—	5491.50	16474.5	TRUE		6.7%	5	16474.5	2802.1
20	2	H	100.0	15	5.00	—	—	—	50	300	—	5670.00	17010	TRUE		6.9%	5	17010.0	2802.1
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4468.90	13406.7	TRUE		5.5%	5	13406.7	2802.1
22	1	H	100.0	30	10.00	—	—	—	50	300	—	5090.10	15270.3	TRUE		3.2%	10	15270.3	5223.5
23	2	H	100.0	30	10.00	—	—	—	50	300	—	5193.90	15581.7	TRUE		3.3%	10	15581.7	5223.5
24	3	H	100.0	30	10.00	—	—	—	50	300	—	6458.00	19374	TRUE		4.0%	10	19374.0	5223.5

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	127.45	382.35	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	113.34	340.02	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	103.98	311.94	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	166.07	498.21	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	191.98	575.94	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	171.44	514.32	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	240.42	721.26	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	250.07	750.21	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	175.40	526.2	FALSE	poor replicate				
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	244.06	732.18	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	271.31	813.93	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	227.90	683.7	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	335.83	1007.49	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	285.72	857.16	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	319.82	959.46	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	514.24	1542.72	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	549.70	1649.1	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	516.58	1549.74	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	891.40	2674.2	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	1254.40	3763.2	FALSE	poor replicate				
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	976.64	2929.92	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1921.30	5763.9	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	2534.10	7602.3	FALSE	poor replicate				
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	1561.00	4683	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11834.9	11834.9	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11538.0	11538	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11549.5	11549.5	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23463.0	23463	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	23725.0	23725	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	23503.0	23503	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	31644.0	31644	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	31136.0	31136	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	32799.0	32799	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	43689.0	43689	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	45406.0	45406	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	46162.0	46162	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	69214.0	69214	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	68931.0	68931	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	69195.0	69195	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	122165	122165	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	120798	120798	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	124571	124571	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	245368	245368	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	245359	245359	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	246338	246338	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	475835	475835	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	486254	486254	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	475914	475914	TRUE					

		Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)												
		Tube Identification			Assay tube contents									
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
484	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
484	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
484	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
484	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
484	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
484	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
484	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
484	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
484	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
484	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
484	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
484	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
484	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
484	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
484	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
484	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
484	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
484	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
484	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
484	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
484	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
484	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
484	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
484	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

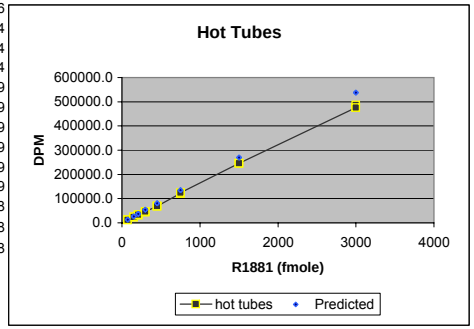
									Number of molecules					Ratio
Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
		(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
484	1	3027.9	344.8	2683.1	11.4%	26.0%	11640.8	8612.9	19	2	17	73	54	0.31
484	2	2944.1	344.8	2599.4	11.7%	25.3%	11640.8	8696.7	18	2	16	73	54	0.30
484	3	3308.1	344.8	2963.3	10.4%	28.4%	11640.8	8332.7	21	2	18	73	52	0.36
484	4	6342.9	529.5	5813.4	8.3%	26.9%	23563.7	17220.8	40	3	36	147	107	0.34
484	5	5389.5	529.5	4860.0	9.8%	22.9%	23563.7	18174.2	34	3	30	147	113	0.27
484	6	6095.7	529.5	5566.2	8.7%	25.9%	23563.7	17468.0	38	3	35	147	109	0.32
484	7	5890.5	735.7	5154.8	12.5%	18.5%	31859.7	25969.2	37	5	32	199	162	0.20
484	8	5583.3	735.7	4847.6	13.2%	17.5%	31859.7	26276.4	35	5	30	199	164	0.18
484	9	5283.6	735.7	4547.9	13.9%	16.6%	31859.7	26576.1	33	5	28	199	166	0.17
484	10		743.3				45085.7			5	-5	281		
484	11	6776.7	743.3	6033.4	11.0%	15.0%	45085.7	38309.0	42	5	38	281	239	0.16
484	12	7239.3	743.3	6496.0	10.3%	16.1%	45085.7	37846.4	45	5	40	281	236	0.17
484	13	8599.2	941.4	7657.8	10.9%	12.4%	69113.3	60514.1	54	6	48	431	377	0.13
484	14	8793.0	941.4	7851.6	10.7%	12.7%	69113.3	60320.3	55	6	49	431	376	0.13
484	15	8960.4	941.4	8019.0	10.5%	13.0%	69113.3	60152.9	56	6	50	431	375	0.13
484	16	12990.3	1580.5	11409.8	12.2%	10.6%	122511.3	109521.0	81	10	71	763	682	0.10
484	17		1580.5				122511.3			10	-10	763		
484	18	13634.4	1580.5	12053.9	11.6%	11.1%	122511.3	108876.9	85	10	75	763	678	0.11
484	19	16474.5	2802.1	13672.4	17.0%	6.7%	245688.3	229213.8	103	17	85	1531	1428	0.06
484	20	17010.0	2802.1	14207.9	16.5%	6.9%	245688.3	228678.3	106	17	89	1531	1425	0.06
484	21	13406.7	2802.1	10604.6	20.9%	5.5%	245688.3	232281.6	84	17	66	1531	1447	0.05
484	22	15270.3	5223.5	10046.9	34.2%	3.2%	479334.3	464064.0	95	33	63	2987	2892	0.02
484	23	15581.7	5223.5	10358.3	33.5%	3.3%	479334.3	463752.6	97	33	65	2987	2890	0.02
484	24	19374.0	5223.5	14150.6	27.0%	4.0%	479334.3	459960.3	121	33	88	2987	2866	0.03

		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
		Tube Identification			Assay tube contents								Scintillation Results	
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
484	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	382.4	344.8
484	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	340.0	344.8
484	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	311.9	344.8
484	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	498.2	529.5
484	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	575.9	529.5
484	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	514.3	529.5
484	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	721.3	735.7
484	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	750.2	735.7
484	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70		735.7
484	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	732.2	743.3
484	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	813.9	743.3
484	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	683.7	743.3
484	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	1007.5	941.4
484	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	857.2	941.4
484	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	959.5	941.4
484	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1542.7	1580.5
484	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1649.1	1580.5
484	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1549.7	1580.5
484	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2674.2	2802.1
484	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500		2802.1
484	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	2929.9	2802.1
484	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	5763.9	5223.5
484	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000		5223.5
484	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	4683.0	5223.5

Free -- Positions 49-72, radiolabeled R1881 without cytosol

Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial (nM)	Hot R1881 Volume (ul)	Molecules of R1881 (fmole)	Counts per Scintillation Vial (dpm)	Experimental number of molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (dpm)	predicted dpm
484	49	1	Hot	c1	10	7.5	75	11834.9	74	11640.8	13449
484	50	2	Hot	c1	10	7.5	75	11538.0	72	11640.8	13449
484	51	3	Hot	c1	10	7.5	75	11549.5	72	11640.8	13449
484	52	1	Hot	c2	10	15	150	23463.0	146	23563.7	26898
484	53	2	Hot	c2	10	15	150	23725.0	148	23563.7	26898
484	54	3	Hot	c2	10	15	150	23503.0	146	23563.7	26898
484	55	1	Hot	c3	10	21	210	31644.0	197	31859.7	37657
484	56	2	Hot	c3	10	21	210	31136.0	194	31859.7	37657
484	57	3	Hot	c3	10	21	210	32799.0	204	31859.7	37657
484	58	1	Hot	c4	10	30	300	43689.0	272	45085.7	53796
484	59	2	Hot	c4	10	30	300	45406.0	283	45085.7	53796
484	60	3	Hot	c4	10	30	300	46162.0	288	45085.7	53796
484	61	1	Hot	c5	10	45	450	69214.0	431	69113.3	80694
484	62	2	Hot	c5	10	45	450	68931.0	430	69113.3	80694
484	63	3	Hot	c5	10	45	450	69195.0	431	69113.3	80694
484	64	1	Hot	c6	100	7.5	750	122165.0	761	122511.3	134489
484	65	2	Hot	c6	100	7.5	750	120798.0	753	122511.3	134489
484	66	3	Hot	c6	100	7.5	750	124571.0	776	122511.3	134489
484	67	1	Hot	c7	100	15	1500	245368.0	1529	245688.3	268979
484	68	2	Hot	c7	100	15	1500	245359.0	1529	245688.3	268979
484	69	3	Hot	c7	100	15	1500	246338.0	1535	245688.3	268979
484	70	1	Hot	c8	100	30	3000	475835.0	2965	479334.3	537958
484	71	2	Hot	c8	100	30	3000	486254.0	3030	479334.3	537958
484	72	3	Hot	c8	100	30	3000	475914.0	2966	479334.3	537958

Computation Check	
7/25/05	specific activity date
80.77	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7932E+14	DPM/mmole
1.7932E+11	DPM/nmole
179.3	DPM/fmole
0.005577	fmole/DPM



Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	160.4753004 dpm/fmole	
1/slope	0.006231489 fmole/dpm	
	x	y
origin	0	0
end point	3030.1	486254
SLOPE function, used if missing HOT tubes		
Slope	160.9 dpm/fmole	
1/slope	0.006214 fmole/dpm	
	x	y
origin	0	0
end point	3021.7	486254

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
4.98757E-11	0.31152	2.16389E-10	4.98757E-11
4.83192E-11	0.29889	2.16389E-10	4.83192E-11
5.50848E-11	0.35563	2.16389E-10	5.50848E-11
1.08064E-10	0.33758	4.38021E-10	1.08064E-10
9.03419E-11	0.26741	4.38021E-10	9.03419E-11
1.03469E-10	0.31865	4.38021E-10	1.03469E-10
9.5821E-11	0.19850	5.92234E-10	9.5821E-11
9.01105E-11	0.18448	5.92234E-10	9.01105E-11
8.45395E-11	0.17113	5.92234E-10	8.45395E-11
		8.3809E-10	
1.12154E-10	0.15749	8.3809E-10	1.12154E-10
1.20754E-10	0.17164	8.3809E-10	1.20754E-10
1.4235E-10	0.12655	1.28474E-09	1.4235E-10
1.45953E-10	0.13017	1.28474E-09	1.45953E-10
1.49064E-10	0.13331	1.28474E-09	1.49064E-10
2.12094E-10	0.10418	2.27734E-09	2.12094E-10
		2.27734E-09	
2.24067E-10	0.11071	2.27734E-09	2.24067E-10
2.54155E-10	0.05965	4.56706E-09	2.54155E-10
2.64109E-10	0.06213	4.56706E-09	2.64109E-10
1.97128E-10	0.04565	4.56706E-09	1.97128E-10
1.86759E-10	0.02165	8.91026E-09	1.86759E-10
1.92548E-10	0.02234	8.91026E-09	1.92548E-10
2.63042E-10	0.03076	8.91026E-09	2.63042E-10

Bmax molar	2.45E-10	KD molar	8.34E-10
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.79E+17	**kd nM =**	8.34E-01
Bmax molar to Bmax moles	7.356E-14		
= DPM/((DPM/mmole)*1000)	7.356E-14		
=Bmax DPM	13190.72016		
assay date	7/25/2005		
Bmax(dpm)	13190.72016		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.77		
DPM/mmole	1.79E+14		
DPM/pmole	1.79E+05		
1/(DPM/mmole)	5.58E-15		
1/(DPM/pmole)	5.58E-06		
SA(dpm/pmole)	1.79E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.073560		
bmax pmole/mg	0.1226		
Bmax fmole/mg	122.6		
Bmax (fmole/100 ug)	12.26		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

- Include the DPM value in column O
- Provide a reason in column R
- The value in column Q will automatically change to FALSE

For your convenience, data reduction is performed in columns

U through BZ, and the values needed for analysis are presented

in columns CF through CN

Cells in column S 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

Laboratory Code:	E	
Run identification:	488	
Assay start date:	8/1/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.69	**Ci/mmole**
Cytosol lot or vial number:	062305	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	9.26E-01	**nM**
Bmax	15.58	**fmole/100 ug**
total volume in tubes	300	**uL**
volume of ethanol counted:	2	**mL**
mulitply DPM in sample by :	3	

Receptor_Notes
diluted to 2 mg/ml for use (0.6 mg/300 ul)

protcol calls for counting decanted EtOH supe

reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	1162.60	3487.8	TRUE		30.9%	0.25	3487.8	323.8
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	1091.71	3275.13	TRUE		29.1%	0.25	3275.1	323.8
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	1080.18	3240.54	TRUE		28.8%	0.25	3240.5	323.8
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1924.60	5773.8	TRUE		24.9%	0.5	5773.8	408.9
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1912.70	5738.1	TRUE		24.8%	0.5	5738.1	408.9
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1899.10	5697.3	TRUE		24.6%	0.5	5697.3	408.9
7	1	H	10.0	21	0.70	—	—	—	50	300	—	2356.40	7069.2	TRUE		22.3%	0.7	7069.2	466.8
8	2	H	10.0	21	0.70	—	—	—	50	300	—	2283.60	6850.8	TRUE		21.7%	0.7	6850.8	466.8
9	3	H	10.0	21	0.70	—	—	—	50	300	—	2370.60	7111.8	TRUE		22.5%	0.7	7111.8	466.8
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2953.40	8860.2	TRUE		19.8%	1	8860.2	612.5
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2887.90	8663.7	TRUE		19.3%	1	8663.7	612.5
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2913.50	8740.5	TRUE		19.5%	1	8740.5	612.5
13	1	H	10.0	45	1.50	—	—	—	50	300	—	3544.00	10632	TRUE		15.8%	1.5	10632.0	756.7
14	2	H	10.0	45	1.50	—	—	—	50	300	—	3499.70	10499.1	TRUE		15.6%	1.5	10499.1	756.7
15	3	H	10.0	45	1.50	—	—	—	50	300	—	3556.90	10670.7	TRUE		15.9%	1.5	10670.7	756.7
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	4298.50	12895.5	TRUE		11.3%	2.5	12895.5	1116.8
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	4267.70	12803.1	TRUE		11.2%	2.5	12803.1	1116.8
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	4308.10	12924.3	TRUE		11.3%	2.5	12924.3	1116.8
19	1	H	100.0	15	5.00	—	—	—	50	300	—	5293.80	15881.4	TRUE		7.0%	5	15881.4	2171.9
20	2	H	100.0	15	5.00	—	—	—	50	300	—	5267.00	15801	TRUE		6.9%	5	15801.0	2171.9
21	3	H	100.0	15	5.00	—	—	—	50	300	—	5232.30	15696.9	TRUE		6.9%	5	15696.9	2171.9
22	1	H	100.0	30	10.00	—	—	—	50	300	—	6154.80	18464.4	TRUE		4.2%	10	18464.4	3870.5
23	2	H	100.0	30	10.00	—	—	—	50	300	—	6295.00	18885	TRUE		4.3%	10	18885.0	3870.5
24	3	H	100.0	30	10.00	—	—	—	50	300	—	6141.30	18423.9	TRUE		4.2%	10	18423.9	3870.5

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	96.84	290.52	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	109.88	329.64	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	117.06	351.18	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	145.32	435.96	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	644.68	1934.04	FALSE	double spike				
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	127.25	381.75	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	140.07	420.21	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	161.26	483.78	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	165.49	496.47	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	208.41	625.23	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	195.14	585.42	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	208.95	626.85	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	289.57	868.71	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	239.95	719.85	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	227.21	681.63	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	372.60	1117.8	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	378.08	1134.24	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	366.16	1098.48	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	735.43	2206.29	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	669.08	2007.24	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	767.37	2302.11	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1171.10	3513.3	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1424.70	4274.1	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	1274.70	3824.1	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11523.6	11523.6	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	10780.5	10780.5	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11505.3	11505.3	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23095.0	23095	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	23256.0	23256	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	23176.0	23176	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	31225.0	31225	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	32084.0	32084	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	31592.0	31592	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	44276.0	44276	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	45361.0	45361	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	44895.0	44895	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	68003.0	68003	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	66087.0	66087	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	67434.0	67434	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	115076	115076	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	112000	112000	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	116283	116283	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	226718	226718	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	228290	228290	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	228240	228240	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	441027	441027	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	433639	433639	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	430899	430899	TRUE					

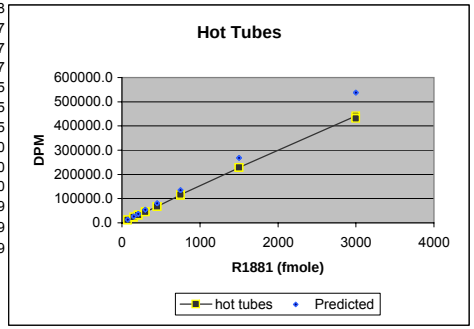
		Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)												
		Tube Identification			Assay tube contents									
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
488	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
488	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
488	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
488	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
488	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
488	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
488	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
488	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
488	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
488	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
488	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
488	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
488	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
488	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
488	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
488	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
488	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
488	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
488	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
488	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
488	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
488	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
488	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
488	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

Run	Position	Total Counts (dpm)	Non Specific Binding (Mean of reps in pos. 25-48) (dpm)	Specific Binding (Total - Non Specific) (dpm)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72) (dpm)	Free (total added - bound) (dpm)	Number of molecules: Total Binding molecules (fmole)	Non Specific Binding molecules (fmole)	Specific Binding molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (fmole)	Free (total added - bound) (fmole)	Ratio: Specific Bound / Free
488	1	3487.8	323.8	3164.0	9.3%	30.9%	11269.8	7782.0	24	2	22	77	53	0.41
488	2	3275.1	323.8	2951.4	9.9%	29.1%	11269.8	7994.7	22	2	20	77	54	0.37
488	3	3240.5	323.8	2916.8	10.0%	28.8%	11269.8	8029.3	22	2	20	77	55	0.36
488	4	5773.8	408.9	5364.9	7.1%	24.9%	23175.7	17401.9	39	3	37	158	119	0.31
488	5	5738.1	408.9	5329.2	7.1%	24.8%	23175.7	17437.6	39	3	36	158	119	0.31
488	6	5697.3	408.9	5288.4	7.2%	24.6%	23175.7	17478.4	39	3	36	158	119	0.30
488	7	7069.2	466.8	6602.4	6.6%	22.3%	31633.7	24564.5	48	3	45	215	167	0.27
488	8	6850.8	466.8	6384.0	6.8%	21.7%	31633.7	24782.9	47	3	43	215	169	0.26
488	9	7111.8	466.8	6645.0	6.6%	22.5%	31633.7	24521.9	48	3	45	215	167	0.27
488	10	8860.2	612.5	8247.7	6.9%	19.8%	44844.0	35983.8	60	4	56	305	245	0.23
488	11	8663.7	612.5	8051.2	7.1%	19.3%	44844.0	36180.3	59	4	55	305	246	0.22
488	12	8740.5	612.5	8128.0	7.0%	19.5%	44844.0	36103.5	60	4	55	305	246	0.23
488	13	10632.0	756.7	9875.3	7.1%	15.8%	67174.7	56542.7	72	5	67	458	385	0.17
488	14	10499.1	756.7	9742.4	7.2%	15.6%	67174.7	56675.6	72	5	66	458	386	0.17
488	15	10670.7	756.7	9914.0	7.1%	15.9%	67174.7	56504.0	73	5	68	458	385	0.18
488	16	12895.5	1116.8	11778.7	8.7%	11.3%	114453.0	101557.5	88	8	80	780	692	0.12
488	17	12803.1	1116.8	11686.3	8.7%	11.2%	114453.0	101649.9	87	8	80	780	692	0.11
488	18	12924.3	1116.8	11807.5	8.6%	11.3%	114453.0	101528.7	88	8	80	780	692	0.12
488	19	15881.4	2171.9	13709.5	13.7%	7.0%	227749.3	211867.9	108	15	93	1551	1443	0.06
488	20	15801.0	2171.9	13629.1	13.7%	6.9%	227749.3	211948.3	108	15	93	1551	1444	0.06
488	21	15696.9	2171.9	13525.0	13.8%	6.9%	227749.3	212052.4	107	15	92	1551	1444	0.06
488	22	18464.4	3870.5	14593.9	21.0%	4.2%	435188.3	416723.9	126	26	99	2964	2839	0.04
488	23	18885.0	3870.5	15014.5	20.5%	4.3%	435188.3	416303.3	129	26	102	2964	2836	0.04
488	24	18423.9	3870.5	14553.4	21.0%	4.2%	435188.3	416764.4	125	26	99	2964	2839	0.03

		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
		Tube Identification			Assay tube contents								Scintillation Results	
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
488	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	290.5	323.8
488	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	329.6	323.8
488	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	351.2	323.8
488	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	436.0	408.9
488	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50		408.9
488	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	381.8	408.9
488	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	420.2	466.8
488	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	483.8	466.8
488	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	496.5	466.8
488	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	625.2	612.5
488	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	585.4	612.5
488	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	626.9	612.5
488	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	868.7	756.7
488	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	719.9	756.7
488	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	681.6	756.7
488	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1117.8	1116.8
488	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1134.2	1116.8
488	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1098.5	1116.8
488	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2206.3	2171.9
488	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	2007.2	2171.9
488	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	2302.1	2171.9
488	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	3513.3	3870.5
488	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	4274.1	3870.5
488	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	3824.1	3870.5

Free -- Positions 49-72, radiolabeled R1881 without cytosol												
Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial	Hot R1881 Volume	Molecules of R1881	Counts per Scintillation Vial	Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)	predicted dpm	
					(nM)	(ul)	(fmole)	(dpm)	(fmole)	(dpm)		
488	49	1	Hot	c1	10	7.5	75	11523.6	78	11269.8	13434	
488	50	2	Hot	c1	10	7.5	75	10780.5	73	11269.8	13434	
488	51	3	Hot	c1	10	7.5	75	11505.3	78	11269.8	13434	
488	52	1	Hot	c2	10	15	150	23095.0	157	23175.7	26869	
488	53	2	Hot	c2	10	15	150	23256.0	158	23175.7	26869	
488	54	3	Hot	c2	10	15	150	23176.0	158	23175.7	26869	
488	55	1	Hot	c3	10	21	210	31225.0	213	31633.7	37617	
488	56	2	Hot	c3	10	21	210	32084.0	219	31633.7	37617	
488	57	3	Hot	c3	10	21	210	31592.0	215	31633.7	37617	
488	58	1	Hot	c4	10	30	300	44276.0	302	44844.0	53738	
488	59	2	Hot	c4	10	30	300	45361.0	309	44844.0	53738	
488	60	3	Hot	c4	10	30	300	44895.0	306	44844.0	53738	
488	61	1	Hot	c5	10	45	450	68003.0	463	67174.7	80607	
488	62	2	Hot	c5	10	45	450	66087.0	450	67174.7	80607	
488	63	3	Hot	c5	10	45	450	67434.0	459	67174.7	80607	
488	64	1	Hot	c6	100	7.5	750	115076.0	784	114453.0	134345	
488	65	2	Hot	c6	100	7.5	750	112000.0	763	114453.0	134345	
488	66	3	Hot	c6	100	7.5	750	116283.0	792	114453.0	134345	
488	67	1	Hot	c7	100	15	1500	226718.0	1544	227749.3	268690	
488	68	2	Hot	c7	100	15	1500	228290.0	1555	227749.3	268690	
488	69	3	Hot	c7	100	15	1500	228240.0	1555	227749.3	268690	
488	70	1	Hot	c8	100	30	3000	441027.0	3004	435188.3	537379	
488	71	2	Hot	c8	100	30	3000	433639.0	2954	435188.3	537379	
488	72	3	Hot	c8	100	30	3000	430899.0	2935	435188.3	537379	

Computation Check	
8/1/05	specific activity date
80.69	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7913E+14	DPM/mmole
1.7913E+11	DPM/nmole
179.1	DPM/fmole
0.005583	fmole/DPM

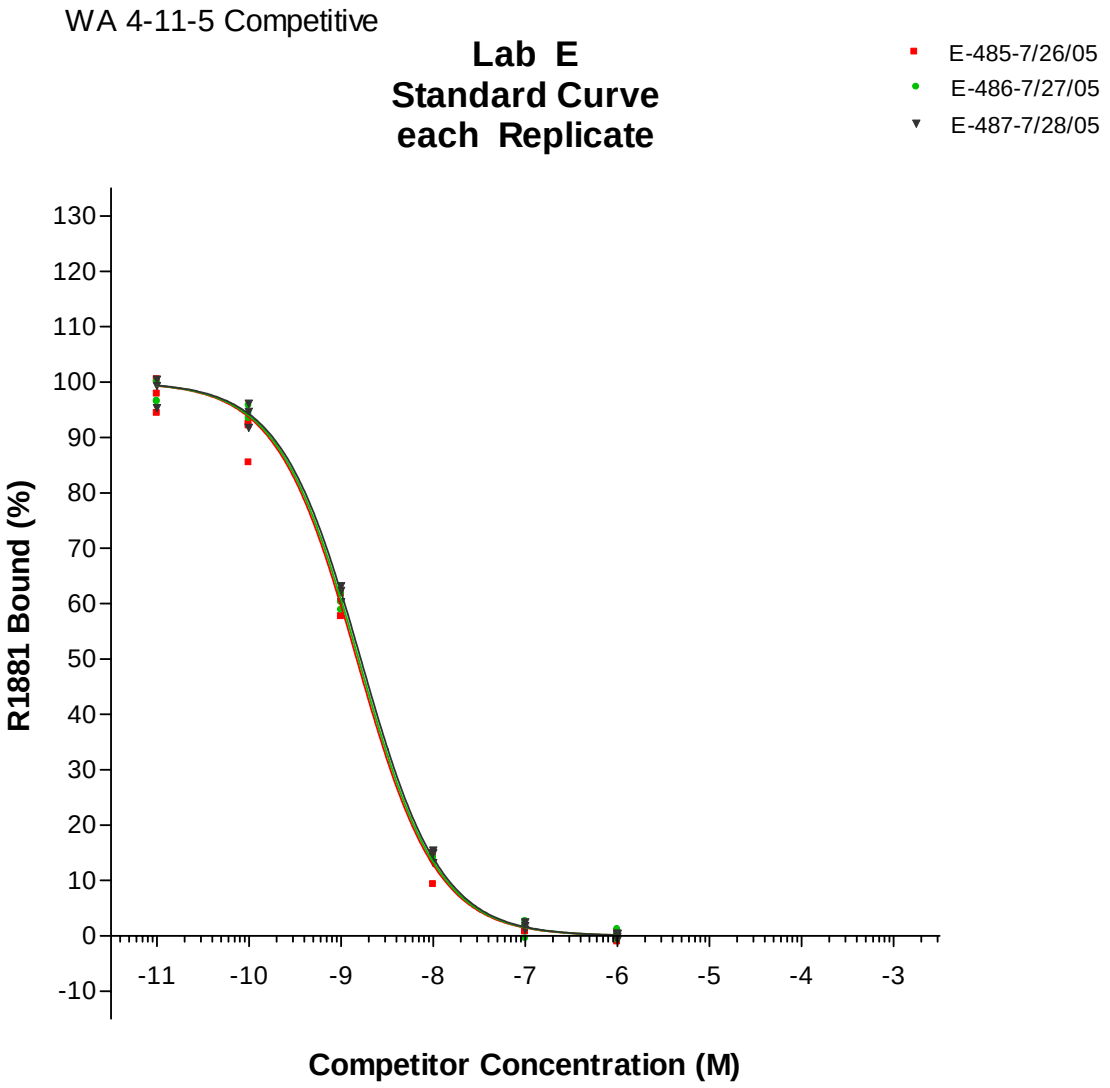


Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	146.8048102 dpm/fmole	
1/slope	0.006811766 fmole/dpm	
	x	y
origin	0	0
end point	3004.2	441027
SLOPE function, used if missing HOT tubes		
Slope	145.6 dpm/fmole	
1/slope	0.006870 fmole/dpm	
	x	y
origin	0	0
end point	3029.8	441027

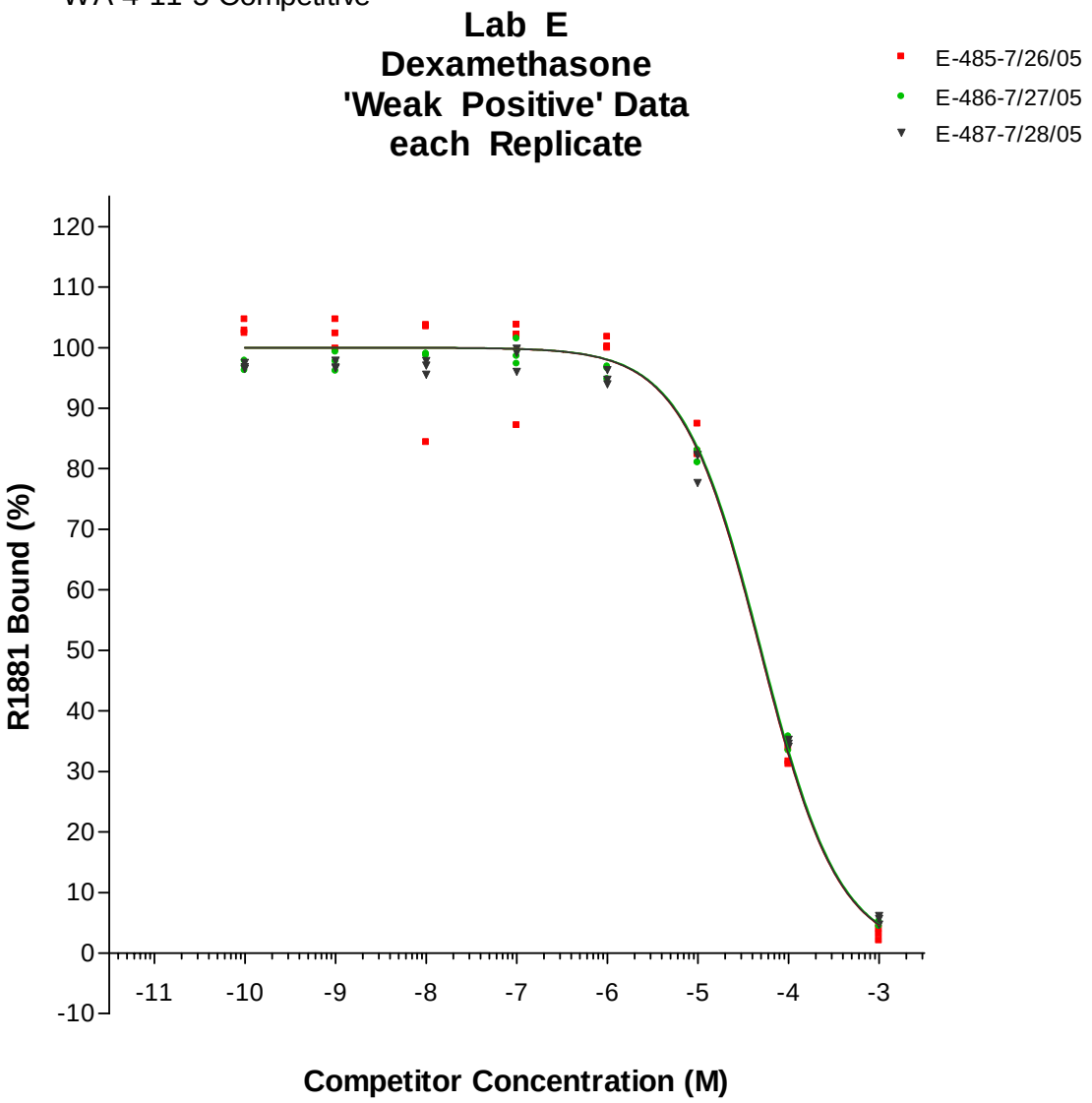
Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
5.88787E-11	0.40658	2.09718E-10	5.88787E-11
5.49212E-11	0.36916	2.09718E-10	5.49212E-11
5.42775E-11	0.36327	2.09718E-10	5.42775E-11
9.98354E-11	0.30830	4.31272E-10	9.98354E-11
9.9171E-11	0.30562	4.31272E-10	9.9171E-11
9.84118E-11	0.30257	4.31272E-10	9.84118E-11
1.22863E-10	0.26878	5.88666E-10	1.22863E-10
1.18798E-10	0.25760	5.88666E-10	1.18798E-10
1.23655E-10	0.27098	5.88666E-10	1.23655E-10
1.5348E-10	0.22921	8.34494E-10	1.5348E-10
1.49823E-10	0.22253	8.34494E-10	1.49823E-10
1.51253E-10	0.22513	8.34494E-10	1.51253E-10
1.83767E-10	0.17465	1.25004E-09	1.83767E-10
1.81294E-10	0.17190	1.25004E-09	1.81294E-10
1.84487E-10	0.17546	1.25004E-09	1.84487E-10
2.19187E-10	0.11598	2.12984E-09	2.19187E-10
2.17468E-10	0.11497	2.12984E-09	2.17468E-10
2.19723E-10	0.11630	2.12984E-09	2.19723E-10
2.55118E-10	0.06471	4.23815E-09	2.55118E-10
2.53622E-10	0.06430	4.23815E-09	2.53622E-10
2.51685E-10	0.06378	4.23815E-09	2.51685E-10
2.71575E-10	0.03502	8.09835E-09	2.71575E-10
2.79402E-10	0.03607	8.09835E-09	2.79402E-10
2.70822E-10	0.03492	8.09835E-09	2.70822E-10

Bmax molar	3.12E-10	KD molar	9.26E-10
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.79E+17	**kd nM =**	9.26E-01
Bmax molar to Bmax moles	9.345E-14		
= DPM/((DPM/mmole)*1000)	9.345E-14		
=Bmax DPM	16739.36373		
assay date	8/1/2005		
Bmax(dpm)	16739.36373		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.69		
DPM/mmole	1.79E+14		
DPM/pmole	1.79E+05		
1/(DPM/mmole)	5.58E-15		
1/(DPM/pmole)	5.58E-06		
SA(dpm/pmole)	1.79E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.093450		
bmax pmole/mg	0.15575		
Bmax fmole/mg	155.75		
Bmax (fmole/100 ug)	15.575		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Appendix 4: Prism Files for Competitive Binding Experiments

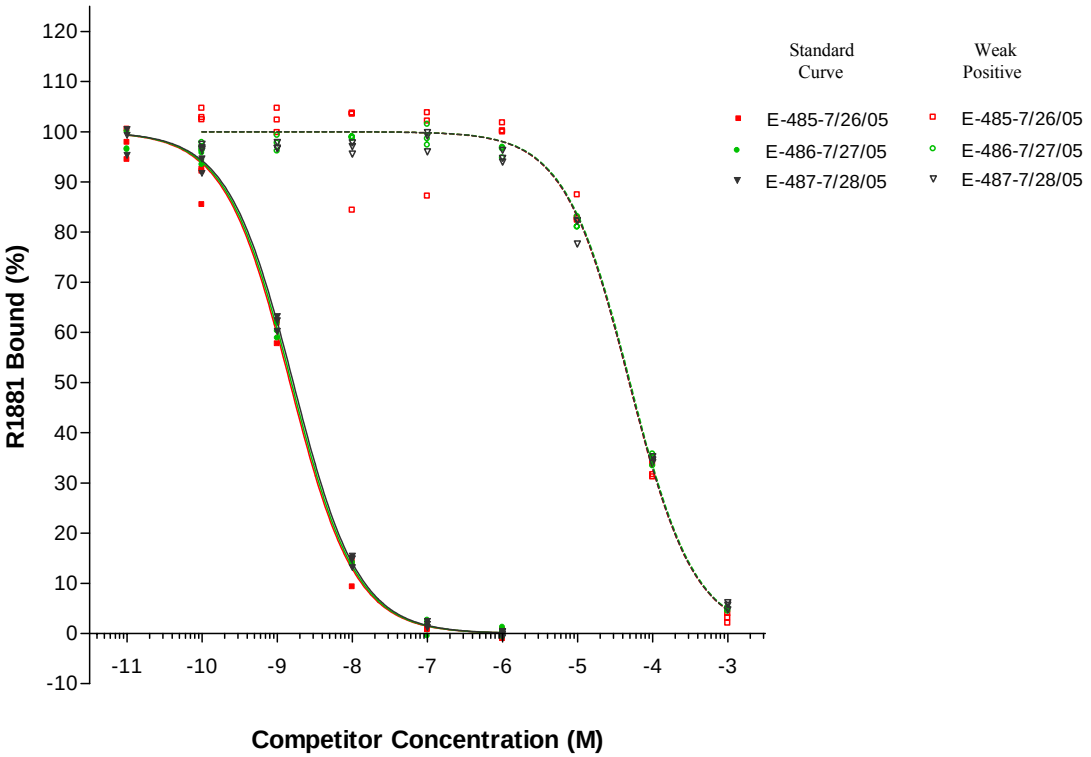


WA 4-11-5 Competitive



WA 4-11-5 Competitive

Lab E
Standard Curve and 'Weak Positive'



Appendix 5: Excel Files for Competitive Binding Experiments

Competitive Assay of a known Weak Positive

57 Assay Tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells
in columns O, AE and AI

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

The value in column Q will automatically change to FALSE

Columns T and U contain values to be analyzed
by nonlinear regression software
They are also presented in table form in columns
AC thorugh AJ

Column O, Rows 10 through 13 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Provide information in all blue cells in this column

Laboratory Code:	**E**	
Run identification:	485	
Assay start date:	7/26/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.76	**Ci/mmole**
Cytosol vial or lot identification:	62305	
Protein (cytosol):	0.75	**micro gram per tube**
Standard Curve IC50:	1.48E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	4.89E-05	**M**
RBA:	0.00302%	

volume of ethanol counted: 2 **mL**

protcol calls for counting decanted EtOH supernate
reflects 100ul of reaction mixture processed

total volume in tubes **3.1E+00** uL

Summary values				
	n	Mean	SD	
EtOH	6	11246.6	379.02	Total Binding, solvent control, tubes
Hot	6	44286.3	781.44	Total hot R1881 added to each tube
NSB	6	624.3	75.17	Nonspecific Binding
Specific EtOH	6	10622.3	379.02	

Assay Characterization Values		
EtOH / Hot	0.25	less than 0.1?
NSB / EtOH	0.06	around 0.25 ?

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Check the 10% rule:	25.40%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3529.80	10942.38	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3645.30	11300.43	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3456.80	10716.08	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	231.26	693.78	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	171.17	513.51	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	193.13	579.39	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	231.29	716.999	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	227.40	704.94	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	249.75	774.225	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	714.34	2214.454	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	680.56	2109.736	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	519.78	1611.318	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2319.20	7189.52	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2179.20	6755.52	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2336.00	7241.6	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3129.90	9702.69	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3357.50	10408.25	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3372.20	10453.82	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3436.80	10654.08	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3645.30	11300.43	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3555.80	11022.98	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)
1	1		—	97.1	10942.38	10318.0	-320.1	97.1
2	2		—	100.5	11300.43	10676.1	-678.1	100.5
3	3		—	95.0	10716.08	10091.7	-93.8	95.0
4	1		-6.0	0.7	693.78	69.4	9928.5	0.7
5	2		-6.0	-1.0	513.51	-110.8	10108.8	-1.0
6	3		-6.0	0.0	579.39	-45.0	10042.9	-0.4
7	1	cold R1881	-7.0	0.9	716.999	92.7	9905.3	0.9
8	2	cold R1881	-7.0	0.8	704.94	80.6	9917.4	0.8
9	3	cold R1881	-7.0	1.4	774.225	149.9	9848.1	1.4
10	1	cold R1881	-8.0	15.0	2214.454	1590.1	8407.9	15.0
11	2	cold R1881	-8.0	14.0	2109.736	1485.4	8512.6	14.0
12	3	cold R1881	-8.0	9.3	1611.318	987.0	9011.0	9.3
13	1	cold R1881	-9.0	61.8	7189.52	6565.2	3432.8	61.8
14	2	cold R1881	-9.0	57.7	6755.52	6131.2	3866.8	57.7
15	3	cold R1881	-9.0	62.3	7241.6	6617.3	3380.7	62.3
16	1	cold R1881	-10.0	85.5	9702.69	9078.4	919.6	85.5
17	2	cold R1881	-10.0	92.1	10408.25	9783.9	214.1	92.1
18	3	cold R1881	-10.0	92.5	10453.82	9829.5	168.5	92.5
19	1	cold R1881	-11.0	94.4	10654.08	10029.7	-31.8	94.4
20	2	cold R1881	-11.0	100.5	11300.43	10676.1	-678.1	100.5
21	3	cold R1881	-11.0	97.9	11022.98	10398.6	-400.7	97.9

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Check the 10% rule:	25.40%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	270.89	839.759	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	337.57	1046.467	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	306.63	950.553	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1285.90	3986.29	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1270.50	3938.55	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1360.50	4217.55	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3196.00	9907.6	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3037.00	9414.7	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3024.20	9375.02	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3626.30	11241.53	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3633.70	11264.47	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3688.50	11434.35	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3701.10	11473.41	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3756.60	11645.46	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3188.10	9883.11	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3092.40	9586.44	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3755.70	11642.67	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3747.40	11616.94	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3788.80	11745.28	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3621.60	11226.96	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3707.50	11493.25	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3725.00	11547.5	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3787.70	11741.87	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3708.90	11497.59	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3666.20	11365.22	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3659.90	11345.69	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3809.70	11810.07	TRUE	
49	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	227.75	683.25	TRUE	
50	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	229.81	689.43	TRUE	
51	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	195.56	586.68	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		45592.00	45592.00	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		44619.00	44619.00	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		43503.00	43503.00	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		44375.00	44375.00	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		43522.00	43522.00	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		44107.00	44107.00	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	Values for analysis by nonlinear regression: percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)
22	1	Weak Positive	-3.0	2.0	839.759	215.4	9782.5	2.0
23	2	Weak Positive	-3.0	4.0	1046.467	422.1	9575.8	4.0
24	3	Weak Positive	-3.0	3.1	950.553	326.2	9671.8	3.1
25	1	Weak Positive	-4.0	31.6	3986.29	3362.0	6636.0	31.6
26	2	Weak Positive	-4.0	31.2	3938.55	3314.2	6683.8	31.2
27	3	Weak Positive	-4.0	33.8	4217.55	3593.2	6404.8	33.8
28	1	Weak Positive	-5.0	87.4	9907.6	9283.3	714.7	87.4
29	2	Weak Positive	-5.0	82.8	9414.7	8790.4	1207.6	82.8
30	3	Weak Positive	-5.0	82.4	9375.02	8750.7	1247.3	82.4
31	1	Weak Positive	-6.0	100.0	11241.53	10617.2	-619.2	100.0
32	2	Weak Positive	-6.0	100.2	11264.47	10640.1	-642.2	100.2
33	3	Weak Positive	-6.0	101.8	11434.35	10810.0	-812.0	101.8
34	1	Weak Positive	-7.0	102.1	11473.41	10849.1	-851.1	102.1
35	2	Weak Positive	-7.0	103.8	11645.46	11021.1	-1023.2	103.8
36	3	Weak Positive	-7.0	87.2	9883.11	9258.8	739.2	87.2
37	1	Weak Positive	-8.0	84.4	9586.44	8962.1	1035.9	84.4
38	2	Weak Positive	-8.0	103.7	11642.67	11018.3	-1020.4	103.7
39	3	Weak Positive	-8.0	103.5	11616.94	10992.6	-994.6	103.5
40	1	Weak Positive	-9.0	104.7	11745.28	11120.9	-1123.0	104.7
41	2	Weak Positive	-9.0	99.8	11226.96	10602.6	-604.7	99.8
42	3	Weak Positive	-9.0	102.3	11493.25	10868.9	-870.9	102.3
43	1	Weak Positive	-10.0	102.8	11547.5	10923.2	-925.2	102.8
44	2	Weak Positive	-10.0	104.7	11741.87	11117.5	-1119.6	104.7
45	3	Weak Positive	-10.0	102.4	11497.59	10873.3	-875.3	102.4
46	1		—	101.1	11365.22	10740.9	-742.9	101.1
47	2		—	100.9	11345.69	10721.4	-723.4	100.9
48	3		—	105.3	11810.07	11185.7	-1187.8	105.3
49	1		-6.0	0.6	683.25	58.9	9939.1	0.6
50	2		-6.0	0.6	689.43	65.1	9932.9	0.6
51	3		-6.0	-0.4	586.68	-37.7	10035.6	-0.4
52	1				45592	44967.7		
53	2				44619	43994.7		
54	3				43503	42878.7		
55	1				44375	43750.7		
56	2				43522	42897.7		
57	3				44107	43482.7		

Prism data

standard curve				weak positive			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC
-6.0	0.65372	-1.04337	-0.42317	-3.0	2.0280	3.9740	3.0710
-6.0	0.55459	0.61277	-0.35454	-4.0	31.6499	31.2005	33.8270
-7.0	0.87231	0.75878	1.41104	-5.0	87.3940	82.7538	82.3802
-8.0	14.96958	13.98374	9.29156	-6.0	99.9518	100.1678	101.7671
-9.0	61.80561	57.71986	62.29590	-7.0	102.1348	103.7545	87.1635
-10.0	85.46497	92.10722	92.53622	-8.0	84.3706	103.7282	103.4860
-11.0	94.42150	100.50634	97.89438	-9.0	104.6942	99.8147	102.3216
				-10.0	102.8323	104.6621	102.3624

Competitive Assay of a known Weak Positive

57 Assay Tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells
in columns O, AE and AI

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

The value in column Q will automatically change to FALSE

Columns T and U contain values to be analyzed
by nonlinear regression software
They are also presented in table form in columns
AC thorugh AJ

Column O, Rows 10 through 13 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Provide information in all blue cells in this column

Laboratory Code:	**E**	
Run identification:	486	
Assay start date:	7/27/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.75	**Ci/mmole**
Cytosol vial or lot identification:	62305	
Protein (cytosol):	0.75	**micro gram per tube**
Standard Curve IC50:	1.55E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	5.04E-05	**M**
RBA:	0.00307%	

volume of ethanol counted: 2 **mL**

protcol calls for counting decanted EtOH supernate
reflects 100ul of reaction mixture processed

total volume in tubes **3.1E+00** uL

Summary values				
	n	Mean	SD	
EtOH	6	11462.4	109.57	Total Binding, solvent control, tubes
Hot	6	45528.3	610.51	Total hot R1881 added to each tube
NSB	6	596.6	78.51	Nonspecific Binding
Specific EtOH	6	10865.8	109.57	

Assay Characterization Values		
EtOH / Hot	0.25	less than 0.1?
NSB / EtOH	0.05	around 0.25 ?

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Check the 10% rule:	25.18%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3738.50	11589.35	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3689.60	11437.76	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3732.10	11569.51	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	175.15	525.45	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	213.74	641.22	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	176.05	528.15	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	285.54	885.174	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	254.25	788.175	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	175.86	545.166	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	675.17	2093.027	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	690.92	2141.852	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	686.98	2129.638	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2360.10	7316.31	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2255.40	6991.74	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2305.10	7145.81	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3499.50	10848.45	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3467.40	10748.94	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3549.00	11001.9	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3578.10	11092.11	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3698.60	11465.66	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3573.40	11077.54	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)
1	1		—	101.2	11589.35	10992.8	-723.5	101.2
2	2		—	99.8	11437.76	10841.2	-571.9	99.8
3	3		—	101.0	11569.51	10972.9	-703.7	101.0
4	1		-6.0	-0.7	525.45	-71.1	10340.4	-0.7
5	2		-6.0	0.4	641.22	44.7	10224.6	0.4
6	3		-6.0	0.0	528.15	-68.4	10337.7	-0.6
7	1	cold R1881	-7.0	2.7	885.174	288.6	9980.7	2.7
8	2	cold R1881	-7.0	1.8	788.175	191.6	10077.7	1.8
9	3	cold R1881	-7.0	-0.5	545.166	-51.4	10320.7	-0.5
10	1	cold R1881	-8.0	13.8	2093.027	1496.5	8772.8	13.8
11	2	cold R1881	-8.0	14.2	2141.852	1545.3	8724.0	14.2
12	3	cold R1881	-8.0	14.1	2129.638	1533.1	8736.2	14.1
13	1	cold R1881	-9.0	61.8	7316.31	6719.7	3549.5	61.8
14	2	cold R1881	-9.0	58.9	6991.74	6395.2	3874.1	58.9
15	3	cold R1881	-9.0	60.3	7145.81	6549.2	3720.0	60.3
16	1	cold R1881	-10.0	94.3	10848.45	10251.9	17.4	94.3
17	2	cold R1881	-10.0	93.4	10748.94	10152.4	116.9	93.4
18	3	cold R1881	-10.0	95.8	11001.9	10405.3	-136.1	95.8
19	1	cold R1881	-11.0	96.6	11092.11	10495.5	-226.3	96.6
20	2	cold R1881	-11.0	100.0	11465.66	10869.1	-599.8	100.0
21	3	cold R1881	-11.0	96.5	11077.54	10481.0	-211.7	96.5

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Check the 10% rule:	25.18%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	347.43	1077.033	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	362.29	1123.099	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	375.15	1162.965	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1445.80	4481.98	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1426.00	4420.6	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1363.50	4226.85	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3032.00	9399.2	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3033.10	9402.61	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3103.20	9619.92	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3589.20	11126.52	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3577.80	11091.18	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3515.60	10898.36	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3604.60	11174.26	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3749.30	11622.83	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3647.10	11306.01	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3661.70	11351.27	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3661.00	11349.1	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3651.50	11319.65	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3672.10	11383.51	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3562.20	11042.82	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3618.80	11218.28	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3565.40	11052.74	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3621.80	11227.58	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3589.90	11128.69	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3682.10	11414.51	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3700.80	11472.48	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3642.20	11290.82	TRUE	
49	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	187.37	562.11	TRUE	
50	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	243.43	730.29	TRUE	
51	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	197.40	592.2	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		45196.00	45196.00	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		45074.00	45074.00	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		45024.00	45024.00	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		45431.00	45431.00	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		45823.00	45823.00	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		46622.00	46622.00	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	Values for analysis by nonlinear regression: percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)
22	1	Weak Positive	-3.0	4.4	1077.033	480.5	9788.8	4.4
23	2	Weak Positive	-3.0	4.8	1123.099	526.5	9742.7	4.8
24	3	Weak Positive	-3.0	5.2	1162.965	566.4	9702.9	5.2
25	1	Weak Positive	-4.0	35.8	4481.98	3885.4	6383.9	35.8
26	2	Weak Positive	-4.0	35.2	4420.6	3824.0	6445.2	35.2
27	3	Weak Positive	-4.0	33.4	4226.85	3630.3	6639.0	33.4
28	1	Weak Positive	-5.0	81.0	9399.2	8802.6	1466.6	81.0
29	2	Weak Positive	-5.0	81.0	9402.61	8806.0	1463.2	81.0
30	3	Weak Positive	-5.0	83.0	9619.92	9023.4	1245.9	83.0
31	1	Weak Positive	-6.0	96.9	11126.52	10530.0	-260.7	96.9
32	2	Weak Positive	-6.0	96.6	11091.18	10494.6	-225.3	96.6
33	3	Weak Positive	-6.0	94.8	10898.36	10301.8	-32.5	94.8
34	1	Weak Positive	-7.0	97.3	11174.26	10577.7	-308.4	97.3
35	2	Weak Positive	-7.0	101.5	11622.83	11026.3	-757.0	101.5
36	3	Weak Positive	-7.0	98.6	11306.01	10709.4	-440.2	98.6
37	1	Weak Positive	-8.0	99.0	11351.27	10754.7	-485.4	99.0
38	2	Weak Positive	-8.0	99.0	11349.1	10752.5	-483.3	99.0
39	3	Weak Positive	-8.0	98.7	11319.65	10723.1	-453.8	98.7
40	1	Weak Positive	-9.0	99.3	11383.51	10786.9	-517.7	99.3
41	2	Weak Positive	-9.0	96.1	11042.82	10446.3	-177.0	96.1
42	3	Weak Positive	-9.0	97.8	11218.28	10621.7	-352.4	97.8
43	1	Weak Positive	-10.0	96.2	11052.74	10456.2	-186.9	96.2
44	2	Weak Positive	-10.0	97.8	11227.58	10631.0	-361.7	97.8
45	3	Weak Positive	-10.0	96.9	11128.69	10532.1	-262.9	96.9
46	1		—	99.6	11414.51	10817.9	-548.7	99.6
47	2		—	100.1	11472.48	10875.9	-606.6	100.1
48	3		—	98.4	11290.82	10694.3	-425.0	98.4
49	1		-6.0	-0.3	562.11	-34.5	10303.7	-0.3
50	2		-6.0	1.2	730.29	133.7	10135.5	1.2
51	3		-6.0	0.0	592.2	-4.4	10273.6	0.0
52	1				45196	44599.4		
53	2				45074	44477.4		
54	3				45024	44427.4		
55	1				45431	44834.4		
56	2				45823	45226.4		
57	3				46622	46025.4		

Prism data

standard curve				weak positive			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC
-6.0	-0.65453	0.41092	-0.62968	-3.0	4.4218	4.8457	5.2126
-6.0	-0.31714	1.23065	-0.04022	-4.0	35.7580	35.1932	33.4100
-7.0	2.65607	1.76337	-0.47308	-5.0	81.0120	81.0434	83.0433
-8.0	13.77213	14.22147	14.10907	-6.0	96.9088	96.5836	94.8090
-9.0	61.84283	58.85576	60.27369	-7.0	97.3482	101.4764	98.5607
-10.0	94.34967	93.43387	95.76190	-8.0	98.9772	98.9572	98.6862
-11.0	96.59212	100.02996	96.45803	-9.0	99.2739	96.1385	97.7533
				-10.0	96.2298	97.8389	96.9288

Competitive Assay of a known Weak Positive

57 Assay Tubes

Please return by eMail to n.a.Holter@pnl.gov

Provide information in all blue cells
in columns O, AE and AI

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R
The value in column Q will automatically change to FALSE

Columns T and U contain values to be analyzed
by nonlinear regression software
They are also presented in table form in columns
AC thorugh AJ

Column O, Rows 10 through 13 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Provide information in all blue cells in this column

Laboratory Code:	**E**	
Run identification:	487	
Assay start date:	7/28/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.74	**Ci/mmole**
Cytosol vial or lot identification:	62305	
Protein (cytosol):	0.75	**micro gram per tube**
Standard Curve IC50:	1.64E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	4.92E-05	**M**
RBA:	0.00332%	

volume of ethanol counted: 2 **mL**

protcol calls for counting decanted EtOH supernate
reflects 100ul of reaction mixture processed

total volume in tubes **3.1E+00** uL

Summary values				
	n	Mean	SD	
EtOH	6	11412.4	304.13	Total Binding, solvent control, tubes
Hot	6	43997.8	748.13	Total hot R1881 added to each tube
NSB	5	525.6	56.98	Nonspecific Binding
Specific EtOH	6	10886.8	304.13	

Assay Characterization Values		
EtOH / Hot	0.26	less than 0.1?
NSB / EtOH	0.05	around 0.25 ?

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Check the 10% rule:	25.94%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3764.00	11668.4	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3658.10	11340.11	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3513.10	10890.61	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	144.88	434.64	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	365.34	1096.02	FALSE	double shot
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	168.08	504.24	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	209.10	648.21	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	231.96	719.076	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	254.57	789.167	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	630.46	1954.426	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	692.62	2147.122	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	710.22	2201.682	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2387.40	7400.94	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2284.80	7082.88	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2357.70	7308.87	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3492.30	10826.13	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3545.90	10992.29	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3390.60	10510.86	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3696.30	11458.53	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3516.30	10900.53	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3656.70	11335.77	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)
1	1		—	102.4	11668.4	11142.8	-781.6	102.4
2	2		—	99.3	11340.11	10814.5	-453.3	99.3
3	3		—	95.2	10890.61	10365.0	-3.8	95.2
4	1		-6.0	-0.8	434.64	-91.0	10452.1	-0.8
5	2		-6.0					
6	3		-6.0	0.0	504.24	-21.4	10382.5	-0.2
7	1	cold R1881	-7.0	1.1	648.21	122.6	10238.6	1.1
8	2	cold R1881	-7.0	1.8	719.076	193.5	10167.7	1.8
9	3	cold R1881	-7.0	2.4	789.167	263.5	10097.6	2.4
10	1	cold R1881	-8.0	13.1	1954.426	1428.8	8932.3	13.1
11	2	cold R1881	-8.0	14.9	2147.122	1621.5	8739.6	14.9
12	3	cold R1881	-8.0	15.4	2201.682	1676.1	8685.1	15.4
13	1	cold R1881	-9.0	63.2	7400.94	6875.3	3485.8	63.2
14	2	cold R1881	-9.0	60.2	7082.88	6557.3	3803.9	60.2
15	3	cold R1881	-9.0	62.3	7308.87	6783.2	3577.9	62.3
16	1	cold R1881	-10.0	94.6	10826.13	10300.5	60.6	94.6
17	2	cold R1881	-10.0	96.1	10992.29	10466.7	-105.5	96.1
18	3	cold R1881	-10.0	91.7	10510.86	9985.2	375.9	91.7
19	1	cold R1881	-11.0	100.4	11458.53	10932.9	-571.8	100.4
20	2	cold R1881	-11.0	95.3	10900.53	10374.9	-13.8	95.3
21	3	cold R1881	-11.0	99.3	11335.77	10810.1	-449.0	99.3

Competitive Assay Tube Layout - One Test Chemical (Weak Positive)

Check the 10% rule:	25.94%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	367.08	1137.948	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	385.58	1195.298	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	335.02	1038.562	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1366.00	4234.6	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1384.60	4292.26	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1406.90	4361.39	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2895.20	8975.12	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3055.00	9470.5	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3060.10	9486.31	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3468.70	10752.97	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3495.20	10835.12	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3551.60	11009.96	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3650.50	11316.55	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3675.70	11394.67	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3540.90	10976.79	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3576.80	11088.08	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3524.30	10925.33	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3603.90	11172.09	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3563.90	11048.09	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3567.30	11058.63	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3604.90	11175.19	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3568.10	11061.11	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3558.20	11030.42	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3593.30	11139.23	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3790.20	11749.62	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3664.30	11359.33	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3698.80	11466.28	TRUE	
49	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	188.45	565.35	TRUE	
50	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	185.95	557.85	TRUE	
51	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	188.68	566.04	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		44169.00	44169.00	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		44087.00	44087.00	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		43379.00	43379.00	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		42891.00	42891.00	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		44531.00	44531.00	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		44930.00	44930.00	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	Values for analysis by nonlinear regression: percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)
22	1	Weak Positive	-3.0	5.6	1137.948	612.3	9748.8	5.6
23	2	Weak Positive	-3.0	6.2	1195.298	669.7	9691.5	6.2
24	3	Weak Positive	-3.0	4.7	1038.562	512.9	9848.2	4.7
25	1	Weak Positive	-4.0	34.1	4234.6	3709.0	6652.2	34.1
26	2	Weak Positive	-4.0	34.6	4292.26	3766.6	6594.5	34.6
27	3	Weak Positive	-4.0	35.2	4361.39	3835.8	6525.4	35.2
28	1	Weak Positive	-5.0	77.6	8975.12	8449.5	1911.6	77.6
29	2	Weak Positive	-5.0	82.2	9470.5	8944.9	1416.3	82.2
30	3	Weak Positive	-5.0	82.3	9486.31	8960.7	1400.5	82.3
31	1	Weak Positive	-6.0	93.9	10752.97	10227.3	133.8	93.9
32	2	Weak Positive	-6.0	94.7	10835.12	10309.5	51.6	94.7
33	3	Weak Positive	-6.0	96.3	11009.96	10484.3	-123.2	96.3
34	1	Weak Positive	-7.0	99.1	11316.55	10790.9	-429.8	99.1
35	2	Weak Positive	-7.0	99.8	11394.67	10869.0	-507.9	99.8
36	3	Weak Positive	-7.0	96.0	10976.79	10451.2	-90.0	96.0
37	1	Weak Positive	-8.0	97.0	11088.08	10562.5	-201.3	97.0
38	2	Weak Positive	-8.0	95.5	10925.33	10399.7	-38.6	95.5
39	3	Weak Positive	-8.0	97.8	11172.09	10646.5	-285.3	97.8
40	1	Weak Positive	-9.0	96.7	11048.09	10522.5	-161.3	96.7
41	2	Weak Positive	-9.0	96.8	11058.63	10533.0	-171.9	96.8
42	3	Weak Positive	-9.0	97.8	11175.19	10649.6	-288.4	97.8
43	1	Weak Positive	-10.0	96.8	11061.11	10535.5	-174.3	96.8
44	2	Weak Positive	-10.0	96.5	11030.42	10504.8	-143.7	96.5
45	3	Weak Positive	-10.0	97.5	11139.23	10613.6	-252.5	97.5
46	1		—	103.1	11749.62	11224.0	-862.9	103.1
47	2		—	99.5	11359.33	10833.7	-472.6	99.5
48	3		—	100.5	11466.28	10940.7	-579.5	100.5
49	1		-6.0	0.4	565.35	39.7	10321.4	0.4
50	2		-6.0	0.3	557.85	32.2	10328.9	0.3
51	3		-6.0	0.4	566.04	40.4	10320.7	0.4
52	1				44169	43643.4		
53	2				44087	43561.4		
54	3				43379	42853.4		
55	1				42891	42365.4		
56	2				44531	44005.4		
57	3				44930	44404.4		

Prism data

standard curve				weak positive			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC
-6.0	-0.83573		-0.19642	-3.0	5.6245	6.1513	4.7116
-6.0	0.36490	0.29601	0.37124	-4.0	34.0687	34.5983	35.2333
-7.0	1.12601	1.77695	2.42076	-5.0	77.6125	82.1628	82.3080
-8.0	13.12421	14.89421	15.39537	-6.0	93.9429	94.6975	96.3035
-9.0	63.15296	60.23143	62.30725	-7.0	99.1196	99.8372	95.9988
-10.0	94.61492	96.14117	91.71901	-8.0	97.0210	95.5261	97.7927
-11.0	100.42380	95.29831	99.29619	-9.0	96.6537	96.7505	97.8212
				-10.0	96.7733	96.4914	97.4909