DRAFT REPORT

Validation of an Androgen Receptor Binding Assay–Task 4

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Draft

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This report contains 95 pages, including seven appendices.

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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

Study Dates and Data Retention

Study initiation date:	21 January 2005
Experimental start date:	18 January 2005
Experimental end date:	2 March 2005
Study completion date:	

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 Environmental Protection Agency. 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 with the exception listed below. 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, Richland Management.

Inspection/Audit Dates:	Study Phase Audited:	Date(s) reported to Study Director and Management:
January 24 & 25, 2006	Audit of Data Quality, Data and Draft Report	January 25,2006

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, tracer, and test or control substances 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 (tracer). Battelle, Richland conducted three separate experiments to evaluate the inhibition of androgen receptor binding of ^{3}H-R1881 by the test substances.

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 0.907, 0.844, and 0.857 nM and the B_{max} observed was 12.76, 12.09, and 12.01 fmoles/100 ug.

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. The IC_{50} values for R1881 were determined to be 1.98, 1.85, and 1.84 nM. The IC_{50} values for dexamethasone were determined to be 42.4, 41.6, and 43.0 μM. The RBA values for dexamethasone in comparison to R1881 were, 0.0047, 0.0044, and 0.0043 %.

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 Substances Information and Preparation

Battelle provided the following test materials at the necessary dilutions:

- Tracer: ^{3}H-R1881 (CAS No. 965-93-5)(Lot # 3538497)
- Reference Substance: Unlabelled R1881–Methyltrienolone (CAS No. 965-93-5) (Lot# 3411228)
- Test Substance: Dexamethasone (CAS No. 50-02-2)(Lot # P4311T)

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

The following were prepared at Battelle, Richland:

- Buffer with Tris(Tris base; Lot# 120K5444 & Tris HCl; Lot# 072K5420), ethylene diamine tetraacetic acid(EDTA; Lot# 99H0095), dithiothreitol (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# 83862B)

The following reagent was obtained from supplier as identified:

- Sustrate: ^{3}H-R1881 (CAS No. 965-93-5) (Perkin-Elmer Cat# NET 590; Lot # 3538497; Specific Activity = 82 Ci/mmol)

R1881 Preparation

Chemical Repository, Sequim, Washington provided the R1881 stock solutions (30 mM) prepared in absolute ethanol. Chemical Repository 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^{-8} 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^{-5} M stock was used to generate a final unlabeled R1881 concentration in the assay tubes of 0.025, 0.05 0.07, 0.10, and 0.15 uM. To generate a final unlabeled R1881 concentration in the assay tubes of 0.25, 0.05 and 0.10 uM, 7.5, 15 or 30 ul of 1.0 x 10^{-7} M stock were used.

Dilutions of the R1881 stock solutions were prepared in ethanol for competitive assays. The final assay target concentrations for R1881 were 1.0 × 10^{-6} (NSB), 1.0 x10^{-7}, 1.0 × 10^{-8}, 1.0 x10^{-9}, 1.9 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 (Sequim, WA) provided the dexamethasone stock solutions (30 mM) prepared in absolute ethanol. The Chemical Repository was responsible for the preparation of the dexamethasone stock.

Dilutions of the dexamethasone stock solutions were prepared in ethanol by Battelle, Richland. The final target concentrations for dexamethasone in assay tubes were 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9}, and 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 solution was 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.

For competitive assays, the [^{3}H]-R1881 was diluted with ethanol to achieve a substrate solution at a concentration of 10 nM. The substrate solution (30 µL) was added to the incubation mixtures to achieve a final concentration of 1 nM [^{3}H]-R1881 in the 300 ul volume for the assay.

Preparation of Rat Ventral Prostate Cytosol

Rat ventral prostate cytosol was prepared by Battelle, Richland.

- 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 190 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 centrigrade. 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 extrapoliation 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 study protocol and specific assay protocols. 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 attached study protocol.
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, 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

Battelle, Richland supplied all raw data in electronic format using Microsoft Excel® spreadsheets and Prism templates.

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 article 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

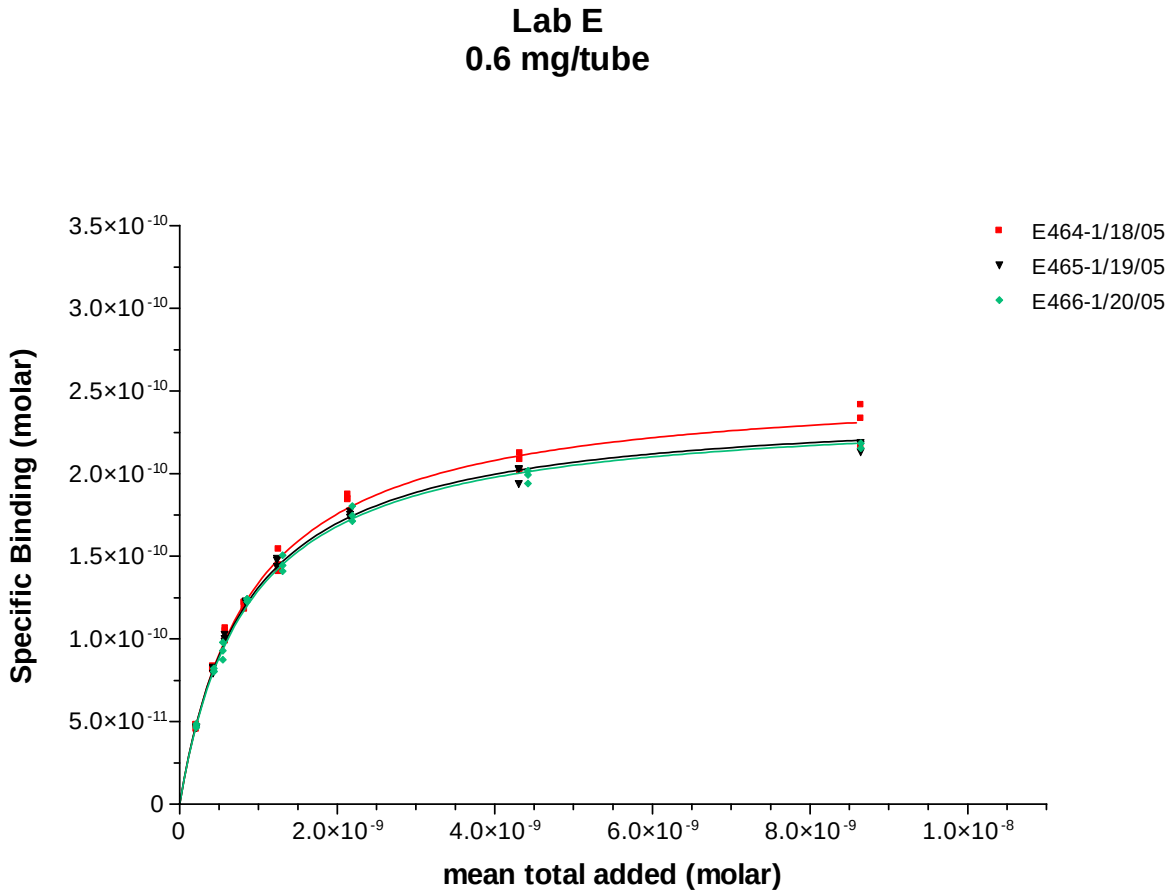
Rat ventral prostate cytosol was prepared by Battelle, Richland at a protein concentration of 5.206 mg/mL. A series of saturation and competitive binding experiments were conducted using the cytosol.

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 0.907, 0.844, and 0.857 nM and the B_{max} observed was 12.76, 12.09, and 12.01 fmoles/100 ug.

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 1.98, 1.85, and 1.84 nM. The IC_{50} values for dexamethasone were determined to be 42.4, 41.6, and 43.0 µM. The RBA values for dexamethasone in comparison to R1881 were, 0.0047, 0.0044, and 0.0043 %.

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

WA 4-11-4 Saturation



Specific bound	E464-1/18/05	E465-1/19/05	E466-1/20/05
BMAX	2.552e-010	2.417e-010	2.401e-010
KD	9.066e-010	8.443e-010	8.567e-010
Std. Error			
BMAX	4.189e-012	2.714e-012	3.165e-012
KD	2.920e-011	1.902e-011	2.223e-011
95% Confidence Intervals			
BMAX	2.465e-010 to 2.638e-010	2.361e-010 to 2.474e-010	2.335e-010 to 2.467e-010
KD	8.460e-010 to 9.671e-010	8.049e-010 to 8.838e-010	8.104e-010 to 9.029e-010
Goodness of Fit			
Degrees of Freedom	22	22	21
R^2 (unweighted)	0.9910	0.9966	0.9959
Weighted Sum of Squares ($1/Y^2$)	0.03069	0.01508	0.01710
Absolute Sum of Squares	7.757e-022	2.492e-022	2.778e-022
Sy.x	5.938e-012	3.366e-012	3.637e-012
Data			
Number of X values	24	24	23
Number of Y replicates	1	1	1
Total number of values	24	24	23
Number of missing values	0	0	0

WA 4-11-4 Saturation

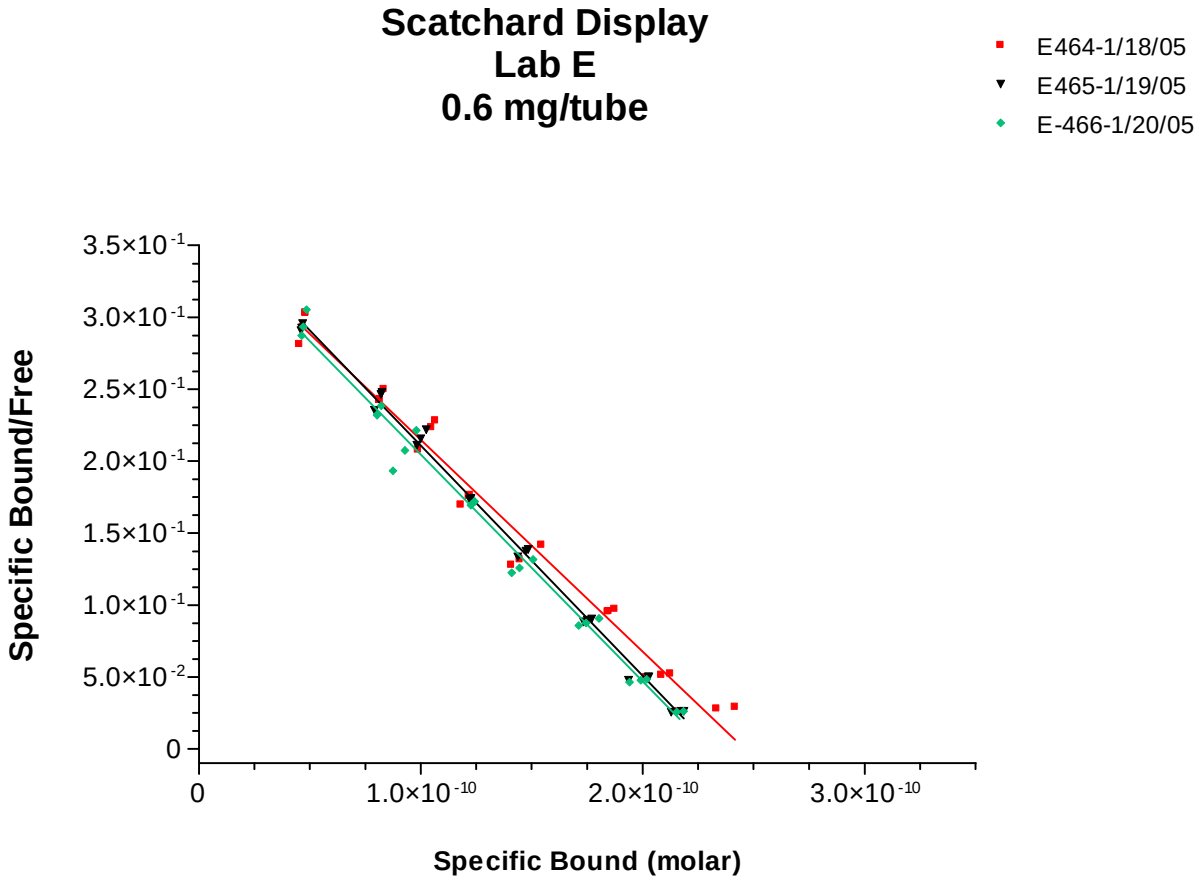


Figure 2: Competitive Binding of R1881 and Dexamethasone (Weak Positive) to the Androgen Receptor

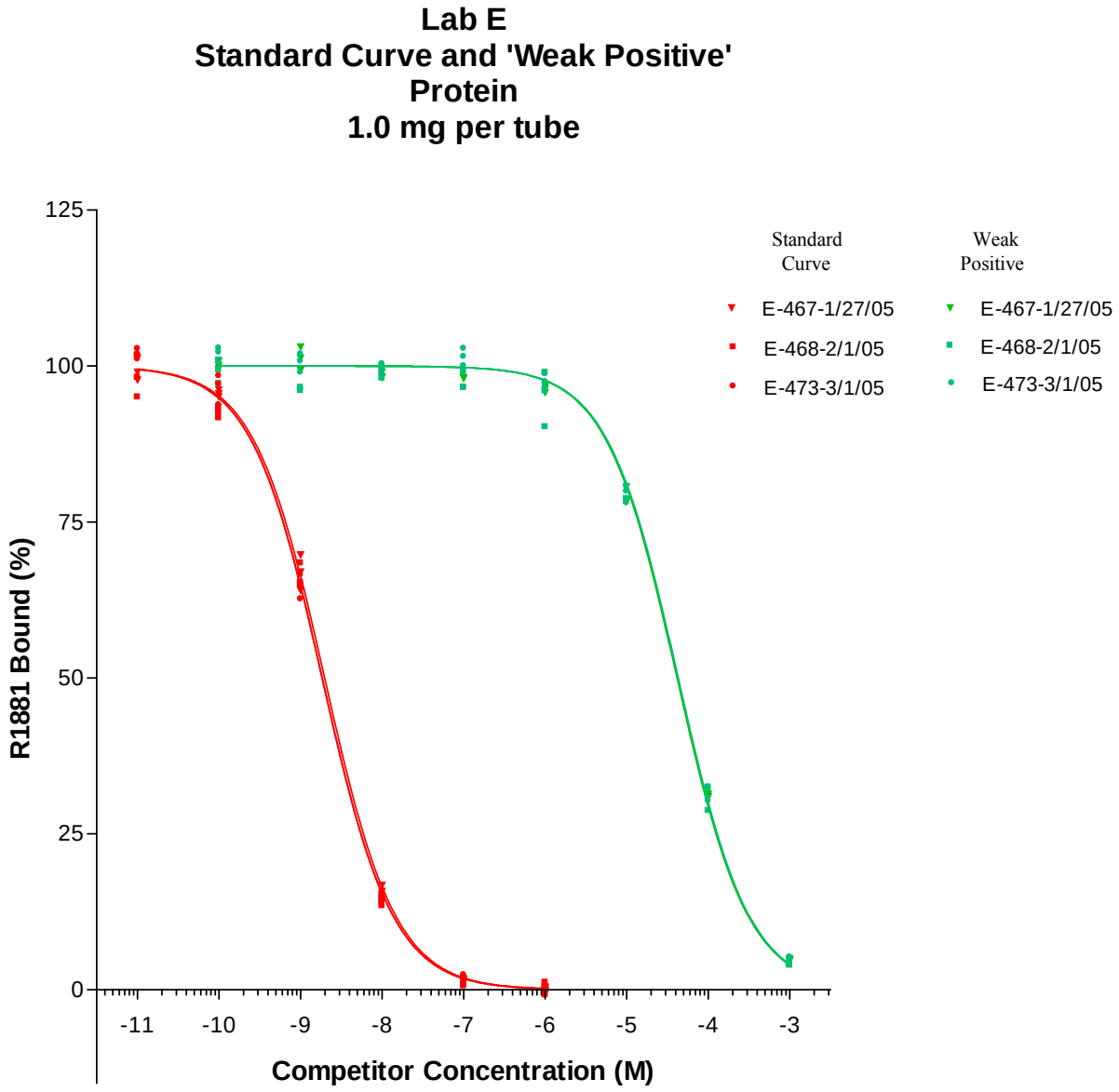


Table 1: Saturation Binding Experiments

Run number	Assay date	Technician	K_d (M)	B_{max} (fmoles/100 ug)
1	18 January 2005	JEC	0.907×10^{-9}	12.76
2	19 January 2005	JEC	0.844×10^{-9}	12.09
3	20 January 2005	JEC	0.857×10^{-9}	12.01
Mean			0.869×10^{-9}	12.28
SD			0.033×10^{-9}	0.41

Table 2: Competitive Binding Experiments

			R1881		Dexamethasone	
Run number	Assay date	Technician	IC_{50} (M)	RBA	IC_{50} (M)	RBA(%)
1	27 January 2005	JEC	1.98×10^{-9}	100	4.24×10^{-5}	0.0047
2	01 February 2005	JEC	1.85×10^{-9}	100	4.16×10^{-5}	0.0044
3	01 March 2005	JEC	1.84×10^{-9}	100	4.30×10^{-5}	0.0043
	Mean		1.89×10^{-9}		4.23×10^{-5}	0.0045
	SD		0.08×10^{-9}		0.07×10^{-5}	0.0002

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 Protocol No. 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 4

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 common preparations of rat prostate cytosol, radiolabeled R 1881, R1881 and dexamethasone (test substance). This protocol is specific to the study to be conducted at Battelle-Richland.

5. **Duration:** approximately 40days

6. **Proposed Study Dates:**

   a. Initiation of *In Vitro* Studies: January 17, 2005
   b. Completion of *In Vitro* Studies: February 25, 2005

7. **Protocol Approval:**

   a. Study Director: James E. Morris Date: 1-21-05
   James E. Morris, Ph.D.

   b. WAL: Michael L. Blanton Date: 1/19/05
   Michael L.Blanton

   c. Sponsor: David P. Houchens Date: 1/14/05
   David P. Houchens, Ph.D.

Reviewed By:

a. MSL QA Representative: Mary E Lynn Date: 1/19/05
Mary E. Lynn

b. EDSP Battelle QAM: Terri L Pollock Date: 1-14-05
Terri L. Pollock

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

a.1 Test Substance: Dexamethasone (CAS # 50-28-2) will be prepared by and supplied by the Chemical Repository, Battelle, Sequim, Washington.

a.2 Reference substance: Radioinert Methyltrienolone (CAS 965-93-5, Perkin-Elmer, NEN, catalog number NLP 005005MG)

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°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 labeled as defined in the assay. Cytosol will be stored at -80^{o}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 Sprague-Dawley male rats (85 to 100 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 degrees centrigrade.

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 and reference substance 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, control 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 substance(s), solvent control response compared to the negative control, K_d and IC_{50} values with confidence limits for R 1881 and test substance, and RBA values for the test substances.

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 substances receipt and use records.

d. Test 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 US 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
Polytron PT 35/10 Tissue Homogenizer
Vacuum Concentrator
Refrigerated General Laboratory Centrifuge
High-Speed Refrigerated Centrifuge (up to 30,000 x *g*)
pH Meter with Tris Compatible Electrode
Scintillation Counter
refrigerators

4.2 Chemicals

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

Triamcinolone Acetonide
Steroids (Steraloids - recrystallized)
Optifluor

4.3 Supplies
20 ml Polypropylene Scintillation Vials
12 x 75 mm Borosilicate Glass Test Tubes
1000 ml graduated cylinders
500 ml Erlenmeyer flasks
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^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^oC. 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 (using 4^oC pH standardizing solutions) the cooled solution to 7.4. Store at 4^oC. Use 1.0 ml/100 ml TEDG buffer = 10mM. (50 mM Tris = 50 ml 1 M Tris/1 L ddH_2O)

5.1.5. *Potassium Chloride Stock Solution*: Add 298.2 g KCL to 600 ml ddH_2O in a 1000 ml volumetric flask; bring the total volume to 1000 ml = 4.0 M. Store at room temperature. Use 10.0 ml per 100 ml high-salt TEDG buffer = 0.4 M.

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
7 50 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°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°C. Check pH of the final solution to make sure it is 7.4 at 4°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. Place in the refrigerator overnight.

5.4.3. The next morning, repeat the washing steps x 2 with fresh 50 mM TRIS buffer.

5.4.4. After the last wash, 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°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^{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 X 10^{-5}	1 X 10^{-6}
S1	3 X 10^{-6}	1 X 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 at 4°C with a Polytron homgenizer using 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. Recool 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 into 5 ml and store -80°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 50ul triamcinolone acetonide (60mM stock) to ALL tubes

7.1.3. For 3 tubes 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 duplicate)

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 1.2 mg per 300 ul assay tube. (In our lab this is usually about a 1:1 dilution or 150 ul cytosol:150 ul TEDG buffer)

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

7.5. Before leaving for the day, prepare the first wash 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 100 ml Erlenmeyer flask. Place a stir bar in the flask and place the flask into 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 2-3 minutes at 4^{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 the supernatant from each tube using 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 at 4^{o}C 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. Add 14 ml of Optifluor scintillation cocktail and 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 mM 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 (nM)*	*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 1.2 mg per 300 ul assay (in our laboratory this was about a 1:1 dilution). 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 (20hr).

9.7 Before leaving for the day, prepare the first wash 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 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 -

$$\frac{\text{X DPM}}{2.22 \times 10^{12} \text{ dpm/Ci} * 83.2 \text{ Ci/mmole}} = \text{X} * 5.4141 \times 10^{-15} \text{ mmole}$$

$$\frac{5.4141 \times 10^{-15} \text{ moles}}{1000 \text{ mmole/mole}} = \text{X} * 5.4141 \times 10^{-18} \text{ moles}$$

$$\text{X} * 5.4141 \times 10^{-18} \text{ moles} = 1.8047 \times 10^{-14} \text{ moles/liter} = \text{X} * (1.8047 \times 10^{-5}) \text{ nM} = 1 \text{ X} 10^{-9} \text{M}$$
$$0.0003 \text{ liters}$$

*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

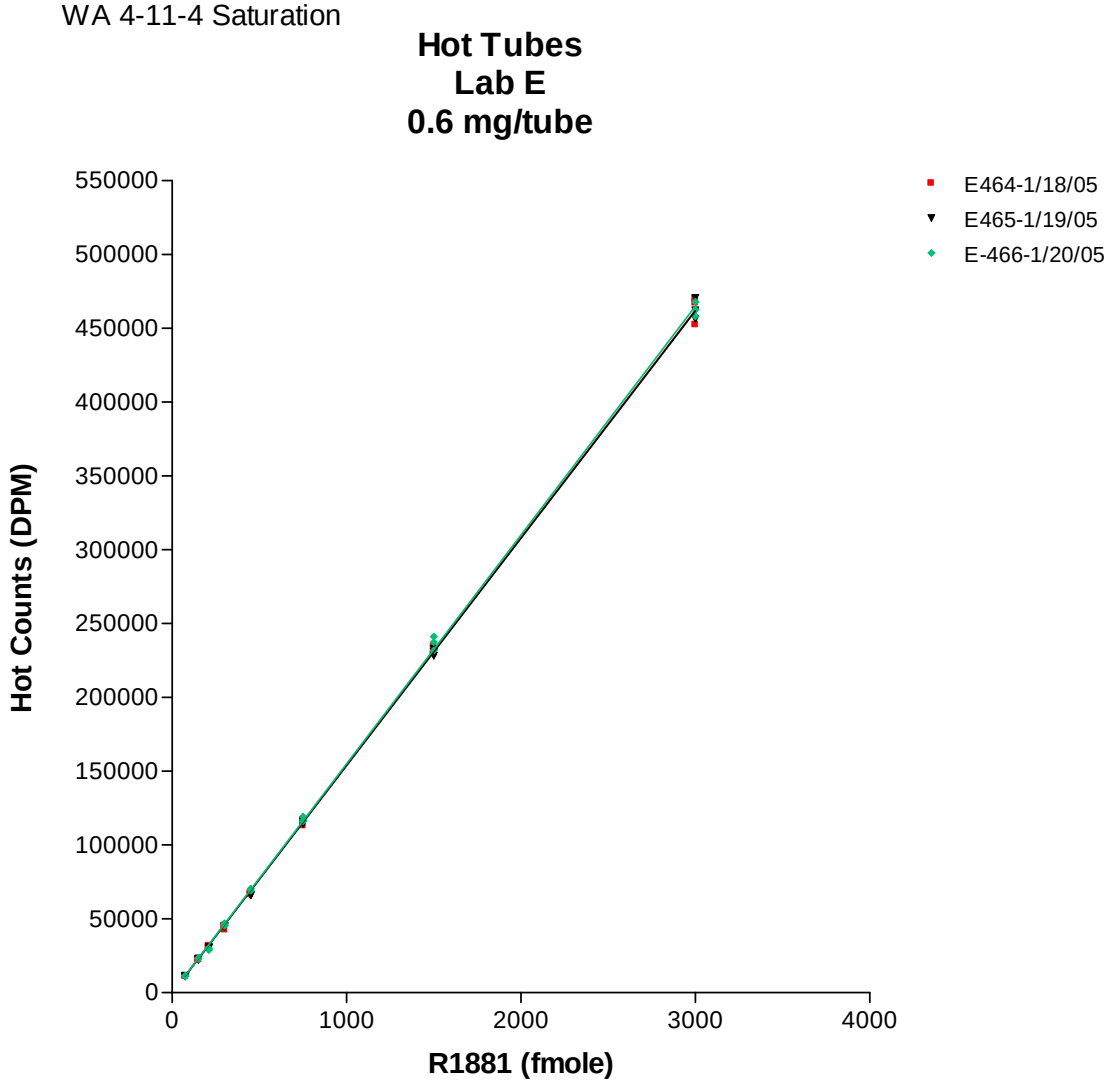
3) Munson, P.J., and Rodbard, D. (1980) *Anal. Biochem.* **107**, 220-239.

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

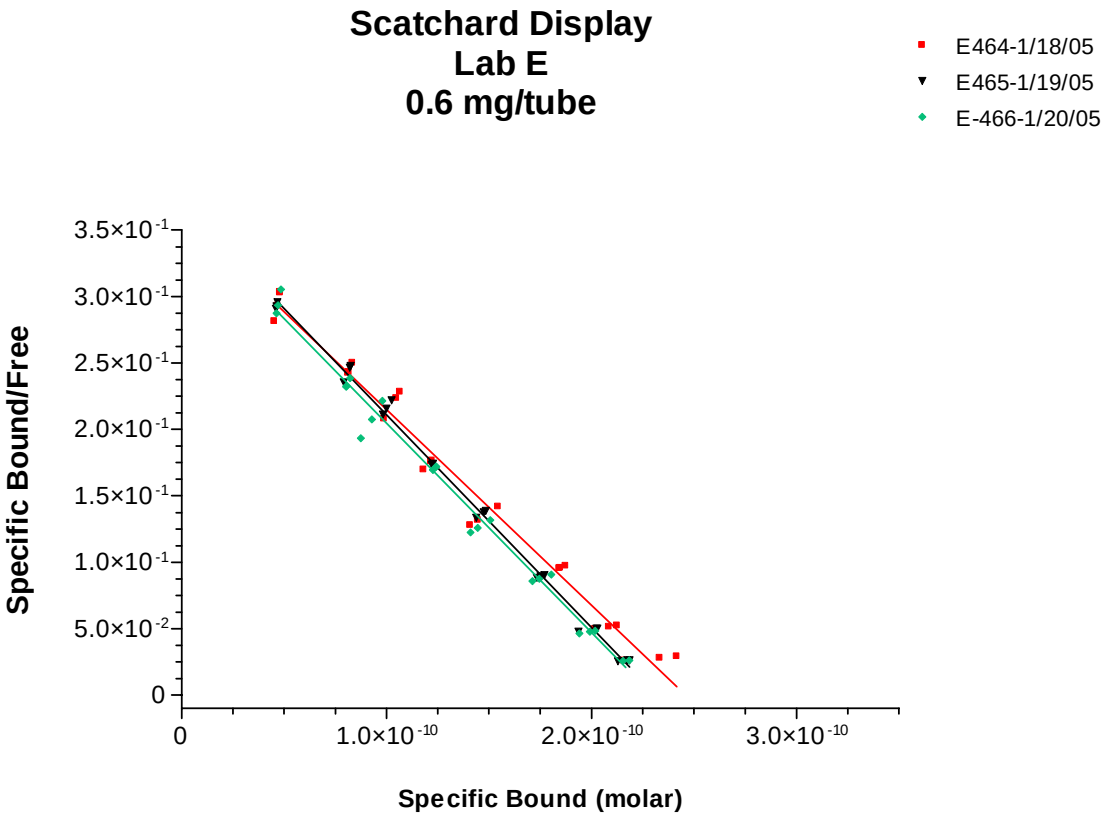
5) Wilson, V.S., Lambright, C.S., Ostby, J. and Gray, Jr., L.E.. *In* vitro and in vivo effects of 17□-trenbolone: A feedlot effluent contaminant. (2002). *Toxicol. Sci. (in prep).*

Appendix 2: Prism Files for Saturation Binding Experiments

WA 4-11-4 Saturation

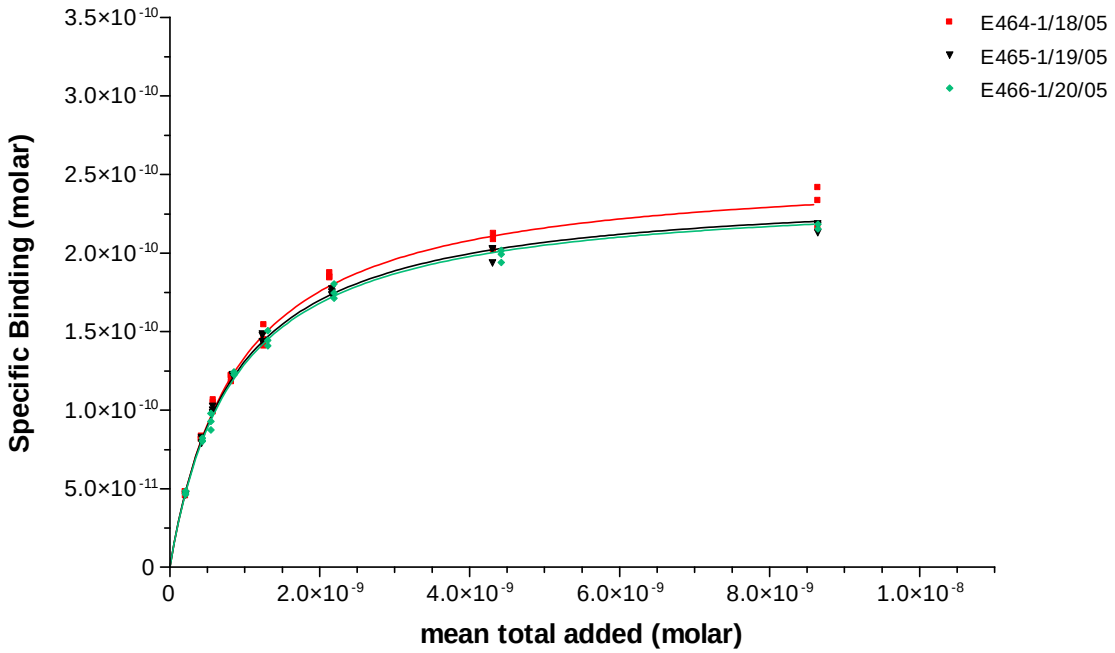


WA 4-11-4 Saturation



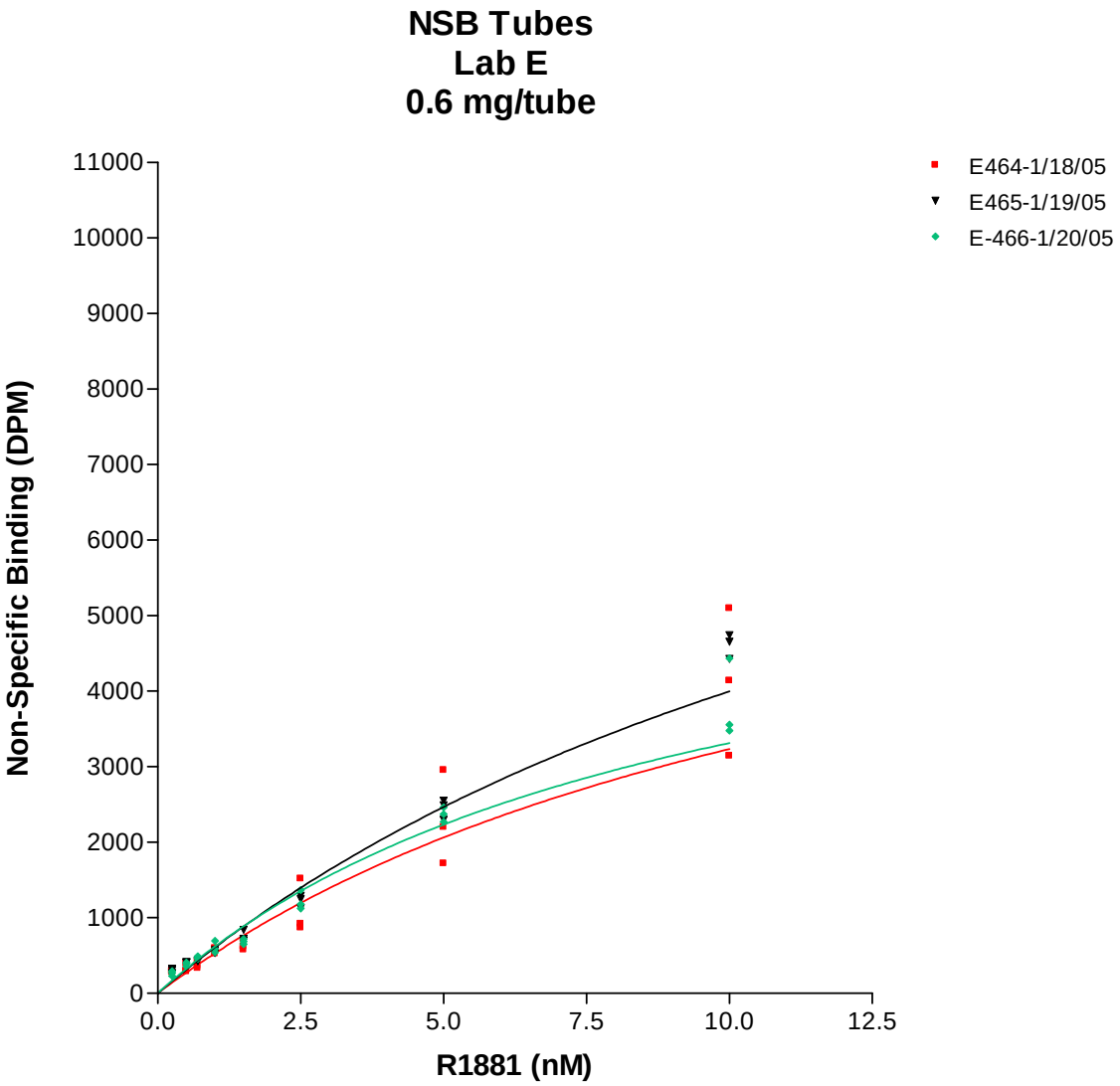
WA 4-11-4 Saturation

Lab E
0.6 mg/tube

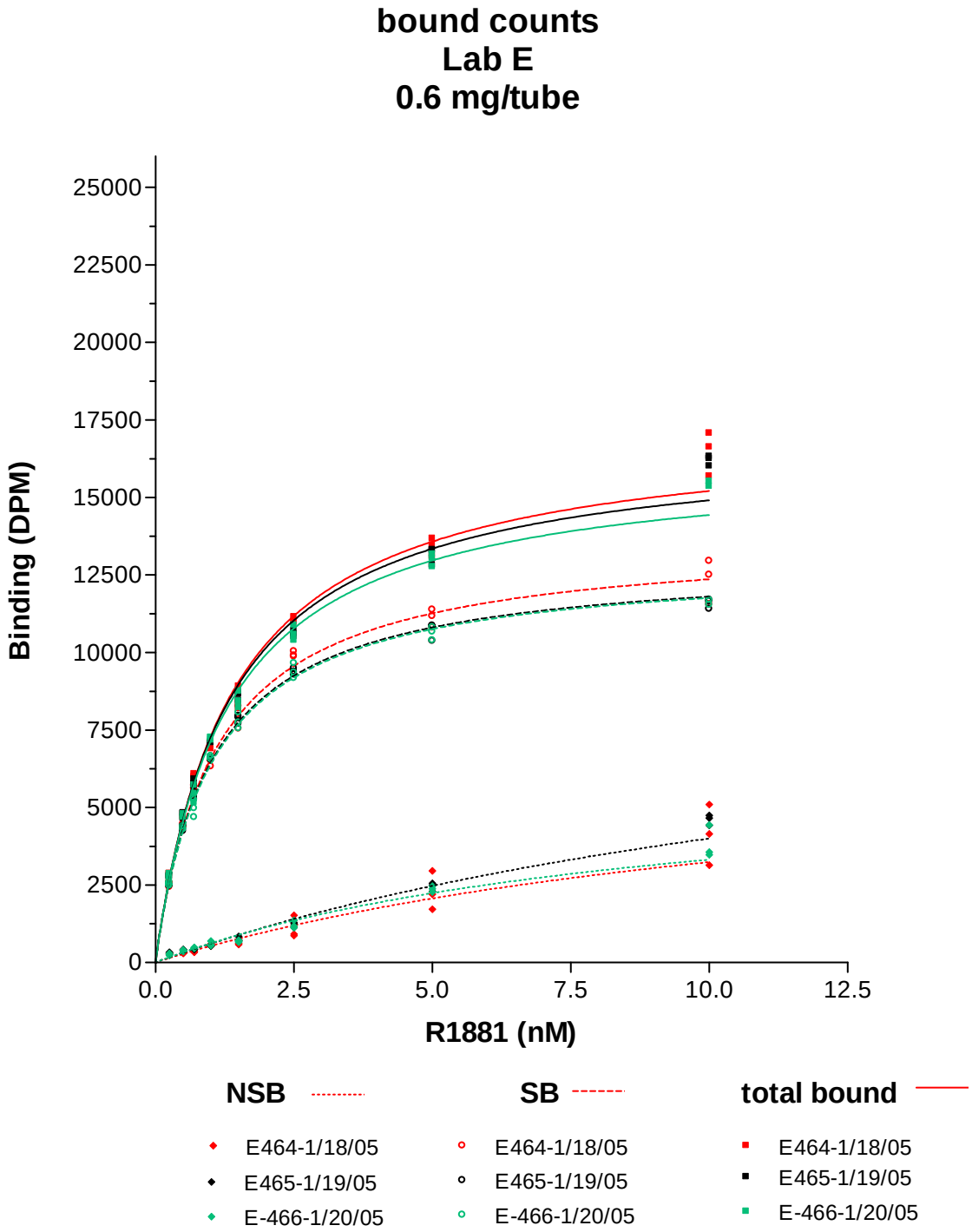


Specific bound	E464-1/18/05	E465-1/19/05	E466-1/20/05
BMAX	2.552e-010	2.417e-010	2.401e-010
KD	9.066e-010	8.443e-010	8.567e-010
Std. Error			
BMAX	4.189e-012	2.714e-012	3.165e-012
KD	2.920e-011	1.902e-011	2.223e-011
95% Confidence Intervals			
BMAX	2.465e-010 to 2.638e-010	2.361e-010 to 2.474e-010	2.335e-010 to 2.467e-010
KD	8.460e-010 to 9.671e-010	8.049e-010 to 8.838e-010	8.104e-010 to 9.029e-010
Goodness of Fit			
Degrees of Freedom	22	22	21
R^2 (unweighted)	0.9910	0.9966	0.9959
Weighted Sum of Squares (1/Y^2)	0.03069	0.01508	0.01710
Absolute Sum of Squares	7.757e-022	2.492e-022	2.778e-022
Sy.x	5.938e-012	3.366e-012	3.637e-012
Data			
Number of X values	24	24	23
Number of Y replicates	1	1	1
Total number of values	24	24	23
Number of missing values	0	0	0

WA 4-11-4 Saturation



WA 4-11-4 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 column O
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 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:	464	
Assay start date:	1/18/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.45	**Ci/mmole**
Cytosol lot or vial number:	102704	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	9.07E-01	**nM**
Bmax	12.76	**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 supernate
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	—	954.35	2863.05	TRUE		25.2%	0.25	2863.1	291.7
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	955.41	2866.23	TRUE		25.2%	0.25	2866.2	291.7
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	906.88	2720.64	TRUE		24.0%	0.25	2720.6	291.7
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1562.8	4688.4	TRUE		20.7%	0.5	4688.4	330.0
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1566.1	4698.3	TRUE		20.7%	0.5	4698.3	330.0
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1599.4	4798.2	TRUE		21.2%	0.5	4798.2	330.0
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1995.1	5985.3	TRUE		19.2%	0.7	5985.3	372.4
8	2	H	10.0	21	0.70	—	—	—	50	300	—	2027.6	6082.8	TRUE		19.5%	0.7	6082.8	372.4
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1887.9	5663.7	TRUE		18.2%	0.7	5663.7	372.4
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2367.8	7103.4	TRUE		16.1%	1	7103.4	560.5
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2295.4	6886.2	TRUE		15.6%	1	6886.2	560.5
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2361.1	7083.3	TRUE		16.0%	1	7083.3	560.5
13	1	H	10.0	45	1.50	—	—	—	50	300	—	2727.7	8183.1	TRUE		12.2%	1.5	8183.1	639.3
14	2	H	10.0	45	1.50	—	—	—	50	300	—	2795	8385	TRUE		12.5%	1.5	8385.0	639.3
15	3	H	10.0	45	1.50	—	—	—	50	300	—	2969.6	8908.8	TRUE		13.3%	1.5	8908.8	639.3
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	3657	10971	TRUE		9.6%	2.5	10971.0	1101.3
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	3713.2	11139.6	TRUE		9.7%	2.5	11139.6	1101.3
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	3664.6	10993.8	TRUE		9.6%	2.5	10993.8	1101.3
19	1	H	100.0	15	5.00	—	—	—	50	300	—	4365	13095	TRUE		5.7%	5	13095.0	2291.2
20	2	H	100.0	15	5.00	—	—	—	50	300	—	4557.1	13671.3	TRUE		5.9%	5	13671.3	2291.2
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4486.4	13459.2	TRUE		5.8%	5	13459.2	2291.2
22	1	H	100.0	30	10.00	—	—	—	50	300	—	5542	16626	TRUE		3.6%	10	16626.0	4126.2
23	2	H	100.0	30	10.00	—	—	—	50	300	—	5227.4	15682.2	TRUE		3.4%	10	15682.2	4126.2
24	3	H	100.0	30	10.00	—	—	—	50	300	—	5690.2	17070.6	TRUE		3.7%	10	17070.6	4126.2

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	95.08	285.24	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	101.62	304.86	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	94.99	284.97	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	131.49	394.47	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	96.34	289.02	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	102.14	306.42	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	110.69	332.07	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	139.99	419.97	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	121.73	365.19	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	198.72	596.16	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	187.05	561.15	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	174.77	524.31	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	206.65	619.95	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	192.67	578.01	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	240.02	720.06	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	306.03	918.09	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	289.19	867.57	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	506.03	1518.09	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	573.17	1719.51	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	733.4	2200.2	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	984.63	2953.89	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1380.5	4141.5	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1047.36	3142.08	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	1698.3	5094.9	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11407.4	11407.4	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11376.8	11376.8	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11284.1	11284.1	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	22448	22448	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	22893	22893	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	22688	22688	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	31857	31857	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	30352	30352	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	31136	31136	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	44975	44975	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	45052	45052	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	42555	42555	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	67786	67786	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	67365	67365	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	66471	66471	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	114513	114513	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	113089	113089	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	115813	115813	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	229950	229950	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	229694	229694	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	234366	234366	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	469926	469926	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	467197	467197	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	452527	452527	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)
464	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
464	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
464	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
464	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
464	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
464	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
464	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
464	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
464	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
464	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
464	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
464	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
464	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
464	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
464	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
464	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
464	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
464	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
464	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
464	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
464	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
464	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
464	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
464	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

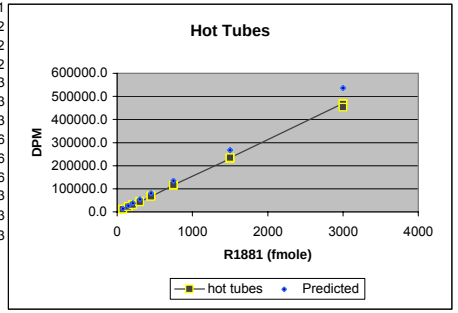
									Number of molecules					Ratio
Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific 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)	
464	1	2863.1	291.7	2571.4	10.2%	25.2%	11356.1	8493.1	19	2	17	74	55	0.30
464	2	2866.2	291.7	2574.5	10.2%	25.2%	11356.1	8489.9	19	2	17	74	55	0.30
464	3	2720.6	291.7	2429.0	10.7%	24.0%	11356.1	8635.5	18	2	16	74	56	0.28
464	4	4688.4	330.0	4358.4	7.0%	20.7%	22676.3	17987.9	30	2	28	147	117	0.24
464	5	4698.3	330.0	4368.3	7.0%	20.7%	22676.3	17978.0	30	2	28	147	117	0.24
464	6	4798.2	330.0	4468.2	6.9%	21.2%	22676.3	17878.1	31	2	29	147	116	0.25
464	7	5985.3	372.4	5612.9	6.2%	19.2%	31115.0	25129.7	39	2	36	202	163	0.22
464	8	6082.8	372.4	5710.4	6.1%	19.5%	31115.0	25032.2	39	2	37	202	162	0.23
464	9	5663.7	372.4	5291.3	6.6%	18.2%	31115.0	25451.3	37	2	34	202	165	0.21
464	10	7103.4	560.5	6542.9	7.9%	16.1%	44194.0	37090.6	46	4	42	287	241	0.18
464	11	6886.2	560.5	6325.7	8.1%	15.6%	44194.0	37307.8	45	4	41	287	242	0.17
464	12	7083.3	560.5	6522.8	7.9%	16.0%	44194.0	37110.7	46	4	42	287	241	0.18
464	13	8183.1	639.3	7543.8	7.8%	12.2%	67207.3	59024.2	53	4	49	436	383	0.13
464	14	8385.0	639.3	7745.7	7.6%	12.5%	67207.3	58822.3	54	4	50	436	382	0.13
464	15	8908.8	639.3	8269.5	7.2%	13.3%	67207.3	58298.5	58	4	54	436	378	0.14
464	16	10971.0	1101.3	9869.8	10.0%	9.6%	114471.7	103500.7	71	7	64	743	672	0.10
464	17	11139.6	1101.3	10038.4	9.9%	9.7%	114471.7	103332.1	72	7	65	743	670	0.10
464	18	10993.8	1101.3	9892.6	10.0%	9.6%	114471.7	103477.9	71	7	64	743	671	0.10
464	19	13095.0	2291.2	10803.8	17.5%	5.7%	231336.7	218241.7	85	15	70	1501	1416	0.05
464	20	13671.3	2291.2	11380.1	16.8%	5.9%	231336.7	217665.4	89	15	74	1501	1412	0.05
464	21	13459.2	2291.2	11168.0	17.0%	5.8%	231336.7	217877.5	87	15	72	1501	1414	0.05
464	22	16626.0	4126.2	12499.8	24.8%	3.6%	463216.7	446590.7	108	27	81	3005	2898	0.03
464	23	15682.2	4126.2	11556.0	26.3%	3.4%	463216.7	447534.5	102	27	75	3005	2904	0.03
464	24	17070.6	4126.2	12944.4	24.2%	3.7%	463216.7	446146.1	111	27	84	3005	2895	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)
464	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		285.2	291.7
464	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		304.9	291.7
464	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		285.0	291.7
464	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50		394.5	330.0
464	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50		289.0	330.0
464	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50		306.4	330.0
464	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70		332.1	372.4
464	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70		420.0	372.4
464	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70		365.2	372.4
464	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100		596.2	560.5
464	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100		561.2	560.5
464	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100		524.3	560.5
464	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150		620.0	639.3
464	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150		578.0	639.3
464	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150		720.1	639.3
464	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		918.1	1101.3
464	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		867.6	1101.3
464	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1518.1	1101.3
464	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500		1719.5	2291.2
464	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500		2200.2	2291.2
464	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500		2953.9	2291.2
464	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000		4141.5	4126.2
464	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000		3142.1	4126.2
464	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000		5094.9	4126.2

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
464	49	1	Hot	c1	10	7.5	75	11407.4	74	11356.1	13395
464	50	2	Hot	c1	10	7.5	75	11376.8	74	11356.1	13395
464	51	3	Hot	c1	10	7.5	75	11284.1	73	11356.1	13395
464	52	1	Hot	c2	10	15	150	22448.0	146	22676.3	26791
464	53	2	Hot	c2	10	15	150	22893.0	149	22676.3	26791
464	54	3	Hot	c2	10	15	150	22688.0	147	22676.3	26791
464	55	1	Hot	c3	10	21	210	31857.0	207	31115.0	37507
464	56	2	Hot	c3	10	21	210	30352.0	197	31115.0	37507
464	57	3	Hot	c3	10	21	210	31136.0	202	31115.0	37507
464	58	1	Hot	c4	10	30	300	44975.0	292	44194.0	53581
464	59	2	Hot	c4	10	30	300	45052.0	292	44194.0	53581
464	60	3	Hot	c4	10	30	300	42555.0	276	44194.0	53581
464	61	1	Hot	c5	10	45	450	67786.0	440	67207.3	80372
464	62	2	Hot	c5	10	45	450	67365.0	437	67207.3	80372
464	63	3	Hot	c5	10	45	450	66471.0	431	67207.3	80372
464	64	1	Hot	c6	100	7.5	750	114513.0	743	114471.7	133953
464	65	2	Hot	c6	100	7.5	750	113089.0	734	114471.7	133953
464	66	3	Hot	c6	100	7.5	750	115813.0	751	114471.7	133953
464	67	1	Hot	c7	100	15	1500	229950.0	1492	231336.7	267906
464	68	2	Hot	c7	100	15	1500	229694.0	1490	231336.7	267906
464	69	3	Hot	c7	100	15	1500	234366.0	1521	231336.7	267906
464	70	1	Hot	c8	100	30	3000	469926.0	3049	463216.7	535813
464	71	2	Hot	c8	100	30	3000	467197.0	3031	463216.7	535813
464	72	3	Hot	c8	100	30	3000	452527.0	2936	463216.7	535813

Computation Check	
1/18/05	specific activity date
80.45	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7860E+14	DPM/mmole
1.7860E+11	DPM/nmole
178.6	DPM/fmole
0.005599	fmole/DPM



Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	154.1235838	dpm/fmole
1/slope	0.0064883	fmole/dpm
	x	y
origin	0	0
end point	3049.0	469926
SLOPE function, used if missing HOT tubes		
Slope	154.8	dpm/fmole
1/slope	0.006458	fmole/dpm
	x	y
origin	0	0
end point	3035.0	469926

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
4.79899E-11	0.30276	2.11942E-10	4.79899E-11
4.80493E-11	0.30325	2.11942E-10	4.80493E-11
4.53321E-11	0.28128	2.11942E-10	4.53321E-11
8.13424E-11	0.24230	4.23214E-10	8.13424E-11
8.15272E-11	0.24298	4.23214E-10	8.15272E-11
8.33917E-11	0.24993	4.23214E-10	8.33917E-11
1.04755E-10	0.22336	5.80707E-10	1.04755E-10
1.06574E-10	0.22812	5.80707E-10	1.06574E-10
9.87526E-11	0.20790	5.80707E-10	9.87526E-11
1.22111E-10	0.17640	8.24803E-10	1.22111E-10
1.18057E-10	0.16955	8.24803E-10	1.18057E-10
1.21736E-10	0.17576	8.24803E-10	1.21736E-10
1.40791E-10	0.12781	1.25431E-09	1.40791E-10
1.44559E-10	0.13168	1.25431E-09	1.44559E-10
1.54335E-10	0.14185	1.25431E-09	1.54335E-10
1.84202E-10	0.09536	2.13641E-09	1.84202E-10
1.87348E-10	0.09715	2.13641E-09	1.87348E-10
1.84627E-10	0.09560	2.13641E-09	1.84627E-10
2.01634E-10	0.04950	4.31749E-09	2.01634E-10
2.1239E-10	0.05228	4.31749E-09	2.1239E-10
2.08431E-10	0.05126	4.31749E-09	2.08431E-10
2.33288E-10	0.02799	8.64512E-09	2.33288E-10
2.15673E-10	0.02582	8.64512E-09	2.15673E-10
2.41585E-10	0.02901	8.64512E-09	2.41585E-10

Bmax molar	2.55E-10	KD molar	9.07E-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 =**	9.07E-01
Bmax molar to Bmax moles	7.6548E-14		
= DPM/((DPM/mmole)*1000)	7.6548E-14		
=Bmax DPM	13671.7945		
assay date	1/18/2005		
Bmax(dpm)	13671.7945		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.45		
DPM/mmole	1.79E+14		
DPM/pmole	1.79E+05		
1/(DPM/mmole)	5.60E-15		
1/(DPM/pmole)	5.60E-06		
SA(dpm/pmole)	1.79E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.076548		
bmax pmole/mg	0.12758		
Bmax fmole/mg	127.58		
Bmax (fmole/100 ug)	12.758		
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 column O
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 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:	465	
Assay start date:	1/19/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.44	**Ci/mmole**
Cytosol lot or vial number:	102704	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	8.44E-01	**nM**
Bmax	12.09	**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 supernate
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	—	927.03	2781.09	TRUE		24.7%	0.25	2781.1	300.6
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	923.13	2769.39	TRUE		24.6%	0.25	2769.4	300.6
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	934.09	2802.27	TRUE		24.9%	0.25	2802.3	300.6
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1608.7	4826.1	TRUE		21.3%	0.5	4826.1	409.6
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1549	4647	TRUE		20.5%	0.5	4647.0	409.6
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1602.3	4806.9	TRUE		21.2%	0.5	4806.9	409.6
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1897.5	5692.5	TRUE		18.6%	0.7	5692.5	428.7
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1971.2	5913.6	TRUE		19.3%	0.7	5913.6	428.7
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1928	5784	TRUE		18.9%	0.7	5784.0	428.7
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2368.3	7104.9	TRUE		15.8%	1	7104.9	548.0
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2372.7	7118.1	TRUE		15.9%	1	7118.1	548.0
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2362.2	7086.6	TRUE		15.8%	1	7086.6	548.0
13	1	H	10.0	45	1.50	—	—	—	50	300	—	2883.7	8651.1	TRUE		13.1%	1.5	8651.1	752.8
14	2	H	10.0	45	1.50	—	—	—	50	300	—	2816.2	8448.6	TRUE		12.8%	1.5	8448.6	752.8
15	3	H	10.0	45	1.50	—	—	—	50	300	—	2901.9	8705.7	TRUE		13.2%	1.5	8705.7	752.8
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	3533.7	10601.1	TRUE		9.2%	2.5	10601.1	1221.0
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	3568.3	10704.9	TRUE		9.3%	2.5	10704.9	1221.0
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	3502.6	10507.8	TRUE		9.1%	2.5	10507.8	1221.0
19	1	H	100.0	15	5.00	—	—	—	50	300	—	4436.1	13308.3	TRUE		5.8%	5	13308.3	2445.4
20	2	H	100.0	15	5.00	—	—	—	50	300	—	4428.5	13285.5	TRUE		5.8%	5	13285.5	2445.4
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4272	12816	TRUE		5.6%	5	12816.0	2445.4
22	1	H	100.0	30	10.00	—	—	—	50	300	—	5418.2	16254.6	TRUE		3.5%	10	16254.6	4609.1
23	2	H	100.0	30	10.00	—	—	—	50	300	—	5438.3	16314.9	TRUE		3.5%	10	16314.9	4609.1
24	3	H	100.0	30	10.00	—	—	—	50	300	—	5336.9	16010.7	TRUE		3.5%	10	16010.7	4609.1

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	102.35	307.05	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	108.64	325.92	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	89.61	268.83	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	136.61	409.83	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	137.11	411.33	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	135.91	407.73	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	143.42	430.26	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	140.86	422.58	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	144.41	433.23	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	191.41	574.23	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	178.61	535.83	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	178.02	534.06	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	232.32	696.96	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	240.57	721.71	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	279.86	839.58	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	415.98	1247.94	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	375.49	1126.47	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	429.55	1288.65	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	850.1	2550.3	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	765.57	2296.71	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	829.77	2489.31	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1476.2	4428.6	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1580.5	4741.5	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	1552.4	4657.2	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11566.6	11566.6	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11243.8	11243.8	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	10971.8	10971.8	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	22080	22080	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	22853	22853	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	23044	23044	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	30527	30527	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	30880	30880	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	30483	30483	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	44646	44646	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	45337	45337	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	44561	44561	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	66242	66242	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	65883	65883	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	66191	66191	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	116907	116907	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	115949	115949	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	114276	114276	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	232812	232812	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	228162	228162	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	230543	230543	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	456139	456139	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	462118	462118	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	470591	470591	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)
465	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
465	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
465	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
465	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
465	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
465	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
465	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
465	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
465	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
465	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
465	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
465	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
465	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
465	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
465	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
465	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
465	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
465	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
465	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
465	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
465	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
465	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
465	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
465	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

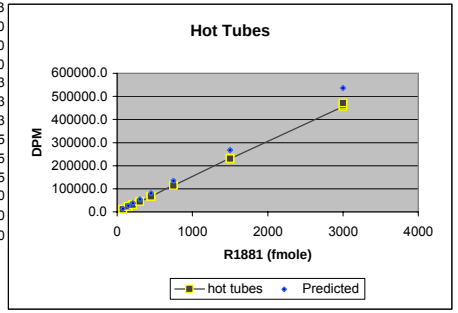
									Number of molecules					Ratio
Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific 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)	
465	1	2781.1	300.6	2480.5	10.8%	24.7%	11260.7	8479.6	18	2	16	73	55	0.29
465	2	2769.4	300.6	2468.8	10.9%	24.6%	11260.7	8491.3	18	2	16	73	55	0.29
465	3	2802.3	300.6	2501.7	10.7%	24.9%	11260.7	8458.5	18	2	16	73	55	0.30
465	4	4826.1	409.6	4416.5	8.5%	21.3%	22659.0	17832.9	31	3	29	147	116	0.25
465	5	4647.0	409.6	4237.4	8.8%	20.5%	22659.0	18012.0	30	3	28	147	117	0.24
465	6	4806.9	409.6	4397.3	8.5%	21.2%	22659.0	17852.1	31	3	29	147	116	0.25
465	7	5692.5	428.7	5263.8	7.5%	18.6%	30630.0	24937.5	37	3	34	199	162	0.21
465	8	5913.6	428.7	5484.9	7.2%	19.3%	30630.0	24716.4	38	3	36	199	160	0.22
465	9	5784.0	428.7	5355.3	7.4%	18.9%	30630.0	24846.0	38	3	35	199	161	0.22
465	10	7104.9	548.0	6556.9	7.7%	15.8%	44848.0	37743.1	46	4	43	291	245	0.17
465	11	7118.1	548.0	6570.1	7.7%	15.9%	44848.0	37729.9	46	4	43	291	245	0.17
465	12	7086.6	548.0	6538.6	7.7%	15.8%	44848.0	37761.4	46	4	42	291	245	0.17
465	13	8651.1	752.8	7898.4	8.7%	13.1%	66105.3	57454.2	56	5	51	429	373	0.14
465	14	8448.6	752.8	7695.9	8.9%	12.8%	66105.3	57656.7	55	5	50	429	374	0.13
465	15	8705.7	752.8	7953.0	8.6%	13.2%	66105.3	57399.6	57	5	52	429	373	0.14
465	16	10601.1	1221.0	9380.1	11.5%	9.2%	115710.7	105109.6	69	8	61	751	683	0.09
465	17	10704.9	1221.0	9483.9	11.4%	9.3%	115710.7	105005.8	70	8	62	751	682	0.09
465	18	10507.8	1221.0	9286.8	11.6%	9.1%	115710.7	105202.9	68	8	60	751	683	0.09
465	19	13308.3	2445.4	10862.9	18.4%	5.8%	230505.7	217197.4	86	16	71	1497	1410	0.05
465	20	13285.5	2445.4	10840.1	18.4%	5.8%	230505.7	217220.2	86	16	70	1497	1411	0.05
465	21	12816.0	2445.4	10370.6	19.1%	5.6%	230505.7	217689.7	83	16	67	1497	1414	0.05
465	22	16254.6	4609.1	11645.5	28.4%	3.5%	462949.3	446694.7	106	30	76	3006	2901	0.03
465	23	16314.9	4609.1	11705.8	28.3%	3.5%	462949.3	446634.4	106	30	76	3006	2900	0.03
465	24	16010.7	4609.1	11401.6	28.8%	3.5%	462949.3	446938.6	104	30	74	3006	2902	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)
465	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		307.1	300.6
465	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		325.9	300.6
465	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		268.8	300.6
465	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50		409.8	409.6
465	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50		411.3	409.6
465	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50		407.7	409.6
465	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70		430.3	428.7
465	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70		422.6	428.7
465	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70		433.2	428.7
465	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100		574.2	548.0
465	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100		535.8	548.0
465	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100		534.1	548.0
465	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150		697.0	752.8
465	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150		721.7	752.8
465	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150		839.6	752.8
465	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1247.9	1221.0
465	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1126.5	1221.0
465	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1288.7	1221.0
465	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500		2550.3	2445.4
465	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500		2296.7	2445.4
465	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500		2489.3	2445.4
465	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000		4428.6	4609.1
465	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000		4741.5	4609.1
465	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000		4657.2	4609.1

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
465	49	1	Hot	c1	10	7.5	75	11566.6	75	11260.7	13393
465	50	2	Hot	c1	10	7.5	75	11243.8	73	11260.7	13393
465	51	3	Hot	c1	10	7.5	75	10971.8	71	11260.7	13393
465	52	1	Hot	c2	10	15	150	22080.0	143	22659.0	26787
465	53	2	Hot	c2	10	15	150	22853.0	148	22659.0	26787
465	54	3	Hot	c2	10	15	150	23044.0	150	22659.0	26787
465	55	1	Hot	c3	10	21	210	30527.0	198	30630.0	37501
465	56	2	Hot	c3	10	21	210	30880.0	201	30630.0	37501
465	57	3	Hot	c3	10	21	210	30483.0	198	30630.0	37501
465	58	1	Hot	c4	10	30	300	44646.0	290	44848.0	53573
465	59	2	Hot	c4	10	30	300	45337.0	294	44848.0	53573
465	60	3	Hot	c4	10	30	300	44561.0	289	44848.0	53573
465	61	1	Hot	c5	10	45	450	66242.0	430	66105.3	80360
465	62	2	Hot	c5	10	45	450	65883.0	428	66105.3	80360
465	63	3	Hot	c5	10	45	450	66191.0	430	66105.3	80360
465	64	1	Hot	c6	100	7.5	750	116907.0	759	115710.7	133933
465	65	2	Hot	c6	100	7.5	750	115949.0	753	115710.7	133933
465	66	3	Hot	c6	100	7.5	750	114276.0	742	115710.7	133933
465	67	1	Hot	c7	100	15	1500	232812.0	1512	230505.7	267865
465	68	2	Hot	c7	100	15	1500	228162.0	1482	230505.7	267865
465	69	3	Hot	c7	100	15	1500	230543.0	1497	230505.7	267865
465	70	1	Hot	c8	100	30	3000	456139.0	2962	462949.3	535730
465	71	2	Hot	c8	100	30	3000	462118.0	3001	462949.3	535730
465	72	3	Hot	c8	100	30	3000	470591.0	3056	462949.3	535730

Computation Check	
1/19/05	specific activity date
80.44	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7858E+14	DPM/mmole
1.7858E+11	DPM/nmole
178.6	DPM/fmole
0.005600	fmole/DPM



Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	153.997894	dpm/fmole
1/slope	0.006493595	fmole/dpm
	x	y
origin	0	0
end point	3055.8	470591

SLOPE function, used if missing HOT tubes		
Slope	154.7	dpm/fmole
1/slope	0.006463	fmole/dpm
	x	y
origin	0	0
end point	3041.5	470591

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
4.63011E-11	0.29252	2.10194E-10	4.63011E-11
4.60827E-11	0.29074	2.10194E-10	4.60827E-11
4.66964E-11	0.29576	2.10194E-10	4.66964E-11
8.24383E-11	0.24766	4.22955E-10	8.24383E-11
7.90952E-11	0.23525	4.22955E-10	7.90952E-11
8.20799E-11	0.24632	4.22955E-10	8.20799E-11
9.82549E-11	0.21108	5.71743E-10	9.82549E-11
1.02382E-10	0.22191	5.71743E-10	1.02382E-10
9.99628E-11	0.21554	5.71743E-10	9.99628E-11
1.22391E-10	0.17372	8.37138E-10	1.22391E-10
1.22637E-10	0.17413	8.37138E-10	1.22637E-10
1.22049E-10	0.17315	8.37138E-10	1.22049E-10
1.47431E-10	0.13747	1.23393E-09	1.47431E-10
1.43652E-10	0.13348	1.23393E-09	1.43652E-10
1.48451E-10	0.13855	1.23393E-09	1.48451E-10
1.7509E-10	0.08924	2.15987E-09	1.7509E-10
1.77027E-10	0.09032	2.15987E-09	1.77027E-10
1.73348E-10	0.08827	2.15987E-09	1.73348E-10
2.02767E-10	0.05001	4.30264E-09	2.02767E-10
2.02342E-10	0.04990	4.30264E-09	2.02342E-10
1.93578E-10	0.04764	4.30264E-09	1.93578E-10
2.17376E-10	0.02607	8.64146E-09	2.17376E-10
2.18502E-10	0.02621	8.64146E-09	2.18502E-10
2.12824E-10	0.02551	8.64146E-09	2.12824E-10

Bmax molar	2.42E-10	KD molar	8.44E-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 =**	8.44E-01
Bmax molar to Bmax moles	7.2522E-14		
= DPM/((DPM/mmole)*1000)	7.2522E-14		
=Bmax DPM	12950.74377		
assay date	1/19/2005		
Bmax(dpm)	12950.74377		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.44		
DPM/mmole	1.79E+14		
DPM/pmole	1.79E+05		
1/(DPM/mmole)	5.60E-15		
1/(DPM/pmole)	5.60E-06		
SA(dpm/pmole)	1.79E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.072522		
bmax pmole/mg	0.12087		
Bmax fmole/mg	120.87		
Bmax (fmole/100 ug)	12.087		
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 column O
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 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:	466	
Assay start date:	1/20/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.43	**Ci/mmole**
Cytosol lot or vial number:	102704	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	8.57E-01	**nM**
Bmax	12.01	**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 supernate
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	—	954.89	2864.67	TRUE		25.2%	0.25	2864.7	264.3
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	929.03	2787.09	TRUE		24.5%	0.25	2787.1	264.3
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	915.63	2746.89	TRUE		24.1%	0.25	2746.9	264.3
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1594.8	4784.4	TRUE		20.6%	0.5	4784.4	386.4
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1562.4	4687.2	TRUE		20.2%	0.5	4687.2	386.4
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1565.7	4697.1	TRUE		20.2%	0.5	4697.1	386.4
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1815.1	5445.3	TRUE		18.5%	0.7	5445.3	474.2
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1906.4	5719.2	TRUE		19.4%	0.7	5719.2	474.2
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1720.1	5160.3	TRUE		17.5%	0.7	5160.3	474.2
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2419.8	7259.4	TRUE		15.8%	1	7259.4	607.9
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2391.1	7173.3	TRUE		15.6%	1	7173.3	607.9
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2418.6	7255.8	TRUE		15.8%	1	7255.8	607.9
13	1	H	10.0	45	1.50	—	—	—	50	300	—	2745.9	8237.7	TRUE		11.8%	1.5	8237.7	686.5
14	2	H	10.0	45	1.50	—	—	—	50	300	—	2807.8	8423.4	TRUE		12.0%	1.5	8423.4	686.5
15	3	H	10.0	45	1.50	—	—	—	50	300	—	2916.2	8748.6	TRUE		12.5%	1.5	8748.6	686.5
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	3464.4	10393.2	TRUE		8.9%	2.5	10393.2	1223.6
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	3627	10881	TRUE		9.3%	2.5	10881.0	1223.6
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	3523.9	10571.7	TRUE		9.0%	2.5	10571.7	1223.6
19	1	H	100.0	15	5.00	—	—	—	50	300	—	4346.3	13038.9	TRUE		5.5%	5	13038.9	2369.2
20	2	H	100.0	15	5.00	—	—	—	50	300	—	4391.7	13175.1	TRUE		5.6%	5	13175.1	2369.2
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4253.6	12760.8	TRUE		5.4%	5	12760.8	2369.2
22	1	H	100.0	30	10.00	—	—	—	50	300	—	5118.3	15354.9	TRUE		3.3%	10	15354.9	3822.2
23	2	H	100.0	30	10.00	—	—	—	50	300	—	5172.9	15518.7	TRUE		3.4%	10	15518.7	3822.2
24	3	H	100.0	30	10.00	—	—	—	50	300	—	6456.9	19370.7	FALSE	over spike		10		3822.2

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	76.85	230.55	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	89.71	269.13	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	97.75	293.25	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	118.99	356.97	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	131.55	394.65	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	135.84	407.52	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	157.28	471.84	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	164.04	492.12	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	152.85	458.55	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	180.27	540.81	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	195.33	585.99	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	232.28	696.84	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	228.74	686.22	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	217.62	652.86	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	240.09	720.27	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	374.36	1123.08	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	455.97	1367.91	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	393.29	1179.87	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	821.05	2463.15	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	791.21	2373.63	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	756.98	2270.94	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1477.8	4433.4	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1185.2	3555.6	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	1159.2	3477.6	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11469.5	11469.5	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11324.9	11324.9	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11355.8	11355.8	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23083	23083	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	23469	23469	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	23103	23103	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	29262	29262	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	30036	30036	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	28945	28945	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	46904	46904	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	45555	45555	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	45378	45378	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	70334	70334	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	70368	70368	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	69169	69169	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	116526	116526	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	116313	116313	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	119192	119192	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	231824	231824	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	241297	241297	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	237414	237414	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	468100	468100	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	462550	462550	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	458242	458242	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)
466	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
466	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
466	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
466	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
466	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
466	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
466	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
466	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
466	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
466	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
466	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
466	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
466	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
466	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
466	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
466	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
466	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
466	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
466	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
466	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
466	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
466	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
466	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
466	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)	
466	1	2864.7	264.3	2600.4	9.2%	25.2%	11383.4	8518.7	18	2	17	73	55	0.31
466	2	2787.1	264.3	2522.8	9.5%	24.5%	11383.4	8596.3	18	2	16	73	55	0.29
466	3	2746.9	264.3	2482.6	9.6%	24.1%	11383.4	8636.5	18	2	16	73	56	0.29
466	4	4784.4	386.4	4398.0	8.1%	20.6%	23218.3	18433.9	31	2	28	150	119	0.24
466	5	4687.2	386.4	4300.8	8.2%	20.2%	23218.3	18531.1	30	2	28	150	120	0.23
466	6	4697.1	386.4	4310.7	8.2%	20.2%	23218.3	18521.2	30	2	28	150	119	0.23
466	7	5445.3	474.2	4971.1	8.7%	18.5%	29414.3	23969.0	35	3	32	190	155	0.21
466	8	5719.2	474.2	5245.0	8.3%	19.4%	29414.3	23695.1	37	3	34	190	153	0.22
466	9	5160.3	474.2	4686.1	9.2%	17.5%	29414.3	24254.0	33	3	30	190	156	0.19
466	10	7259.4	607.9	6651.5	8.4%	15.8%	45945.7	38686.3	47	4	43	296	250	0.17
466	11	7173.3	607.9	6565.4	8.5%	15.6%	45945.7	38772.4	46	4	42	296	250	0.17
466	12	7255.8	607.9	6647.9	8.4%	15.8%	45945.7	38689.9	47	4	43	296	250	0.17
466	13	8237.7	686.5	7551.3	8.3%	11.8%	69957.0	61719.3	53	4	49	451	398	0.12
466	14	8423.4	686.5	7737.0	8.1%	12.0%	69957.0	61533.6	54	4	50	451	397	0.13
466	15	8748.6	686.5	8062.2	7.8%	12.5%	69957.0	61208.4	56	4	52	451	395	0.13
466	16	10393.2	1223.6	9169.6	11.8%	8.9%	117343.7	106950.5	67	8	59	757	690	0.09
466	17	10881.0	1223.6	9657.4	11.2%	9.3%	117343.7	106462.7	70	8	62	757	687	0.09
466	18	10571.7	1223.6	9348.1	11.6%	9.0%	117343.7	106772.0	68	8	60	757	689	0.09
466	19	13038.9	2369.2	10669.7	18.2%	5.5%	236845.0	223806.1	84	15	69	1528	1444	0.05
466	20	13175.1	2369.2	10805.9	18.0%	5.6%	236845.0	223669.9	85	15	70	1528	1443	0.05
466	21	12760.8	2369.2	10391.6	18.6%	5.4%	236845.0	224084.2	82	15	67	1528	1445	0.05
466	22	15354.9	3822.2	11532.7	24.9%	3.3%	462964.0	447609.1	99	25	74	2986	2887	0.03
466	23	15518.7	3822.2	11696.5	24.6%	3.4%	462964.0	447445.3	100	25	75	2986	2886	0.03
466	24		3822.2				462964.0			25	-25	2986		

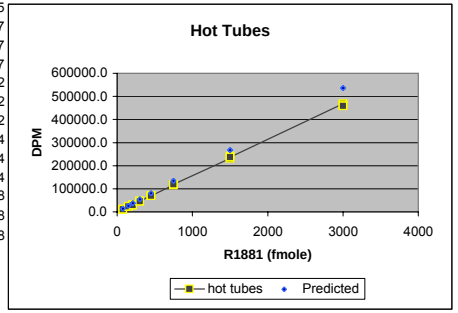
		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol													
		Tube Identification			Assay tube contents									Scintillation Results	
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	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)
466	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		230.6	264.3
466	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		269.1	264.3
466	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		293.3	264.3
466	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50		357.0	386.4
466	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50		394.7	386.4
466	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50		407.5	386.4
466	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70		471.8	474.2
466	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70		492.1	474.2
466	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70		458.6	474.2
466	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100		540.8	607.9
466	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100		586.0	607.9
466	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100		696.8	607.9
466	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150		686.2	686.5
466	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150		652.9	686.5
466	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150		720.3	686.5
466	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1123.1	1223.6
466	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1367.9	1223.6
466	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1179.9	1223.6
466	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500		2463.2	2369.2
466	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500		2373.6	2369.2
466	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500		2270.9	2369.2
466	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000		4433.4	3822.2
466	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000		3555.6	3822.2
466	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000		3477.6	3822.2

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
466	49	1	Hot	c1	10	7.5	75	11469.5	74	11383.4	13391
466	50	2	Hot	c1	10	7.5	75	11324.9	73	11383.4	13391
466	51	3	Hot	c1	10	7.5	75	11355.8	73	11383.4	13391
466	52	1	Hot	c2	10	15	150	23083.0	149	23218.3	26782
466	53	2	Hot	c2	10	15	150	23469.0	151	23218.3	26782
466	54	3	Hot	c2	10	15	150	23103.0	149	23218.3	26782
466	55	1	Hot	c3	10	21	210	29262.0	189	29414.3	37495
466	56	2	Hot	c3	10	21	210	30036.0	194	29414.3	37495
466	57	3	Hot	c3	10	21	210	28945.0	187	29414.3	37495
466	58	1	Hot	c4	10	30	300	46904.0	303	45945.7	53565
466	59	2	Hot	c4	10	30	300	45555.0	294	45945.7	53565
466	60	3	Hot	c4	10	30	300	45378.0	293	45945.7	53565
466	61	1	Hot	c5	10	45	450	70334.0	454	69957.0	80347
466	62	2	Hot	c5	10	45	450	70368.0	454	69957.0	80347
466	63	3	Hot	c5	10	45	450	69169.0	446	69957.0	80347
466	64	1	Hot	c6	100	7.5	750	116526.0	752	117343.7	133912
466	65	2	Hot	c6	100	7.5	750	116313.0	750	117343.7	133912
466	66	3	Hot	c6	100	7.5	750	119192.0	769	117343.7	133912
466	67	1	Hot	c7	100	15	1500	231824.0	1495	236845.0	267824
466	68	2	Hot	c7	100	15	1500	241297.0	1556	236845.0	267824
466	69	3	Hot	c7	100	15	1500	237414.0	1531	236845.0	267824
466	70	1	Hot	c8	100	30	3000	468100.0	3019	462964.0	535648
466	71	2	Hot	c8	100	30	3000	462550.0	2983	462964.0	535648
466	72	3	Hot	c8	100	30	3000	458242.0	2956	462964.0	535648

Computation Check

1/20/05	specific activity date
80.43	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7855E+14	DPM/mmole
1.7855E+11	DPM/nmole
178.5	DPM/fmole
0.005601	fmole/DPM



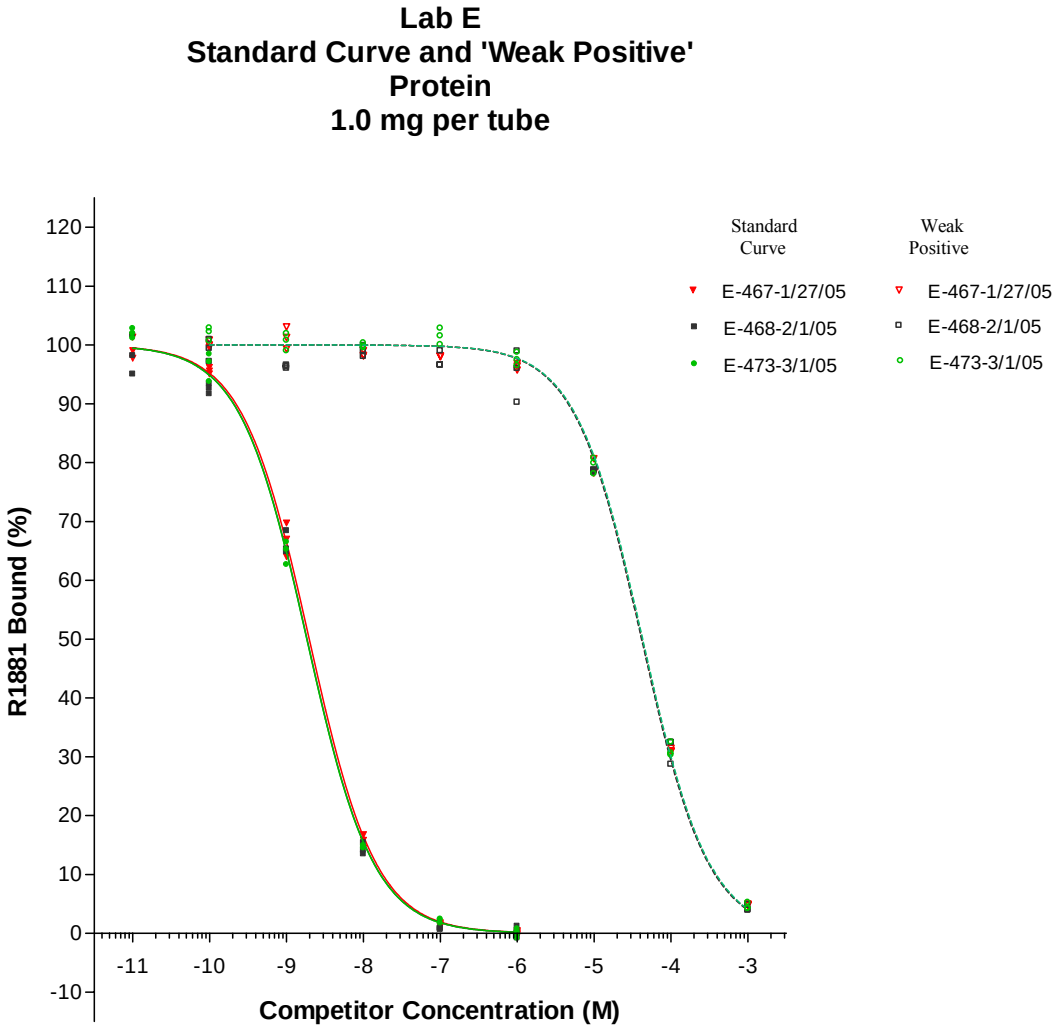
Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	155.039027	dpm/fmole
1/slope	0.006449989	fmole/dpm
	x	y
origin	0	0
end point	3019.2	468100
SLOPE function, used if missing HOT tubes		
Slope	155.1	dpm/fmole
1/slope	0.006447	fmole/dpm
	x	y
origin	0	0
end point	3017.9	468100

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
4.85461E-11	0.30525	2.12516E-10	4.85461E-11
4.70977E-11	0.29347	2.12516E-10	4.70977E-11
4.63472E-11	0.28745	2.12516E-10	4.63472E-11
8.21065E-11	0.23858	4.33463E-10	8.21065E-11
8.02919E-11	0.23209	4.33463E-10	8.02919E-11
8.04767E-11	0.23274	4.33463E-10	8.04767E-11
9.28059E-11	0.20740	5.49136E-10	9.28059E-11
9.79193E-11	0.22135	5.49136E-10	9.79193E-11
8.74853E-11	0.19321	5.49136E-10	8.74853E-11
1.24177E-10	0.17193	8.57759E-10	1.24177E-10
1.2257E-10	0.16933	8.57759E-10	1.2257E-10
1.2411E-10	0.17183	8.57759E-10	1.2411E-10
1.40974E-10	0.12235	1.30603E-09	1.40974E-10
1.44441E-10	0.12574	1.30603E-09	1.44441E-10
1.50512E-10	0.13172	1.30603E-09	1.50512E-10
1.71187E-10	0.08574	2.19069E-09	1.71187E-10
1.80293E-10	0.09071	2.19069E-09	1.80293E-10
1.74519E-10	0.08755	2.19069E-09	1.74519E-10
1.99192E-10	0.04767	4.42165E-09	1.99192E-10
2.01734E-10	0.04831	4.42165E-09	2.01734E-10
1.94E-10	0.04637	4.42165E-09	1.94E-10
2.15304E-10	0.02577	8.64306E-09	2.15304E-10
2.18362E-10	0.02614	8.64306E-09	2.18362E-10
		8.64306E-09	

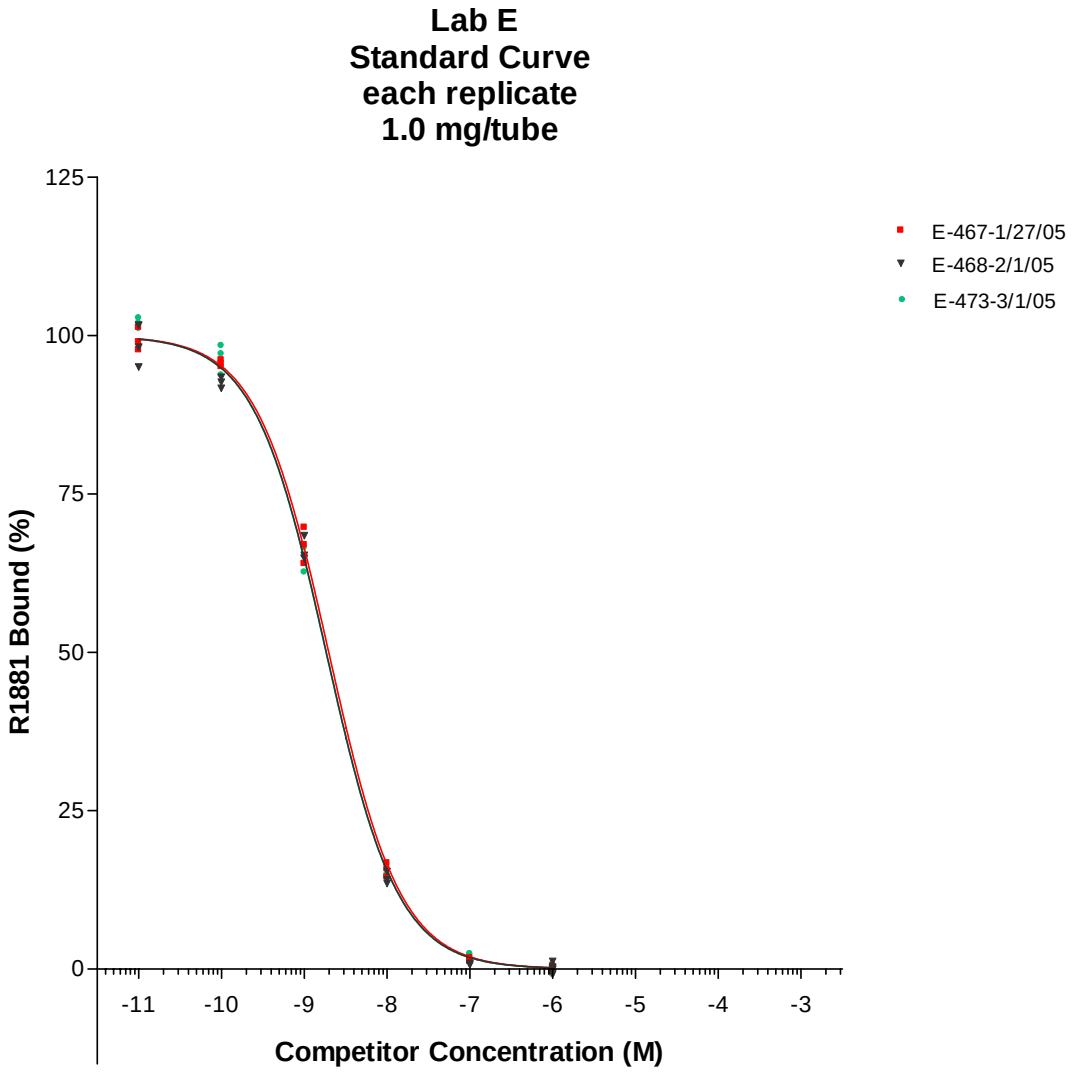
Bmax molar	2.40E-10	KD molar	8.57E-10
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.79E+17	**kd nM =**	8.57E-01
Bmax molar to Bmax moles	7.2039E-14		
= DPM/((DPM/mmole)*1000)	7.2039E-14		
=Bmax DPM	12862.51455		
assay date	1/20/2005		
Bmax(dpm)	12862.51455		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.43		
DPM/mmole	1.79E+14		
DPM/pmole	1.79E+05		
1/(DPM/mmole)	5.60E-15		
1/(DPM/pmole)	5.60E-06		
SA(dpm/pmole)	1.79E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.072039		
bmax pmole/mg	0.120065		
Bmax fmole/mg	120.065		
Bmax (fmole/100 ug)	12.0065		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Appendix 4: Prism Files for Competitive Binding Experiments

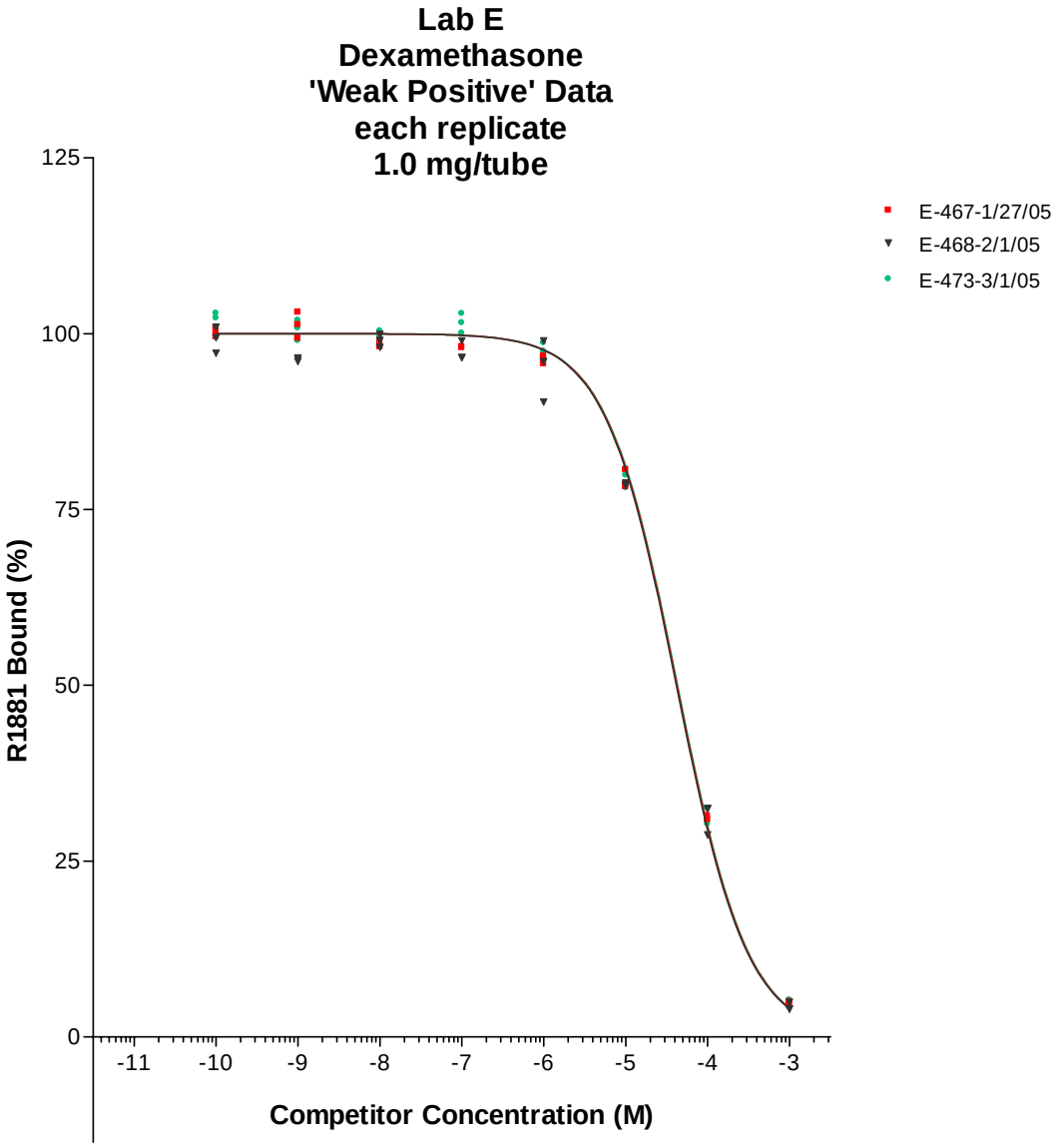
WA 4-11-4 Competitive



WA 4-11-4 Competitive



WA 4-11-4 Competitive



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 column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Change the "TRUE" in column Q to "FALSE"

Provide a reason in column R

Columns T and U contain values to be analyzed by nonlinear regression software

Provide information in all blue cells in this column

Laboratory Code:	**E**	
Run identification:	467	
Assay start date:	1/27/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.34	**Ci/mmole**
Cytosol vial or lot identification:		
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.98E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	4.24E-05	**M**
RBA:	4.67E-05	

volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 13 will contain output parameters from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values	n	Mean	SD	
EtOH	6	10477.9	267.08	Total Binding, solvent control, tubes
Hot	6	44979.7	893.18	Total hot R1881 added to each tube
NSB	6	506.4	35.29	Nonspecific Binding
Specific EtOH	6	9971.5	267.08	

Assay Characterization Values		
EtOH / Hot	0.23	less than 0.1?
NSB / EtOH	0.05	around 0.25 ?

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

Check the 10% rule:	23.29%	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-E supplied by Battelle to laboratory "E"	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	3266.00	10124.6	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3425.00	10617.5	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3392.60	10517.06	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	151.17	453.51	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	178.62	535.86	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	182.94	548.82	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	209.77	650.287	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	201.09	623.379	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	219.47	680.357	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	628.52	1948.412	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	671.03	2080.193	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	701.97	2176.107	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2317.90	7185.49	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2406.40	7459.84	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2222.10	6888.51	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3237.80	10037.18	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3221.80	9987.58	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3256.40	10094.84	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3348.70	10380.97	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3307.90	10254.49	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3422.70	10610.37	TRUE	

Position	Replicate		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)	Ratio Total binding/ Hot
				Values for analysis by nonlinear regression					
1	1		—	96.5	10124.6	9618.2	-153.1	96.5	22.50928
2	2		—	101.4	10617.5	10111.1	-646.0	101.4	23.60511
3	3		—	100.4	10517.06	10010.6	-545.6	100.4	23.38181
4	1		-6.0	-0.5	453.51	-52.9	9517.9	-0.5	1.008256
5	2		-6.0	0.3	535.86	29.4	9435.6	0.3	1.191338
6	3		-6.0	0.0	548.82	42.4	9422.6	0.4	1.220151
7	1	cold R1881	-7.0	1.4	650.287	143.8	9321.2	1.4	1.445735
8	2	cold R1881	-7.0	1.2	623.379	116.9	9348.1	1.2	1.385913
9	3	cold R1881	-7.0	1.7	680.357	173.9	9291.1	1.7	1.512588
10	1	cold R1881	-8.0	14.5	1948.412	1442.0	8023.0	14.5	4.331762
11	2	cold R1881	-8.0	15.8	2080.193	1573.7	7891.3	15.8	4.624741
12	3	cold R1881	-8.0	16.7	2176.107	1669.7	7795.3	16.7	4.837979
13	1	cold R1881	-9.0	67.0	7185.49	6679.0	2786.0	67.0	15.97497
14	2	cold R1881	-9.0	69.7	7459.84	6953.4	2511.6	69.7	16.58492
15	3	cold R1881	-9.0	64.0	6888.51	6382.1	3082.9	64.0	15.31472
16	1	cold R1881	-10.0	95.6	10037.18	9530.7	-65.7	95.6	22.31493
17	2	cold R1881	-10.0	95.1	9987.58	9481.1	-16.1	95.1	22.20466
18	3	cold R1881	-10.0	96.2	10094.84	9588.4	-123.4	96.2	22.44312
19	1	cold R1881	-11.0	99.0	10380.97	9874.5	-409.5	99.0	23.07925
20	2	cold R1881	-11.0	97.8	10254.49	9748.0	-283.0	97.8	22.79806
21	3	cold R1881	-11.0	101.3	10610.37	10103.9	-638.9	101.3	23.58926

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

Check the 10% rule:	23.29%	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-E supplied by Battelle to laboratory "E"	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	313.56	972.036	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	323.73	1003.563	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	319.29	989.799	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1173.80	3638.78	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1157.10	3587.01	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1159.90	3595.69	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2758.10	8550.11	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2679.10	8305.21	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2689.00	8335.9	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3264.30	10119.33	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3277.40	10159.94	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3242.60	10052.06	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3318.50	10287.35	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3316.20	10280.22	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3320.20	10292.62	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3349.10	10382.21	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3323.00	10301.3	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3319.50	10290.45	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3478.40	10783.04	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3421.50	10606.65	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3358.40	10411.04	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3366.20	10435.22	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3380.50	10479.55	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3406.60	10560.46	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3404.70	10554.57	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3293.70	10210.47	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3497.80	10843.18	TRUE	
49	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	166.67	500.01	TRUE	
50	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	172.73	518.19	TRUE	
51	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	160.76	482.28	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		46037.00	46037.00	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		43727.00	43727.00	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		44506.00	44506.00	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		45643.00	45643.00	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		44418.00	44418.00	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		45547.00	45547.00	TRUE	

Values for analysis by nonlinear regression

Position	Replicate		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)	Ratio Total binding/ Hot
22	1	Weak Positive	-3.0	4.7	972.036	465.6	8999.4	4.7	2.161056
23	2	Weak Positive	-3.0	5.0	1003.563	497.1	8967.9	5.0	2.231148
24	3	Weak Positive	-3.0	4.8	989.799	483.4	8981.7	4.8	2.200548
25	1	Weak Positive	-4.0	31.4	3638.78	3132.3	6332.7	31.4	8.089833
26	2	Weak Positive	-4.0	30.9	3587.01	3080.6	6384.4	30.9	7.974737
27	3	Weak Positive	-4.0	31.0	3595.69	3089.2	6375.8	31.0	7.994034
28	1	Weak Positive	-5.0	80.7	8550.11	8043.7	1421.3	80.7	19.00883
29	2	Weak Positive	-5.0	78.2	8305.21	7798.8	1666.2	78.2	18.46437
30	3	Weak Positive	-5.0	78.5	8335.9	7829.5	1635.6	78.5	18.5326
31	1	Weak Positive	-6.0	96.4	10119.33	9612.9	-147.9	96.4	22.49757
32	2	Weak Positive	-6.0	96.8	10159.94	9653.5	-188.5	96.8	22.58785
33	3	Weak Positive	-6.0	95.7	10052.06	9545.6	-80.6	95.7	22.34801
34	1	Weak Positive	-7.0	98.1	10287.35	9780.9	-315.9	98.1	22.87111
35	2	Weak Positive	-7.0	98.0	10280.22	9773.8	-308.8	98.0	22.85526
36	3	Weak Positive	-7.0	98.1	10292.62	9786.2	-321.2	98.1	22.88283
37	1	Weak Positive	-8.0	99.0	10382.21	9875.8	-410.8	99.0	23.08201
38	2	Weak Positive	-8.0	98.2	10301.3	9794.9	-329.8	98.2	22.90213
39	3	Weak Positive	-8.0	98.1	10290.45	9784.0	-319.0	98.1	22.878
40	1	Weak Positive	-9.0	103.1	10783.04	10276.6	-811.6	103.1	23.97314
41	2	Weak Positive	-9.0	101.3	10606.65	10100.2	-635.2	101.3	23.58099
42	3	Weak Positive	-9.0	99.3	10411.04	9904.6	-439.6	99.3	23.1461
43	1	Weak Positive	-10.0	99.6	10435.22	9928.8	-463.8	99.6	23.19986
44	2	Weak Positive	-10.0	100.0	10479.55	9973.1	-508.1	100.0	23.29842
45	3	Weak Positive	-10.0	100.8	10560.46	10054.0	-589.0	100.8	23.4783
46	1		—	100.8	10554.57	10048.1	-583.1	100.8	23.4652
47	2		—	97.3	10210.47	9704.0	-239.0	97.3	22.70019
48	3		—	103.7	10843.18	10336.7	-871.7	103.7	24.10685
49	1		-6.0	-0.1	500.01	-6.4	9471.4	-0.1	1.111636
50	2		-6.0	0.1	518.19	11.7	9453.3	0.1	1.152054
51	3		-6.0	-0.2	482.28	-24.2	9489.2	-0.2	1.072218
52	1				46037	45530.6			
53	2				43727	43220.6			
54	3				44506	43999.6			
55	1				45643	45136.6			
56	2				44418	43911.6			
57	3				45547	45040.6			

Prism data

standard curve				weak positive			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC
-6.0	-0.53087	0.29499	0.42496	-3.0	4.6692	4.9854	4.8474
-6.0	-0.06453	0.11779	-0.24234	-4.0	31.4130	30.8938	30.9809
-7.0	1.44254	1.17269	1.74410	-5.0	80.6669	78.2109	78.5187
-8.0	14.46095	15.78254	16.74442	-6.0	96.4041	96.8113	95.7294
-9.0	66.98167	69.73303	64.00337	-7.0	98.0891	98.0176	98.1419
-10.0	95.58022	95.08280	96.15847	-8.0	99.0404	98.2290	98.1202
-11.0	99.02796	97.75954	101.32853	-9.0	103.0602	101.2912	99.3295
				-10.0	99.5720	100.0166	100.8280

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 column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Change the "TRUE" in column Q to "FALSE"

Provide a reason in column R

Columns T and U contain values to be analyzed by nonlinear regression software

Provide information in all blue cells in this column

Laboratory Code:	**E**	
Run identification:	468	
Assay start date:	2/1/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.28	**Ci/mmole**
Cytosol vial or lot identification:		
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.85E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	4.16E-05	**M**
RBA:	4.44E-05	

volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 13 will contain output parameters from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values				
	n	Mean	SD	
EtOH	6	10355.8	172.65	Total Binding, solvent control, tubes
Hot	6	45804.0	803.69	Total hot R1881 added to each tube
NSB	6	654.9	68.14	Nonspecific Binding
Specific EtOH	6	9700.9	172.65	

Assay Characterization Values		
EtOH / Hot	0.23	less than 0.1?
NSB / EtOH	0.06	around 0.25 ?

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

Check the 10% rule:	22.61%	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-E supplied by Battelle to laboratory "E"*	*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	3285.20	10184.12	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3370.20	10447.62	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3378.60	10473.66	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	199.34	598.02	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	213.80	641.4	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	192.24	576.72	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	230.78	715.418	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	239.48	742.388	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	235.53	730.143	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	693.03	2148.393	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	633.31	1963.261	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	652.35	2022.285	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2256.00	6993.6	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2352.80	7293.68	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2239.90	6943.69	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3081.00	9551.1	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3111.10	9644.41	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3135.60	9720.36	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3393.00	10518.3	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3185.50	9875.05	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3283.00	10177.3	TRUE	

Position	Replicate		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)	Ratio Total binding/ Hot
			Values for analysis by nonlinear regression						
1	1		—	98.2	10184.12	9529.3	-483.2	98.2	22.23413
2	2		—	100.9	10447.62	9792.8	-746.7	100.9	22.80941
3	3		—	101.2	10473.66	9818.8	-772.7	101.2	22.86626
4	1		-6.0	-0.6	598.02	-56.8	9102.9	-0.6	1.305606
5	2		-6.0	-0.1	641.4	-13.5	9059.5	-0.1	1.400314
6	3		-6.0	0.0	576.72	-78.1	9124.2	-0.8	1.259104
7	1	cold R1881	-7.0	0.6	715.418	60.6	8985.5	0.6	1.561912
8	2	cold R1881	-7.0	0.9	742.388	87.5	8958.6	0.9	1.620793
9	3	cold R1881	-7.0	0.8	730.143	75.3	8970.8	0.8	1.594059
10	1	cold R1881	-8.0	15.4	2148.393	1493.5	7552.6	15.4	4.690405
11	2	cold R1881	-8.0	13.5	1963.261	1308.4	7737.7	13.5	4.286222
12	3	cold R1881	-8.0	14.1	2022.285	1367.4	7678.7	14.1	4.415084
13	1	cold R1881	-9.0	65.3	6993.6	6338.7	2707.3	65.3	15.26854
14	2	cold R1881	-9.0	68.4	7293.68	6638.8	2407.3	68.4	15.92367
15	3	cold R1881	-9.0	64.8	6943.69	6288.8	2757.3	64.8	15.15957
16	1	cold R1881	-10.0	91.7	9551.1	8896.2	149.8	91.7	20.85211
17	2	cold R1881	-10.0	92.7	9644.41	8989.6	56.5	92.7	21.05582
18	3	cold R1881	-10.0	93.4	9720.36	9065.5	-19.4	93.4	21.22164
19	1	cold R1881	-11.0	101.7	10518.3	9863.4	-817.4	101.7	22.96371
20	2	cold R1881	-11.0	95.0	9875.05	9220.2	-174.1	95.0	21.55936
21	3	cold R1881	-11.0	98.2	10177.3	9522.4	-476.4	98.2	22.21924

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

Check the 10% rule:	22.61%	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-E supplied by Battelle to laboratory "E"	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	334.23	1036.113	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	364.86	1131.066	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	336.43	1042.933	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1227.30	3804.63	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1225.20	3798.12	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1109.70	3440.07	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2670.50	8278.55	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2675.60	8294.36	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2663.70	8257.47	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3308.80	10257.28	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3035.50	9410.05	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3215.90	9969.29	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3233.10	10022.61	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3234.30	10026.33	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3309.10	10258.21	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3336.70	10343.77	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3279.30	10165.83	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3309.60	10259.76	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3226.60	10002.46	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3232.80	10021.68	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3216.80	9972.08	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3322.40	10299.44	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3252.30	10082.13	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3369.80	10446.38	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3379.50	10476.45	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3254.60	10089.26	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3375.40	10463.74	TRUE	
49	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	220.98	662.94	TRUE	
50	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	256.17	768.51	TRUE	
51	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	227.19	681.57	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		47201.00	47201.00	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		44911.00	44911.00	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		45158.00	45158.00	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		45858.00	45858.00	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		45680.00	45680.00	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		46016.00	46016.00	TRUE	

Position	Replicate		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)	Ratio Total binding/ Hot
			Values for analysis by nonlinear regression						
22	1	Weak Positive	-3.0	3.9	1036.113	381.3	8664.8	3.9	2.262058
23	2	Weak Positive	-3.0	4.9	1131.066	476.2	8569.9	4.9	2.469361
24	3	Weak Positive	-3.0	4.0	1042.933	388.1	8658.0	4.0	2.276947
25	1	Weak Positive	-4.0	32.5	3804.63	3149.8	5896.3	32.5	8.306327
26	2	Weak Positive	-4.0	32.4	3798.12	3143.3	5902.8	32.4	8.292114
27	3	Weak Positive	-4.0	28.7	3440.07	2785.2	6260.9	28.7	7.510414
28	1	Weak Positive	-5.0	78.6	8278.55	7623.7	1422.4	78.6	18.07386
29	2	Weak Positive	-5.0	78.8	8294.36	7639.5	1406.6	78.8	18.10837
30	3	Weak Positive	-5.0	78.4	8257.47	7602.6	1443.5	78.4	18.02784
31	1	Weak Positive	-6.0	99.0	10257.28	9602.4	-556.3	99.0	22.39385
32	2	Weak Positive	-6.0	90.3	9410.05	8755.2	290.9	90.3	20.54417
33	3	Weak Positive	-6.0	96.0	9969.29	9314.4	-268.3	96.0	21.76511
34	1	Weak Positive	-7.0	96.6	10022.61	9367.8	-321.7	96.6	21.88152
35	2	Weak Positive	-7.0	96.6	10026.33	9371.5	-325.4	96.6	21.88964
36	3	Weak Positive	-7.0	99.0	10258.21	9603.4	-557.3	99.0	22.39588
37	1	Weak Positive	-8.0	99.9	10343.77	9688.9	-642.8	99.9	22.58268
38	2	Weak Positive	-8.0	98.0	10165.83	9511.0	-464.9	98.0	22.1942
39	3	Weak Positive	-8.0	99.0	10259.76	9604.9	-558.8	99.0	22.39927
40	1	Weak Positive	-9.0	96.4	10002.46	9347.6	-301.5	96.4	21.83753
41	2	Weak Positive	-9.0	96.6	10021.68	9366.8	-320.7	96.6	21.87949
42	3	Weak Positive	-9.0	96.0	9972.08	9317.2	-271.1	96.0	21.7712
43	1	Weak Positive	-10.0	99.4	10299.44	9644.6	-598.5	99.4	22.4859
44	2	Weak Positive	-10.0	97.2	10082.13	9427.3	-381.2	97.2	22.01146
45	3	Weak Positive	-10.0	100.9	10446.38	9791.5	-745.4	100.9	22.8067
46	1		—	101.2	10476.45	9821.6	-775.5	101.2	22.87235
47	2		—	97.3	10089.26	9434.4	-388.3	97.3	22.02703
48	3		—	101.1	10463.74	9808.9	-762.8	101.1	22.8446
49	1		-6.0	0.1	662.94	8.1	9038.0	0.1	1.447341
50	2		-6.0	1.2	768.51	113.7	8932.4	1.2	1.677823
51	3		-6.0	0.3	681.57	26.7	9019.4	0.3	1.488014
52	1				47201	46546.1			
53	2				44911	44256.1			
54	3				45158	44503.1			
55	1				45858	45203.1			
56	2				45680	45025.1			
57	3				46016	45361.1			

Prism data

standard curve				weak positive			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	***concentration (log)***	y1-PC	y2-PC	y3-PC
-6.0	-0.58592	-0.13875	-0.80549	-3.0	3.9301	4.9089	4.0004
-6.0	0.08329	1.17153	0.27533	-4.0	32.4687	32.4016	28.7107
-7.0	0.62425	0.90226	0.77604	-5.0	78.5871	78.7500	78.3698
-8.0	15.39574	13.48735	14.09579	-6.0	98.9843	90.2509	96.0157
-9.0	65.34145	68.43475	64.82696	-7.0	96.5653	96.6036	98.9939
-10.0	91.70485	92.66671	93.44963	-8.0	99.8759	98.0417	99.0099
-11.0	101.67501	95.04421	98.15989	-9.0	96.3576	96.5557	96.0444
				-10.0	99.4189	97.1788	100.9336

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 column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Change the "TRUE" in column Q to "FALSE"

Provide a reason in column R

Columns T and U contain values to be analyzed by nonlinear regression software

Provide information in all blue cells in this column

Laboratory Code:	**E**	
Run identification:	473	
Assay start date:	3/1/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.93	**Ci/mmole**
Cytosol vial or lot identification:	102705	
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.84E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	4.30E-05	**M**
RBA:	4.27E-05	

volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH s
mulitply DPM in sample by :	3		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 13 will contain output parameters from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values				
	n	Mean	SD	
EtOH	6	10883.5	252.97	Total Binding, solvent control, tubes
Hot	6	43457.5	412.01	Total hot R1881 added to each tube
NSB	6	550.2	56.34	Nonspecific Binding
Specific EtOH	6	10333.3	252.97	

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

supernate

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

Check the 10% rule:	25.04%	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-E supplied by Battelle to laboratory "E"	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	3425.10	10617.81	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3460.40	10727.24	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3432.60	10641.06	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	179.59	538.77	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	210.02	630.06	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	193.77	581.31	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	234.09	725.679	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	253.99	787.369	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	258.17	800.327	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	674.09	2089.679	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	660.03	2046.093	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	666.29	2065.499	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2266.70	7026.77	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2352.50	7292.75	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2394.70	7423.57	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3415.00	10586.5	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3458.40	10721.04	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3303.30	10240.23	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3603.90	11172.09	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3549.10	11002.21	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3576.80	11088.08	TRUE	

Position	Replicate		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)	Ratio Total binding/ Hot
			Values for analysis by nonlinear regression						
1	1		—	97.4	10617.81	10067.6	-284.5	97.4	24.43263
2	2		—	98.5	10727.24	10177.0	-393.9	98.5	24.68444
3	3		—	97.7	10641.06	10090.9	-307.7	97.7	24.48613
4	1		-6.0	-0.1	538.77	-11.4	9794.6	-0.1	1.239763
5	2		-6.0	0.8	630.06	79.9	9703.3	0.8	1.44983
6	3		-6.0	0.0	581.31	31.1	9752.0	0.3	1.337652
7	1	cold R1881	-7.0	1.7	725.679	175.5	9607.6	1.7	1.669859
8	2	cold R1881	-7.0	2.3	787.369	237.2	9546.0	2.3	1.811814
9	3	cold R1881	-7.0	2.4	800.327	250.1	9533.0	2.4	1.841631
10	1	cold R1881	-8.0	14.9	2089.679	1539.5	8243.6	14.9	4.808558
11	2	cold R1881	-8.0	14.5	2046.093	1495.9	8287.2	14.5	4.708262
12	3	cold R1881	-8.0	14.7	2065.499	1515.3	8267.8	14.7	4.752917
13	1	cold R1881	-9.0	62.7	7026.77	6476.6	3306.6	62.7	16.16929
14	2	cold R1881	-9.0	65.3	7292.75	6742.5	3040.6	65.3	16.78134
15	3	cold R1881	-9.0	66.5	7423.57	6873.4	2909.8	66.5	17.08237
16	1	cold R1881	-10.0	97.1	10586.5	10036.3	-253.2	97.1	24.36058
17	2	cold R1881	-10.0	98.4	10721.04	10170.8	-387.7	98.4	24.67017
18	3	cold R1881	-10.0	93.8	10240.23	9690.0	93.1	93.8	23.56378
19	1	cold R1881	-11.0	102.8	11172.09	10621.9	-838.8	102.8	25.70808
20	2	cold R1881	-11.0	101.1	11002.21	10452.0	-668.9	101.1	25.31717
21	3	cold R1881	-11.0	102.0	11088.08	10537.9	-754.8	102.0	25.51477

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

Check the 10% rule:	25.04%	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-E supplied by Battelle to laboratory "E"	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	320.72	994.232	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	351.66	1090.146	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	317.21	983.351	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1198.20	3714.42	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1261.00	3909.1	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1186.60	3678.46	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2840.90	8806.79	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2869.90	8896.69	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2781.00	8621.1	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3427.50	10625.25	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3393.50	10519.85	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3468.50	10752.35	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3562.50	11043.75	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3606.20	11179.22	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3512.80	10889.68	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3523.10	10921.61	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3495.60	10836.36	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3503.70	10861.47	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3574.40	11080.64	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3537.30	10965.63	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3478.00	10781.8	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3606.90	11181.39	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3532.50	10950.75	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3585.50	11115.05	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3554.60	11019.26	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3616.40	11210.84	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3575.80	11084.98	TRUE	
49	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	187.41	562.23	TRUE	
50	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	154.19	462.57	TRUE	
51	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	175.44	526.32	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		42948.00	42948.00	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		43425.00	43425.00	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		44132.00	44132.00	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		43627.00	43627.00	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		43135.00	43135.00	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		43478.00	43478.00	TRUE	

Position	Replicate		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)	Ratio Total binding/ Hot
			Values for analysis by nonlinear regression						
22	1	Weak Positive	-3.0	4.3	994.232	444.0	9339.1	4.3	2.287826
23	2	Weak Positive	-3.0	5.2	1090.146	539.9	9243.2	5.2	2.508534
24	3	Weak Positive	-3.0	4.2	983.351	433.1	9350.0	4.2	2.262788
25	1	Weak Positive	-4.0	30.6	3714.42	3164.2	6618.9	30.6	8.547247
26	2	Weak Positive	-4.0	32.5	3909.1	3358.9	6424.2	32.5	8.995225
27	3	Weak Positive	-4.0	30.3	3678.46	3128.3	6654.9	30.3	8.4645
28	1	Weak Positive	-5.0	79.9	8806.79	8256.6	1526.5	79.9	20.26529
29	2	Weak Positive	-5.0	80.8	8896.69	8346.5	1436.6	80.8	20.47216
30	3	Weak Positive	-5.0	78.1	8621.1	8070.9	1712.2	78.1	19.838
31	1	Weak Positive	-6.0	97.5	10625.25	10075.0	-291.9	97.5	24.44975
32	2	Weak Positive	-6.0	96.5	10519.85	9969.6	-186.5	96.5	24.20721
33	3	Weak Positive	-6.0	98.7	10752.35	10202.1	-419.0	98.7	24.74222
34	1	Weak Positive	-7.0	101.6	11043.75	10493.5	-710.4	101.6	25.41276
35	2	Weak Positive	-7.0	102.9	11179.22	10629.0	-845.9	102.9	25.72449
36	3	Weak Positive	-7.0	100.1	10889.68	10339.5	-556.4	100.1	25.05823
37	1	Weak Positive	-8.0	100.4	10921.61	10371.4	-588.3	100.4	25.1317
38	2	Weak Positive	-8.0	99.5	10836.36	10286.2	-503.0	99.5	24.93553
39	3	Weak Positive	-8.0	99.8	10861.47	10311.3	-528.1	99.8	24.99332
40	1	Weak Positive	-9.0	101.9	11080.64	10530.4	-747.3	101.9	25.49765
41	2	Weak Positive	-9.0	100.8	10965.63	10415.4	-632.3	100.8	25.233
42	3	Weak Positive	-9.0	99.0	10781.8	10231.6	-448.5	99.0	24.80999
43	1	Weak Positive	-10.0	102.9	11181.39	10631.2	-848.1	102.9	25.72948
44	2	Weak Positive	-10.0	100.7	10950.75	10400.5	-617.4	100.7	25.19876
45	3	Weak Positive	-10.0	102.2	11115.05	10564.8	-781.7	102.2	25.57683
46	1		—	101.3	11019.26	10469.1	-685.9	101.3	25.35641
47	2		—	103.2	11210.84	10660.6	-877.5	103.2	25.79725
48	3		—	101.9	11084.98	10534.8	-751.7	101.9	25.50763
49	1		-6.0	0.1	562.23	12.0	9771.1	0.1	1.293747
50	2		-6.0	-0.8	462.57	-87.6	9870.8	-0.8	1.064419
51	3		-6.0	-0.2	526.32	-23.9	9807.0	-0.2	1.211114
52	1				42948	42397.8			
53	2				43425	42874.8			
54	3				44132	43581.8			
55	1				43627	43076.8			
56	2				43135	42584.8			
57	3				43478	42927.8			

Prism data

standard curve			
concentration (log)	Y1-SC	Y2-SC	Y3-SC
-6.0	-0.11071	0.77274	0.30097
-6.0	0.11632	-0.84813	-0.23119
-7.0	1.69809	2.29509	2.42049
-8.0	14.89810	14.47630	14.66410
-9.0	62.67646	65.25046	66.51646
-10.0	97.12550	98.42750	93.77449
-11.0	102.79250	101.14850	101.97950

weak positive			
concentration (log)	y1-PC	y2-PC	y3-PC
-3.0	4.2970	5.2252	4.1917
-4.0	30.6214	32.5054	30.2734
-5.0	79.9025	80.7725	78.1055
-6.0	97.5005	96.4805	98.7305
-7.0	101.5505	102.8615	100.0595
-8.0	100.3685	99.5435	99.7865
-9.0	101.9075	100.7945	99.0155
-10.0	102.8825	100.6505	102.2405