DRAFT REPORT

Validation of an Androgen Receptor Binding Assay–Task 6

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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
Quality Assurance :	Mary Lynn
	Janet Cloutier

Study Dates and Data Retention

Study initiation date:	26 September, 2005
Experimental start date:	08 November, 2005
Experimental end date:	22 December, 2005

Richland, Battelle will retain all supporting documentation, including raw data and written records, for a period of up to five years following submission of the final report to 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, dexamethasone and some of the test chemicals 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:

Mary Lynn

Janet Cloutier

Quality Assurance	Signature	Date

Glossary of Abbreviations

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

Summary

The objective of this study was to provide data to establish laboratory variability among independent laboratories using the same androgen receptor (AR) assay when each laboratory prepared their own cytosol. 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 a series of competitive binding 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. Saturation binding assays were performed under Task 5 and 7 to establish the acceptability of the prepared cytosol. For this task only competitive binding experiments were conducted using the identified cytosols.

In task 5, a total of three saturation experiments were conducted and the amount of cytosolic protein used for each incubation in the saturation binding experiments was 0.6 mg. The K_d observed across the three saturation experiments was 0.930, 0.834, and 0.926 nM and the B_{max} observed was 14.40, 12.26, and 15.58 fmoles/100ug of protein.
In task 7, 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 0.92, 0.90, and 0.92 nM and the B_{max} observed was 11.85, 11.34, and 11.11 fmoles/100 ug.

A series of competitive binding experiments were conducted using 1.0 mg of cytosolic protein for each assay tube. The IC_{50} values for R1881 were determined to be 1.52, 1.77, 1.46, 1.77, 1.90 and 1.69 nM. The IC_{50} values for dexamethasone were determined to be 29.5, 28.4, 43.4, 40.0, 28.3, and 33.6 µM. The RBA values for dexamethasone in comparison to R1881 were, 0.0051, 0.0062, 0.0034, 0.0044, 0.0067 and 0.0050 %.

For the unknown substances the following data was derived from the series of competitive assays in which each unknown was measured in three separate assay runs.

The IC_{50} values for CR42400 were determined to be 1.61, 2.05 and 2.27 x10^{-9}M. The RBA values for CR424000 in comparison to R1881 were 94.17, 86.31, and 64.23 %.

The IC_{50} values for CR42401 were determined to be 7.22, 9.57 and 9.22 x10^{-9}M. The RBA values for CR42401 in comparison to R1881 were 21.01, 18.51 and 15.80 %.

The IC_{50} values for CR42402 were determined to be 3.05, 2.84 and 4.14 x10^{-7}M. The RBA values for CR42402 in comparison to R1881 were 0.4975, 0.6238 and 0.3514%.

The IC_{50} values for CR42403 were determined to be 1.74, 1.49 and 1.68 x10^{-8}M. The RBA values for CR42403 in comparison to R1881 were 10.15, 12.69 and 10.11 %.

The IC_{50} values for CR42404 were determined to be 7.41, 9.46 and 1.02 $X10^{-4}$M. The RBA values for CR42404 in comparison to R1881 were 0.0020, 0.0019 and 0.0014 %.

The IC_{50} values for CR42405 were determined to be 1.56, 1.56 and 1.84 $x10^{-5}$M. The RBA values for CR42405 in comparison to R1881 were 0.0098, 0.0114 and 0.0079 %.

The IC_{50} values for CR42406 were determined to be 1.33, 0.90 and 1.20 $x10^{-7}$M. The RBA values for CR42406 in comparison to R1881 were 1.33, 2.11 and 1.41 %.

The IC_{50} values for CR42407 were determined to be 2.34, 2.30 and 2.51 $X10^{-7}$M. The RBA values for CR42407 in comparison to R1881 were 0.7549, 0.8254 and 0.6734 %.

The following test substance did not exhibit any significant inhibition of the binding of [^{3}H] R1881 to the androgen receptor.

The IC_{50} and RBA values for CR42408 were not determined because of insufficient activity.
The IC_{50} and RBA values for CR42409 were not determined because of insufficient activity.

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:

- 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 (DTT) (Lot# 51K17412), glycerol (Lot# 10655KA and 03240LC), Sodium molybdate (Lot # 30K0210) and phenylmethylsulfonyl fluoride (PMSF) (Lot# 60K0701)- (TEDG + PMSF Buffer) (pH 7.4)

- Triamcinolone acetonide (TRIAM) (Sigma; Lot # 40K1266)
- Absolute ethanol (AAPER ; Lot# 03K10UA)

- Hydroxylapatite (HAP) (lot# 251604 and 210001176)

The following reagent was obtained from supplier as identified:

- Substrate: ^{3}H-R1881 (CAS No. 965-93-5) (Perkin-Elmer Cat# NET 590; Lot # 3559-507; 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 of the R1881 stocks.
Dilutions of the unlabeled R1881 stock were prepared in ethanol for the saturation assays at 1.0 x 10^{-6} and 1.0 x 10^{-5} M. In the assay, 7.5 ul, 15.0 ul, 21.0 ul, 30.0 and 45.0 ul of the 1.0 x 10^{-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^{-6} 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.

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 with the protein (BSA) standards.

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}). (See data generated for cytosol preparation for Task 5 and 7 in Figure 1).

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 (trace, 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 acceptable data obtained were reported.

Results

Rat ventral prostate cytosols were prepared by Battelle, Richland at a protein concentration of 5.532 and 4.280 mg/mL. A series of competitive binding experiments were conducted using these cytosol preparations.

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. In task 5, the K_d observed across the three saturation experiments being reported was 0.930, 0.834, and 0.926 nM and the B_{max} observed was 14.40, 12.26, and 15.58 fmoles/100ug of protein. In Task 7, the K_d observed across the three saturation experiments was 0.92, 0.90, and 0.92 nM and the B_{max} observed was 11.85, 11.34, and 11.11 fmoles/100 ug.

A series of competitive binding experiments were conducted using 1.0 mg of cytosolic protein for each assay tube. The IC_{50} values for R1881 were determined to be 1.52, 1.77, 1.46, 1.77, 1.90 and 1.69 nM. The IC_{50} values for dexamethasone were determined to be 29.5, 28.4, 43.4, 40.0, 28.3, and 33.6 µM. The RBA values for dexamethasone in comparison to R1881 were, 0.0051, 0.0062, 0.0034, 0.0044, 0.0067 and 0.0050 %.

For the unknown substances the following data was derived from the series of competitive assays in which each unknown was measured in three separate assay runs.

The IC_{50} values for CR42400 were determined to be 1.61, 2.05 and 2.27 $x10^{-9}$M. The RBA values for CR42400 in comparison to R1881 were 94.17, 86.31, and 64.23 %.

The IC_{50} values for CR42401 were determined to be 7.22, 9.57 and 9.22 $x10^{-9}$M. The RBA values for CR42401 in comparison to R1881 were 21.01, 18.51 and 15.80 %.

The IC_{50} values for CR42402 were determined to be 3.05, 2.84 and 4.14 $x10^{-7}$M. The RBA values for CR42402 in comparison to R1881 were 0.4975, 0.6238 and 0.3514%.

The IC_{50} values for CR42403 were determined to be 1.74, 1.49 and 1.68 $x10^{-8}$M. The RBA values for CR42403 in comparison to R1881 were 10.15, 12.69 and 10.11 %.

The IC_{50} values for CR42404 were determined to be 7.41, 9.46 and 1.02 $X10^{-4}$M. The RBA values for CR42404 in comparison to R1881 were 0.0020, 0.0019 and 0.0014 %.

The IC_{50} values for CR42405 were determined to be 1.56, 1.56 and 1.84 $x10^{-5}$M. The RBA values for CR42405 in comparison to R1881 were 0.0098, 0.0114 and 0.0079 %.

The IC_{50} values for CR42406 were determined to be 1.33, 0.90 and 1.20 $x10^{-7}$M. The RBA values for CR42406 in comparison to R1881 were 1.33, 2.11 and 1.41 %.

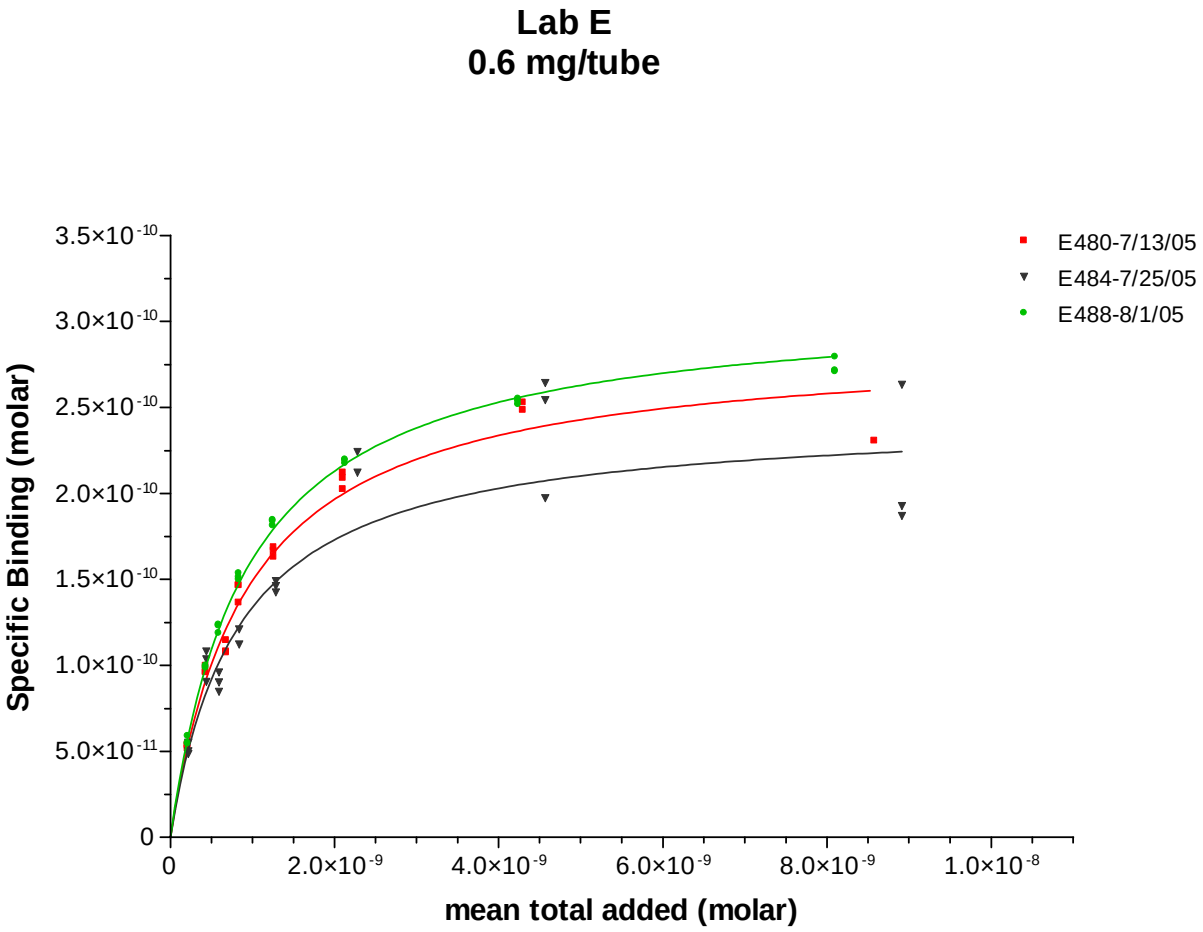
The IC_{50} values for CR42407 were determined to be 2.34, 2.30 and 2.51 $X10^{-7}$M. The RBA values for CR42407 in comparison to R1881 were 0.7549, 0.8254 and 0.6734 %.

The following test substance did not exhibit any significant inhibition of the binding of [^{3}H] R1881 to the androgen receptor.

The IC_{50} and RBA values for CR42408 were not determined because of insufficient activity.
The IC_{50} and RBA values for CR42409 were not determined because of insufficient activity.

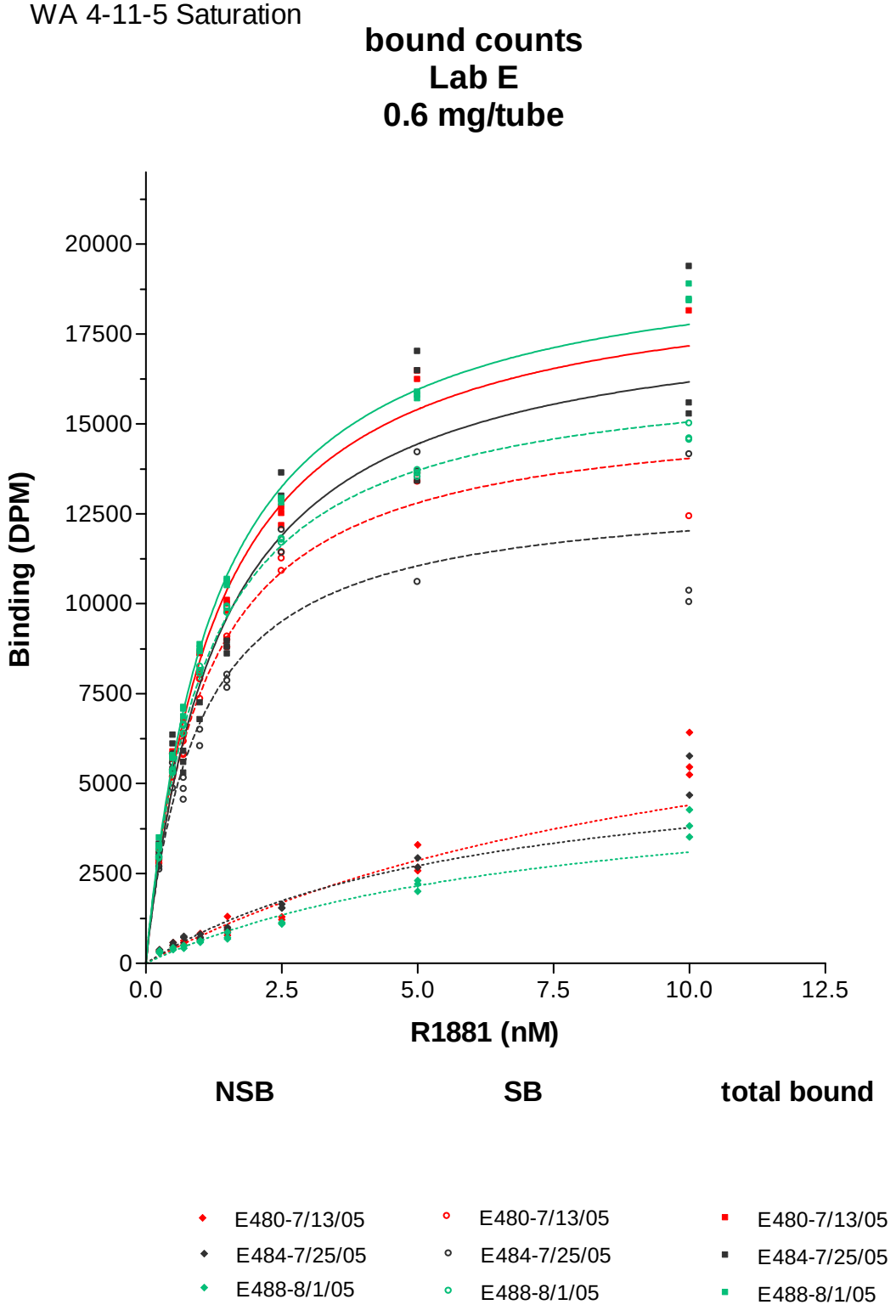
Figure 1: Saturation Binding and Scatchard Plots of ^{3}H-R1881 to the Androgen Receptor

WA 4-11-5 Saturation

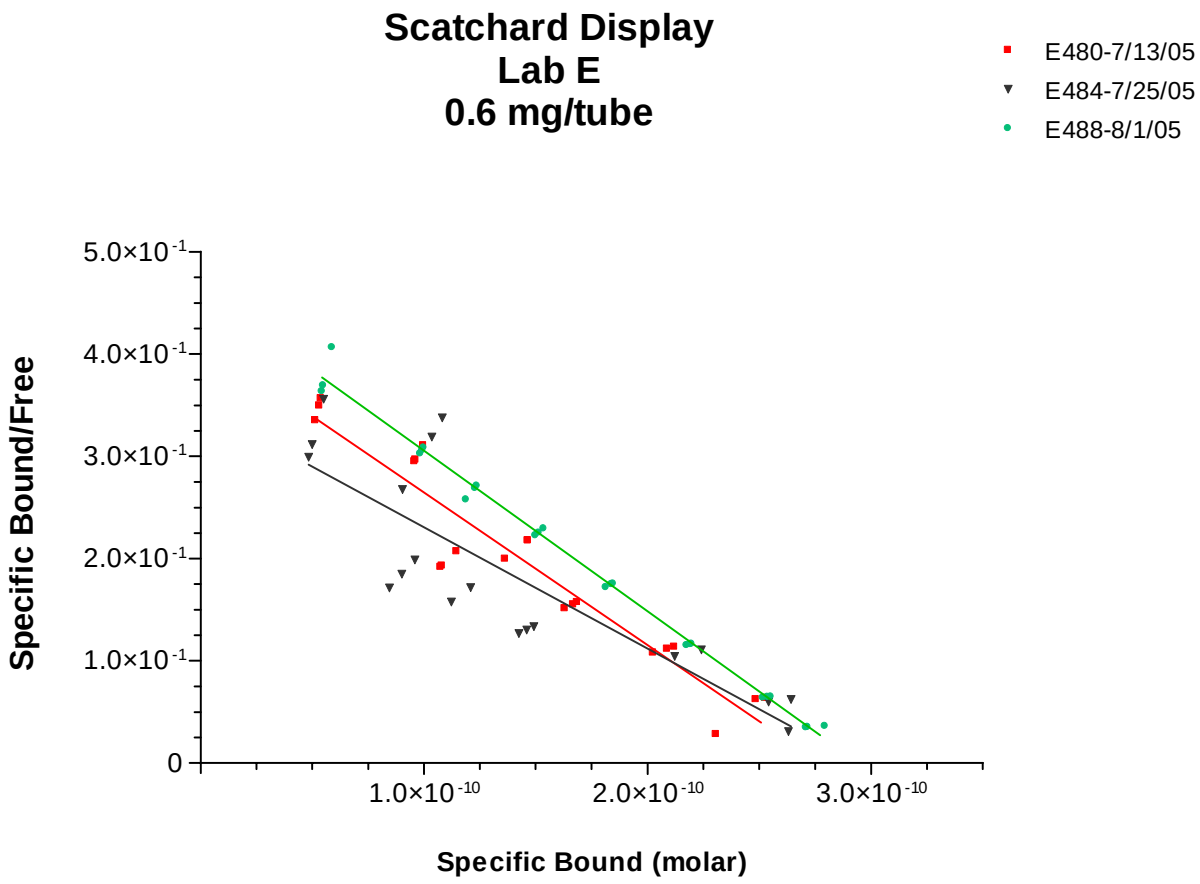


	E480-7/13/05	E484-7/25/05	E488-8/1/05
BMAX	2.880e-010	2.452e-010	3.115e-010
KD	9.302e-010	8.337e-010	9.257e-010
Std. Error			
BMAX	1.109e-011	1.554e-011	3.465e-012
KD	6.621e-011	1.064e-010	2.006e-011
95% Confidence Intervals			
BMAX	2.648e-010 to 3.112e-010	2.128e-010 to 2.776e-010	3.043e-010 to 3.187e-010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

WA 4-11-5 Saturation



WA 4-11-5 Saturation



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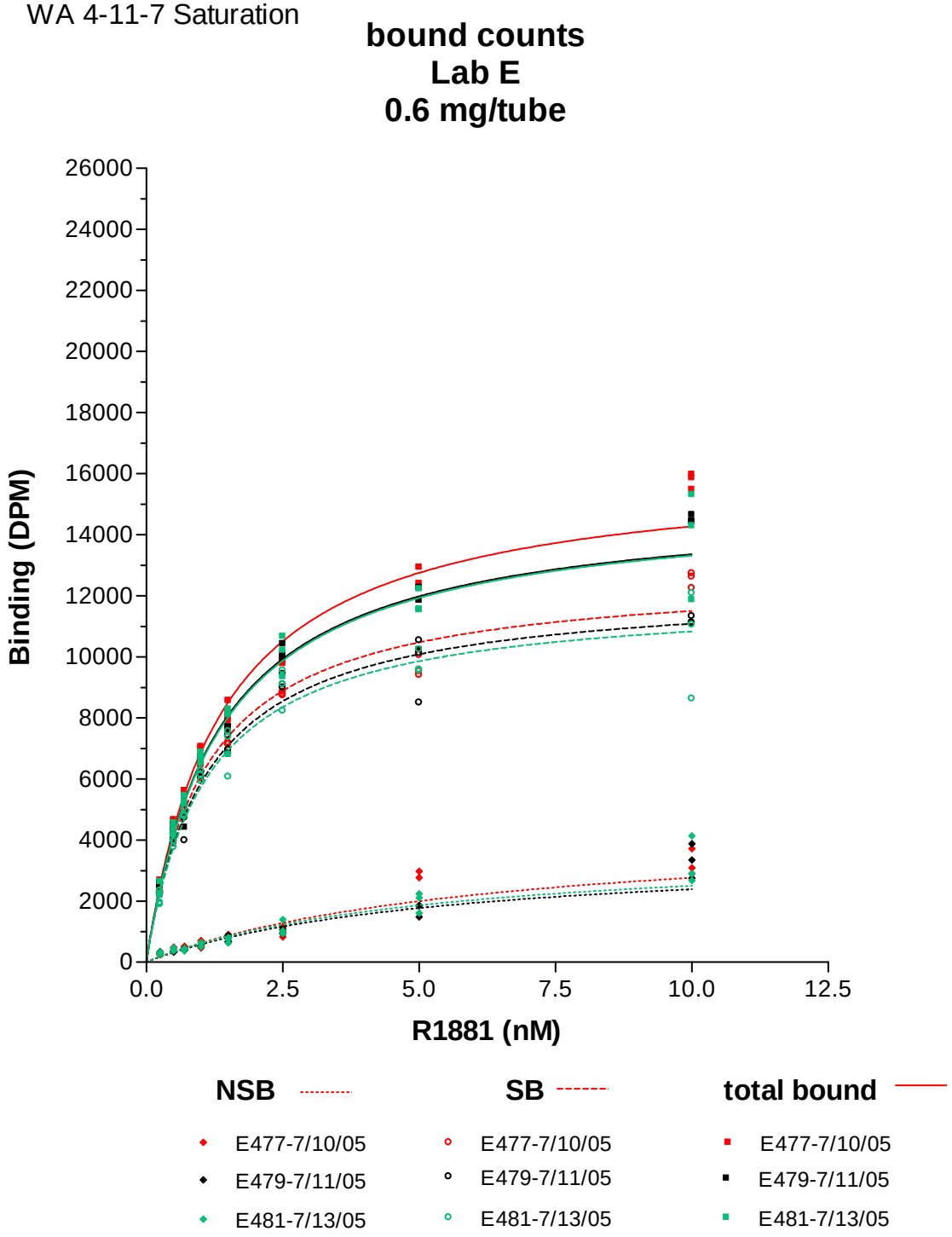
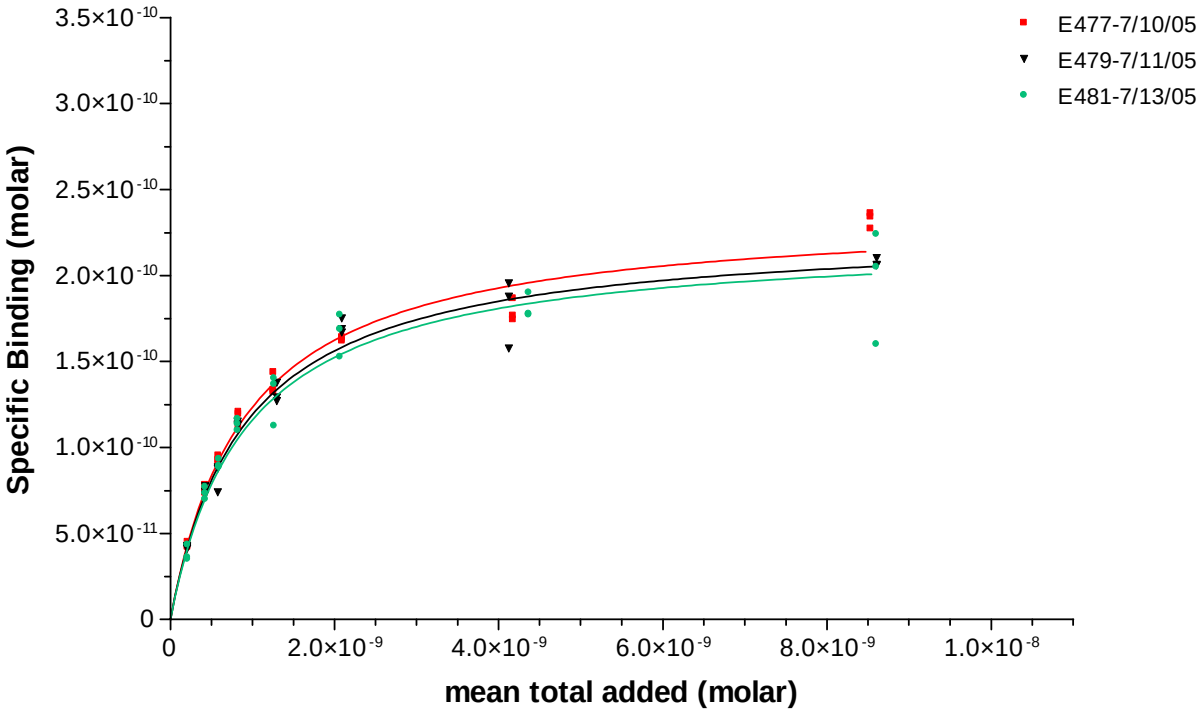


WA 4-11-7 Saturation

Lab E
0.6 mg/tube



Specific bound	E477-7/10/05	E479-7/11/05	E481-7/13/05
BMAX	2.370e-010	2.268e-010	2.222e-010
KD	9.194e-010	9.039e-010	9.156e-010
Std. Error			
BMAX	5.754e-012	7.052e-012	9.612e-012
KD	4.358e-011	5.508e-011	7.680e-011
95% Confidence Intervals			
BMAX	2.251e-010 to 2.490e-010	2.122e-010 to 2.414e-010	2.023e-010 to 2.422e-010
KD	8.290e-010 to 1.010e-009	7.897e-010 to 1.018e-009	7.563e-010 to 1.075e-009
Goodness of Fit			
Degrees of Freedom	22	22	22
R^2 (unweighted)	0.9735	0.9742	0.9417
Weighted Sum of Squares ($1/Y^2$)	0.06464	0.1082	0.2198
Absolute Sum of Squares	2.078e-021	1.823e-021	3.947e-021
Sy.x	9.718e-012	9.102e-012	1.339e-011
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	24	24	24
Number of missing values	0	0	0

WA 4-11-7 Saturation

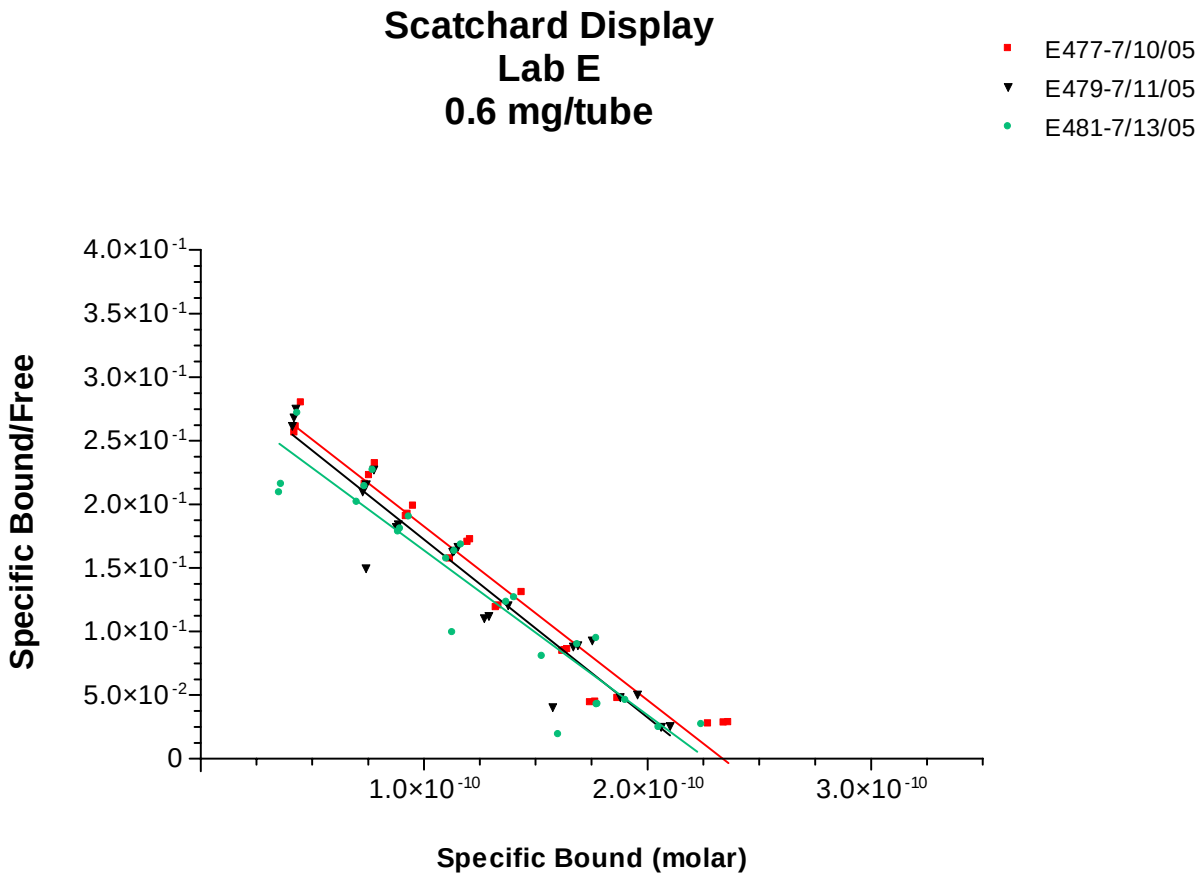


Figure 2: Competitive Binding of R1881 and Dexamethasone (Weak Positive) to the Androgen Receptor

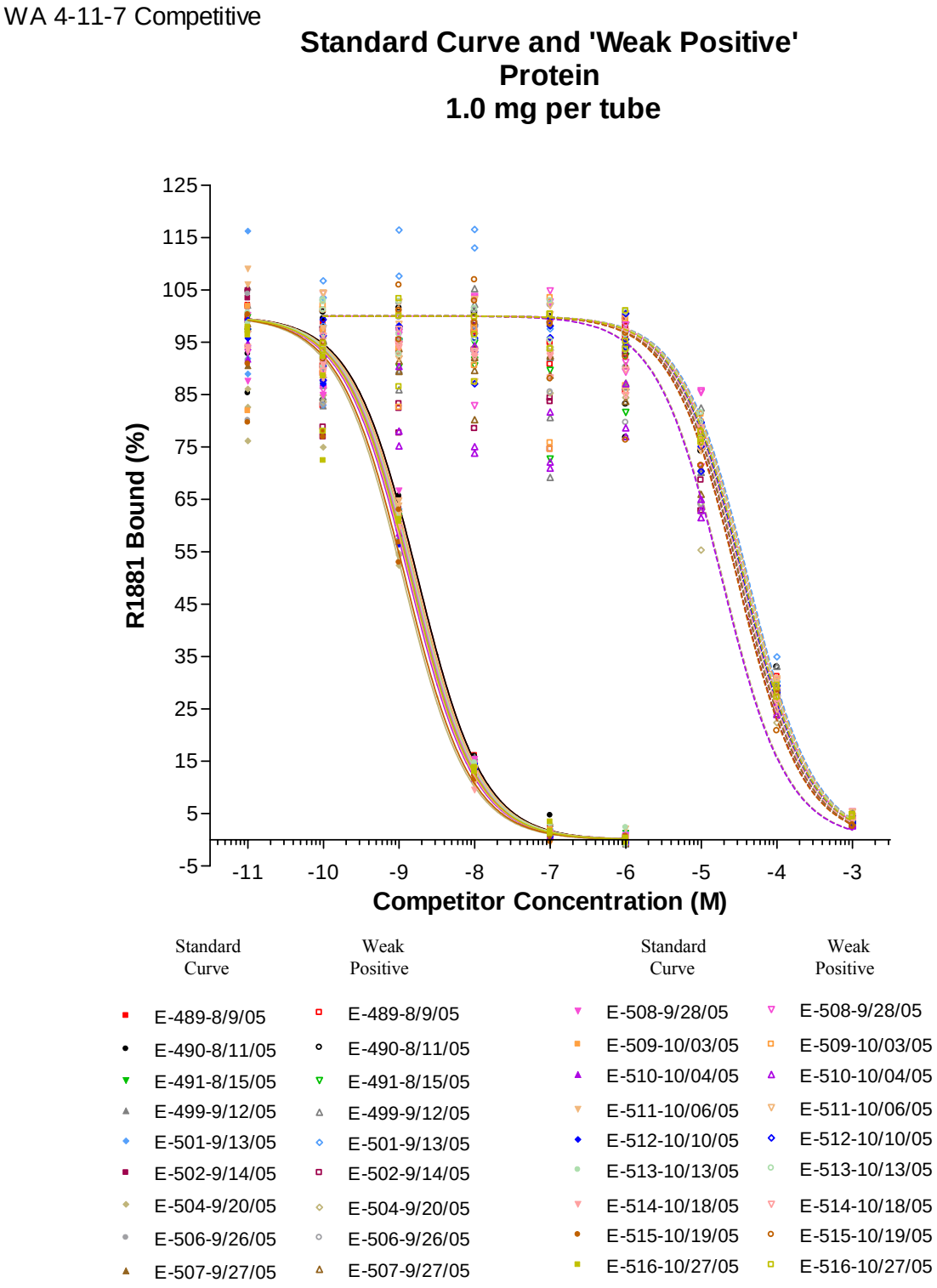


Table 1: Saturation Binding Experiments

Task 5: AR Saturation Binding Data (Cytosol

Run number	Assay date	Technician	K_d (M)	B_{max} (fmoles/100 ug)
480	13 July 2005	JEC	0.93×10^{-9}	14.40
484	25 July 2005	JEC	0.83×10^{-9}	12.26
488	1 August 2005	JEC	0.93×10^{-9}	15.58
Mean			0.90×10^{-9}	14.08
SD			0.05×10^{-9}	1.68

Task 7: AR Saturation Binding Data

Run number	Assay date	Technician	K_d (M)	B_{max} (fmoles/100 ug)
477	10 July 2005	JEC	0.92×10^{-9}	11.85
479	11 July 2005	JEC	0.90×10^{-9}	11.34
481	13 July 2005	JEC	0.92×10^{-9}	11.11
Mean			0.91×10^{-9}	11.43
SD			0.01×10^{-9}	0.38

Table 2: Competitive Binding Experiments

Run number	Assay date	Technician	R1881		Dexamethasone	
			IC_{50} (M)	RBA(%)	IC_{50} (M)	RBA(%)
517	08 November, 2005	JEC	1.52×10^{-9}	100	2.95×10^{-5}	0.0051
518	10 November, 2005	JEC	1.77×10^{-9}	100	2.84×10^{-5}	0.0062
519	17 November, 2005	JEC	1.46×10^{-9}	100	4.34×10^{-5}	0.0034
520	22 November 2005	JEC	1.77×10^{-9}	100	4.00×10^{-5}	0.0044
522	08 December 2005	JEC	1.90×10^{-9}	100	2.83×10^{-5}	0.0067
523	20 December 2005	JEC	1.69×10^{-9}	100	3.63×10^{-5}	0.0050
	Mean		1.68×10^{-9}		3.39×10^{-5}	0.0051
	SD		0.17×10^{-9}		0.65×10^{-5}	0.0012

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

Table 3: Summary Table (IC50 and RBA)

Run identification:	517	518	519	520	522	523			coefficient of variation
Assay start date:	11/8/2005	11/10/2005	11/17/2005	11/22/2005	12/8/2005	12/20/2005	**average**	**SD**	
Standard Curve IC50:	1.52E-09	1.77E-09	1.46E-09	1.77E-09	1.90E-09	1.69E-09	1.68E-09	1.67E-10	9.91%
Weak Positive IC50:	2.95E-05	2.84E-05	4.34E-05	4.00E-05	2.83E-05	3.36E-05	3.39E-05	6.45E-06	19.05%
RBA:	0.0051%	0.0062%	0.0034%	0.0044%	0.0067%	0.0050%	0.0051%	0.0012%	23.54%
RBA of means:							0.0050%		
CR42400									
IC50:	1.61E-09	2.05E-09	2.27E-09				1.98E-09	3.34E-10	16.92%
RBA:	94.1651%	86.3060%	64.2258%				81.5657%	15.5223%	19.03%
RBA of means:							80.0000%		
CR42401									
IC50:	7.22E-09	9.57E-09	9.22E-09				8.67E-09	1.27E-09	14.60%
RBA:	21.0082%	18.5096%	15.7969%				18.4382%	2.6064%	14.14%
RBA of means:							18.2419%		
CR42402									
IC50:	3.05E-07	2.84E-07	4.14E-07				3.34E-07	7.01E-08	20.95%
RBA:	0.4975%	0.6238%	0.3514%				0.4909%	0.1364%	27.78%
RBA of means:							0.4729%		
CR42403									
IC50:				1.74E-08	1.49E-08	1.68E-08	1.64E-08	1.27E-09	7.77%
RBA:				10.1495%	12.6908%	10.1075%	10.9826%	1.4795%	13.47%
RBA of means:							10.0306%		
CR42404									
IC50:	7.41E-05	9.46E-05	1.02E-04				9.02E-05	1.43E-05	15.88%
RBA:	0.0020%	0.0019%	0.0014%				0.0018%	0.0003%	17.76%
RBA of means:							0.0018%		
CR42405									
IC50:	1.56E-05	1.56E-05	1.84E-05				1.65E-05	1.63E-06	9.87%
RBA:	0.0098%	0.0114%	0.0079%				0.0097%	0.0017%	17.88%
RBA of means:							0.0096%		

Run identification:	517	518	519	520	522	523			coefficient of variation
Assay start date:	11/8/2005	11/10/2005	11/17/2005	11/22/2005	12/8/2005	12/20/2005	**average**	**SD**	
CR42406									
IC50:				1.33E-07	8.99E-08	1.20E-07	1.14E-07	2.21E-08	19.29%
RBA:				1.3291%	2.1085%	1.4061%	1.6146%	0.4295%	26.60%
RBA of means:							1.5604%		
CR42407									
IC50:				2.34E-07	2.30E-07	2.51E-07	2.38E-07	1.15E-08	4.84%
RBA:				0.7549%	0.8254%	0.6734%	0.7513%	0.0761%	10.12%
RBA of means:							0.7489%		
CR42408									
IC50:				7.85E-03	2.14E-02	1.46E-02	1.46E-02	6.78E-03	46.43%
RBA:				0.0000%	0.0000%	0.0000%	0.0000%	0.0000%	50.29%
RBA of means:							0.0000%		
CR42409									
IC50:				4.65E-03	7.90E-03	6.17E-03	6.24E-03	1.62E-03	26.02%
RBA:				0.0000%	0.0000%	0.0000%	0.0000%	0.0000%	24.36%
RBA of means:							0.0000%		

Appendix 1: Copy of Battelle, Richland Protocol No. SR-04-WA 4-11-06 and protocol amendment

Assay Protocol	Battelle Pacific Northwest Laboratories P.O. Box 999 Battelle Boulevard Richland, Washington 99352	SR-04-WA 4-11-06

PROTOCOL

1. **Title:** Validation of an Androgen Receptor Binding Assay – Task 6

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

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

4. **Objective:** To provide data to establish laboratory variability among five laboratories using the same assay and rat prostate cytosol prepared in each participating laboratory, radiolabeled R1881, R1881 and test substances (dexamethasone and 10 bar coded unknown test substance). This protocol is specific to the study to be conducted at Battelle-Richland.

5. **Duration:** approximately 45 days

6. **Proposed Study Dates:**

   a. Initiation of *In Vitro* Studies: September 26, 2005
   b. Completion of *In Vitro* Studies: November 11, 2005

7. **Protocol Approval:**

   a. Study Director: James E. Morris Date: 9/20/05
   James E. Morris, Ph.D.

   b. WAL: Michael L. Blanton Date: 9/16/05
   Michael L.Blanton

   c. Sponsor: David P. Houchens Date: 9/14/05
   David P. Houchens, Ph.D.

Assay Protocol	Battelle Pacific Northwest Laboratories P.O. Box 999 Battelle Boulevard Richland, Washington 99352	SR-04-WA 4-11-06

Reviewed By:

a. MSL QA Representative: Mary E Lynn Date: 9/19/05
Mary E. Lynn

b. EDSP Battelle QAM: Terri Pollock Date: 9-14-05
Terri L. Pollock

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

a.1 Test Substances: Dexamethasone (CAS # 50-02-2) (also, identified as weak positive). There are 10 additional test substances identified by the following bar codes (code numbers): **CR42400, CR42401, CR42402, CR42403, CR42404, CR42405, CR42406, CR42407, CR42408, CR42409.** All test substances 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) will be prepared by and supplied by the Chemical Repository, Battelle, Sequim, Washington.

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

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

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

9. **Test System:**

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

10. **Experimental Design:**

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

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

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

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

11. **Quality Assurance:** This study will be audited by the Quality Assurance Unit to assure adherence to Good Laboratory Practice Regulations, adherence to the study protocol and compliance with Battelle Standard Operating Procedures. The Quality Assurance Unit will conduct a review of the raw data for accuracy and traceability and will audit the final report.

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

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

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

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

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

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

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

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

Appendix 1

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

1.0 Purpose and Applicability

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

2.0 Safety and Operating Precautions

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

3.0 Animal Use

Follow U.S. EPA approved animal use protocols

4.0 Equipment and Materials

4.1 Equipment

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

4.2 Chemicals

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

4.3 Supplies

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

5.0 Stock Preparations

5.1. Preparation of Stock Solutions for making TEDG Buffer

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

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

5.1.3. *Sodium Molybdate Stock:* Add 2.419 g sodium molybdate to 8.0 ml ddH_2O in a 10 ml volumetric flask; bring the total volume to 10 mls = 1.0 M. Store at 4^{o}C. 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^{o}C and pH the cooled solution to 7.4. Store at 4+ 2 ^{o} C. Use 1.0 ml/100 ml TEDG buffer = 10mM. (50 mM Tris = 50 ml 1 M Tris/1 L ddH_2O)

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

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

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

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

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

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

5.3 Preparation of 50 mM TRIS Buffer

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

5.4 Preparation of 60% Hydroxylapatite (HAP) Slurry

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

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

5.4.3. Place in the refrigerator overnight.

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

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

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

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

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

5.6 Calculation Check and Dilutions

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

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

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

5.7 Preparation of 100X Radioinert R1881 Solutions

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

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

5.8 Compound Stock Preparations

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

EXAMPLE:

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

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

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

Table 1: Standard Curve

Standards	*Initial R1881 Concentration (Molar)*	**Final R1881 Concentration (Molar) in AR assay tube*
Negative Control	0	
0	0 (EtOH)	0
NSB	1 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 using an ice bath with a Polytron homgenizer using three to five 5-sec bursts of the Polytron. [Note: place probe of the Polytron in TEDG buffer in an ice-water bath to cool it down prior to its use for homogenization. Re-cool probe as needed.]

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

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

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

7.0. Assay procedure for chemicals: Day 1

7.1. Set up tubes: 12x75 mm glass tubes

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

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

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

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

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

Position	*Replicate*	*Competitor*	*Competitor Code*	*Concentration Code*	*Labels on vials in set 1-1-E supplied by Battelle to laboratory "E"*	*Competitor Initial Concentration (M)*	*Cytosol (uL)*	*Tracer (Hot R1881) Volume (uL)*	*Competitor Volume (uL)*	*triamcelenone Volume (uL)*	*Competitor Final Concentration (M)*	*Aliquot (uL)*	*HAP (500 ul)*
1	1	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
2	2	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
3	3	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	1.0E-06	100	500
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	1.0E-07	100	500
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	1.0E-07	100	500
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	1.0E-07	100	500
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	1.0E-08	100	500
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	1.0E-08	100	500
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	1.0E-08	100	500
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	1.0E-09	100	500

14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	1.0E-09	100	500
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	1.0E-09	100	500
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	1.0E-10	100	500
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	1.0E-10	100	500
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	1.0E-10	100	500
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	1.0E-11	100	500
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	1.0E-11	100	500
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	1.0E-11	100	500
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	1.E-03	100	500
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	1.E-03	100	500
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	1.E-03	100	500
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	1.E-04	100	500
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	1.E-04	100	500
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	1.E-04	100	500
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	1.E-05	100	500
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	1.E-05	100	500
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	1.E-05	100	500
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	1.E-06	100	500
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	1.E-06	100	500
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	1.E-06	100	500
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	1.E-07	100	500
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	1.0E-07	100	500
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	1.0E-07	100	500
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	1.0E-08	100	500
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	1.0E-08	100	500
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	1.0E-08	100	500
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	1.0E-09	100	500
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	1.0E-09	100	500
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	1.0E-09	100	500
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	1.0E-10	100	500
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	1.0E-10	100	500
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	1.0E-10	100	500
46	1	unknown 1	C	1	**E-1-C11**	3.00E-02	300	30	10	50	1.0E-03	100	500
47	2	unknown 1	C	1	**E-1-C11**	3.00E-02	300	30	10	50	1.0E-03	100	500
48	3	unknown 1	C	1	**E-1-C11**	3.00E-02	300	30	10	50	1.0E-03	100	500
49	1	unknown 1	C	2	**E-1-C12**	3.00E-03	300	30	10	50	1.0E-04	100	500
50	2	unknown 1	C	2	**E-1-C12**	3.00E-03	300	30	10	50	1.0E-04	100	500
51	3	unknown 1	C	2	**E-1-C12**	3.00E-03	300	30	10	50	1.0E-04	100	500
52	1	unknown 1	C	3	**E-1-C13**	3.00E-04	300	30	10	50	1.0E-05	100	500
53	2	unknown 1	C	3	**E-1-C13**	3.00E-04	300	30	10	50	1.0E-05	100	500
54	3	unknown 1	C	3	**E-1-C13**	3.00E-04	300	30	10	50	1.0E-05	100	500
55	1	unknown 1	C	4	**E-1-C14**	3.00E-05	300	30	10	50	1.0E-06	100	500
56	2	unknown 1	C	4	**E-1-C14**	3.00E-05	300	30	10	50	1.0E-06	100	500

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

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

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

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

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

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

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

8.0 Assay procedure: Day 2

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

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

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

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

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

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

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

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

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

9.0. Saturation Radioligand Binding Assay

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

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

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

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

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

Saturation Assay Tube Layout

Position	*Replicate*	*Tube Type Code*	*Hot Initial Concentration (nM)*	*Hot R1881 Volume (uL)*	*Hot Final Concentration (nM)*	*Cold Initial Concentration (uM)*	*Cold Volume (uL)*	*Cold Final Concentration (nM)*	*Triamcelenone Acetate (uL)*	*Cytosol (ul)*
1	1	H	10.0	7.5	0.25	—	—	—	50	300
2	2	H	10.0	7.5	0.25	—	—	—	50	300
3	3	H	10.0	7.5	0.25	—	—	—	50	300
4	1	H	10.0	15	0.50	—	—	—	50	300
5	2	H	10.0	15	0.50	—	—	—	50	300
6	3	H	10.0	15	0.50	—	—	—	50	300
7	1	H	10.0	21	0.70	—	—	—	50	300
8	2	H	10.0	21	0.70	—	—	—	50	300
9	3	H	10.0	21	0.70	—	—	—	50	300
10	1	H	10.0	30	1.00	—	—	—	50	300
11	2	H	10.0	30	1.00	—	—	—	50	300
12	3	H	10.0	30	1.00	—	—	—	50	300
13	1	H	10.0	45	1.50	—	—	—	50	300
14	2	H	10.0	45	1.50	—	—	—	50	300
15	3	H	10.0	45	1.50	—	—	—	50	300
16	1	H	100.0	7.5	2.50	—	—	—	50	300
17	2	H	100.0	7.5	2.50	—	—	—	50	300
18	3	H	100.0	7.5	2.50	—	—	—	50	300
19	1	H	100.0	15	5.00	—	—	—	50	300
20	2	H	100.0	15	5.00	—	—	—	50	300
21	3	H	100.0	15	5.00	—	—	—	50	300
22	1	H	100.0	30	10.00	—	—	—	50	300
23	2	H	100.0	30	10.00	—	—	—	50	300
24	3	H	100.0	30	10.00	—	—	—	50	300
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
28	1	HC	10.0	15	0.5	1.00	15	50	50	300
29	2	HC	10.0	15	0.5	1.00	15	50	50	300
30	3	HC	10.0	15	0.5	1.00	15	50	50	300
31	1	HC	10.0	21	0.7	1.00	21	70	50	300
32	2	HC	10.0	21	0.7	1.00	21	70	50	300
33	3	HC	10.0	21	0.7	1.00	21	70	50	300
34	1	HC	10.0	30	1	1.00	30	100	50	300
35	2	HC	10.0	30	1	1.00	30	100	50	300
36	3	HC	10.0	30	1	1.00	30	100	50	300

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

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

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

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

Day 2

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

10.0 Data Processing

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

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

Calculation Check -

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

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

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

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

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

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

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

10.3 Graphical Presentation of the Data

10.3.1. Standard Curve and Test Chemical Competitive Binding Curves: Data for the standard curve and each test chemical will be plotted as the percent 3H_R1881 bound versus the molar concentration. Estimates of the IC_{50}s will be determined using appropriate non linear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA). A Scatchard Analysis may also be performed for the standard curve using R1881 to demonstrate that the assay meets acceptable QA standards.

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

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

10.4 References

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

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

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

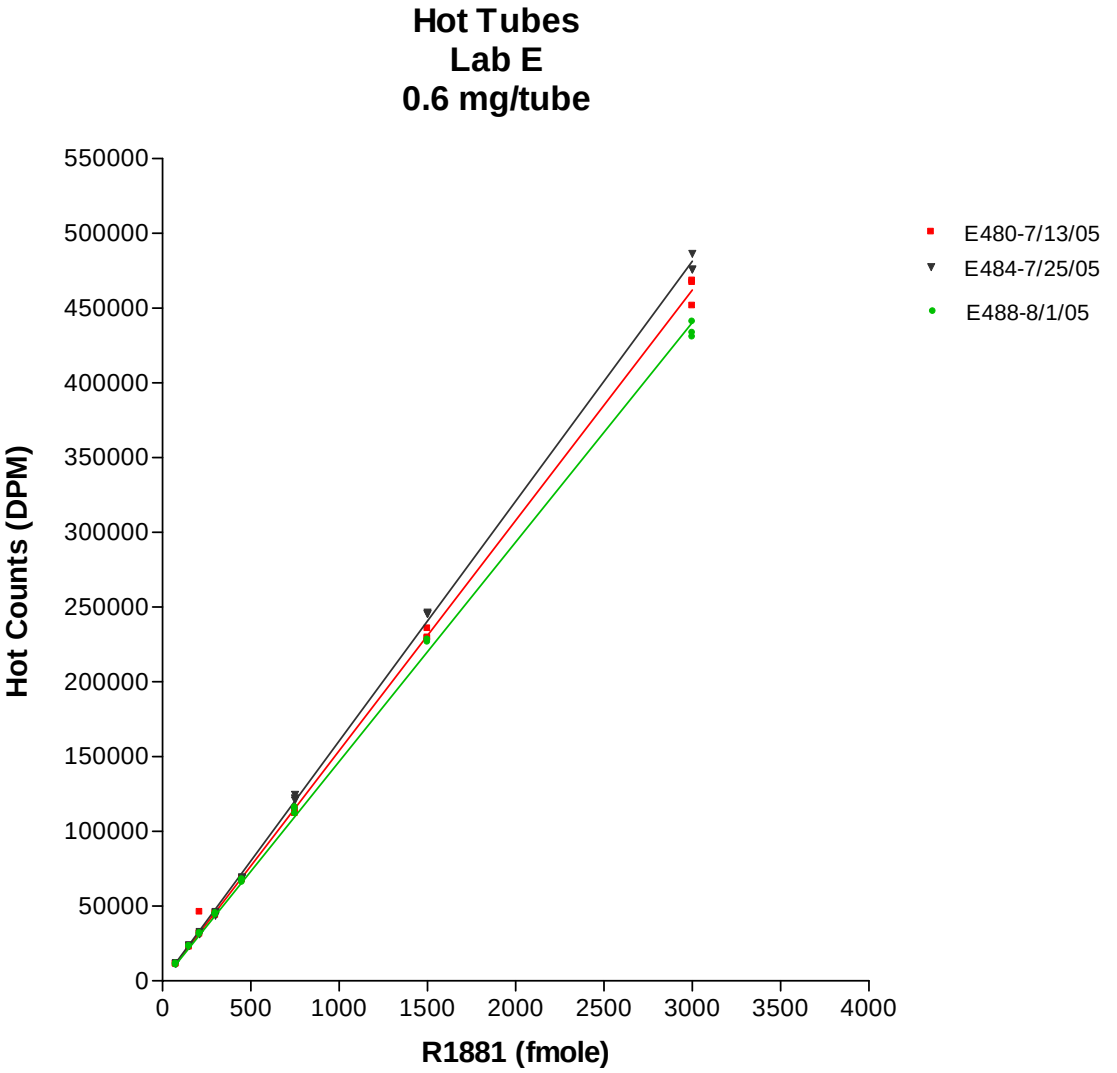
4) Tekpetey, F.R., and Amann, R.P. (1988) *Biol. Reprod.* **38**, 1051-1060.

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

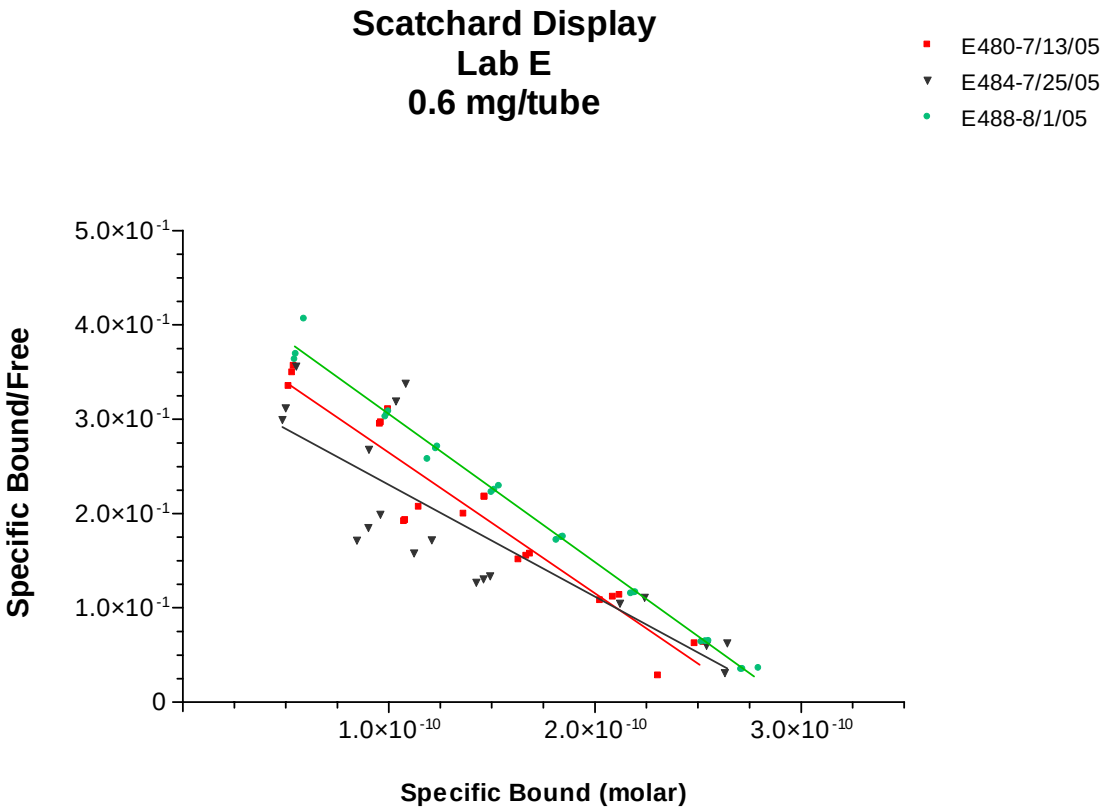
6) Bradford, M. (1976). *Anal. Biochem.* **72**, 248-254.

Appendix 2: Prism Files for Saturation Binding Experiments

WA 4-11-5 Saturation

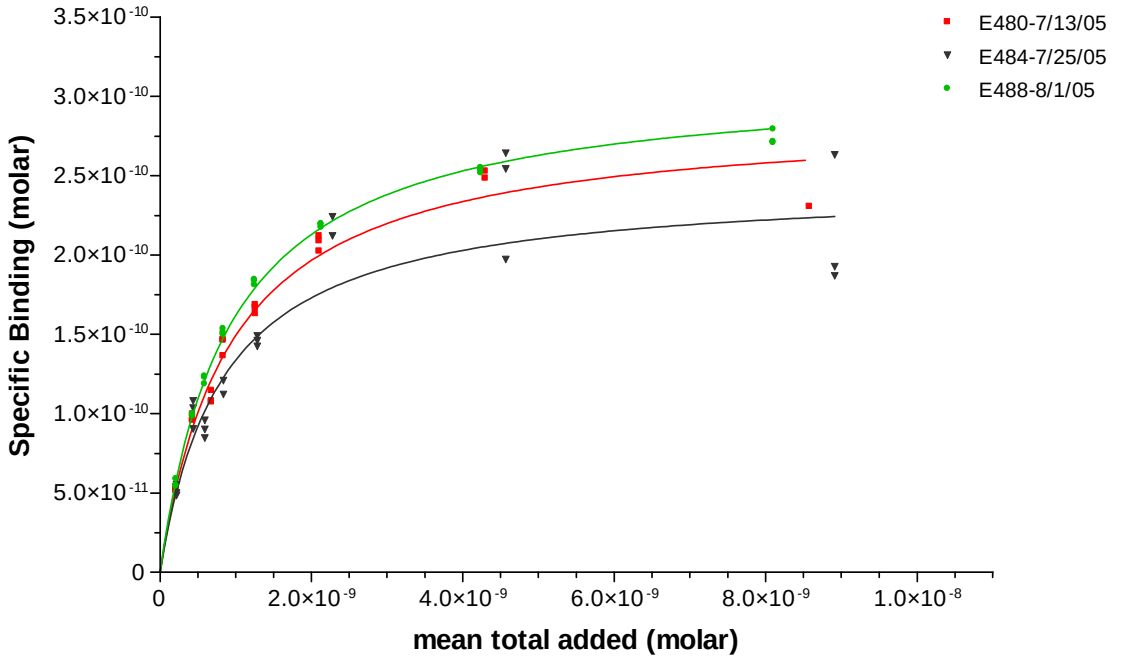


WA 4-11-5 Saturation



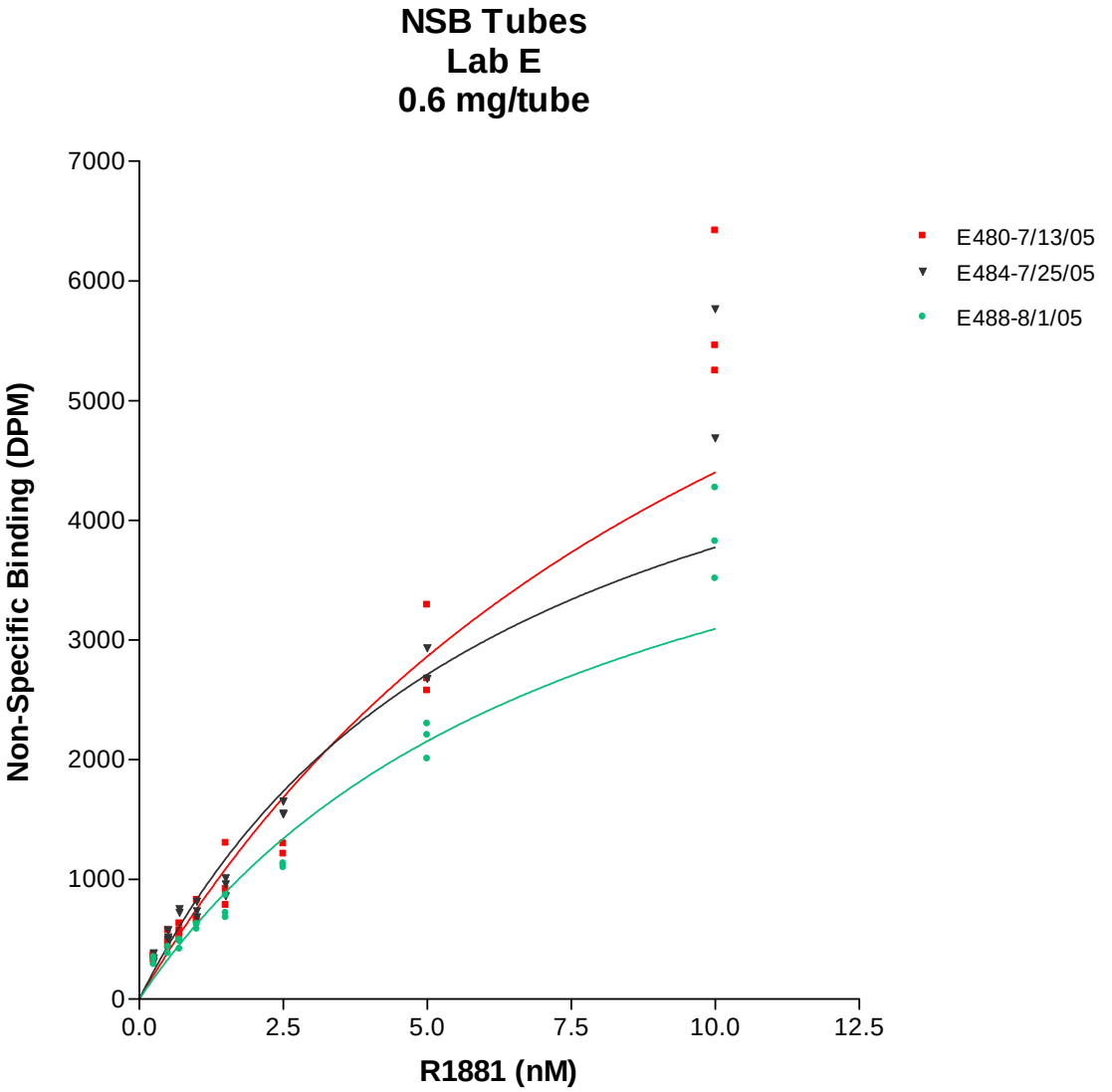
WA 4-11-5 Saturation

Lab E
0.6 mg/tube

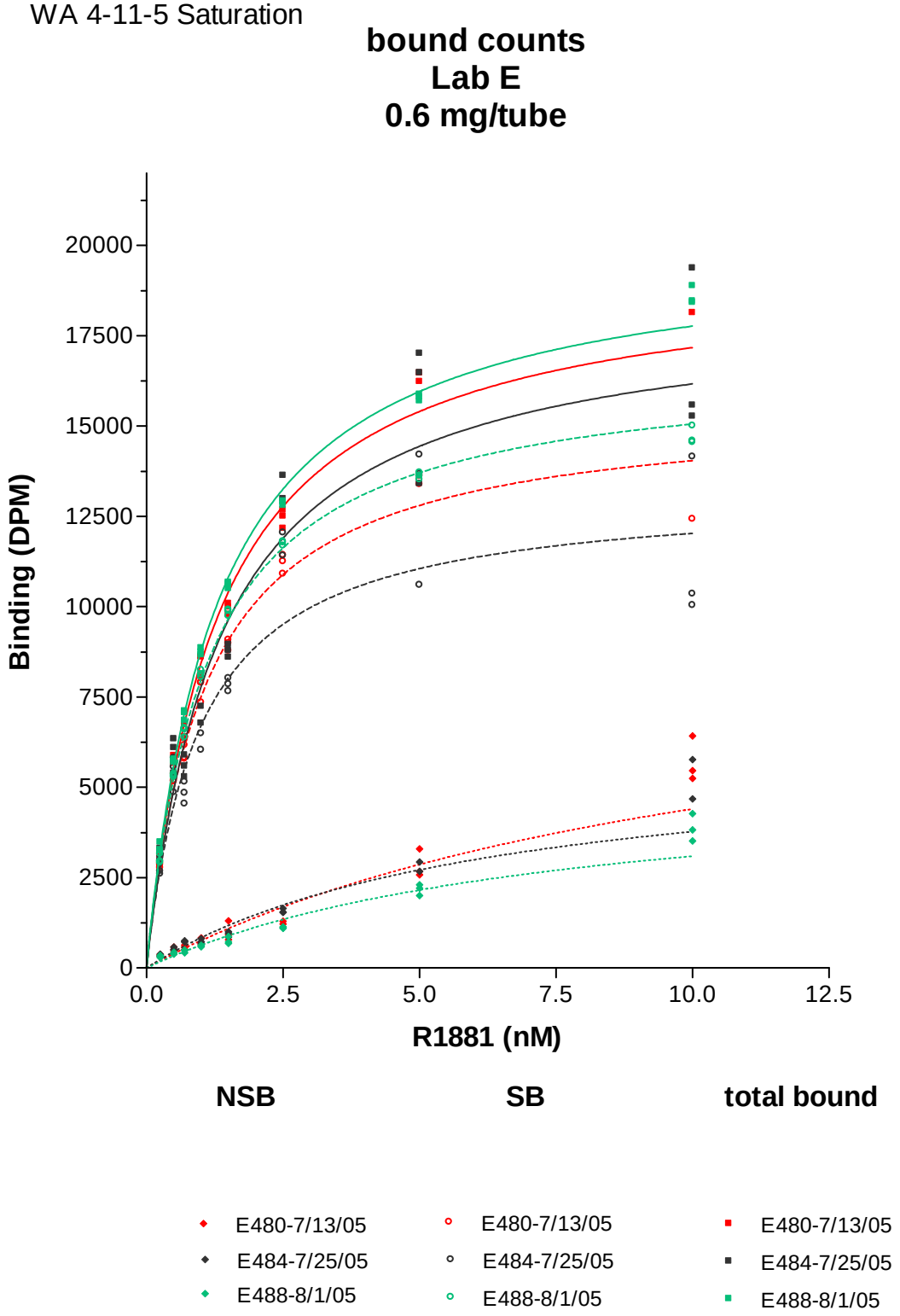


	E480-7/13/05	E484-7/25/05	E488-8/1/05
BMAX	2.880e-010	2.452e-010	3.115e-010
KD	9.302e-010	8.337e-010	9.257e-010
Std. Error			
BMAX	1.109e-011	1.554e-011	3.465e-012
KD	6.621e-011	1.064e-010	2.006e-011
95% Confidence Intervals			
BMAX	2.648e-010 to 3.112e-010	2.128e-010 to 2.776e-010	3.043e-010 to 3.187e-010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

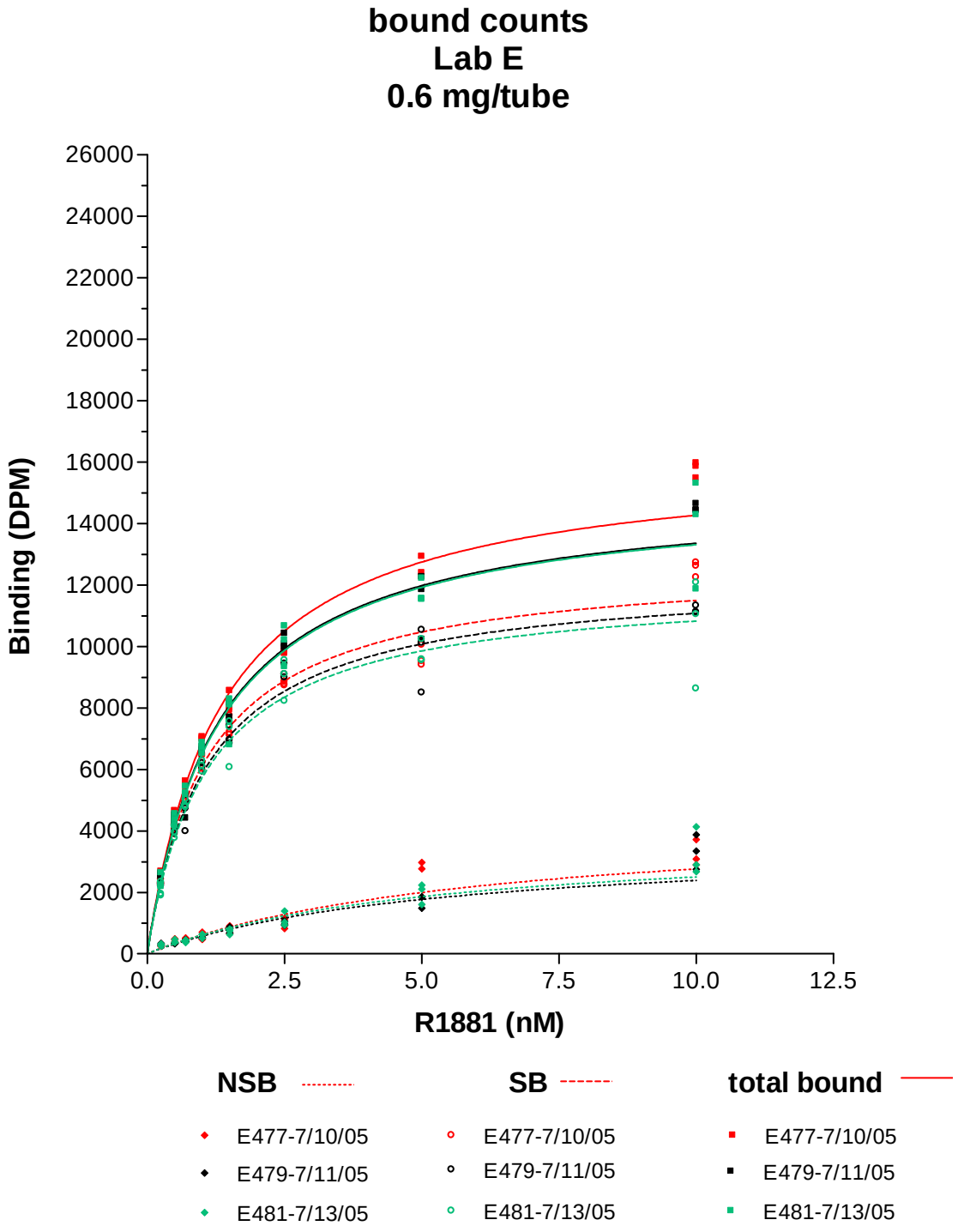
WA 4-11-5 Saturation



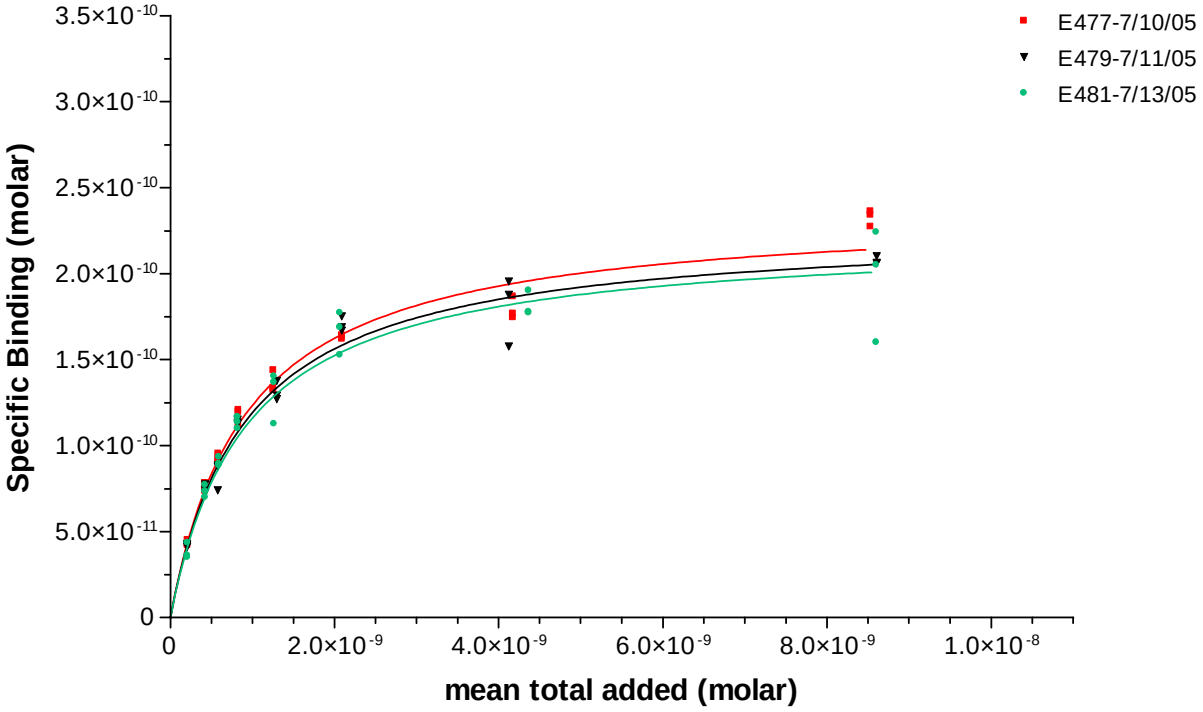
WA 4-11-5 Saturation



WA 4-11 Task 7 Saturation

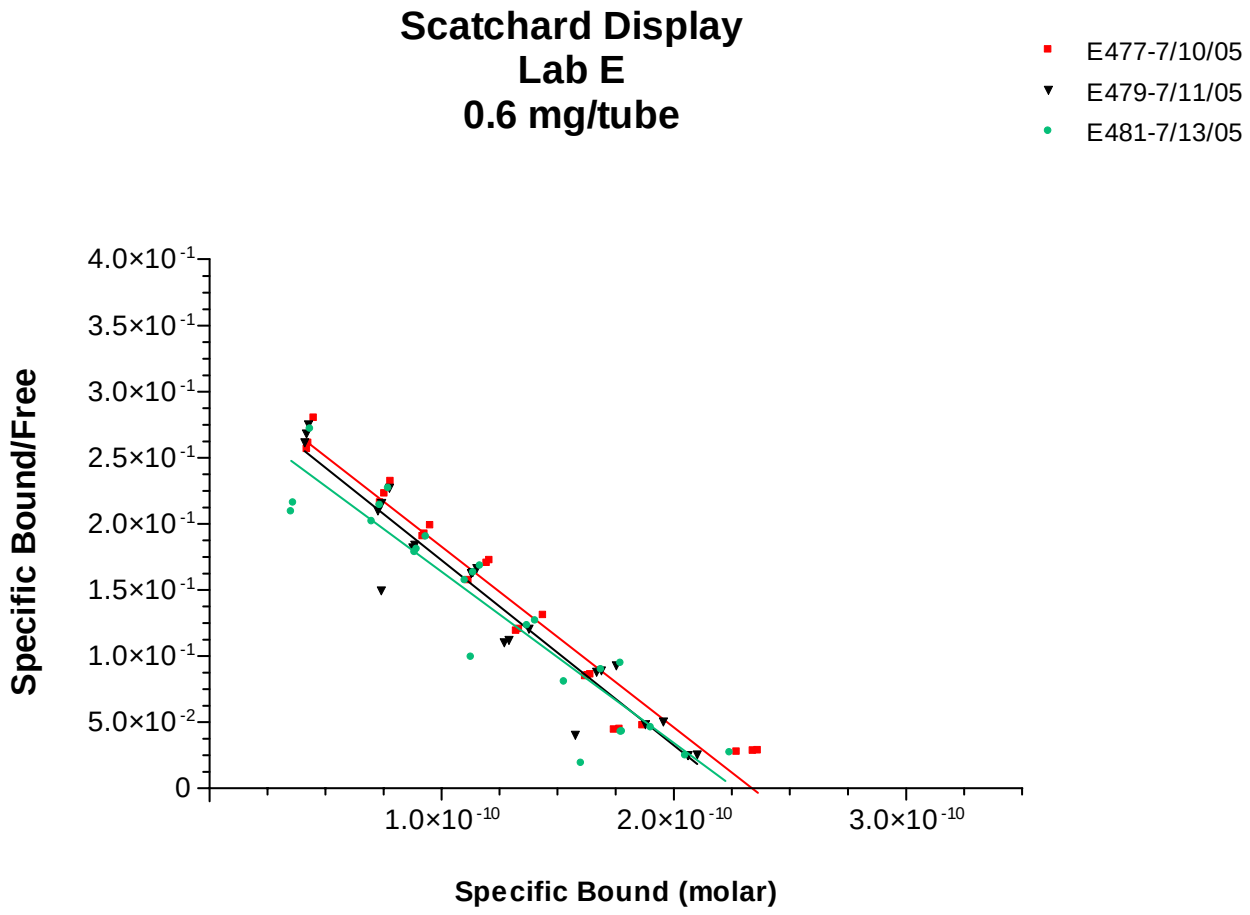


Lab E
0.6 mg/tube

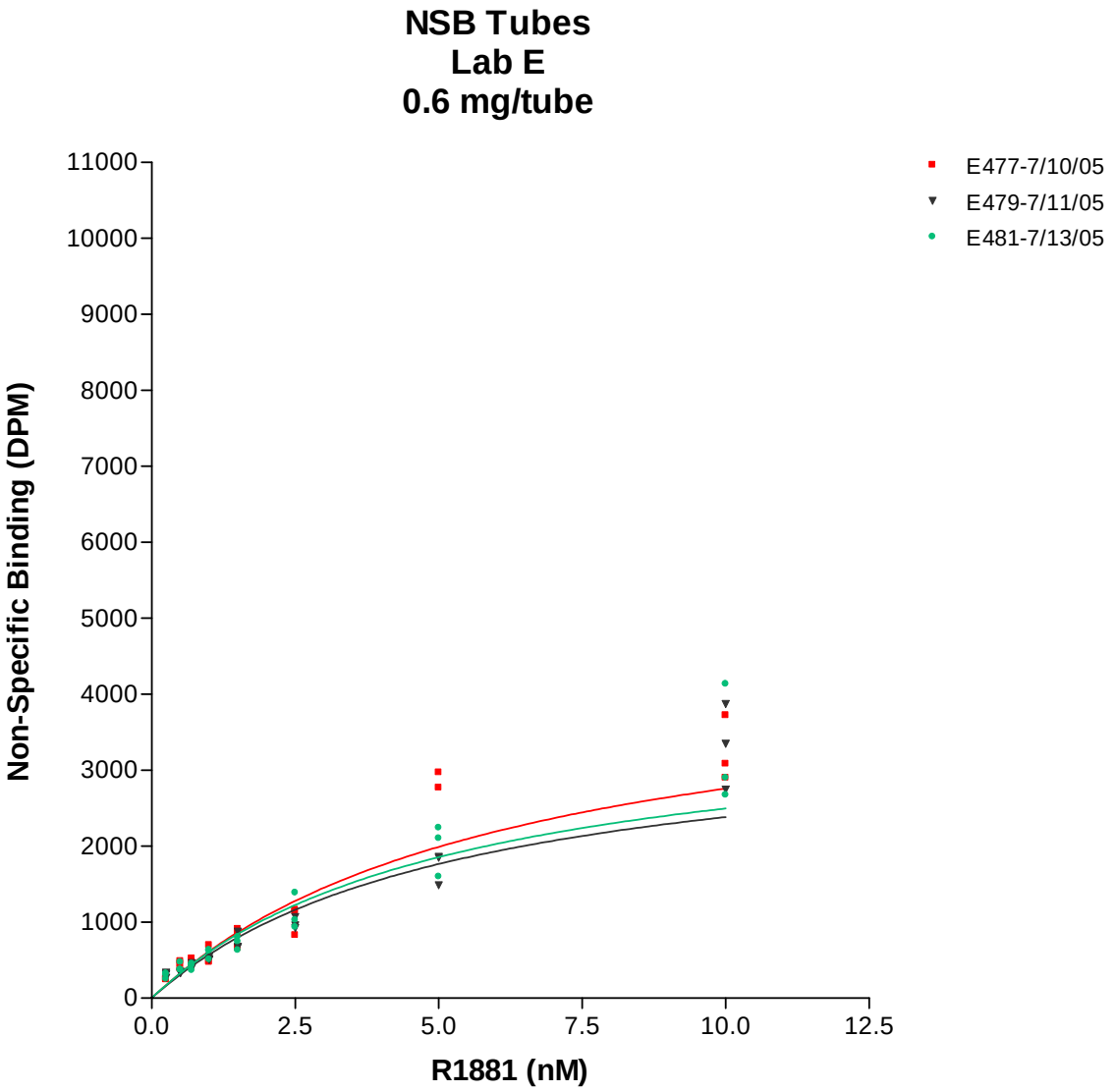


Specific bound	E477-7/10/05	E479-7/11/05	E481-7/13/05
BMAX	2.370e-010	2.268e-010	2.222e-010
KD	9.194e-010	9.039e-010	9.156e-010
Std. Error			
BMAX	5.754e-012	7.052e-012	9.612e-012
KD	4.358e-011	5.508e-011	7.680e-011
95% Confidence Intervals			
BMAX	2.251e-010 to 2.490e-010	2.122e-010 to 2.414e-010	2.023e-010 to 2.422e-010
KD	8.290e-010 to 1.010e-009	7.897e-010 to 1.018e-009	7.563e-010 to 1.075e-009
Goodness of Fit			
Degrees of Freedom	22	22	22
R^2 (unweighted)	0.9735	0.9742	0.9417
Weighted Sum of Squares ($1/Y^2$)	0.06464	0.1082	0.2198
Absolute Sum of Squares	2.078e-021	1.823e-021	3.947e-021
Sy.x	9.718e-012	9.102e-012	1.339e-011
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	24	24	24
Number of missing values	0	0	0

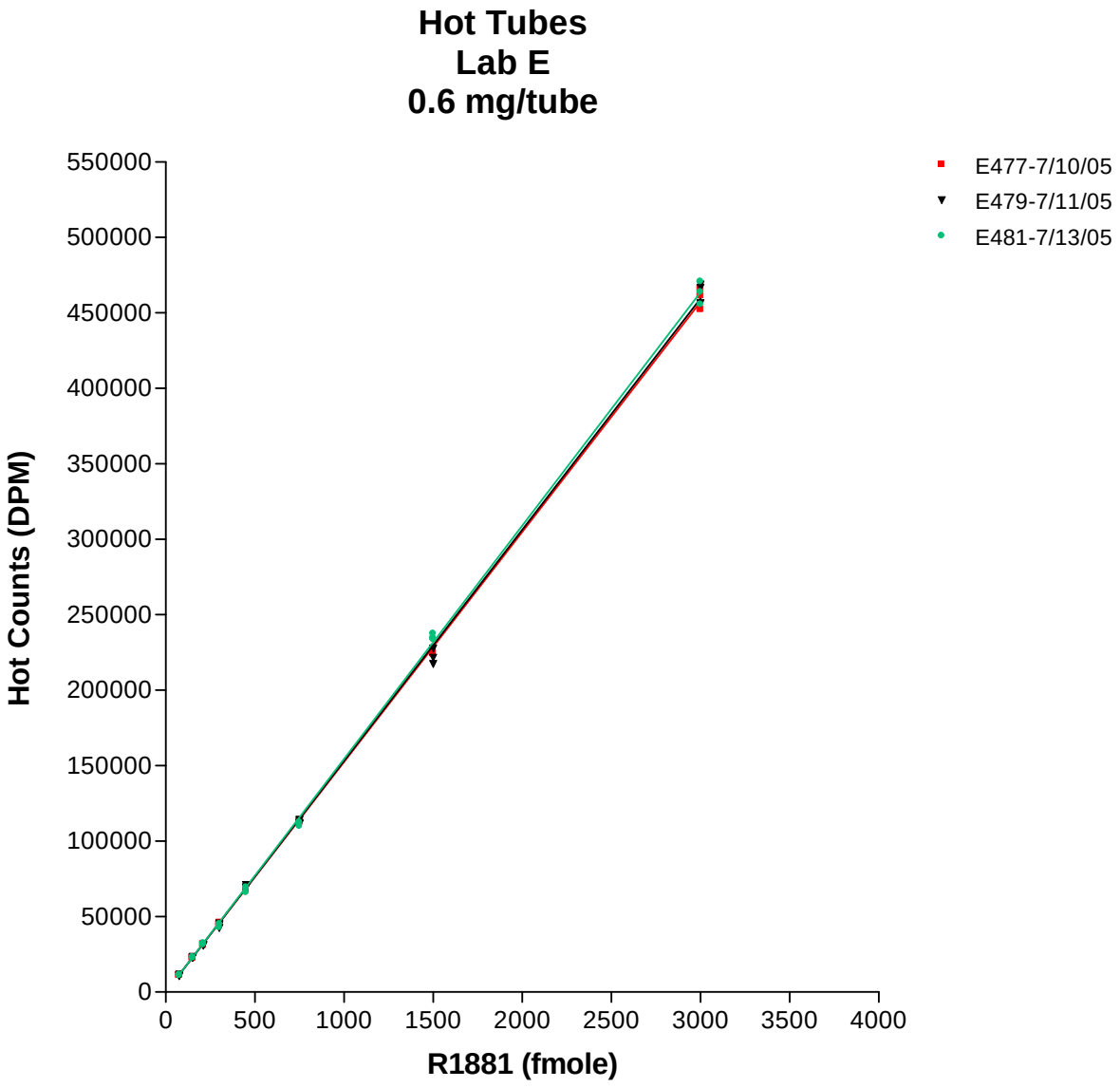
WA 4-11 Task 7 Saturation



WA 4-11 Task 7 Saturation



WA 4-11 Task 7 Saturation



Appendix 3: Excel Files for Saturation Binding Experiments:

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

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

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

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

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

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

protcol calls for counting decanted EtOH supe

reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

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

Saturation Assay Tube Layout

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

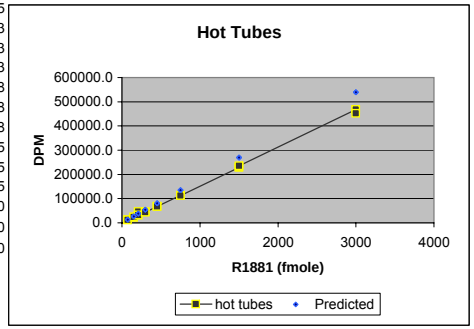
		Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)												
		Tube Identification			Assay tube contents									
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final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

										Number of molecules					Ratio
Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
		(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
480	1	3213.7	351.8	2861.9	10.9%	28.2%	11405.0	8191.3	21	2	19	74	53	0.35
480	2	3124.8	351.8	2773.0	11.3%	27.4%	11405.0	8280.2	20	2	18	74	54	0.33
480	3	3257.0	351.8	2905.2	10.8%	28.6%	11405.0	8148.0	21	2	19	74	53	0.36
480	4	5877.6	504.1	5373.5	8.6%	25.4%	23178.7	17301.1	38	3	35	151	112	0.31
480	5	5689.2	504.1	5185.1	8.9%	24.5%	23178.7	17489.5	37	3	34	151	114	0.30
480	6	5667.0	504.1	5162.9	8.9%	24.4%	23178.7	17511.7	37	3	34	151	114	0.29
480	7	6363.6	575.3	5788.3	9.0%	17.4%	36580.3	30216.7	41	4	38	238	196	0.19
480	8	6396.9	575.3	5821.6	9.0%	17.5%	36580.3	30183.4	42	4	38	238	196	0.19
480	9	6745.5	575.3	6170.2	8.5%	18.4%	36580.3	29834.8	44	4	40	238	194	0.21
480	10	8615.4	716.6	7898.8	8.3%	19.2%	44896.7	36281.3	56	5	51	292	236	0.22
480	11	8064.3	716.6	7347.7	8.9%	18.0%	44896.7	36832.4	52	5	48	292	239	0.20
480	12	8608.2	716.6	7891.6	8.3%	19.2%	44896.7	36288.5	56	5	51	292	236	0.22
480	13	9783.6	1002.6	8781.0	10.2%	14.4%	67919.3	58135.7	64	7	57	441	377	0.15
480	14	10088.1	1002.6	9085.5	9.9%	14.9%	67919.3	57831.2	66	7	59	441	376	0.16
480	15	9987.3	1002.6	8984.7	10.0%	14.7%	67919.3	57932.0	65	7	58	441	376	0.16
480	16	12682.5	1256.5	11426.0	9.9%	11.2%	113362.0	100679.5	82	8	74	736	654	0.11
480	17	12509.1	1256.5	11252.6	10.0%	11.0%	113362.0	100852.9	81	8	73	736	655	0.11
480	18	12167.1	1256.5	10910.6	10.3%	10.7%	113362.0	101194.9	79	8	71	736	657	0.11
480	19	16236.0	2849.1	13386.9	17.5%	7.0%	231408.0	215172.0	105	18	87	1503	1397	0.06
480	20	16469.4	2849.1	13620.3	17.3%	7.1%	231408.0	214938.6	107	18	88	1503	1396	0.06
480	21		2849.1				231408.0			18	-18	1503		
480	22		5711.3				462413.0			37	-37	3002		
480	23		5711.3				462413.0			37	-37	3002		
480	24	18141.3	5711.3	12430.0	31.5%	3.9%	462413.0	444271.7	118	37	81	3002	2885	0.03

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

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

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



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

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

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

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

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

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

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

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

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

protcol calls for counting decanted EtOH supe

reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

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

Saturation Assay Tube Layout

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

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

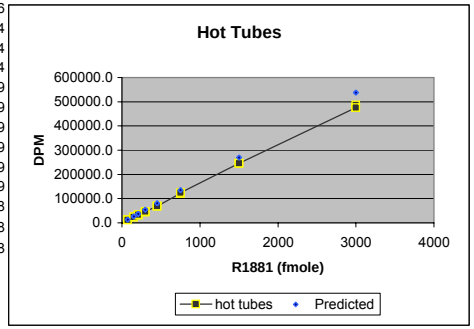
									Number of molecules					Ratio
Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific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

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

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

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

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



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

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

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

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

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

For your convenience, data reduction is performed in columns

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

in columns CF through CN

Cells in column S are presented with a grey background

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

the cytosol concentration is probably too high for good competitive assays

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

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

protcol calls for counting decanted EtOH supe

reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

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

Saturation Assay Tube Layout

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

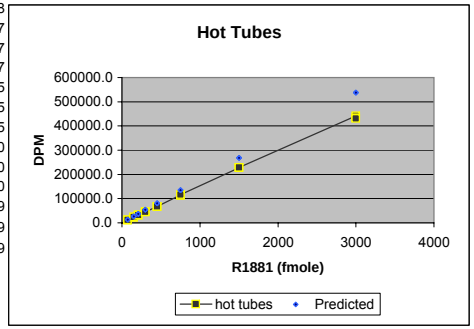
		Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)												
		Tube Identification			Assay tube contents									
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final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

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

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

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

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



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

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

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

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

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

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

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

Laboratory Code:	E	
Run identification:	477	
Assay start date:	7/10/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.96	**Ci/mmole**
Cytosol lot or vial number:	051905	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	9.19E-01	**nM**
Bmax	11.85	**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)	Significant portion of label on Vial	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	—	851.52	2554.56	TRUE		22.5%	0.25	2554.6	255.1
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	894.52	2683.56	TRUE		23.6%	0.25	2683.6	255.1
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	840.57	2521.71	TRUE		22.2%	0.25	2521.7	255.1
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1505.40	4516.2	TRUE		19.8%	0.5	4516.2	444.4
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1551.10	4653.3	TRUE		20.4%	0.5	4653.3	444.4
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1471.10	4413.3	TRUE		19.4%	0.5	4413.3	444.4
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1812.30	5436.9	TRUE		17.3%	0.7	5436.9	482.7
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1872.40	5617.2	TRUE		17.9%	0.7	5617.2	482.7
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1825.50	5476.5	TRUE		17.4%	0.7	5476.5	482.7
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2351.90	7055.7	TRUE		15.7%	1	7055.7	551.9
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2189.80	6569.4	TRUE		14.7%	1	6569.4	551.9
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2332.20	6996.6	TRUE		15.6%	1	6996.6	551.9
13	1	H	10.0	45	1.50	—	—	—	50	300	—	2646.30	7938.9	TRUE		11.7%	1.5	7938.9	812.5
14	2	H	10.0	45	1.50	—	—	—	50	300	—	2855.10	8565.3	TRUE		12.6%	1.5	8565.3	812.5
15	3	H	10.0	45	1.50	—	—	—	50	300	—	2668.00	8004	TRUE		11.8%	1.5	8004.0	812.5
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	3275.40	9826.2	TRUE		8.7%	2.5	9826.2	1042.8
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	3297.80	9893.4	TRUE		8.8%	2.5	9893.4	1042.8
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	3257.70	9773.1	TRUE		8.7%	2.5	9773.1	1042.8
19	1	H	100.0	15	5.00	—	—	—	50	300	—	4309.70	12929.1	TRUE		5.7%	5	12929.1	2870.2
20	2	H	100.0	15	5.00	—	—	—	50	300	—	4132.30	12396.9	TRUE		5.5%	5	12396.9	2870.2
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4090.50	12271.5	TRUE		5.5%	5	12271.5	2870.2
22	1	H	100.0	30	10.00	—	—	—	50	300	—	5286.20	15858.6	TRUE		3.4%	10	15858.6	3234.8
23	2	H	100.0	30	10.00	—	—	—	50	300	—	5323.70	15971.1	TRUE		3.5%	10	15971.1	3234.8
24	3	H	100.0	30	10.00	—	—	—	50	300	—	5161.00	15483	TRUE		3.4%	10	15483.0	3234.8

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)	Significant portion of label on Vial	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	81.83	245.49	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	82.78	248.34	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	90.50	271.5	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	153.02	459.06	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	161.77	485.31	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	129.65	388.95	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	173.58	520.74	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	145.57	436.71	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	163.59	490.77	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	157.77	473.31	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	233.46	700.38	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	160.67	482.01	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	220.16	660.48	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	288.58	865.74	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	303.72	911.16	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	387.68	1163.04	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	275.68	827.04	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	379.43	1138.29	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	511.17	1533.51	FALSE	spill				
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	990.14	2970.42	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	923.31	2769.93	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	965.92	2897.76	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1240.30	3720.9	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	1028.58	3085.74	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11380.7	11380.7	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11321.1	11321.1	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11385.1	11385.1	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23012.0	23012	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	22959.0	22959	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	22401.0	22401	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	31011.0	31011	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	31801.0	31801	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	31527.0	31527	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	44722.0	44722	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	43823.0	43823	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	45910.0	45910	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	67656.0	67656	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	68111.0	68111	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	67622.0	67622	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	112317	112317	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	113821	113821	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	112310	112310	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	226752	226752	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	224386	224386	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	224037	224037	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	461331	461331	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	466101	466101	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	452231	452231	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)
477	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
477	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
477	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
477	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
477	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
477	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
477	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
477	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
477	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
477	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
477	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
477	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
477	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
477	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
477	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
477	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
477	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
477	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
477	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
477	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
477	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
477	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
477	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
477	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

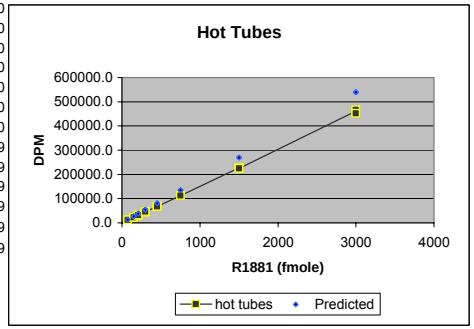
									Number of molecules					Ratio
Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific 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)	
477	1	2554.6	255.1	2299.5	10.0%	22.5%	11362.3	8807.7	17	2	15	75	58	0.26
477	2	2683.6	255.1	2428.5	9.5%	23.6%	11362.3	8678.7	18	2	16	75	57	0.28
477	3	2521.7	255.1	2266.6	10.1%	22.2%	11362.3	8840.6	17	2	15	75	58	0.26
477	4	4516.2	444.4	4071.8	9.8%	19.8%	22790.7	18274.5	30	3	27	149	120	0.22
477	5	4653.3	444.4	4208.9	9.6%	20.4%	22790.7	18137.4	31	3	28	149	119	0.23
477	6	4413.3	444.4	3968.9	10.1%	19.4%	22790.7	18377.4	29	3	26	149	121	0.22
477	7	5436.9	482.7	4954.2	8.9%	17.3%	31446.3	26009.4	36	3	32	206	171	0.19
477	8	5617.2	482.7	5134.5	8.6%	17.9%	31446.3	25829.1	37	3	34	206	169	0.20
477	9	5476.5	482.7	4993.8	8.8%	17.4%	31446.3	25969.8	36	3	33	206	170	0.19
477	10	7055.7	551.9	6503.8	7.8%	15.7%	44818.3	37762.6	46	4	43	294	248	0.17
477	11	6569.4	551.9	6017.5	8.4%	14.7%	44818.3	38248.9	43	4	39	294	251	0.16
477	12	6996.6	551.9	6444.7	7.9%	15.6%	44818.3	37821.7	46	4	42	294	248	0.17
477	13	7938.9	812.5	7126.4	10.2%	11.7%	67796.3	59857.4	52	5	47	445	393	0.12
477	14	8565.3	812.5	7752.8	9.5%	12.6%	67796.3	59231.0	56	5	51	445	388	0.13
477	15	8004.0	812.5	7191.5	10.2%	11.8%	67796.3	59792.3	52	5	47	445	392	0.12
477	16	9826.2	1042.8	8783.4	10.6%	8.7%	112816.0	102989.8	64	7	58	740	675	0.09
477	17	9893.4	1042.8	8850.6	10.5%	8.8%	112816.0	102922.6	65	7	58	740	675	0.09
477	18	9773.1	1042.8	8730.3	10.7%	8.7%	112816.0	103042.9	64	7	57	740	676	0.08
477	19	12929.1	2870.2	10058.9	22.2%	5.7%	225058.3	212129.2	85	19	66	1476	1391	0.05
477	20	12396.9	2870.2	9526.7	23.2%	5.5%	225058.3	212661.4	81	19	62	1476	1395	0.04
477	21	12271.5	2870.2	9401.3	23.4%	5.5%	225058.3	212786.8	80	19	62	1476	1396	0.04
477	22	15858.6	3234.8	12623.8	20.4%	3.4%	459887.7	444029.1	104	21	83	3016	2912	0.03
477	23	15971.1	3234.8	12736.3	20.3%	3.5%	459887.7	443916.6	105	21	84	3016	2911	0.03
477	24	15483.0	3234.8	12248.2	20.9%	3.4%	459887.7	444404.7	102	21	80	3016	2915	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)
477	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		245.5	255.1
477	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		248.3	255.1
477	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		271.5	255.1
477	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50		459.1	444.4
477	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50		485.3	444.4
477	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50		389.0	444.4
477	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70		520.7	482.7
477	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70		436.7	482.7
477	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70		490.8	482.7
477	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100		473.3	551.9
477	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100		700.4	551.9
477	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100		482.0	551.9
477	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150		660.5	812.5
477	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150		865.7	812.5
477	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150		911.2	812.5
477	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1163.0	1042.8
477	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		827.0	1042.8
477	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		1138.3	1042.8
477	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500			2870.2
477	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500		2970.4	2870.2
477	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500		2769.9	2870.2
477	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000		2897.8	3234.8
477	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000		3720.9	3234.8
477	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000		3085.7	3234.8

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
477	49	1	Hot	c1	10	7.5	75	11380.7	75	11362.3	13480
477	50	2	Hot	c1	10	7.5	75	11321.1	74	11362.3	13480
477	51	3	Hot	c1	10	7.5	75	11385.1	75	11362.3	13480
477	52	1	Hot	c2	10	15	150	23012.0	151	22790.7	26960
477	53	2	Hot	c2	10	15	150	22959.0	151	22790.7	26960
477	54	3	Hot	c2	10	15	150	22401.0	147	22790.7	26960
477	55	1	Hot	c3	10	21	210	31011.0	203	31446.3	37744
477	56	2	Hot	c3	10	21	210	31801.0	209	31446.3	37744
477	57	3	Hot	c3	10	21	210	31527.0	207	31446.3	37744
477	58	1	Hot	c4	10	30	300	44722.0	293	44818.3	53920
477	59	2	Hot	c4	10	30	300	43823.0	287	44818.3	53920
477	60	3	Hot	c4	10	30	300	45910.0	301	44818.3	53920
477	61	1	Hot	c5	10	45	450	67656.0	444	67796.3	80880
477	62	2	Hot	c5	10	45	450	68111.0	447	67796.3	80880
477	63	3	Hot	c5	10	45	450	67622.0	444	67796.3	80880
477	64	1	Hot	c6	100	7.5	750	112317.0	737	112816.0	134800
477	65	2	Hot	c6	100	7.5	750	113821.0	747	112816.0	134800
477	66	3	Hot	c6	100	7.5	750	112310.0	737	112816.0	134800
477	67	1	Hot	c7	100	15	1500	226752.0	1487	225058.3	269599
477	68	2	Hot	c7	100	15	1500	224386.0	1472	225058.3	269599
477	69	3	Hot	c7	100	15	1500	224037.0	1469	225058.3	269599
477	70	1	Hot	c8	100	30	3000	461331.0	3026	459887.7	539199
477	71	2	Hot	c8	100	30	3000	466101.0	3057	459887.7	539199
477	72	3	Hot	c8	100	30	3000	452231.0	2966	459887.7	539199

Computation Check	
7/10/05	specific activity date
80.96	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7973E+14	DPM/mmole
1.7973E+11	DPM/nmole
179.7	DPM/fmole
0.005564	fmole/DPM



Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	152.4724108 dpm/fmole	
1/slope	0.006558564 fmole/dpm	
	x	y
origin	0	0
end point	3057.0	466101
SLOPE function, used if missing HOT tubes		
Slope	153.1 dpm/fmole	
1/slope	0.006533 fmole/dpm	
	x	y
origin	0	0
end point	3045.1	466101

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
4.26457E-11	0.26107	2.10726E-10	4.26457E-11
4.50381E-11	0.27982	2.10726E-10	4.50381E-11
4.20364E-11	0.25639	2.10726E-10	4.20364E-11
7.5515E-11	0.22281	4.22676E-10	7.5515E-11
7.80576E-11	0.23205	4.22676E-10	7.80576E-11
7.36066E-11	0.21596	4.22676E-10	7.36066E-11
9.188E-11	0.19048	5.83205E-10	9.188E-11
9.52238E-11	0.19879	5.83205E-10	9.52238E-11
9.26144E-11	0.19229	5.83205E-10	9.26144E-11
1.2062E-10	0.17223	8.31202E-10	1.2062E-10
1.11601E-10	0.15732	8.31202E-10	1.11601E-10
1.19524E-10	0.17040	8.31202E-10	1.19524E-10
1.32167E-10	0.11906	1.25735E-09	1.32167E-10
1.43784E-10	0.13089	1.25735E-09	1.43784E-10
1.33375E-10	0.12028	1.25735E-09	1.33375E-10
1.62897E-10	0.08528	2.09229E-09	1.62897E-10
1.64144E-10	0.08599	2.09229E-09	1.64144E-10
1.61913E-10	0.08473	2.09229E-09	1.61913E-10
1.86553E-10	0.04742	4.17394E-09	1.86553E-10
1.76683E-10	0.04480	4.17394E-09	1.76683E-10
1.74357E-10	0.04418	4.17394E-09	1.74357E-10
2.34121E-10	0.02843	8.52909E-09	2.34121E-10
2.36208E-10	0.02869	8.52909E-09	2.36208E-10
2.27155E-10	0.02756	8.52909E-09	2.27155E-10

Bmax molar	2.37E-10	KD molar	9.19E-10
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.80E+17	**kd nM =**	9.19E-01
Bmax molar to Bmax moles	7.1112E-14		
= DPM/((DPM/mmole)*1000)	7.1112E-14		
=Bmax DPM	12781.17279		
assay date	7/10/2005		
Bmax(dpm)	12781.17279		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.96		
DPM/mmole	1.80E+14		
DPM/pmole	1.80E+05		
1/(DPM/mmole)	5.56E-15		
1/(DPM/pmole)	5.56E-06		
SA(dpm/pmole)	1.80E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.071112		
bmax pmole/mg	0.11852		
Bmax fmole/mg	118.52		
Bmax (fmole/100 ug)	11.852		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

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

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

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

Laboratory Code:	E	
Run identification:	479	
Assay start date:	7/11/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.95	**Ci/mmole**
Cytosol lot or vial number:	051905	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	9.04E-01	**nM**
Bmax	11.34	**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)	Significant portion of label on Vial	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	—	869.35	2608.05	TRUE		23.7%	0.25	2608.1	306.8
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	854.35	2563.05	TRUE		23.3%	0.25	2563.1	306.8
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	839.25	2517.75	TRUE		22.9%	0.25	2517.8	306.8
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1416.70	4250.1	TRUE		18.6%	0.5	4250.1	337.9
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1504.30	4512.9	TRUE		19.7%	0.5	4512.9	337.9
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1445.40	4336.2	TRUE		18.9%	0.5	4336.2	337.9
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1472.10	4416.3	TRUE		14.2%	0.7	4416.3	427.0
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1716.60	5149.8	TRUE		16.5%	0.7	5149.8	427.0
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1731.60	5194.8	TRUE		16.7%	0.7	5194.8	427.0
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2243.10	6729.3	TRUE		15.3%	1	6729.3	522.5
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2211.50	6634.5	TRUE		15.0%	1	6634.5	522.5
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2200.70	6602.1	TRUE		15.0%	1	6602.1	522.5
13	1	H	10.0	45	1.50	—	—	—	50	300	—	2528.90	7586.7	TRUE		10.9%	1.5	7586.7	741.2
14	2	H	10.0	45	1.50	—	—	—	50	300	—	2565.60	7696.8	TRUE		11.0%	1.5	7696.8	741.2
15	3	H	10.0	45	1.50	—	—	—	50	300	—	2719.50	8158.5	TRUE		11.7%	1.5	8158.5	741.2
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	3325.10	9975.3	TRUE		8.9%	2.5	9975.3	983.2
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	3475.20	10425.6	TRUE		9.3%	2.5	10425.6	983.2
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	3360.80	10082.4	TRUE		9.0%	2.5	10082.4	983.2
19	1	H	100.0	15	5.00	—	—	—	50	300	—	3409.20	10227.6	TRUE		4.6%	5	10227.6	1730.5
20	2	H	100.0	15	5.00	—	—	—	50	300	—	3949.70	11849.1	TRUE		5.3%	5	11849.1	1730.5
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4089.00	12267	TRUE		5.5%	5	12267.0	1730.5
22	1	H	100.0	30	10.00	—	—	—	50	300	—	4812.40	14437.2	TRUE		3.1%	10	14437.2	3322.4
23	2	H	100.0	30	10.00	—	—	—	50	300	—	4883.40	14650.2	TRUE		3.2%	10	14650.2	3322.4
24	3	H	100.0	30	10.00	—	—	—	50	300	—	4808.60	14425.8	TRUE		3.1%	10	14425.8	3322.4

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)	Significant portion of label on Vial	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	104.60	313.8	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	112.05	336.15	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	90.13	270.39	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	112.41	337.23	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	109.13	327.39	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	116.36	349.08	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	140.92	422.76	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	154.61	463.83	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	131.49	394.47	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	171.04	513.12	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	182.67	548.01	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	168.80	506.4	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	224.84	674.52	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	224.23	672.69	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	292.09	876.27	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	355.69	1067.07	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	307.34	922.02	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	320.14	960.42	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	619.69	1859.07	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	495.15	1485.45	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	615.70	1847.1	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1290.50	3871.5	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1116.00	3348	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	915.87	2747.61	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11492.2	11492.2	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	10478.2	10478.2	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	10974.0	10974	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23303.0	23303	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	22698.0	22698	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	22712.0	22712	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	30789.0	30789	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	31196.0	31196	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	31430.0	31430	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	45413.0	45413	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	44380.0	44380	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	42457.0	42457	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	70912.0	70912	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	69410.0	69410	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	69246.0	69246	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	111379	111379	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	112051	112051	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	114006	114006	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	221591	221591	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	227633	227633	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	217422	217422	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	456488	456488	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	468707	468707	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	466608	466608	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)
479	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
479	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
479	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
479	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
479	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
479	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
479	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
479	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
479	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
479	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
479	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
479	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
479	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
479	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
479	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
479	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
479	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
479	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
479	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
479	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
479	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
479	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
479	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
479	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)	
479	1	2608.1	306.8	2301.3	11.8%	23.7%	10981.5	8373.4	17	2	15	72	55	0.27
479	2	2563.1	306.8	2256.3	12.0%	23.3%	10981.5	8418.4	17	2	15	72	55	0.27
479	3	2517.8	306.8	2211.0	12.2%	22.9%	10981.5	8463.7	16	2	14	72	55	0.26
479	4	4250.1	337.9	3912.2	8.0%	18.6%	22904.3	18654.2	28	2	26	150	122	0.21
479	5	4512.9	337.9	4175.0	7.5%	19.7%	22904.3	18391.4	29	2	27	150	120	0.23
479	6	4336.2	337.9	3998.3	7.8%	18.9%	22904.3	18568.1	28	2	26	150	121	0.22
479	7	4416.3	427.0	3989.3	9.7%	14.2%	31138.3	26722.0	29	3	26	203	174	0.15
479	8	5149.8	427.0	4722.8	8.3%	16.5%	31138.3	25988.5	34	3	31	203	170	0.18
479	9	5194.8	427.0	4767.8	8.2%	16.7%	31138.3	25943.5	34	3	31	203	169	0.18
479	10	6729.3	522.5	6206.8	7.8%	15.3%	44083.3	37354.0	44	3	41	288	244	0.17
479	11	6634.5	522.5	6112.0	7.9%	15.0%	44083.3	37448.8	43	3	40	288	245	0.16
479	12	6602.1	522.5	6079.6	7.9%	15.0%	44083.3	37481.2	43	3	40	288	245	0.16
479	13	7586.7	741.2	6845.5	9.8%	10.9%	69856.0	62269.3	50	5	45	456	407	0.11
479	14	7696.8	741.2	6955.6	9.6%	11.0%	69856.0	62159.2	50	5	45	456	406	0.11
479	15	8158.5	741.2	7417.3	9.1%	11.7%	69856.0	61697.5	53	5	48	456	403	0.12
479	16	9975.3	983.2	8992.1	9.9%	8.9%	112478.7	102503.4	65	6	59	734	669	0.09
479	17	10425.6	983.2	9442.4	9.4%	9.3%	112478.7	102053.1	68	6	62	734	666	0.09
479	18	10082.4	983.2	9099.2	9.8%	9.0%	112478.7	102396.3	66	6	59	734	669	0.09
479	19	10227.6	1730.5	8497.1	16.9%	4.6%	222215.3	211987.7	67	11	55	1451	1384	0.04
479	20	11849.1	1730.5	10118.6	14.6%	5.3%	222215.3	210366.2	77	11	66	1451	1374	0.05
479	21	12267.0	1730.5	10536.5	14.1%	5.5%	222215.3	209948.3	80	11	69	1451	1371	0.05
479	22	14437.2	3322.4	11114.8	23.0%	3.1%	463934.3	449497.1	94	22	73	3029	2935	0.02
479	23	14650.2	3322.4	11327.8	22.7%	3.2%	463934.3	449284.1	96	22	74	3029	2934	0.03
479	24	14425.8	3322.4	11103.4	23.0%	3.1%	463934.3	449508.5	94	22	73	3029	2935	0.02

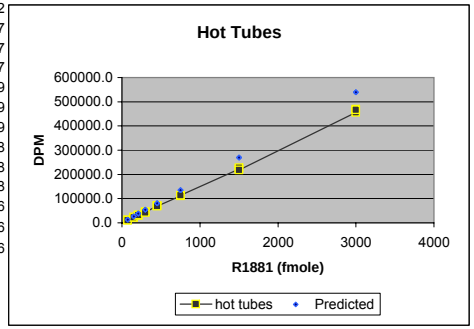
		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
		Tube Identification			Assay tube contents								Scintillation Results	
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	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)
479	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	313.8	306.8
479	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	336.2	306.8
479	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	270.4	306.8
479	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	337.2	337.9
479	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	327.4	337.9
479	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	349.1	337.9
479	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	422.8	427.0
479	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	463.8	427.0
479	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	394.5	427.0
479	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	513.1	522.5
479	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	548.0	522.5
479	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	506.4	522.5
479	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	674.5	741.2
479	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	672.7	741.2
479	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	876.3	741.2
479	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1067.1	983.2
479	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	922.0	983.2
479	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	960.4	983.2
479	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	1859.1	1730.5
479	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	1485.5	1730.5
479	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	1847.1	1730.5
479	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	3871.5	3322.4
479	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	3348.0	3322.4
479	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	2747.6	3322.4

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
479	49	1	Hot	c1	10	7.5	75	11492.2	75	10981.5	13478
479	50	2	Hot	c1	10	7.5	75	10478.2	68	10981.5	13478
479	51	3	Hot	c1	10	7.5	75	10974.0	72	10981.5	13478
479	52	1	Hot	c2	10	15	150	23303.0	152	22904.3	26956
479	53	2	Hot	c2	10	15	150	22698.0	148	22904.3	26956
479	54	3	Hot	c2	10	15	150	22712.0	148	22904.3	26956
479	55	1	Hot	c3	10	21	210	30789.0	201	31138.3	37738
479	56	2	Hot	c3	10	21	210	31196.0	204	31138.3	37738
479	57	3	Hot	c3	10	21	210	31430.0	205	31138.3	37738
479	58	1	Hot	c4	10	30	300	45413.0	297	44083.3	53912
479	59	2	Hot	c4	10	30	300	44380.0	290	44083.3	53912
479	60	3	Hot	c4	10	30	300	42457.0	277	44083.3	53912
479	61	1	Hot	c5	10	45	450	70912.0	463	69856.0	80867
479	62	2	Hot	c5	10	45	450	69410.0	453	69856.0	80867
479	63	3	Hot	c5	10	45	450	69246.0	452	69856.0	80867
479	64	1	Hot	c6	100	7.5	750	111379.0	727	112478.7	134779
479	65	2	Hot	c6	100	7.5	750	112051.0	732	112478.7	134779
479	66	3	Hot	c6	100	7.5	750	114006.0	744	112478.7	134779
479	67	1	Hot	c7	100	15	1500	221591.0	1447	222215.3	269558
479	68	2	Hot	c7	100	15	1500	227633.0	1486	222215.3	269558
479	69	3	Hot	c7	100	15	1500	217422.0	1420	222215.3	269558
479	70	1	Hot	c8	100	30	3000	456488.0	2981	463934.3	539116
479	71	2	Hot	c8	100	30	3000	468707.0	3060	463934.3	539116
479	72	3	Hot	c8	100	30	3000	466608.0	3047	463934.3	539116

Computation Check

7/11/05	specific activity date
80.95	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7971E+14	DPM/mmole
1.7971E+11	DPM/nmole
179.7	DPM/fmole
0.005565	fmole/DPM



Linear regression results (LINEST function)

(Regression line forced through 0,0)

Slope	153.1501233 dpm/fmole	
1/slope	0.006529541 fmole/dpm	
	x	y
origin	0	0
end point	3060.4	468707

SLOPE function, used if missing HOT tubes

Slope	154.1 dpm/fmole	
1/slope	0.006491 fmole/dpm	
	x	y
origin	0	0
end point	3042.3	468707

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
4.2686E-11	0.27483	2.03694E-10	4.2686E-11
4.18513E-11	0.26802	2.03694E-10	4.18513E-11
4.1011E-11	0.26123	2.03694E-10	4.1011E-11
7.25669E-11	0.20972	4.2485E-10	7.25669E-11
7.74416E-11	0.22701	4.2485E-10	7.74416E-11
7.4164E-11	0.21533	4.2485E-10	7.4164E-11
7.39967E-11	0.14929	5.77581E-10	7.39967E-11
8.76023E-11	0.18173	5.77581E-10	8.76023E-11
8.8437E-11	0.18378	5.77581E-10	8.8437E-11
1.15129E-10	0.16616	8.17696E-10	1.15129E-10
1.13371E-10	0.16321	8.17696E-10	1.13371E-10
1.1277E-10	0.16220	8.17696E-10	1.1277E-10
1.26977E-10	0.10993	1.29575E-09	1.26977E-10
1.29019E-10	0.11190	1.29575E-09	1.29019E-10
1.37583E-10	0.12022	1.29575E-09	1.37583E-10
1.66794E-10	0.08773	2.08635E-09	1.66794E-10
1.75146E-10	0.09252	2.08635E-09	1.75146E-10
1.68781E-10	0.08886	2.08635E-09	1.68781E-10
1.57611E-10	0.04008	4.12185E-09	1.57611E-10
1.87688E-10	0.04810	4.12185E-09	1.87688E-10
1.9544E-10	0.05019	4.12185E-09	1.9544E-10
2.06168E-10	0.02473	8.60546E-09	2.06168E-10
2.10119E-10	0.02521	8.60546E-09	2.10119E-10
2.05956E-10	0.02470	8.60546E-09	2.05956E-10

Bmax molar	2.27E-10	KD molar	9.04E-10
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.80E+17	**kd nM =**	9.04E-01
Bmax molar to Bmax moles	6.804E-14		
= DPM/((DPM/mmole)*1000)	6.804E-14		
=Bmax DPM	12227.15402		
assay date	7/11/2005		
Bmax(dpm)	12227.15402		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.95		
DPM/mmole	1.80E+14		
DPM/pmole	1.80E+05		
1/(DPM/mmole)	5.56E-15		
1/(DPM/pmole)	5.56E-06		
SA(dpm/pmole)	1.80E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.068040		
bmax pmole/mg	0.1134		
Bmax fmole/mg	113.4		
Bmax (fmole/100 ug)	11.34		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Laboratory E

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in columns O and DK

If the DPM value for a tube was judged unreliable,

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

For your convenience, data reduction is performed in columns

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

in columns CF through CN

Cells in column S are presented with a grey background

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

the cytosol concentration is probably too high for good competitive assays

Laboratory Code:	E	
Run identification:	481	
Assay start date:	7/13/2005	
Tracer lot number:	3559-507	
Specific activity on day of assay:	80.92	**Ci/mmole**
Cytosol lot or vial number:	051905	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	9.16E-01	**nM**
Bmax	11.11	**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)	Significant portion of label on Vial	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	—	730.51	2191.53	TRUE		19.5%	0.25	2191.5	296.4
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	747.45	2242.35	TRUE		19.9%	0.25	2242.4	296.4
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	878.85	2636.55	TRUE		23.4%	0.25	2636.6	296.4
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1392.20	4176.6	TRUE		18.3%	0.5	4176.6	408.0
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1455.50	4366.5	TRUE		19.1%	0.5	4366.5	408.0
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1519.50	4558.5	TRUE		19.9%	0.5	4558.5	408.0
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1728.20	5184.6	TRUE		16.3%	0.7	5184.6	418.8
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1744.20	5232.6	TRUE		16.4%	0.7	5232.6	418.8
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1816.20	5448.6	TRUE		17.1%	0.7	5448.6	418.8
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2291.20	6873.6	TRUE		15.5%	1	6873.6	590.6
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2173.50	6520.5	TRUE		14.7%	1	6520.5	590.6
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2237.80	6713.4	TRUE		15.2%	1	6713.4	590.6
13	1	H	10.0	45	1.50	—	—	—	50	300	—	2266.50	6799.5	TRUE		10.0%	1.5	6799.5	726.6
14	2	H	10.0	45	1.50	—	—	—	50	300	—	2700.60	8101.8	TRUE		11.9%	1.5	8101.8	726.6
15	3	H	10.0	45	1.50	—	—	—	50	300	—	2762.80	8288.4	TRUE		12.2%	1.5	8288.4	726.6
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	3115.70	9347.1	TRUE		8.4%	2.5	9347.1	1117.1
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	3555.50	10666.5	TRUE		9.6%	2.5	10666.5	1117.1
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	3403.20	10209.6	TRUE		9.2%	2.5	10209.6	1117.1
19	1	H	100.0	15	5.00	—	—	—	50	300	—	3853.00	11559	TRUE		4.9%	5	11559.0	1982.8
20	2	H	100.0	15	5.00	—	—	—	50	300	—	3845.40	11536.2	TRUE		4.9%	5	11536.2	1982.8
21	3	H	100.0	15	5.00	—	—	—	50	300	—	4075.20	12225.6	TRUE		5.2%	5	12225.6	1982.8
22	1	H	100.0	30	10.00	—	—	—	50	300	—	5105.60	15316.8	TRUE		3.3%	10	15316.8	3237.3
23	2	H	100.0	30	10.00	—	—	—	50	300	—	3955.10	11865.3	TRUE		2.6%	10	11865.3	3237.3
24	3	H	100.0	30	10.00	—	—	—	50	300	—	4761.80	14285.4	TRUE		3.1%	10	14285.4	3237.3

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	108.37	325.11	TRUE					
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	84.59	253.77	TRUE					
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	103.46	310.38	TRUE					
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	157.82	473.46	TRUE					
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	124.82	374.46	TRUE					
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	125.38	376.14	TRUE					
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	123.29	369.87	TRUE					
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	151.81	455.43	TRUE					
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	143.71	431.13	TRUE					
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	207.97	623.91	TRUE					
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	212.25	636.75	TRUE					
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	170.37	511.11	TRUE					
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	210.01	630.03	TRUE					
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	248.20	744.6	TRUE					
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	268.36	805.08	TRUE					
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	463.12	1389.36	TRUE					
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	311.35	934.05	TRUE					
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	342.66	1027.98	TRUE					
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	701.26	2103.78	TRUE					
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	534.00	1602	TRUE					
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	747.49	2242.47	TRUE					
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	966.63	2899.89	TRUE					
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	1378.60	4135.8	TRUE					
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	892.02	2676.06	TRUE					
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11449.5	11449.5	TRUE					
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11204.8	11204.8	TRUE					
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11100.3	11100.3	TRUE					
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23154.0	23154	TRUE					
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	22697.0	22697	TRUE					
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	22707.0	22707	TRUE					
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	31416.0	31416	TRUE					
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	31867.0	31867	TRUE					
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	32339.0	32339	TRUE					
58	1	Hot	10.0	30	1	—	—	—	—	—	—	42879.0	42879	TRUE					
59	2	Hot	10.0	30	1	—	—	—	—	—	—	45013.0	45013	TRUE					
60	3	Hot	10.0	30	1	—	—	—	—	—	—	44787.0	44787	TRUE					
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	69306.0	69306	TRUE					
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	68642.0	68642	TRUE					
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	66107.0	66107	TRUE					
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	111878	111878	TRUE					
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	112465	112465	TRUE					
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	110005	110005	TRUE					
67	1	Hot	100.0	15	5	—	—	—	—	—	—	237414	237414	TRUE					
68	2	Hot	100.0	15	5	—	—	—	—	—	—	233806	233806	TRUE					
69	3	Hot	100.0	15	5	—	—	—	—	—	—	234692	234692	TRUE					
70	1	Hot	100.0	30	10	—	—	—	—	—	—	455700	455700	TRUE					
71	2	Hot	100.0	30	10	—	—	—	—	—	—	470855	470855	TRUE					
72	3	Hot	100.0	30	10	—	—	—	—	—	—	463848	463848	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)
481	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
481	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
481	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
481	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
481	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
481	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
481	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
481	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
481	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
481	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
481	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
481	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
481	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
481	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
481	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
481	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
481	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
481	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
481	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
481	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
481	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
481	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
481	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
481	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)	
481	1	2191.5	296.4	1895.1	13.5%	19.5%	11251.5	9060.0	14	2	12	73	59	0.21
481	2	2242.4	296.4	1945.9	13.2%	19.9%	11251.5	9009.2	15	2	13	73	58	0.22
481	3	2636.6	296.4	2340.1	11.2%	23.4%	11251.5	8615.0	17	2	15	73	56	0.27
481	4	4176.6	408.0	3768.6	9.8%	18.3%	22852.7	18676.1	27	3	24	148	121	0.20
481	5	4366.5	408.0	3958.5	9.3%	19.1%	22852.7	18486.2	28	3	26	148	120	0.21
481	6	4558.5	408.0	4150.5	9.0%	19.9%	22852.7	18294.2	29	3	27	148	118	0.23
481	7	5184.6	418.8	4765.8	8.1%	16.3%	31874.0	26689.4	34	3	31	206	173	0.18
481	8	5232.6	418.8	4813.8	8.0%	16.4%	31874.0	26641.4	34	3	31	206	172	0.18
481	9	5448.6	418.8	5029.8	7.7%	17.1%	31874.0	26425.4	35	3	33	206	171	0.19
481	10	6873.6	590.6	6283.0	8.6%	15.5%	44226.3	37352.7	44	4	41	286	242	0.17
481	11	6520.5	590.6	5929.9	9.1%	14.7%	44226.3	37705.8	42	4	38	286	244	0.16
481	12	6713.4	590.6	6122.8	8.8%	15.2%	44226.3	37512.9	43	4	40	286	243	0.16
481	13	6799.5	726.6	6072.9	10.7%	10.0%	68018.3	61218.8	44	5	39	440	396	0.10
481	14	8101.8	726.6	7375.2	9.0%	11.9%	68018.3	59916.5	52	5	48	440	388	0.12
481	15	8288.4	726.6	7561.8	8.8%	12.2%	68018.3	59729.9	54	5	49	440	387	0.13
481	16	9347.1	1117.1	8230.0	12.0%	8.4%	111449.3	102102.2	60	7	53	721	661	0.08
481	17	10666.5	1117.1	9549.4	10.5%	9.6%	111449.3	100782.8	69	7	62	721	652	0.09
481	18	10209.6	1117.1	9092.5	10.9%	9.2%	111449.3	101239.7	66	7	59	721	655	0.09
481	19	11559.0	1982.8	9576.3	17.2%	4.9%	235304.0	223745.0	75	13	62	1523	1448	0.04
481	20	11536.2	1982.8	9553.5	17.2%	4.9%	235304.0	223767.8	75	13	62	1523	1448	0.04
481	21	12225.6	1982.8	10242.9	16.2%	5.2%	235304.0	223078.4	79	13	66	1523	1444	0.05
481	22	15316.8	3237.3	12079.6	21.1%	3.3%	463467.7	448150.9	99	21	78	2999	2900	0.03
481	23	11865.3	3237.3	8628.1	27.3%	2.6%	463467.7	451602.4	77	21	56	2999	2922	0.02
481	24	14285.4	3237.3	11048.2	22.7%	3.1%	463467.7	449182.3	92	21	71	2999	2907	0.02

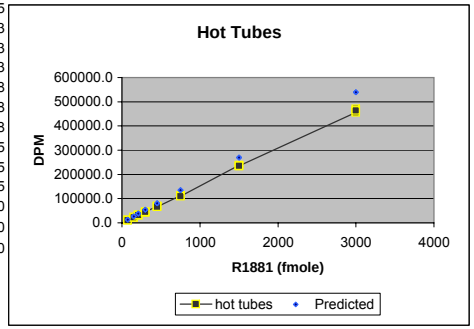
		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
		Tube Identification			Assay tube contents								Scintillation Results	
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	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)
481	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	325.1	296.4
481	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	253.8	296.4
481	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	310.4	296.4
481	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	473.5	408.0
481	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	374.5	408.0
481	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	376.1	408.0
481	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	369.9	418.8
481	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	455.4	418.8
481	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	431.1	418.8
481	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	623.9	590.6
481	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	636.8	590.6
481	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	511.1	590.6
481	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	630.0	726.6
481	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	744.6	726.6
481	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	805.1	726.6
481	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1389.4	1117.1
481	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	934.1	1117.1
481	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1028.0	1117.1
481	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2103.8	1982.8
481	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	1602.0	1982.8
481	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	2242.5	1982.8
481	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	2899.9	3237.3
481	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	4135.8	3237.3
481	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	2676.1	3237.3

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
481	49	1	Hot	c1	10	7.5	75	11449.5	74	11251.5	13474
481	50	2	Hot	c1	10	7.5	75	11204.8	73	11251.5	13474
481	51	3	Hot	c1	10	7.5	75	11100.3	72	11251.5	13474
481	52	1	Hot	c2	10	15	150	23154.0	150	22852.7	26948
481	53	2	Hot	c2	10	15	150	22697.0	147	22852.7	26948
481	54	3	Hot	c2	10	15	150	22707.0	147	22852.7	26948
481	55	1	Hot	c3	10	21	210	31416.0	203	31874.0	37727
481	56	2	Hot	c3	10	21	210	31867.0	206	31874.0	37727
481	57	3	Hot	c3	10	21	210	32339.0	209	31874.0	37727
481	58	1	Hot	c4	10	30	300	42879.0	277	44226.3	53895
481	59	2	Hot	c4	10	30	300	45013.0	291	44226.3	53895
481	60	3	Hot	c4	10	30	300	44787.0	290	44226.3	53895
481	61	1	Hot	c5	10	45	450	69306.0	448	68018.3	80843
481	62	2	Hot	c5	10	45	450	68642.0	444	68018.3	80843
481	63	3	Hot	c5	10	45	450	66107.0	428	68018.3	80843
481	64	1	Hot	c6	100	7.5	750	111878.0	724	111449.3	134738
481	65	2	Hot	c6	100	7.5	750	112465.0	728	111449.3	134738
481	66	3	Hot	c6	100	7.5	750	110005.0	712	111449.3	134738
481	67	1	Hot	c7	100	15	1500	237414.0	1536	235304.0	269475
481	68	2	Hot	c7	100	15	1500	233806.0	1513	235304.0	269475
481	69	3	Hot	c7	100	15	1500	234692.0	1519	235304.0	269475
481	70	1	Hot	c8	100	30	3000	455700.0	2949	463467.7	538950
481	71	2	Hot	c8	100	30	3000	470855.0	3047	463467.7	538950
481	72	3	Hot	c8	100	30	3000	463848.0	3002	463467.7	538950

Computation Check

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

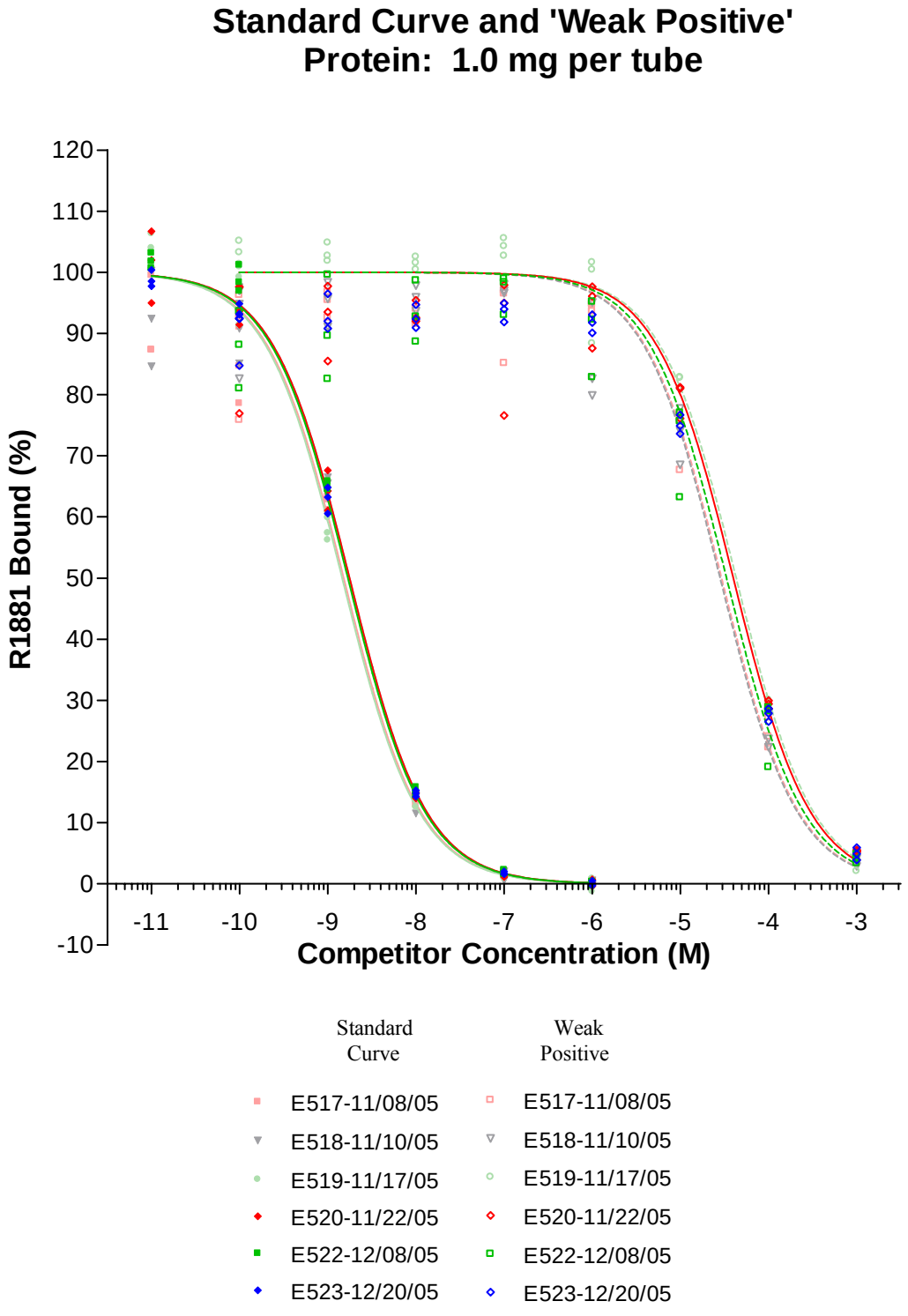


Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	154.5333567 dpm/fmole	
1/slope	0.006471095 fmole/dpm	
	x	y
origin	0	0
end point	3046.9	470855
SLOPE function, used if missing HOT tubes		
Slope	155.2 dpm/fmole	
1/slope	0.006442 fmole/dpm	
	x	y
origin	0	0
end point	3033.5	470855

Prism input for bound/free		Prism input for specific bound	
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*
3.5163E-11	0.20917	2.08767E-10	3.5163E-11
3.61059E-11	0.21599	2.08767E-10	3.61059E-11
4.34201E-11	0.27163	2.08767E-10	4.34201E-11
6.99244E-11	0.20179	4.24022E-10	6.99244E-11
7.34479E-11	0.21413	4.24022E-10	7.34479E-11
7.70104E-11	0.22687	4.24022E-10	7.70104E-11
8.84272E-11	0.17856	5.91409E-10	8.84272E-11
8.93179E-11	0.18069	5.91409E-10	8.93179E-11
9.33256E-11	0.19034	5.91409E-10	9.33256E-11
1.16579E-10	0.16821	8.20601E-10	1.16579E-10
1.10027E-10	0.15727	8.20601E-10	1.10027E-10
1.13606E-10	0.16322	8.20601E-10	1.13606E-10
1.12681E-10	0.09920	1.26205E-09	1.12681E-10
1.36844E-10	0.12309	1.26205E-09	1.36844E-10
1.40307E-10	0.12660	1.26205E-09	1.40307E-10
1.52704E-10	0.08061	2.0679E-09	1.52704E-10
1.77185E-10	0.09475	2.0679E-09	1.77185E-10
1.68707E-10	0.08981	2.0679E-09	1.68707E-10
1.77683E-10	0.04280	4.36597E-09	1.77683E-10
1.7726E-10	0.04269	4.36597E-09	1.7726E-10
1.90052E-10	0.04592	4.36597E-09	1.90052E-10
2.24131E-10	0.02695	8.59945E-09	2.24131E-10
1.6009E-10	0.01911	8.59945E-09	1.6009E-10
2.04994E-10	0.02460	8.59945E-09	2.04994E-10

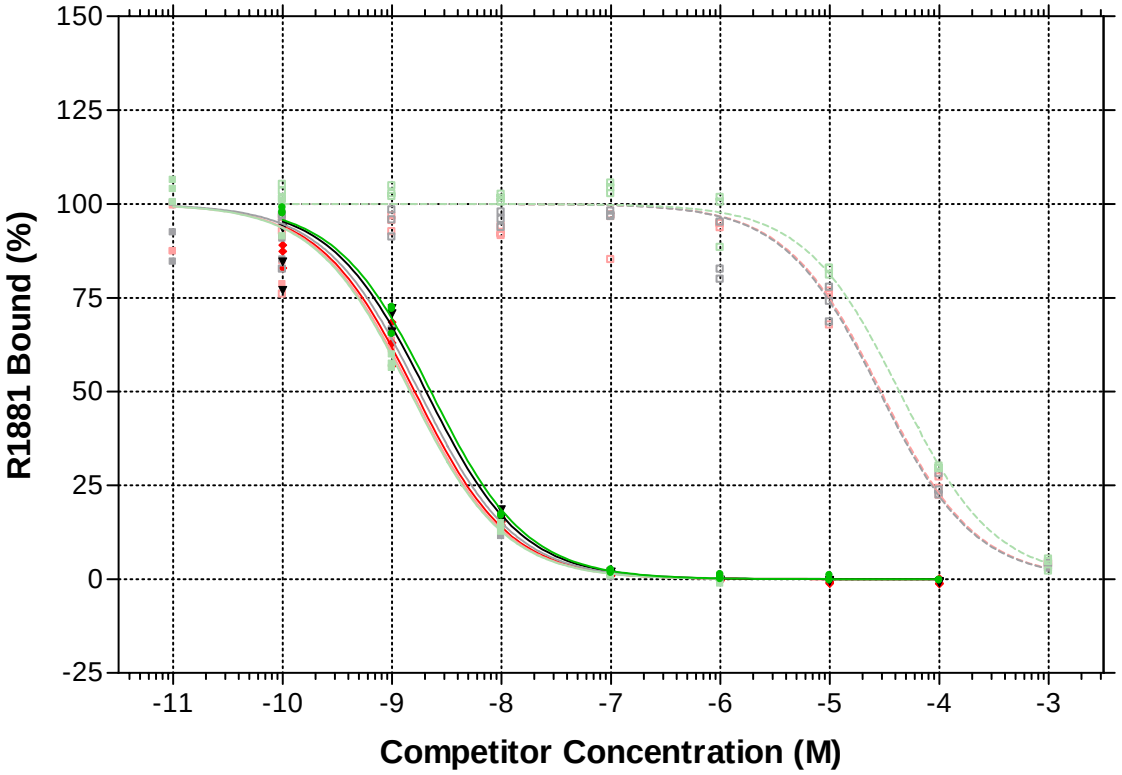
Bmax molar	2.22E-10	KD molar	9.16E-10
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.80E+17	**kd nM =**	9.16E-01
Bmax molar to Bmax moles	6.666E-14		
= DPM/((DPM/mmole)*1000)	6.666E-14		
=Bmax DPM	11975.47965		
assay date	7/13/2005		
Bmax(dpm)	11975.47965		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.92		
DPM/mmole	1.80E+14		
DPM/pmole	1.80E+05		
1/(DPM/mmole)	5.57E-15		
1/(DPM/pmole)	5.57E-06		
SA(dpm/pmole)	1.80E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.066660		
bmax pmole/mg	0.1111		
Bmax fmole/mg	111.1		
Bmax (fmole/100 ug)	11.11		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Appendix 4: Prism Files for Competitive Binding Experiments



WA 4-11 Competitive

Lab E CR42400
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive

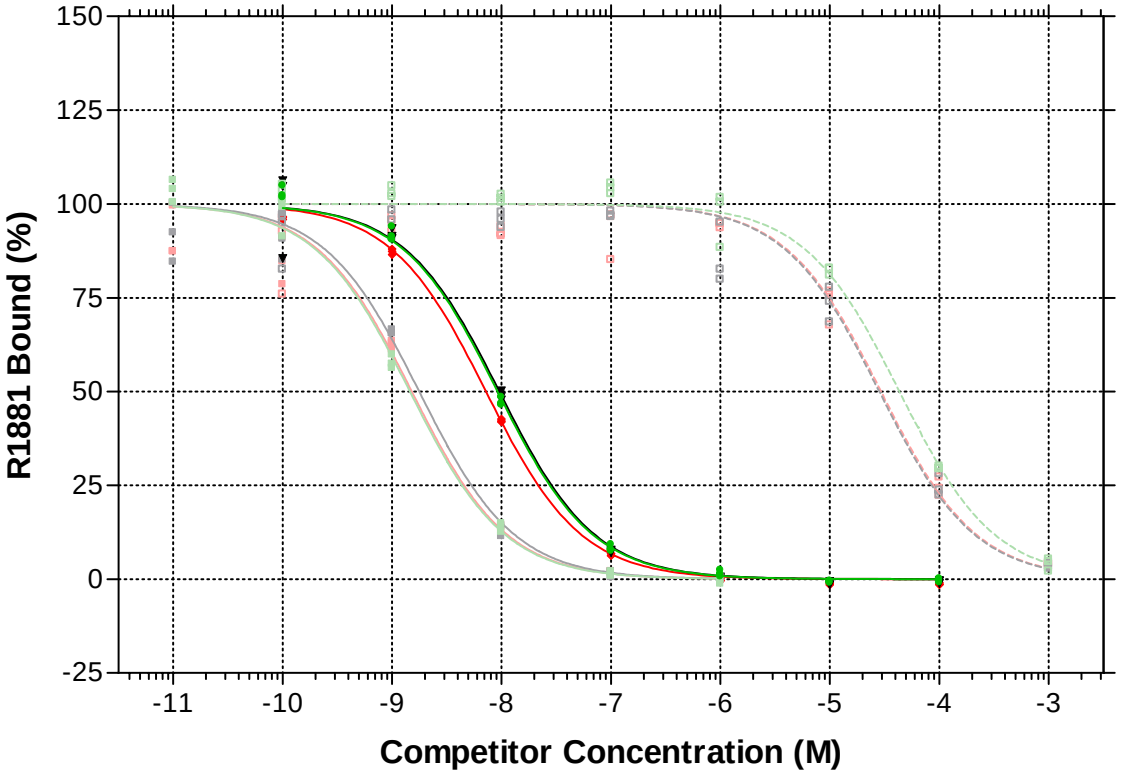


- E517-11/08/05
- E518-11/10/05
- E519-11/17/05

CR42400	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
E517-11/08/05	0.000	0.000	100.000	0.000	-8.793	0.035
E518-11/10/05	0.000	0.000	100.000	0.000	-8.688	0.047
E519-11/17/05	0.000	0.000	100.000	0.000	-8.645	0.016

WA 4-11 Competitive

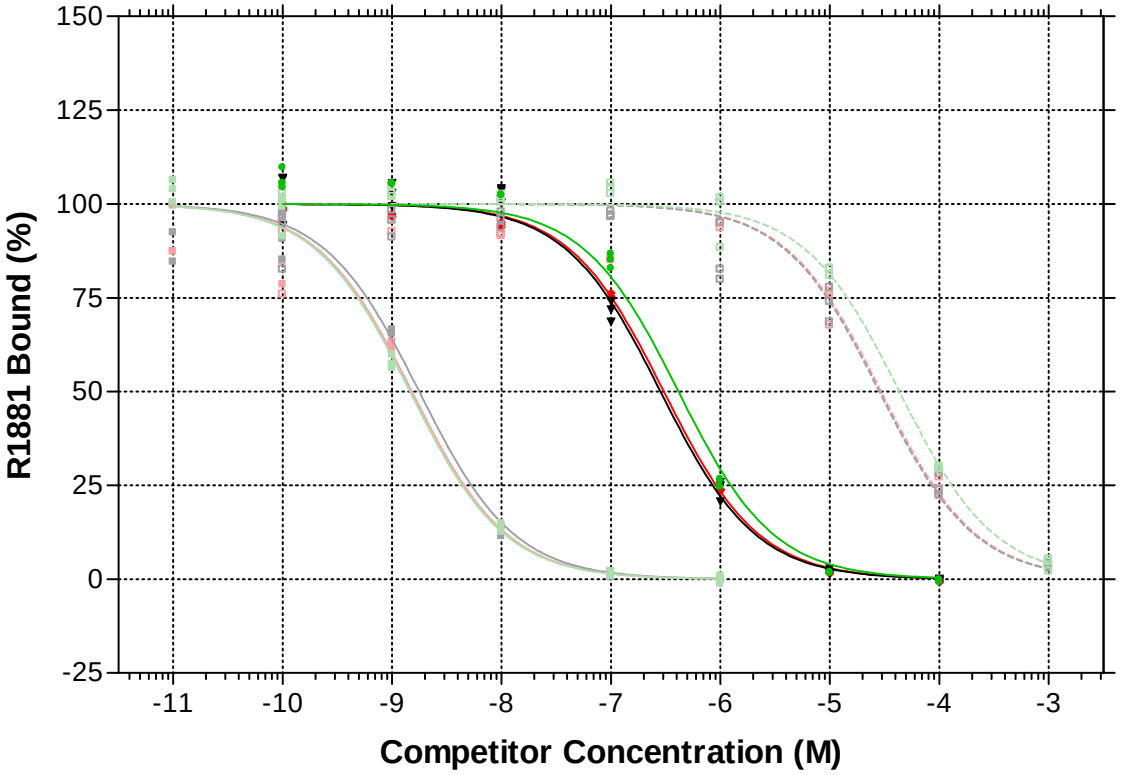
Lab E CR42401
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42401	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
◆ E517-11/08/05	E517-11/08/05	0.000	0.000	100.000	0.000	-8.141	0.012
▼ E518-11/10/05	E518-11/10/05	0.000	0.000	100.000	0.000	-8.019	0.035
● E519-11/17/05	E519-11/17/05	0.000	0.000	100.000	0.000	-8.035	0.018

WA 4-11 Competitive

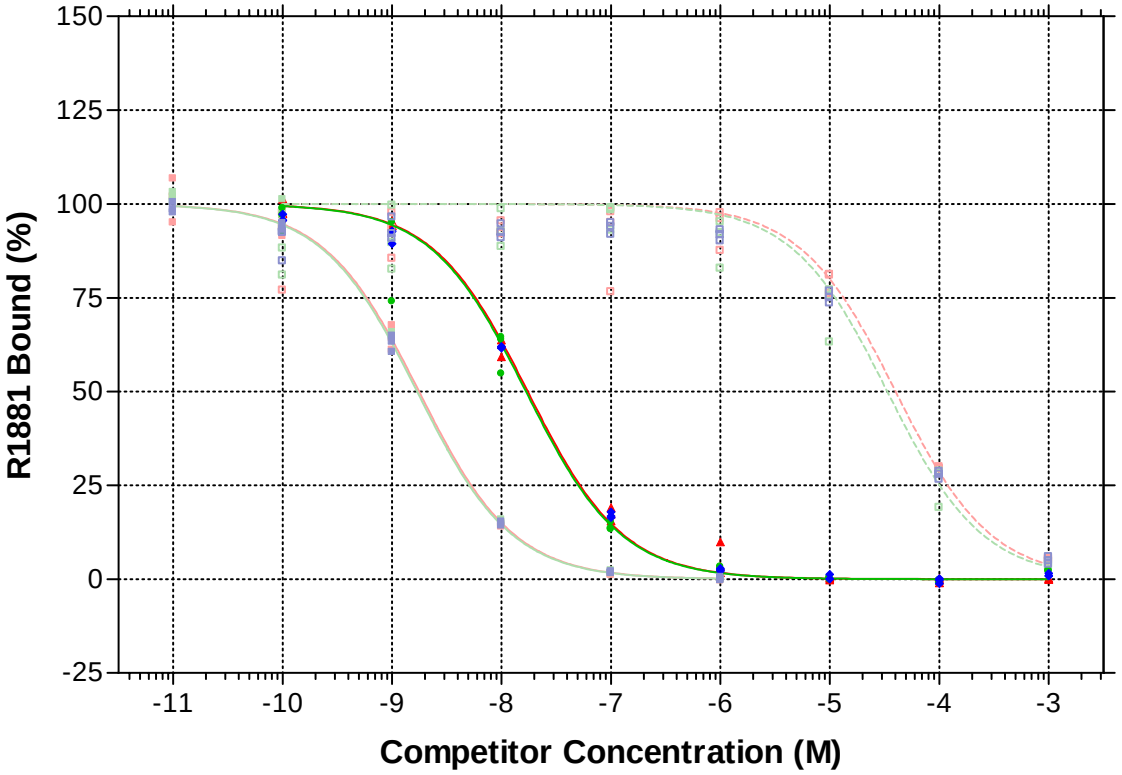
Lab E CR42402
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42402	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
◆ E517-11/08/05	E517-11/08/05	0.000	0.000	100.000	0.000	-6.516	0.014
▼ E518-11/10/05	E518-11/10/05	0.000	0.000	100.000	0.000	-6.547	0.040
● E519-11/17/05	E519-11/17/05	0.000	0.000	100.000	0.000	-6.383	0.044

WA 4-11 Competitive

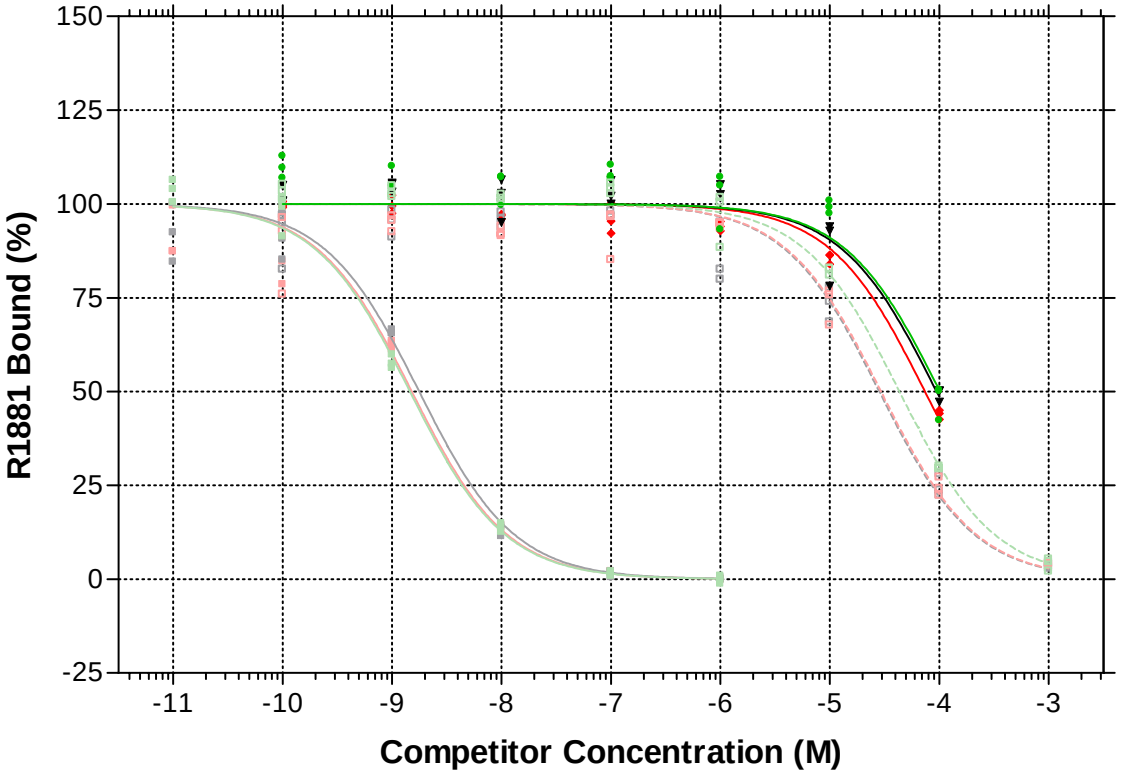
Lab E CR42403
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42403	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
▲ E520-11/22/05	E520-11/22/05	0.000	0.000	100.000	0.000	-7.760	0.022
● E522-12/08/05	E522-12/08/05	0.000	0.000	100.000	0.000	-7.826	0.044
◆ E523-12/20/05	E523-12/20/05	0.000	0.000	100.000	0.000	-7.776	0.019

WA 4-11 Competitive

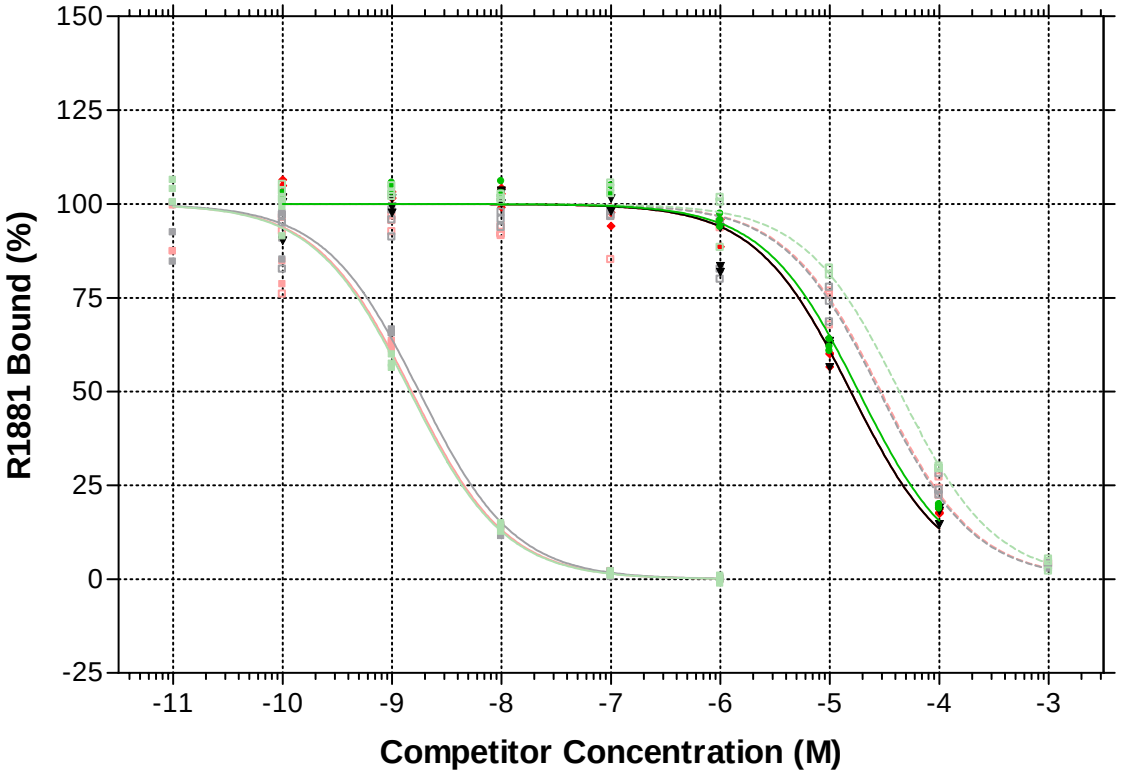
Lab E CR42404
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42404	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
◆ E517-11/08/05	E517-11/08/05	0.000	0.000	100.000	0.000	-4.130	0.031
▼ E518-11/10/05	E518-11/10/05	0.000	0.000	100.000	0.000	-4.024	0.046
● E519-11/17/05	E519-11/17/05	0.000	0.000	100.000	0.000	-3.993	0.073

WA 4-11 Competitive

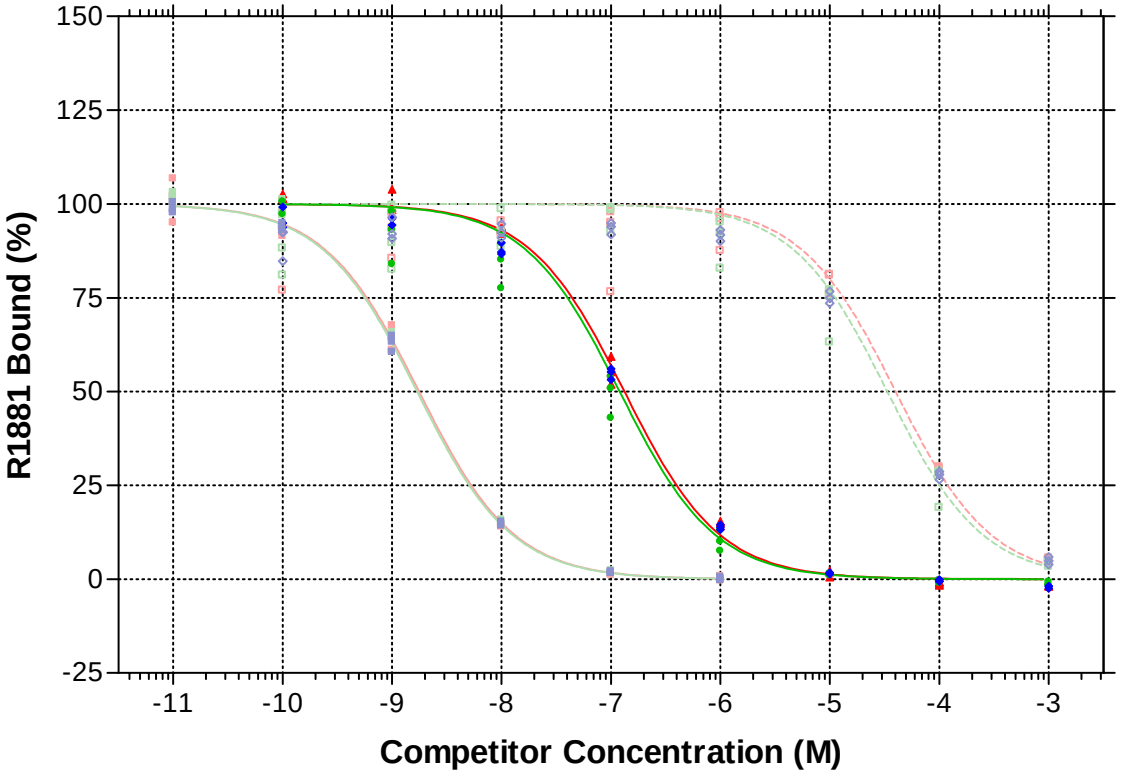
Lab E CR42405
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42405	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
◆ E517-11/08/05	E517-11/08/05	0.000	0.000	100.000	0.000	-4.808	0.033
▼ E518-11/10/05	E518-11/10/05	0.000	0.000	100.000	0.000	-4.808	0.045
● E519-11/17/05	E519-11/17/05	0.000	0.000	100.000	0.000	-4.736	0.034

WA 4-11 Competitive

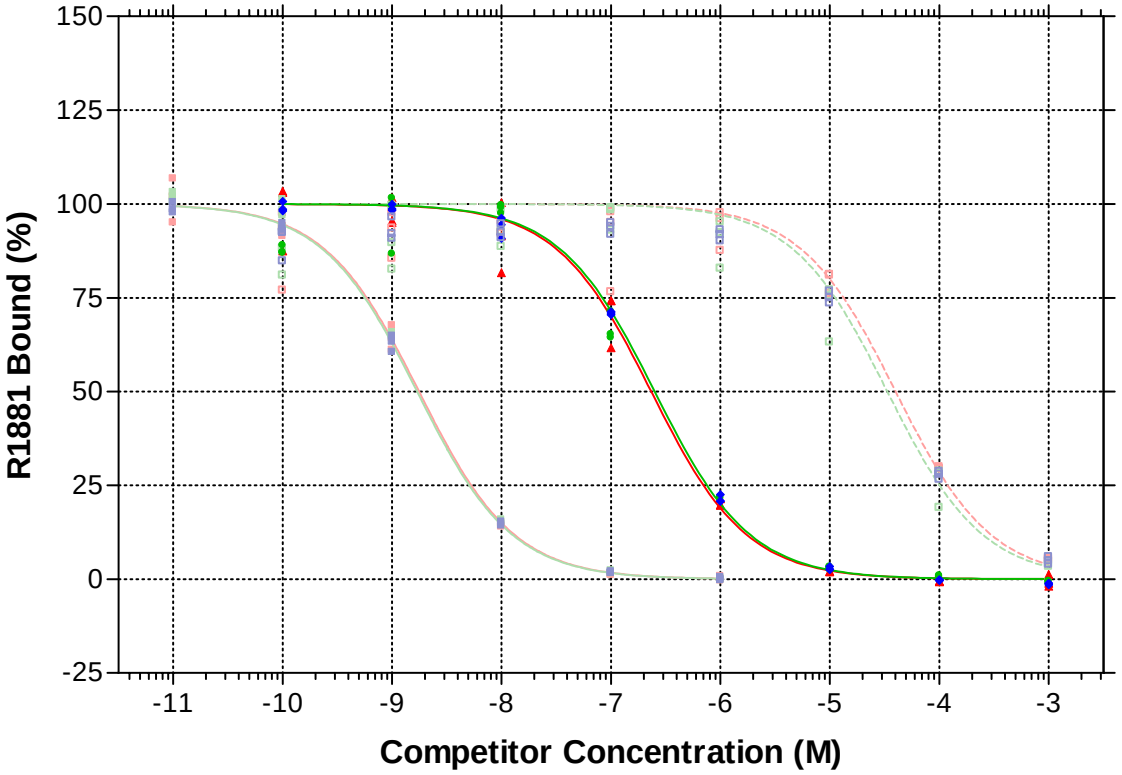
Lab E CR42406
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42406	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
▲ E520-11/22/05	E520-11/22/05	0.000	0.000	100.000	0.000	-6.877	0.021
● E522-12/08/05	E522-12/08/05	0.000	0.000	100.000	0.000	-7.046	0.046
◆ E523-12/20/05	E523-12/20/05	0.000	0.000	100.000	0.000	-6.920	0.028

WA 4-11 Competitive

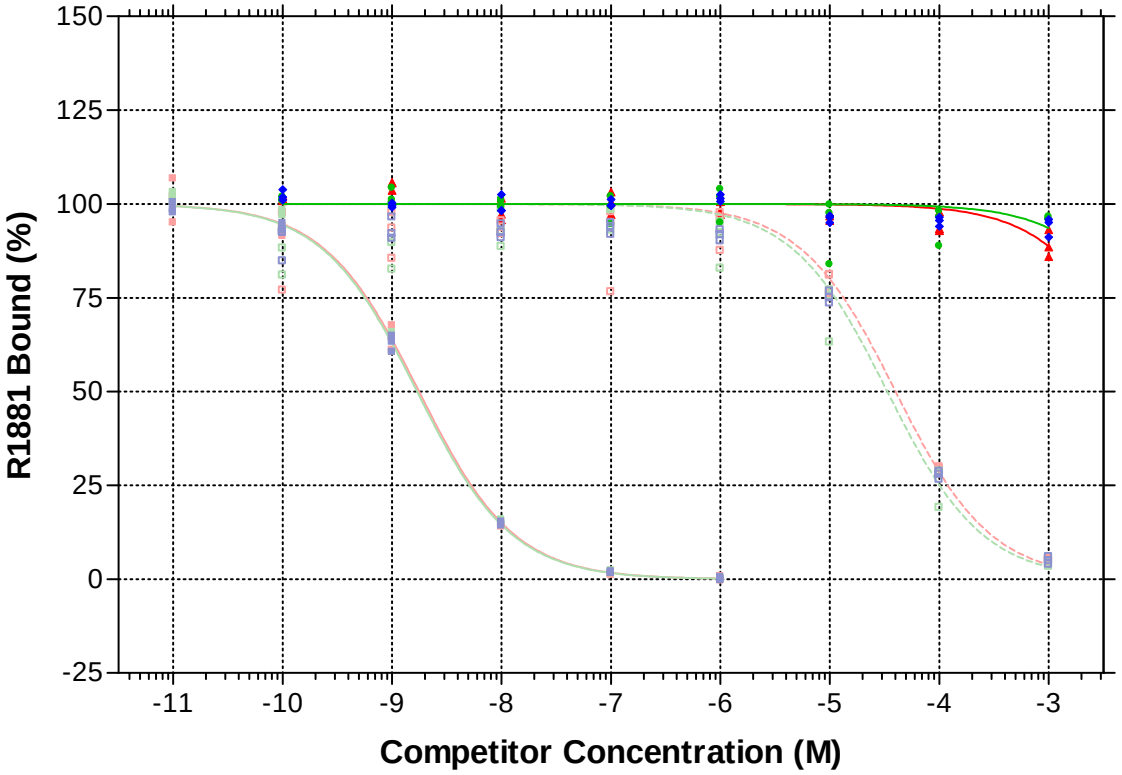
Lab E CR42407
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42407	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
▲ E520-11/22/05	E520-11/22/05	0.000	0.000	100.000	0.000	-6.631	0.048
● E522-12/08/05	E522-12/08/05	0.000	0.000	100.000	0.000	-6.639	0.053
◆ E523-12/20/05	E523-12/20/05	0.000	0.000	100.000	0.000	-6.600	0.015

WA 4-11 Competitive

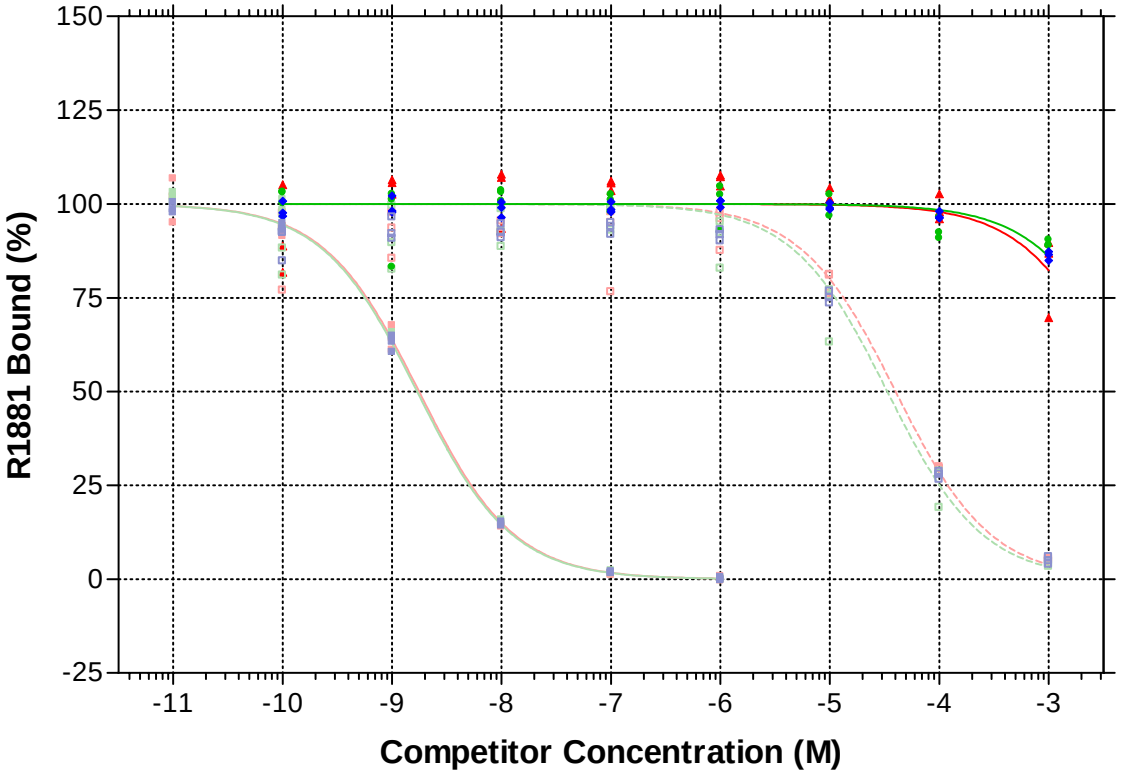
Lab E CR42408
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42408	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
▲ E520-11/22/05	E520-11/22/05	0.000	0.000	100.000	0.000	-2.105	0.084
● E522-12/08/05	E522-12/08/05	0.000	0.000	100.000	0.000	-1.670	0.271
◆ E523-12/20/05	E523-12/20/05	0.000	0.000	100.000	0.000	-1.837	0.107

WA 4-11 Competitive

Lab E CR42409
Protein: 1.0 mg per tube
Solid squares = Standard Curve, Open squares = Weak Positive



	CR42409	BOTTOM		TOP		LOGEC50	
		Y	SEM	Y	SEM	Y	SEM
▲ E520-11/22/05	E520-11/22/05	0.000	0.000	100.000	0.000	-2.332	0.124
● E522-12/08/05	E522-12/08/05	0.000	0.000	100.000	0.000	-2.102	0.122
◆ E523-12/20/05	E523-12/20/05	0.000	0.000	100.000	0.000	-2.209	0.036

Appendix 5: Excel Files for Competitive Binding Experiments

Competitive Assay of a known Weak Positive

177 Assay Tubes

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

Provide information in all blue cells
in columns O and P, and row 45, AE through BC
If the DPM value for a tube was judged unreliable,
Include the DPM value in column O
Provide a reason in column R
The value in column Q will
automatically change to FALSE

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

They are also presented in table form in columns

AC thorugh BD

Provide information in all blue cells in this column

Laboratory Code:	**E**		
Run identification:	517		
Assay start date:	11/8/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	79.47	**Ci/mmole**	
Cytosol vial or lot identification:	051905		
Protein (cytosol):	100	**micro gram per tube**	
Standard Curve IC50:	1.52E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	2.95E-05	**M**	
RBA:	5.14936E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	1.61E-09	**CR42400**	
RBA, Unknown 1:	94.16511%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	7.22E-09	**CR42401**	
RBA, Unknown 2:	21.00817%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	3.05E-07	**CR42402**	
RBA, Unknown 3:	0.49754%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	7.41E-05	**CR42404**	
RBA, Unknown 4:	0.00205%		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	1.56E-05	**CR42405**	
RBA, Unknown 5:	0.00976%		
volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3.1		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values	n	Mean	SD	
EtOH	6	11667.5	327.63	Total Binding, solvent control, tubes
Hot	6	43435.8	1237.92	Total hot R1881 added to each tube
NSB	6	503.7	28.59	Nonspecific Binding
Specific EtOH	6	11163.8	327.63	

Assay Characterization Values		
EtOH / Hot	0.27	less than 0.1?
NSB / EtOH	0.04	around 0.25 ?

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

Check the 10% rule:	26.86%	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	3770.10	11687.31	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3701.50	11474.65	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3591.40	11133.34	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	172.59	517.77	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	173.63	520.89	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	160.58	481.74	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	235.09	728.78	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	238.39	739.01	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	220.23	682.71	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	678.11	2102.14	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	653.81	2026.81	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	628.74	1949.09	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2363.20	7325.92	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2445.30	7580.43	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2423.80	7513.78	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3212.10	9957.51	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2991.70	9274.27	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3440.90	10666.79	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3306.60	10250.46	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3746.70	11614.77	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3308.70	10256.97	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	315.56	946.68	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	326.88	980.64	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	312.91	938.73	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1065.03	3195.09	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	997.92	2993.76	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1179.90	3539.70	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3021.30	9063.90	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2985.80	8957.40	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2686.30	8058.90	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3698.50	11095.50	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3650.70	10952.10	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3692.60	11077.80	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3336.40	10009.20	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3777.10	11331.30	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3760.90	11282.70	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3573.90	10721.70	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3667.00	11001.00	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3592.40	10777.20	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3721.30	11163.90	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3768.30	11304.90	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3613.40	10840.20	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3751.80	11255.40	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	2992.00	8976.00	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3616.80	10850.40	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
1	1		—	100.2	11687.31	11183.6	-523.5	100.2	26.90707
2	2		—	98.3	11474.65	10971.0	-310.9	98.3	26.41747
3	3		—	95.2	11133.34	10629.7	30.4	95.2	25.63169
4	1		-6.00	0.1	517.77	14.1	10646.0	0.1	1.192034
5	2		-6.00	0.2	520.89	17.2	10642.9	0.2	1.199217
6	3		-6.00	0.0	481.74	-21.9	10682.0	-0.2	1.109084
7	1	cold R1881	-7.01	2.0	728.779	225.1	10435.0	2.0	1.677829
8	2	cold R1881	-7.01	2.1	739.009	235.3	10424.8	2.1	1.701381
9	3	cold R1881	-7.01	1.6	682.713	179.0	10481.1	1.6	1.571774
10	1	cold R1881	-8.01	14.3	2102.141	1598.5	9061.6	14.3	4.839647
11	2	cold R1881	-8.01	13.6	2026.811	1523.1	9137.0	13.6	4.666219
12	3	cold R1881	-8.01	12.9	1949.094	1445.4	9214.7	12.9	4.487295
13	1	cold R1881	-9.01	61.1	7325.92	6822.2	3837.9	61.1	16.86607
14	2	cold R1881	-9.01	63.4	7580.43	7076.7	3583.4	63.4	17.45202
15	3	cold R1881	-9.01	62.8	7513.78	7010.1	3650.0	62.8	17.29857
16	1	cold R1881	-10.01	84.7	9957.51	9453.8	1206.3	84.7	22.92464
17	2	cold R1881	-10.01	78.6	9274.27	8770.6	1889.5	78.6	21.35166
18	3	cold R1881	-10.01	91.0	10666.79	10163.1	497.0	91.0	24.55758
19	1	cold R1881	-11.01	87.3	10250.46	9746.8	913.3	87.3	23.59909
20	2	cold R1881	-11.01	99.5	11614.77	11111.1	-451.0	99.5	26.74006
21	3	cold R1881	-11.01	87.4	10256.97	9753.3	906.8	87.4	23.61407
22	1	Weak Positive	-3	4.0	946.68	443.0	10217.1	4.0	2.179491
23	2	Weak Positive	-3	4.3	980.64	477.0	10183.1	4.3	2.257675
24	3	Weak Positive	-3	3.9	938.73	435.0	10225.1	3.9	2.161188
25	1	Weak Positive	-4	24.1	3195.09	2691.4	7968.7	24.1	7.355885
26	2	Weak Positive	-4	22.3	2993.76	2490.1	8170.0	22.3	6.892374
27	3	Weak Positive	-4	27.2	3539.7	3036.0	7624.1	27.2	8.149262
28	1	Weak Positive	-5	76.7	9063.9	8560.2	2099.9	76.7	20.86733
29	2	Weak Positive	-5	75.7	8957.4	8453.7	2206.4	75.7	20.62214
30	3	Weak Positive	-5	67.7	8058.9	7555.2	3104.9	67.7	18.55358
31	1	Weak Positive	-6	94.9	11095.5	10591.8	68.3	94.9	25.54458
32	2	Weak Positive	-6	93.6	10952.1	10448.4	211.7	93.6	25.21444
33	3	Weak Positive	-6	94.7	11077.8	10574.1	86.0	94.7	25.50383
34	1	Weak Positive	-7	85.1	10009.2	9505.5	1154.6	85.1	23.04365
35	2	Weak Positive	-7	97.0	11331.3	10827.6	-167.5	97.0	26.08745
36	3	Weak Positive	-7	96.6	11282.7	10779.0	-118.9	96.6	25.97556
37	1	Weak Positive	-8	91.5	10721.7	10218.0	442.1	91.5	24.684
38	2	Weak Positive	-8	94.0	11001	10497.3	162.8	94.0	25.32701
39	3	Weak Positive	-8	92.0	10777.2	10273.5	386.6	92.0	24.81177
40	1	Weak Positive	-9	95.5	11163.9	10660.2	-0.1	95.5	25.70205
41	2	Weak Positive	-9	96.8	11304.9	10801.2	-141.1	96.8	26.02667
42	3	Weak Positive	-9	92.6	10840.2	10336.5	323.6	92.6	24.95681
43	1	Weak Positive	-10	96.3	11255.4	10751.7	-91.6	96.3	25.91271
44	2	Weak Positive	-10	75.9	8976	8472.3	2187.8	75.9	20.66497
45	3	Weak Positive	-10	92.7	10850.4	10346.7	313.4	92.7	24.9803

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

Check the 10% rule:	26.86%	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"
46	1	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	373.04	1156.42	FALSE	precipitate in tube
47	2	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	283.60	879.16	FALSE	precipitate in tube
48	3	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	833.74	2584.59	FALSE	precipitate in tube
49	1	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	125.28	388.37	TRUE	
50	2	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	118.60	367.66	TRUE	
51	3	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	169.48	525.39	TRUE	
52	1	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	135.77	420.89	TRUE	
53	2	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	119.62	370.82	TRUE	
54	3	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	129.17	400.43	TRUE	
55	1	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	162.96	505.18	TRUE	
56	2	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	154.36	478.52	TRUE	
57	3	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	149.87	464.60	TRUE	
58	1	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	203.41	630.57	TRUE	
59	2	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	195.23	605.21	TRUE	
60	3	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	227.12	704.07	TRUE	
61	1	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	680.82	2110.54	TRUE	
62	2	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	752.16	2331.70	TRUE	
63	3	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	714.23	2214.11	TRUE	
64	1	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2208.90	6847.59	TRUE	
65	2	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2629.40	8151.14	TRUE	
66	3	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2429.60	7531.76	TRUE	
67	1	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	3149.00	9761.90	TRUE	
68	2	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	3370.80	10449.48	TRUE	
69	3	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	3309.50	10259.45	TRUE	
70	1	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	114.98	356.44	FALSE	precipitate in tube
71	2	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	126.31	391.56	FALSE	precipitate in tube
72	3	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	116.99	362.67	FALSE	precipitate in tube
73	1	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	111.72	346.33	TRUE	
74	2	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	127.44	395.06	TRUE	
75	3	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	112.24	347.94	TRUE	
76	1	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	130.84	405.60	TRUE	
77	2	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	120.01	372.03	TRUE	
78	3	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	109.57	339.67	TRUE	
79	1	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	149.72	464.13	TRUE	
80	2	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	167.69	519.84	TRUE	
81	3	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	147.68	457.81	TRUE	
82	1	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	389.23	1206.61	TRUE	
83	2	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	452.71	1403.40	TRUE	
84	3	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	406.78	1261.02	TRUE	
85	1	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1699.80	5269.38	TRUE	
86	2	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1671.30	5181.03	TRUE	
87	3	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1681.40	5212.34	TRUE	
88	1	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	3277.40	10159.94	TRUE	
89	2	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	3309.10	10258.21	TRUE	
90	3	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	3330.60	10324.86	TRUE	
91	1	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3636.40	11272.84	TRUE	
92	2	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3614.50	11204.95	TRUE	
93	3	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3641.30	11288.03	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	
46	1	Unknown 1	-3.01424							
47	2	Unknown 1	-3.01424							
48	3	Unknown 1	-3.01424							
49	1	Unknown 1	-4.01424	-1.0	388.368	-115.3	10775.4	-1.0	0.894119	
50	2	Unknown 1	-4.01424	-1.2	367.66	-136.0	10796.1	-1.2	0.846444	
51	3	Unknown 1	-4.01424	0.2	525.388	21.7	10638.4	0.2	1.209573	
52	1	Unknown 1	-5.01424	-0.7	420.887	-82.8	10742.9	-0.7	0.968986	
53	2	Unknown 1	-5.01424	-1.2	370.822	-132.9	10793.0	-1.2	0.853724	
54	3	Unknown 1	-5.01424	-0.9	400.427	-103.3	10763.4	-0.9	0.921882	
55	1	Unknown 1	-6.01424	0.0	505.176	1.5	10658.6	0.0	1.16304	
56	2	Unknown 1	-6.01424	-0.2	478.516	-25.2	10685.3	-0.2	1.101662	
57	3	Unknown 1	-6.01424	-0.4	464.597	-39.1	10699.2	-0.4	1.069617	
58	1	Unknown 1	-7.01424	1.1	630.571	126.9	10533.2	1.1	1.45173	
59	2	Unknown 1	-7.01424	0.9	605.213	101.5	10558.6	0.9	1.39335	
60	3	Unknown 1	-7.01424	1.8	704.072	200.4	10459.7	1.8	1.620947	
61	1	Unknown 1	-8.01424	14.4	2110.542	1606.9	9053.2	14.4	4.858988	
62	2	Unknown 1	-8.01424	16.4	2331.696	1828.0	8832.1	16.4	5.368139	
63	3	Unknown 1	-8.01424	15.3	2214.113	1710.4	8949.7	15.3	5.097434	
64	1	Unknown 1	-9.01424	56.8	6847.59	6343.9	4316.2	56.8	15.76484	
65	2	Unknown 1	-9.01424	68.5	8151.14	7647.5	3012.6	68.5	18.76593	
66	3	Unknown 1	-9.01424	63.0	7531.76	7028.1	3632.0	63.0	17.33997	
67	1	Unknown 1	-10.01424	82.9	9761.9	9258.2	1401.9	82.9	22.4743	
68	2	Unknown 1	-10.01424	89.1	10449.48	9945.8	714.3	89.1	24.05728	
69	3	Unknown 1	-10.01424	87.4	10259.45	9755.8	904.3	87.4	23.61978	
70	1	Unknown 2	-3.01424							
71	2	Unknown 2	-3.01424							
72	3	Unknown 2	-3.01424							
73	1	Unknown 2	-4.01424	-1.4	346.332	-157.4	10817.5	-1.4	0.797342	
74	2	Unknown 2	-4.01424	-1.0	395.064	-108.6	10768.7	-1.0	0.909535	
75	3	Unknown 2	-4.01424	-1.4	347.944	-155.7	10815.8	-1.4	0.801053	
76	1	Unknown 2	-5.01424	-0.9	405.604	-98.1	10758.2	-0.9	0.9338	
77	2	Unknown 2	-5.01424	-1.2	372.031	-131.7	10791.8	-1.2	0.856507	
78	3	Unknown 2	-5.01424	-1.5	339.667	-164.0	10824.1	-1.5	0.781997	
79	1	Unknown 2	-6.01424	-0.4	464.132	-39.6	10699.7	-0.4	1.068546	
80	2	Unknown 2	-6.01424	0.1	519.839	16.2	10643.9	0.1	1.196798	
81	3	Unknown 2	-6.01424	-0.4	457.808	-45.9	10706.0	-0.4	1.053987	
82	1	Unknown 2	-7.01424	6.3	1206.613	702.9	9957.2	6.3	2.777921	
83	2	Unknown 2	-7.01424	8.1	1403.401	899.7	9760.4	8.1	3.230975	
84	3	Unknown 2	-7.01424	6.8	1261.018	757.3	9902.8	6.8	2.903174	
85	1	Unknown 2	-8.01424	42.7	5269.38	4765.7	5894.4	42.7	12.13141	
86	2	Unknown 2	-8.01424	41.9	5181.03	4677.3	5982.8	41.9	11.92801	
87	3	Unknown 2	-8.01424	42.2	5212.34	4708.7	5951.4	42.2	12.00009	
88	1	Unknown 2	-9.01424	86.5	10159.94	9656.3	1003.8	86.5	23.39069	
89	2	Unknown 2	-9.01424	87.4	10258.21	9754.5	905.6	87.4	23.61693	
90	3	Unknown 2	-9.01424	88.0	10324.86	9821.2	838.9	88.0	23.77037	
91	1	Unknown 2	-10.01424	96.5	11272.84	10769.2	-109.1	96.5	25.95286	
92	2	Unknown 2	-10.01424	95.9	11204.95	10701.3	-41.2	95.9	25.79656	
93	3	Unknown 2	-10.01424	96.6	11288.03	10784.3	-124.2	96.6	25.98783	

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

Check the 10% rule:	26.86%	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"
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2311.00	7164.10	FALSE	precipitate in tube
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2400.50	7441.55	FALSE	precipitate in tube
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2225.90	6900.29	FALSE	precipitate in tube
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	153.57	476.07	TRUE	
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	142.91	443.02	TRUE	
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	132.42	410.50	TRUE	
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	228.15	707.27	TRUE	
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	213.89	663.06	TRUE	
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	233.36	723.42	TRUE	
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1025.41	3178.77	TRUE	
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1026.25	3181.38	TRUE	
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1013.14	3140.73	TRUE	
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2877.40	8919.94	TRUE	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2909.30	9018.83	TRUE	
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2851.80	8840.58	TRUE	
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3563.90	11048.09	TRUE	
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3541.70	10979.27	TRUE	
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3589.40	11127.14	TRUE	
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3669.50	11375.45	TRUE	
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3642.30	11291.13	TRUE	
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3756.10	11643.91	TRUE	
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3705.10	11485.81	TRUE	
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3767.30	11678.63	TRUE	
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3725.70	11549.67	TRUE	
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	783.71	2429.50	FALSE	precipitate in tube/ faint
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	632.82	1961.74	FALSE	precipitate in tube/ faint
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	871.39	2701.31	FALSE	precipitate in tube/ faint
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1785.20	5534.12	TRUE	
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1753.50	5435.85	TRUE	
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1694.80	5253.88	TRUE	
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3273.30	10147.23	TRUE	
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3274.60	10151.26	TRUE	
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3185.60	9875.36	TRUE	
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3550.00	11005.00	TRUE	
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3597.20	11151.32	TRUE	
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3505.90	10868.29	TRUE	
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3600.10	11160.31	TRUE	
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3769.50	11685.45	TRUE	
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3483.30	10798.23	TRUE	
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3664.30	11359.33	TRUE	
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3653.40	11325.54	TRUE	
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3618.90	11218.59	TRUE	
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3848.60	11930.66	TRUE	
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3745.00	11609.50	TRUE	
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3675.60	11394.36	TRUE	
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3790.00	11749.00	TRUE	
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3744.20	11607.02	TRUE	
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3731.60	11567.96	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
94	1	Unknown 3	-3.01424						
95	2	Unknown 3	-3.01424						
96	3	Unknown 3	-3.01424						
97	1	Unknown 3	-4.01424	-0.2	476.067	-27.6	10687.7	-0.2	1.096024
98	2	Unknown 3	-4.01424	-0.5	443.021	-60.7	10720.8	-0.5	1.019944
99	3	Unknown 3	-4.01424	-0.8	410.502	-93.2	10753.3	-0.8	0.945077
100	1	Unknown 3	-5.01424	1.8	707.265	203.6	10456.5	1.8	1.628298
101	2	Unknown 3	-5.01424	1.4	663.059	159.4	10500.7	1.4	1.526525
102	3	Unknown 3	-5.01424	2.0	723.416	219.7	10440.4	2.0	1.665482
103	1	Unknown 3	-6.01424	24.0	3178.771	2675.1	7985.0	24.0	7.318315
104	2	Unknown 3	-6.01424	24.0	3181.375	2677.7	7982.4	24.0	7.32431
105	3	Unknown 3	-6.01424	23.6	3140.734	2637.0	8023.1	23.6	7.230744
106	1	Unknown 3	-7.01424	75.4	8919.94	8416.3	2243.8	75.4	20.5359
107	2	Unknown 3	-7.01424	76.3	9018.83	8515.1	2145.0	76.3	20.76357
108	3	Unknown 3	-7.01424	74.7	8840.58	8336.9	2323.2	74.7	20.3532
109	1	Unknown 3	-8.01424	94.5	11048.09	10544.4	115.7	94.5	25.43543
110	2	Unknown 3	-8.01424	93.8	10979.27	10475.6	184.5	93.8	25.27699
111	3	Unknown 3	-8.01424	95.2	11127.14	10623.5	36.6	95.2	25.61742
112	1	Unknown 3	-9.01424	97.4	11375.45	10871.8	-211.7	97.4	26.18909
113	2	Unknown 3	-9.01424	96.6	11291.13	10787.4	-127.3	96.6	25.99497
114	3	Unknown 3	-9.01424	99.8	11643.91	11140.2	-480.1	99.8	26.80715
115	1	Unknown 3	-10.01424	98.4	11485.81	10982.1	-322.0	98.4	26.44317
116	2	Unknown 3	-10.01424	100.1	11678.63	11174.9	-514.8	100.1	26.88709
117	3	Unknown 3	-10.01424	98.9	11549.67	11046.0	-385.9	98.9	26.59019
118	1	Unknown 4	-3.01424						
119	2	Unknown 4	-3.01424						
120	3	Unknown 4	-3.01424						
121	1	Unknown 4	-4.01424	45.1	5534.12	5030.4	5629.7	45.1	12.74091
122	2	Unknown 4	-4.01424	44.2	5435.85	4932.2	5727.9	44.2	12.51467
123	3	Unknown 4	-4.01424	42.6	5253.88	4750.2	5909.9	42.6	12.09573
124	1	Unknown 4	-5.01424	86.4	10147.23	9643.5	1016.6	86.4	23.36143
125	2	Unknown 4	-5.01424	86.4	10151.26	9647.6	1012.5	86.4	23.3707
126	3	Unknown 4	-5.01424	83.9	9875.36	9371.7	1288.4	83.9	22.73551
127	1	Unknown 4	-6.01424	94.1	11005	10501.3	158.8	94.1	25.33622
128	2	Unknown 4	-6.01424	95.4	11151.32	10647.6	12.5	95.4	25.67309
129	3	Unknown 4	-6.01424	92.8	10868.29	10364.6	295.5	92.8	25.02148
130	1	Unknown 4	-7.01424	95.5	11160.31	10656.6	3.5	95.5	25.69379
131	2	Unknown 4	-7.01424	100.2	11685.45	11181.8	-521.7	100.2	26.90279
132	3	Unknown 4	-7.01424	92.2	10798.23	10294.5	365.6	92.2	24.86019
133	1	Unknown 4	-8.01424	97.2	11359.33	10855.6	-195.5	97.2	26.15198
134	2	Unknown 4	-8.01424	96.9	11325.54	10821.9	-161.8	96.9	26.07419
135	3	Unknown 4	-8.01424	96.0	11218.59	10714.9	-54.8	96.0	25.82796
136	1	Unknown 4	-9.01424	102.4	11930.66	11427.0	-766.9	102.4	27.46732
137	2	Unknown 4	-9.01424	99.5	11609.5	11105.8	-445.7	99.5	26.72793
138	3	Unknown 4	-9.01424	97.6	11394.36	10890.7	-230.6	97.6	26.23263
139	1	Unknown 4	-10.01424	100.7	11749	11245.3	-585.2	100.7	27.0491
140	2	Unknown 4	-10.01424	99.5	11607.02	11103.3	-443.2	99.5	26.72222
141	3	Unknown 4	-10.01424	99.1	11567.96	11064.3	-404.2	99.1	26.6323

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

															Check the 10% rule:	26.86%	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"*
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	579.05	1795.06	FALSE	precipitate in tube
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	533.20	1652.92	FALSE	precipitate in tube
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	539.99	1673.97	FALSE	precipitate in tube
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	835.76	2590.86	TRUE	
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	790.68	2451.11	TRUE	
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	804.66	2494.45	TRUE	
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2322.30	7199.13	TRUE	
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2333.90	7235.09	TRUE	
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2201.50	6824.65	TRUE	
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3614.90	11206.19	TRUE	
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3553.60	11016.16	TRUE	
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3355.10	10400.81	TRUE	
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3683.10	11417.61	TRUE	
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3551.00	11008.10	TRUE	
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3728.00	11556.80	TRUE	
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3917.80	12145.18	TRUE	
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3859.60	11964.76	TRUE	
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3732.60	11571.06	TRUE	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3878.70	12023.97	TRUE	
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3821.60	11846.96	TRUE	
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3818.70	11837.97	TRUE	
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3855.60	11952.36	TRUE	
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3997.40	12391.94	TRUE	
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3940.40	12215.24	TRUE	
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3888.10	12053.11	TRUE	
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3789.10	11746.21	TRUE	
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3842.00	11910.20	TRUE	
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	155.18	465.54	TRUE	
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	181.00	543.00	TRUE	
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	164.39	493.17	TRUE	
172	1	none	Hot		**—**	—	—	30	—	—	—	—		43867.00	43867.00	TRUE	
173	2	none	Hot		**—**	—	—	30	—	—	—	—		43004.00	43004.00	TRUE	
174	3	none	Hot		**—**	—	—	30	—	—	—	—		41141.00	41141.00	TRUE	
175	1	none	Hot		**—**	—	—	30	—	—	—	—		43927.00	43927.00	TRUE	
176	2	none	Hot		**—**	—	—	30	—	—	—	—		44060.00	44060.00	TRUE	
177	3	none	Hot		**—**	—	—	30	—	—	—	—		44616.00	44616.00	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	
142	1	Unknown 5	-3.01424							
143	2	Unknown 5	-3.01424							
144	3	Unknown 5	-3.01424							
145	1	Unknown 5	-4.01424	18.7	2590.856	2087.2	8572.9	18.7	5.964789	
146	2	Unknown 5	-4.01424	17.4	2451.108	1947.4	8712.7	17.4	5.643055	
147	3	Unknown 5	-4.01424	17.8	2494.446	1990.8	8669.3	17.8	5.74283	
148	1	Unknown 5	-5.01424	60.0	7199.13	6695.4	3964.7	60.0	16.57417	
149	2	Unknown 5	-5.01424	60.3	7235.09	6731.4	3928.7	60.3	16.65696	
150	3	Unknown 5	-5.01424	56.6	6824.65	6321.0	4339.1	56.6	15.71203	
151	1	Unknown 5	-6.01424	95.9	11206.19	10702.5	-42.4	95.9	25.79941	
152	2	Unknown 5	-6.01424	94.2	11016.16	10512.5	147.6	94.2	25.36192	
153	3	Unknown 5	-6.01424	88.7	10400.81	9897.1	763.0	88.7	23.94523	
154	1	Unknown 5	-7.01424	97.8	11417.61	10913.9	-253.8	97.8	26.28615	
155	2	Unknown 5	-7.01424	94.1	11008.1	10504.4	155.7	94.1	25.34336	
156	3	Unknown 5	-7.01424	99.0	11556.8	11053.1	-393.0	99.0	26.6066	
157	1	Unknown 5	-8.01424	104.3	12145.18	11641.5	-981.4	104.3	27.9612	
158	2	Unknown 5	-8.01424	102.7	11964.76	11461.1	-801.0	102.7	27.54583	
159	3	Unknown 5	-8.01424	99.1	11571.06	11067.4	-407.3	99.1	26.63943	
160	1	Unknown 5	-9.01424	103.2	12023.97	11520.3	-860.2	103.2	27.68214	
161	2	Unknown 5	-9.01424	101.6	11846.96	11343.3	-683.2	101.6	27.27462	
162	3	Unknown 5	-9.01424	101.5	11837.97	11334.3	-674.2	101.5	27.25393	
163	1	Unknown 5	-10.01424	102.6	11952.36	11448.7	-788.6	102.6	27.51728	
164	2	Unknown 5	-10.01424	106.5	12391.94	11888.3	-1228.2	106.5	28.5293	
165	3	Unknown 5	-10.01424	104.9	12215.24	11711.6	-1051.5	104.9	28.12249	
166	1		—	103.5	12053.11	11549.4	-889.3	103.5	27.74923	
167	2		—	100.7	11746.21	11242.5	-582.4	100.7	27.04267	
168	3		—	102.2	11910.2	11406.5	-746.4	102.2	27.42022	
169	1		-6.00	-0.3	465.54	-38.1	10698.2	-0.3	1.071788	
170	2		-6.00	0.4	543	39.3	10620.8	0.4	1.25012	
171	3		-6.00	-0.1	493.17	-10.5	10670.6	-0.1	1.135399	
172	1				43867	43363.3				
173	2				43004	42500.3				
174	3				41141	40637.3				
175	1				43927	43423.3				
176	2				44060	43556.3				
177	3				44616	44112.3				

Prism data

standard curve				weak positive				CR42400				CR42401			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-6.0	0.12617	0.15411	-0.19657	-3	3.9681	4.2723	3.8969	-3.0				-3.0			
-6.0	-0.34169	0.35217	-0.09419	-4	24.1084	22.3049	27.1952	-4.0	-1.0330	-1.2184	0.1944	-4.0	-1.4095	-0.9730	-1.3951
-7.0	2.01629	2.10792	1.60365	-5	76.6784	75.7245	67.6761	-5.0	-0.7417	-1.1901	-0.9249	-5.0	-0.8786	-1.1793	-1.4692
-8.0	14.31823	13.64346	12.94730	-6	94.8766	93.5920	94.7180	-6.0	0.0134	-0.2255	-0.3501	-6.0	-0.3543	0.1447	-0.4109
-9.0	61.11041	63.39019	62.79317	-7	85.1460	96.9887	96.5534	-7.0	1.1366	0.9094	1.7950	-7.0	6.2965	8.0592	6.7838
-10.0	84.68297	78.56283	91.03637	-8	91.5282	94.0301	92.0254	-8.0	14.3935	16.3745	15.3212	-8.0	42.6889	41.8975	42.1779
-11.0	87.30708	99.52794	87.36540	-9	95.4893	96.7523	92.5897	-9.0	56.8258	68.5023	62.9542	-9.0	86.4962	87.3765	87.9735
				-10	96.3089	75.8911	92.6811	-10.0	82.9308	89.0898	87.3876	-10.0	96.4651	95.8570	96.6012

CR42402				CR42404				CR42405			
concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-3.0				-3.0				-3.0			
-4.0	-0.2474	-0.5434	-0.8347	-4.0	45.0603	44.1800	42.5500	-4.0	18.6959	17.4441	17.8323
-5.0	1.8236	1.4276	1.9682	-5.0	86.3824	86.4185	83.9471	-5.0	59.9747	60.2968	56.6203
-6.0	23.9622	23.9855	23.6215	-6.0	94.0659	95.3766	92.8413	-6.0	95.8681	94.1659	88.6538
-7.0	75.3889	76.2747	74.6780	-7.0	95.4571	100.1611	92.2138	-7.0	97.7619	94.0937	99.0087
-8.0	94.4519	93.8354	95.1600	-8.0	97.2398	96.9371	95.9791	-8.0	104.2791	102.6630	99.1364
-9.0	97.3842	96.6289	99.7890	-9.0	102.3575	99.4807	97.5536	-9.0	103.1934	101.6078	101.5273
-10.0	98.3728	100.1000	98.9448	-10.0	100.7303	99.4585	99.1086	-10.0	102.5519	106.4895	104.9067

Competitive Assay of a known Weak Positive

177 Assay Tubes

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

Provide information in all blue cells
in columns O and P, and row 45, AE through BC
If the DPM value for a tube was judged unreliable,
Include the DPM value in column O
Provide a reason in column R
The value in column Q will
automatically change to FALSE

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

They are also presented in table form in columns

AC thorugh BD

Provide information in all blue cells in this column

Laboratory Code:	**E**		
Run identification:	518		
Assay start date:	11/10/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	79.44	**Ci/mmole**	
Cytosol vial or lot identification:	051905		
Protein (cytosol):	100	**micro gram per tube**	
Standard Curve IC50:	1.77E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	2.84E-05	**M**	
RBA:	6.23592E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	2.05E-09	**CR42400**	
RBA, Unknown 1:	86.30604%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	9.57E-09	**CR42401**	
RBA, Unknown 2:	18.50962%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	2.84E-07	**CR42402**	
RBA, Unknown 3:	0.62381%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	9.46E-05	**CR42404**	
RBA, Unknown 4:	0.00187%		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	1.56E-05	**CR42405**	
RBA, Unknown 5:	0.01139%		
volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3.1		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values	n	Mean	SD	
EtOH	6	11006.0	579.50	Total Binding, solvent control, tubes
Hot	6	43627.2	962.44	Total hot R1881 added to each tube
NSB	6	465.4	29.43	Nonspecific Binding
Specific EtOH	6	10540.6	579.50	

Assay Characterization Values		
EtOH / Hot	0.25	less than 0.1?
NSB / EtOH	0.04	around 0.25 ?

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

Check the 10% rule:	25.23%	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	3642.60	11292.06	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3539.50	10972.45	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3573.10	11076.61	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	155.01	465.03	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	174.14	522.42	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	152.70	458.10	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	218.78	678.22	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	216.98	672.64	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	167.17	518.23	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	640.99	1987.07	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	540.41	1675.27	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	629.01	1949.93	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2377.40	7369.94	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2377.60	7370.56	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2409.80	7470.38	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3467.10	10748.01	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3236.00	10031.60	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3043.80	9435.78	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3291.10	10202.41	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3027.90	9386.49	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3560.20	11036.62	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	299.47	898.41	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	256.09	768.27	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	274.61	823.83	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1140.30	3420.90	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	987.12	2961.36	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	941.21	2823.63	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2562.60	7687.80	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2887.30	8661.90	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2756.30	8268.90	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3057.50	9172.50	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	2961.80	8885.40	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3496.60	10489.80	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3548.40	10645.20	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3599.40	10798.20	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3561.90	10685.70	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3524.60	10573.80	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3445.90	10337.70	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3592.00	10776.00	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3516.90	10550.70	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3356.40	10069.20	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3611.30	10833.90	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3057.80	9173.40	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3562.70	10688.10	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3486.10	10458.30	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
1	1		—	102.7	11292.06	10826.7	-751.5	102.7	25.88309
2	2		—	99.7	10972.45	10507.0	-431.9	99.7	25.1505
3	3		—	100.7	11076.61	10611.2	-536.0	100.7	25.38925
4	1		-6.00	0.0	465.03	-0.4	10075.5	0.0	1.065918
5	2		-6.00	0.5	522.42	57.0	10018.2	0.5	1.197465
6	3		-6.00	0.0	458.1	-7.3	10082.5	-0.1	1.050034
7	1	cold R1881	-7.01	2.0	678.218	212.8	9862.4	2.0	1.554577
8	2	cold R1881	-7.01	2.0	672.638	207.2	9867.9	2.0	1.541787
9	3	cold R1881	-7.01	0.5	518.227	52.8	10022.3	0.5	1.187854
10	1	cold R1881	-8.01	14.4	1987.069	1521.7	8553.5	14.4	4.55466
11	2	cold R1881	-8.01	11.5	1675.271	1209.9	8865.3	11.5	3.839972
12	3	cold R1881	-8.01	14.1	1949.931	1484.5	8590.6	14.1	4.469534
13	1	cold R1881	-9.01	65.5	7369.94	6904.5	3170.6	65.5	16.89301
14	2	cold R1881	-9.01	65.5	7370.56	6905.2	3170.0	65.5	16.89443
15	3	cold R1881	-9.01	66.5	7470.38	7005.0	3070.2	66.5	17.12323
16	1	cold R1881	-10.01	97.6	10748.01	10282.6	-207.4	97.6	24.63605
17	2	cold R1881	-10.01	90.8	10031.6	9566.2	509.0	90.8	22.99393
18	3	cold R1881	-10.01	85.1	9435.78	8970.4	1104.8	85.1	21.62822
19	1	cold R1881	-11.01	92.4	10202.41	9737.0	338.2	92.4	23.38545
20	2	cold R1881	-11.01	84.6	9386.49	8921.1	1154.1	84.6	21.51524
21	3	cold R1881	-11.01	100.3	11036.62	10571.2	-496.0	100.3	25.29759
22	1	Weak Positive	-3	4.1	898.41	433.0	9642.2	4.1	2.05929
23	2	Weak Positive	-3	2.9	768.27	302.9	9772.3	2.9	1.76099
24	3	Weak Positive	-3	3.4	823.83	358.4	9716.7	3.4	1.888342
25	1	Weak Positive	-4	28.0	3420.9	2955.5	7119.7	28.0	7.841215
26	2	Weak Positive	-4	23.7	2961.36	2496.0	7579.2	23.7	6.787881
27	3	Weak Positive	-4	22.4	2823.63	2358.2	7716.9	22.4	6.472183
28	1	Weak Positive	-5	68.5	7687.8	7222.4	2852.8	68.5	17.62159
29	2	Weak Positive	-5	77.8	8661.9	8196.5	1878.7	77.8	19.85437
30	3	Weak Positive	-5	74.0	8268.9	7803.5	2271.7	74.0	18.95356
31	1	Weak Positive	-6	82.6	9172.5	8707.1	1368.1	82.6	21.02474
32	2	Weak Positive	-6	79.9	8885.4	8420.0	1655.2	79.9	20.36667
33	3	Weak Positive	-6	95.1	10489.8	10024.4	50.8	95.1	24.04419
34	1	Weak Positive	-7	96.6	10645.2	10179.8	-104.6	96.6	24.40039
35	2	Weak Positive	-7	98.0	10798.2	10332.8	-257.6	98.0	24.75109
36	3	Weak Positive	-7	97.0	10685.7	10220.3	-145.1	97.0	24.49322
37	1	Weak Positive	-8	95.9	10573.8	10108.4	-33.2	95.9	24.23673
38	2	Weak Positive	-8	93.7	10337.7	9872.3	202.9	93.7	23.69556
39	3	Weak Positive	-8	97.8	10776	10310.6	-235.4	97.8	24.70021
40	1	Weak Positive	-9	95.7	10550.7	10085.3	-10.1	95.7	24.18378
41	2	Weak Positive	-9	91.1	10069.2	9603.8	471.4	91.1	23.08011
42	3	Weak Positive	-9	98.4	10833.9	10368.5	-293.3	98.4	24.83292
43	1	Weak Positive	-10	82.6	9173.4	8708.0	1367.2	82.6	21.02681
44	2	Weak Positive	-10	97.0	10688.1	10222.7	-147.5	97.0	24.49873
45	3	Weak Positive	-10	94.8	10458.3	9992.9	82.3	94.8	23.97199

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

Check the 10% rule:	25.23%	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"
46	1	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	561.89	1741.86	FALSE	precipitate in tube
47	2	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	602.63	1868.15	FALSE	precipitate in tube
48	3	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	584.64	1812.38	FALSE	precipitate in tube
49	1	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	125.93	390.38	TRUE	
50	2	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	116.18	360.16	TRUE	
51	3	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	127.35	394.79	TRUE	
52	1	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	127.44	395.06	TRUE	
53	2	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	139.65	432.92	TRUE	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	137.77	427.09	TRUE	
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	130.73	405.26	TRUE	
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	144.56	448.14	TRUE	
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	128.03	396.89	TRUE	
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	214.80	665.88	TRUE	
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	214.80	665.88	TRUE	
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	190.95	591.95	TRUE	
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	780.02	2418.06	TRUE	
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	662.92	2055.05	TRUE	
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	700.89	2172.76	TRUE	
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2555.20	7921.12	TRUE	
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2603.60	8071.16	TRUE	
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2393.50	7419.85	TRUE	
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3329.90	10322.69	TRUE	
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3027.30	9384.63	TRUE	
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2766.30	8575.53	TRUE	
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	99.59	308.73	FALSE	precipitate in tube
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	101.32	314.09	FALSE	precipitate in tube
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	91.24	282.84	FALSE	precipitate in tube
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	109.59	339.73	TRUE	
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	138.04	427.92	TRUE	
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	130.46	404.43	TRUE	
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	113.77	352.69	TRUE	
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	129.28	400.77	TRUE	
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	99.08	307.15	TRUE	
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	150.01	465.03	TRUE	
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	170.57	528.77	TRUE	
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	155.09	480.78	TRUE	
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	412.90	1279.99	TRUE	
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	406.84	1261.20	TRUE	
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	389.58	1207.70	TRUE	
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	1806.10	5598.91	TRUE	
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	1773.10	5496.61	TRUE	
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	1857.90	5759.49	TRUE	
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3258.40	10101.04	TRUE	
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3238.50	10039.35	TRUE	
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3325.60	10309.36	TRUE	
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3760.10	11656.31	TRUE	
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3056.00	9473.60	TRUE	
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3710.00	11501.00	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	
46	1	Unknown 1	-3.01424							
47	2	Unknown 1	-3.01424							
48	3	Unknown 1	-3.01424							
49	1	Unknown 1	-4.01424	-0.7	390.383	-75.0	10150.2	-0.7	0.894816	
50	2	Unknown 1	-4.01424	-1.0	360.158	-105.2	10180.4	-1.0	0.825536	
51	3	Unknown 1	-4.01424	-0.7	394.785	-70.6	10145.8	-0.7	0.904906	
52	1	Unknown 1	-5.01424	-0.7	395.064	-70.3	10145.5	-0.7	0.905546	
53	2	Unknown 1	-5.01424	-0.3	432.915	-32.5	10107.7	-0.3	0.992306	
54	3	Unknown 1	-5.01424	-0.4	427.087	-38.3	10113.5	-0.4	0.978947	
55	1	Unknown 1	-6.01424	-0.6	405.263	-60.1	10135.3	-0.6	0.928923	
56	2	Unknown 1	-6.01424	-0.2	448.136	-17.3	10092.4	-0.2	1.027195	
57	3	Unknown 1	-6.01424	-0.6	396.893	-68.5	10143.7	-0.6	0.909738	
58	1	Unknown 1	-7.01424	1.9	665.88	200.5	9874.7	1.9	1.526297	
59	2	Unknown 1	-7.01424	1.9	665.88	200.5	9874.7	1.9	1.526297	
60	3	Unknown 1	-7.01424	1.2	591.945	126.5	9948.6	1.2	1.356827	
61	1	Unknown 1	-8.01424	18.5	2418.062	1952.7	8122.5	18.5	5.54256	
62	2	Unknown 1	-8.01424	15.1	2055.052	1589.6	8485.5	15.1	4.710487	
63	3	Unknown 1	-8.01424	16.2	2172.759	1707.4	8367.8	16.2	4.980289	
64	1	Unknown 1	-9.01424	70.7	7921.12	7455.7	2619.5	70.7	18.15639	
65	2	Unknown 1	-9.01424	72.2	8071.16	7605.8	2469.4	72.2	18.50031	
66	3	Unknown 1	-9.01424	66.0	7419.85	6954.4	3120.7	66.0	17.00741	
67	1	Unknown 1	-10.01424	93.5	10322.69	9857.3	217.9	93.5	23.66115	
68	2	Unknown 1	-10.01424	84.6	9384.63	8919.2	1155.9	84.6	21.51098	
69	3	Unknown 1	-10.01424	76.9	8575.53	8110.1	1965.0	76.9	19.6564	
70	1	Unknown 2	-3.01424							
71	2	Unknown 2	-3.01424							
72	3	Unknown 2	-3.01424							
73	1	Unknown 2	-4.01424	-1.2	339.729	-125.7	10200.8	-1.2	0.77871	
74	2	Unknown 2	-4.01424	-0.4	427.924	-37.5	10112.7	-0.4	0.980866	
75	3	Unknown 2	-4.01424	-0.6	404.426	-61.0	10136.2	-0.6	0.927005	
76	1	Unknown 2	-5.01424	-1.1	352.687	-112.7	10187.9	-1.1	0.808411	
77	2	Unknown 2	-5.01424	-0.6	400.768	-64.6	10139.8	-0.6	0.91862	
78	3	Unknown 2	-5.01424	-1.5	307.148	-158.3	10233.4	-1.5	0.704029	
79	1	Unknown 2	-6.01424	0.0	465.031	-0.4	10075.5	0.0	1.065921	
80	2	Unknown 2	-6.01424	0.6	528.767	63.4	10011.8	0.6	1.212013	
81	3	Unknown 2	-6.01424	0.1	480.779	15.4	10059.8	0.1	1.102017	
82	1	Unknown 2	-7.01424	7.7	1279.99	814.6	9260.6	7.7	2.933929	
83	2	Unknown 2	-7.01424	7.5	1261.204	795.8	9279.4	7.5	2.890868	
84	3	Unknown 2	-7.01424	7.0	1207.698	742.3	9332.9	7.0	2.768225	
85	1	Unknown 2	-8.01424	48.7	5598.91	5133.5	4941.7	48.7	12.83354	
86	2	Unknown 2	-8.01424	47.7	5496.61	5031.2	5044.0	47.7	12.59905	
87	3	Unknown 2	-8.01424	50.2	5759.49	5294.1	4781.1	50.2	13.20161	
88	1	Unknown 2	-9.01424	91.4	10101.04	9635.6	439.5	91.4	23.1531	
89	2	Unknown 2	-9.01424	90.8	10039.35	9573.9	501.2	90.8	23.01169	
90	3	Unknown 2	-9.01424	93.4	10309.36	9844.0	231.2	93.4	23.6306	
91	1	Unknown 2	-10.01424	106.2	11656.31	11190.9	-1115.7	106.2	26.71801	
92	2	Unknown 2	-10.01424	85.5	9473.6	9008.2	1067.0	85.5	21.71491	
93	3	Unknown 2	-10.01424	104.7	11501	11035.6	-960.4	104.7	26.36201	

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

Check the 10% rule:	25.23%	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"
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2463.00	7635.30	FALSE	precipitate in tube
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	1846.00	5722.60	FALSE	precipitate in tube
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2339.90	7253.69	FALSE	precipitate in tube
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	125.57	389.27	TRUE	
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	151.31	469.06	TRUE	
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	141.42	438.40	TRUE	
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	211.07	654.32	TRUE	
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	243.95	756.25	TRUE	
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	203.82	631.84	TRUE	
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1026.50	3182.15	TRUE	
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	850.64	2636.98	TRUE	
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	996.92	3090.45	TRUE	
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2670.80	8279.48	TRUE	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2592.20	8035.82	TRUE	
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2481.00	7691.10	TRUE	
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3690.20	11439.62	TRUE	
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3558.10	11030.11	TRUE	
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3672.50	11384.75	TRUE	
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3736.90	11584.39	TRUE	
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3648.90	11311.59	TRUE	
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3525.20	10928.12	TRUE	
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3354.90	10400.19	TRUE	
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3777.10	11709.01	TRUE	
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3786.20	11737.22	TRUE	
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	750.72	2327.23	FALSE	precipitate in tube
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	573.14	1776.73	FALSE	precipitate in tube
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	756.63	2345.55	FALSE	precipitate in tube
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1856.10	5753.91	TRUE	
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1857.40	5757.94	TRUE	
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1756.40	5444.84	TRUE	
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	2807.30	8702.63	TRUE	
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3345.30	10370.43	TRUE	
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3305.70	10247.67	TRUE	
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3725.10	11547.81	TRUE	
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3635.40	11269.74	TRUE	
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	2947.90	9138.49	FALSE	spill
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3620.50	11223.55	TRUE	
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3763.10	11665.61	TRUE	
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3550.00	11005.00	TRUE	
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3769.70	11686.07	TRUE	
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3648.10	11309.11	TRUE	
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3383.00	10487.30	TRUE	
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3732.90	11571.99	TRUE	
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3660.30	11346.93	TRUE	
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3740.60	11595.86	TRUE	
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3721.00	11535.10	TRUE	
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3575.40	11083.74	TRUE	
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3579.70	11097.07	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
94	1	Unknown 3	-3.01424						
95	2	Unknown 3	-3.01424						
96	3	Unknown 3	-3.01424						
97	1	Unknown 3	-4.01424	-0.7	389.267	-76.1	10151.3	-0.7	0.892258
98	2	Unknown 3	-4.01424	0.0	469.061	3.7	10071.5	0.0	1.075158
99	3	Unknown 3	-4.01424	-0.3	438.402	-27.0	10102.2	-0.3	1.004883
100	1	Unknown 3	-5.01424	1.8	654.317	188.9	9886.3	1.8	1.499793
101	2	Unknown 3	-5.01424	2.8	756.245	290.8	9784.3	2.8	1.733427
102	3	Unknown 3	-5.01424	1.6	631.842	166.4	9908.7	1.6	1.448276
103	1	Unknown 3	-6.01424	25.8	3182.15	2716.7	7358.4	25.8	7.293964
104	2	Unknown 3	-6.01424	20.6	2636.984	2171.6	7903.6	20.6	6.044362
105	3	Unknown 3	-6.01424	24.9	3090.452	2625.0	7450.1	24.9	7.083779
106	1	Unknown 3	-7.01424	74.1	8279.48	7814.1	2261.1	74.1	18.97781
107	2	Unknown 3	-7.01424	71.8	8035.82	7570.4	2504.8	71.8	18.4193
108	3	Unknown 3	-7.01424	68.6	7691.1	7225.7	2849.5	68.6	17.62915
109	1	Unknown 3	-8.01424	104.1	11439.62	10974.2	-899.0	104.1	26.22132
110	2	Unknown 3	-8.01424	100.2	11030.11	10564.7	-489.5	100.2	25.28266
111	3	Unknown 3	-8.01424	103.6	11384.75	10919.3	-844.2	103.6	26.09555
112	1	Unknown 3	-9.01424	105.5	11584.39	11119.0	-1043.8	105.5	26.55316
113	2	Unknown 3	-9.01424	102.9	11311.59	10846.2	-771.0	102.9	25.92786
114	3	Unknown 3	-9.01424	99.3	10928.12	10462.7	-387.5	99.3	25.04889
115	1	Unknown 3	-10.01424	94.3	10400.19	9934.8	140.4	94.3	23.83879
116	2	Unknown 3	-10.01424	106.7	11709.01	11243.6	-1168.4	106.7	26.8388
117	3	Unknown 3	-10.01424	106.9	11737.22	11271.8	-1196.6	106.9	26.90347
118	1	Unknown 4	-3.01424						
119	2	Unknown 4	-3.01424						
120	3	Unknown 4	-3.01424						
121	1	Unknown 4	-4.01424	50.2	5753.91	5288.5	4786.7	50.2	13.18882
122	2	Unknown 4	-4.01424	50.2	5757.94	5292.5	4782.6	50.2	13.19806
123	3	Unknown 4	-4.01424	47.2	5444.84	4979.4	5095.7	47.2	12.48039
124	1	Unknown 4	-5.01424	78.1	8702.63	8237.2	1837.9	78.1	19.94773
125	2	Unknown 4	-5.01424	94.0	10370.43	9905.0	170.1	94.0	23.77058
126	3	Unknown 4	-5.01424	92.8	10247.67	9782.3	292.9	92.8	23.48919
127	1	Unknown 4	-6.01424	105.1	11547.81	11082.4	-1007.2	105.1	26.46931
128	2	Unknown 4	-6.01424	102.5	11269.74	10804.3	-729.2	102.5	25.83193
129	3	Unknown 4	-6.01424						
130	1	Unknown 4	-7.01424	102.1	11223.55	10758.1	-683.0	102.1	25.72606
131	2	Unknown 4	-7.01424	106.3	11665.61	11200.2	-1125.0	106.3	26.73933
132	3	Unknown 4	-7.01424	100.0	11005	10539.6	-464.4	100.0	25.22511
133	1	Unknown 4	-8.01424	106.5	11686.07	11220.7	-1145.5	106.5	26.78622
134	2	Unknown 4	-8.01424	102.9	11309.11	10843.7	-768.5	102.9	25.92217
135	3	Unknown 4	-8.01424	95.1	10487.3	10021.9	53.3	95.1	24.03846
136	1	Unknown 4	-9.01424	105.4	11571.99	11106.6	-1031.4	105.4	26.52473
137	2	Unknown 4	-9.01424	103.2	11346.93	10881.5	-806.4	103.2	26.00886
138	3	Unknown 4	-9.01424	105.6	11595.86	11130.5	-1055.3	105.6	26.57945
139	1	Unknown 4	-10.01424	105.0	11535.1	11069.7	-994.5	105.0	26.44018
140	2	Unknown 4	-10.01424	100.7	11083.74	10618.3	-543.2	100.7	25.40559
141	3	Unknown 4	-10.01424	100.9	11097.07	10631.7	-556.5	100.9	25.43615

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

															DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
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)					
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100		537.56	1666.44	FALSE	precipitate in tube
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100		481.65	1493.12	FALSE	precipitate in tube
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100		515.58	1598.30	FALSE	precipitate in tube
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100		795.20	2465.12	TRUE	
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100		768.05	2380.96	TRUE	
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100		645.90	2002.29	TRUE	
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100		2278.90	7064.59	TRUE	
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100		2307.10	7152.01	TRUE	
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100		2068.70	6412.97	TRUE	
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100		2986.20	9257.22	TRUE	
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100		2932.70	9091.37	TRUE	
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100		3333.70	10334.47	TRUE	
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100		3501.50	10854.65	TRUE	
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100		3599.60	11158.76	TRUE	
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100		3485.40	10804.74	TRUE	
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100		3553.40	11015.54	TRUE	
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100		3667.80	11370.18	TRUE	
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100		3652.40	11322.44	TRUE	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100		3600.70	11162.17	TRUE	
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100		3522.10	10918.51	TRUE	
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100		3471.20	10760.72	TRUE	
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100		3565.10	11051.81	TRUE	
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100		3215.90	9969.29	TRUE	
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100		3606.90	11181.39	TRUE	
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100		3187.40	9880.94	TRUE	
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100		3665.30	11362.43	TRUE	
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100		3694.00	11451.40	TRUE	
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100		150.48	451.44	TRUE	
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100		145.85	437.55	TRUE	
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100		152.63	457.89	TRUE	
172	1	none	Hot		**—**	—	—	30	—	—	—	—			43835.00	43835.00	TRUE	
173	2	none	Hot		**—**	—	—	30	—	—	—	—			44455.00	44455.00	TRUE	
174	3	none	Hot		**—**	—	—	30	—	—	—	—			42064.00	42064.00	TRUE	
175	1	none	Hot		**—**	—	—	30	—	—	—	—			43734.00	43734.00	TRUE	
176	2	none	Hot		**—**	—	—	30	—	—	—	—			44662.00	44662.00	TRUE	
177	3	none	Hot		**—**	—	—	30	—	—	—	—			43013.00	43013.00	TRUE	

Check the 10% rule:	25.23%	If the ratio of EtOH / Hot is > 10% then there are problems with the assay

			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
142	1	Unknown 5	-3.01424						
143	2	Unknown 5	-3.01424						
144	3	Unknown 5	-3.01424						
145	1	Unknown 5	-4.01424	19.0	2465.12	1999.7	8075.5	19.0	5.650424
146	2	Unknown 5	-4.01424	18.2	2380.955	1915.6	8159.6	18.2	5.457505
147	3	Unknown 5	-4.01424	14.6	2002.29	1536.9	8538.3	14.6	4.589549
148	1	Unknown 5	-5.01424	62.6	7064.59	6599.2	3476.0	62.6	16.1931
149	2	Unknown 5	-5.01424	63.4	7152.01	6686.6	3388.6	63.4	16.39348
150	3	Unknown 5	-5.01424	56.4	6412.97	5947.6	4127.6	56.4	14.69949
151	1	Unknown 5	-6.01424	83.4	9257.22	8791.8	1283.4	83.4	21.21893
152	2	Unknown 5	-6.01424	81.8	9091.37	8626.0	1449.2	81.8	20.83878
153	3	Unknown 5	-6.01424	93.6	10334.47	9869.1	206.1	93.6	23.68815
154	1	Unknown 5	-7.01424	98.6	10854.65	10389.2	-314.1	98.6	24.88048
155	2	Unknown 5	-7.01424	101.4	11158.76	10693.4	-618.2	101.4	25.57755
156	3	Unknown 5	-7.01424	98.1	10804.74	10339.3	-264.2	98.1	24.76608
157	1	Unknown 5	-8.01424	100.1	11015.54	10550.1	-475.0	100.1	25.24927
158	2	Unknown 5	-8.01424	103.5	11370.18	10904.8	-829.6	103.5	26.06216
159	3	Unknown 5	-8.01424	103.0	11322.44	10857.0	-781.9	103.0	25.95273
160	1	Unknown 5	-9.01424	101.5	11162.17	10696.8	-621.6	101.5	25.58537
161	2	Unknown 5	-9.01424	99.2	10918.51	10453.1	-377.9	99.2	25.02686
162	3	Unknown 5	-9.01424	97.7	10760.72	10295.3	-220.1	97.7	24.66518
163	1	Unknown 5	-10.01424	100.4	11051.81	10586.4	-511.2	100.4	25.3324
164	2	Unknown 5	-10.01424	90.2	9969.29	9503.9	571.3	90.2	22.85111
165	3	Unknown 5	-10.01424	101.7	11181.39	10716.0	-640.8	101.7	25.62942
166	1		—	89.3	9880.94	9415.5	659.6	89.3	22.64859
167	2		—	103.4	11362.43	10897.0	-821.9	103.4	26.04439
168	3		—	104.2	11451.4	10986.0	-910.8	104.2	26.24832
169	1		-6.00	-0.1	451.44	-14.0	10089.1	-0.1	1.034768
170	2		-6.00	-0.3	437.55	-27.9	10103.0	-0.3	1.00293
171	3		-6.00	-0.1	457.89	-7.5	10082.7	-0.1	1.049552
172	1				43835	43369.6			
173	2				44455	43989.6			
174	3				42064	41598.6			
175	1				43734	43268.6			
176	2				44662	44196.6			
177	3				43013	42547.6			

Prism data

standard curve				weak positive				CR42400				CR42401			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-6.0	-0.00356	0.54091	-0.06930	-3	4.1080	2.8733	3.4004	-3.0				-3.0			
-6.0	-0.13249	-0.26426	-0.07130	-4	28.0392	23.6795	22.3728	-4.0	-0.7117	-0.9985	-0.6700	-4.0	-1.1923	-0.3556	-0.5785
-7.0	2.01899	1.96605	0.50113	-5	68.5199	77.7614	74.0329	-5.0	-0.6673	-0.3082	-0.3635	-5.0	-1.0694	-0.6132	-1.5014
-8.0	14.43625	11.47818	14.08392	-6	82.6055	79.8817	95.1029	-6.0	-0.5706	-0.1638	-0.6500	-6.0	-0.0035	0.6011	0.1459
-9.0	65.50434	65.51022	66.45723	-7	96.5772	98.0287	96.9614	-7.0	1.9019	1.9019	1.2005	-7.0	7.7281	7.5499	7.0422
-10.0	97.55258	90.75590	85.10327	-8	95.8998	93.6599	97.8181	-8.0	18.5251	15.0812	16.1979	-8.0	48.7023	47.7318	50.2258
-11.0	92.37640	84.63564	100.29067	-9	95.6807	91.1126	98.3674	-9.0	70.7335	72.1569	65.9778	-9.0	91.4147	90.8294	93.3910
				-10	82.6140	96.9842	94.8041	-10.0	93.5175	84.6180	76.9419	-10.0	106.1698	85.4621	104.6963

CR42402				CR42404				CR42405			
concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-3.0				-3.0				-3.0			
-4.0	-0.7223	0.0347	-0.2562	-4.0	50.1728	50.2111	47.2406	-4.0	18.9716	18.1731	14.5807
-5.0	1.7922	2.7592	1.5790	-5.0	78.1478	93.9704	92.8058	-5.0	62.6074	63.4368	56.4254
-6.0	25.7742	20.6021	24.9042	-6.0	105.1404	102.5023		-6.0	83.4092	81.8358	93.6293
-7.0	74.1333	71.8216	68.5512	-7.0	102.0641	106.2580	99.9907	-7.0	98.5643	101.4494	98.0908
-8.0	104.1140	100.2289	103.5934	-8.0	106.4521	102.8758	95.0792	-8.0	100.0907	103.4552	103.0023
-9.0	105.4874	102.8994	99.2613	-9.0	105.3698	103.2346	105.5963	-9.0	101.4818	99.1701	97.6732
-10.0	94.2528	106.6697	106.9374	-10.0	105.0198	100.7377	100.8642	-10.0	100.4348	90.1648	101.6641

Competitive Assay of a known Weak Positive

177 Assay Tubes

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

Provide information in all blue cells
in columns O and P, and row 45, AE through BC
If the DPM value for a tube was judged unreliable,
Include the DPM value in column O
Provide a reason in column R
The value in column Q will
automatically change to FALSE

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

They are also presented in table form in columns

AC thorugh BD

Provide information in all blue cells in this column

Laboratory Code:	**E**		
Run identification:	519		
Assay start date:	11/17/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	79.36	**Ci/mmole**	
Cytosol vial or lot identification:	051905		
Protein (cytosol):	100	**micro gram per tube**	
Standard Curve IC50:	1.46E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	4.34E-05	**M**	
RBA:	3.35871E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	2.27E-09	**CR42400**	
RBA, Unknown 1:	64.22585%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	9.22E-09	**CR42401**	
RBA, Unknown 2:	15.79690%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	4.14E-07	**CR42402**	
RBA, Unknown 3:	0.35135%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	1.02E-04	**CR42404**	
RBA, Unknown 4:	0.00143%		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	1.84E-05	**CR42405**	
RBA, Unknown 5:	0.00793%		
volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3.1		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values	n	Mean	SD	
EtOH	6	10644.9	510.72	Total Binding, solvent control, tubes
Hot	6	44120.2	628.34	Total hot R1881 added to each tube
NSB	6	435.2	76.10	Nonspecific Binding
Specific EtOH	6	10209.7	510.72	

Assay Characterization Values		
EtOH / Hot	0.24	less than 0.1?
NSB / EtOH	0.04	around 0.25 ?

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

Check the 10% rule:	24.13%	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	3491.60	10823.96	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3107.60	9633.56	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3437.60	10656.56	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	164.61	493.83	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	136.97	410.91	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	103.38	310.14	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	204.65	634.42	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	188.42	584.10	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	169.84	526.50	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	554.38	1718.58	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	594.17	1841.93	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	628.24	1947.54	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2111.70	6546.27	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	1992.50	6176.75	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2029.60	6291.76	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3407.90	10564.49	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3148.60	9760.66	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3463.40	10736.54	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3642.90	11292.99	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3564.70	11050.57	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3448.00	10688.80	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	215.94	647.82	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	301.08	903.24	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	325.86	977.58	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1148.90	3446.70	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1168.80	3506.40	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1144.30	3432.90	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2901.70	8705.10	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2960.80	8882.40	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2963.60	8890.80	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3562.50	10687.50	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3152.80	9458.40	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3603.30	10809.90	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3639.50	10918.50	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3736.90	11210.70	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3693.80	11081.40	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3600.10	10800.30	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3633.90	10901.70	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3562.20	10686.60	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3712.60	11137.80	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3640.10	10920.30	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3612.40	10837.20	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3659.80	10979.40	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3594.20	10782.60	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3722.80	11168.40	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
1	1		—	101.8	10823.96	10388.7	-614.3	101.8	24.53291
2	2		—	90.1	9633.56	9198.3	576.1	90.1	21.83482
3	3		—	100.1	10656.56	10221.3	-446.9	100.1	24.15349
4	1		-6.00	0.6	493.83	58.6	9715.8	0.6	1.119284
5	2		-6.00	-0.2	410.91	-24.3	9798.8	-0.2	0.931343
6	3		-6.00	0.0	310.14	-125.1	9899.5	-1.2	0.702944
7	1	cold R1881	-7.01	2.0	634.415	199.2	9575.2	2.0	1.437925
8	2	cold R1881	-7.01	1.5	584.102	148.9	9625.6	1.5	1.323889
9	3	cold R1881	-7.01	0.9	526.504	91.3	9683.2	0.9	1.193341
10	1	cold R1881	-8.01	12.6	1718.578	1283.4	8491.1	12.6	3.895221
11	2	cold R1881	-8.01	13.8	1841.927	1406.7	8367.7	13.8	4.174796
12	3	cold R1881	-8.01	14.8	1947.544	1512.3	8262.1	14.8	4.414181
13	1	cold R1881	-9.01	59.9	6546.27	6111.1	3663.4	59.9	14.83736
14	2	cold R1881	-9.01	56.2	6176.75	5741.5	4032.9	56.2	13.99983
15	3	cold R1881	-9.01	57.4	6291.76	5856.5	3917.9	57.4	14.26051
16	1	cold R1881	-10.01	99.2	10564.49	10129.3	-354.8	99.2	23.94481
17	2	cold R1881	-10.01	91.3	9760.66	9325.4	449.0	91.3	22.1229
18	3	cold R1881	-10.01	100.9	10736.54	10301.3	-526.9	100.9	24.33477
19	1	cold R1881	-11.01	106.3	11292.99	10857.8	-1083.3	106.3	25.59598
20	2	cold R1881	-11.01	104.0	11050.57	10615.4	-840.9	104.0	25.04653
21	3	cold R1881	-11.01	100.4	10688.8	10253.6	-479.1	100.4	24.22656
22	1	Weak Positive	-3	2.1	647.82	212.6	9561.8	2.1	1.468308
23	2	Weak Positive	-3	4.6	903.24	468.0	9306.4	4.6	2.047227
24	3	Weak Positive	-3	5.3	977.58	542.4	9232.1	5.3	2.215721
25	1	Weak Positive	-4	29.5	3446.7	3011.5	6763.0	29.5	7.812074
26	2	Weak Positive	-4	30.1	3506.4	3071.2	6703.3	30.1	7.947386
27	3	Weak Positive	-4	29.4	3432.9	2997.7	6776.8	29.4	7.780796
28	1	Weak Positive	-5	81.0	8705.1	8269.9	1504.6	81.0	19.73043
29	2	Weak Positive	-5	82.7	8882.4	8447.2	1327.3	82.7	20.13229
30	3	Weak Positive	-5	82.8	8890.8	8455.6	1318.9	82.8	20.15133
31	1	Weak Positive	-6	100.4	10687.5	10252.3	-477.8	100.4	24.22362
32	2	Weak Positive	-6	88.4	9458.4	9023.2	751.3	88.4	21.43782
33	3	Weak Positive	-6	101.6	10809.9	10374.7	-600.2	101.6	24.50104
34	1	Weak Positive	-7	102.7	10918.5	10483.3	-708.8	102.7	24.74719
35	2	Weak Positive	-7	105.5	11210.7	10775.5	-1001.0	105.5	25.40947
36	3	Weak Positive	-7	104.3	11081.4	10646.2	-871.7	104.3	25.11641
37	1	Weak Positive	-8	101.5	10800.3	10365.1	-590.6	101.5	24.47928
38	2	Weak Positive	-8	102.5	10901.7	10466.5	-692.0	102.5	24.70911
39	3	Weak Positive	-8	100.4	10686.6	10251.4	-476.9	100.4	24.22158
40	1	Weak Positive	-9	104.8	11137.8	10702.6	-928.1	104.8	25.24424
41	2	Weak Positive	-9	102.7	10920.3	10485.1	-710.6	102.7	24.75127
42	3	Weak Positive	-9	101.9	10837.2	10402.0	-627.5	101.9	24.56292
43	1	Weak Positive	-10	103.3	10979.4	10544.2	-769.7	103.3	24.88522
44	2	Weak Positive	-10	101.3	10782.6	10347.4	-572.9	101.3	24.43916
45	3	Weak Positive	-10	105.1	11168.4	10733.2	-958.7	105.1	25.31359

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

Check the 10% rule:	24.13%	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"
46	1	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	501.35	1554.19	FALSE	precipitate in tube
47	2	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	837.51	2596.28	FALSE	precipitate in tube
48	3	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	703.22	2179.98	FALSE	precipitate in tube
49	1	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	130.76	405.36	TRUE	
50	2	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	132.83	411.77	TRUE	
51	3	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	128.61	398.69	TRUE	
52	1	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	134.40	416.64	TRUE	
53	2	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	175.45	543.90	TRUE	
54	3	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	127.23	394.41	TRUE	
55	1	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	182.66	566.25	TRUE	
56	2	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	162.51	503.78	TRUE	
57	3	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	140.62	435.92	TRUE	
58	1	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	202.60	628.06	TRUE	
59	2	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	194.15	601.87	TRUE	
60	3	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	222.51	689.78	TRUE	
61	1	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	695.85	2157.14	TRUE	
62	2	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	706.08	2188.85	TRUE	
63	3	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	704.18	2182.96	TRUE	
64	1	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2529.10	7840.21	TRUE	
65	2	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2293.80	7110.78	TRUE	
66	3	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2512.60	7789.06	TRUE	
67	1	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	3348.80	10381.28	TRUE	
68	2	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	3402.10	10546.51	TRUE	
69	3	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	3362.10	10422.51	TRUE	
70	1	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	114.54	355.07	FALSE	precipitate in tube
71	2	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	92.01	285.23	FALSE	precipitate in tube
72	3	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	101.22	313.78	FALSE	precipitate in tube
73	1	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	145.00	449.50	TRUE	
74	2	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	112.16	347.70	TRUE	
75	3	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	131.28	406.97	TRUE	
76	1	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	125.76	389.86	TRUE	
77	2	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	112.65	349.22	TRUE	
78	3	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	107.60	333.56	TRUE	
79	1	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	162.63	504.15	TRUE	
80	2	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	219.43	680.23	TRUE	
81	3	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	186.30	577.53	TRUE	
82	1	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	444.36	1377.52	TRUE	
83	2	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	401.21	1243.75	TRUE	
84	3	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	388.20	1203.42	TRUE	
85	1	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1668.80	5173.28	TRUE	
86	2	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1680.70	5210.17	TRUE	
87	3	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1738.50	5389.35	TRUE	
88	1	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	3149.70	9764.07	TRUE	
89	2	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	3234.70	10027.57	TRUE	
90	3	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	3119.40	9670.14	TRUE	
91	1	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3490.40	10820.24	TRUE	
92	2	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3593.60	11140.16	TRUE	
93	3	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3508.90	10877.59	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	Values for analysis by nonlinear regression: percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
46	1	Unknown 1	-3.01424						
47	2	Unknown 1	-3.01424						
48	3	Unknown 1	-3.01424						
49	1	Unknown 1	-4.01424	-0.3	405.356	-29.9	9804.3	-0.3	0.918754
50	2	Unknown 1	-4.01424	-0.2	411.773	-23.4	9797.9	-0.2	0.933299
51	3	Unknown 1	-4.01424	-0.4	398.691	-36.5	9811.0	-0.4	0.903648
52	1	Unknown 1	-5.01424	-0.2	416.64	-18.6	9793.0	-0.2	0.94433
53	2	Unknown 1	-5.01424	1.1	543.895	108.7	9665.8	1.1	1.232758
54	3	Unknown 1	-5.01424	-0.4	394.413	-40.8	9815.3	-0.4	0.893952
55	1	Unknown 1	-6.01424	1.3	566.246	131.0	9643.4	1.3	1.283418
56	2	Unknown 1	-6.01424	0.7	503.781	68.6	9705.9	0.7	1.141838
57	3	Unknown 1	-6.01424	0.0	435.922	0.7	9773.7	0.0	0.988033
58	1	Unknown 1	-7.01424	1.9	628.06	192.8	9581.6	1.9	1.423521
59	2	Unknown 1	-7.01424	1.6	601.865	166.6	9607.8	1.6	1.364149
60	3	Unknown 1	-7.01424	2.5	689.781	254.6	9519.9	2.5	1.563414
61	1	Unknown 1	-8.01424	16.9	2157.135	1721.9	8052.5	16.9	4.889227
62	2	Unknown 1	-8.01424	17.2	2188.848	1753.6	8020.8	17.2	4.961105
63	3	Unknown 1	-8.01424	17.1	2182.958	1747.7	8026.7	17.1	4.947756
64	1	Unknown 1	-9.01424	72.5	7840.21	7405.0	2369.5	72.5	17.77013
65	2	Unknown 1	-9.01424	65.4	7110.78	6675.6	3098.9	65.4	16.11685
66	3	Unknown 1	-9.01424	72.0	7789.06	7353.8	2420.6	72.0	17.65419
67	1	Unknown 1	-10.01424	97.4	10381.28	9946.1	-171.6	97.4	23.52956
68	2	Unknown 1	-10.01424	99.0	10546.51	10111.3	-336.8	99.0	23.90406
69	3	Unknown 1	-10.01424	97.8	10422.51	9987.3	-212.8	97.8	23.62301
70	1	Unknown 2	-3.01424						
71	2	Unknown 2	-3.01424						
72	3	Unknown 2	-3.01424						
73	1	Unknown 2	-4.01424	0.1	449.5	14.3	9760.2	0.1	1.018808
74	2	Unknown 2	-4.01424	-0.9	347.696	-87.5	9862.0	-0.9	0.788066
75	3	Unknown 2	-4.01424	-0.3	406.968	-28.3	9802.7	-0.3	0.922408
76	1	Unknown 2	-5.01424	-0.4	389.856	-45.4	9819.8	-0.4	0.883623
77	2	Unknown 2	-5.01424	-0.8	349.215	-86.0	9860.4	-0.8	0.791509
78	3	Unknown 2	-5.01424	-1.0	333.56	-101.7	9876.1	-1.0	0.756026
79	1	Unknown 2	-6.01424	0.7	504.153	68.9	9705.5	0.7	1.142682
80	2	Unknown 2	-6.01424	2.4	680.233	245.0	9529.4	2.4	1.541773
81	3	Unknown 2	-6.01424	1.4	577.53	142.3	9632.1	1.4	1.308993
82	1	Unknown 2	-7.01424	9.2	1377.516	942.3	8832.1	9.2	3.122191
83	2	Unknown 2	-7.01424	7.9	1243.751	808.5	8965.9	7.9	2.819008
84	3	Unknown 2	-7.01424	7.5	1203.42	768.2	9006.2	7.5	2.727596
85	1	Unknown 2	-8.01424	46.4	5173.28	4738.1	5036.4	46.4	11.72543
86	2	Unknown 2	-8.01424	46.8	5210.17	4775.0	4999.5	46.8	11.80904
87	3	Unknown 2	-8.01424	48.5	5389.35	4954.1	4820.3	48.5	12.21516
88	1	Unknown 2	-9.01424	91.4	9764.07	9328.9	445.6	91.4	22.13063
89	2	Unknown 2	-9.01424	94.0	10027.57	9592.4	182.1	94.0	22.72786
90	3	Unknown 2	-9.01424	90.5	9670.14	9234.9	539.5	90.5	21.91773
91	1	Unknown 2	-10.01424	101.7	10820.24	10385.0	-610.6	101.7	24.52448
92	2	Unknown 2	-10.01424	104.9	11140.16	10704.9	-930.5	104.9	25.24959
93	3	Unknown 2	-10.01424	102.3	10877.59	10442.4	-667.9	102.3	24.65446

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

Check the 10% rule:	24.13%	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"
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2142.60	6642.06	FALSE	precipitate in tube
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2138.40	6629.04	FALSE	precipitate in tube
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2515.10	7796.81	FALSE	precipitate in tube
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	112.65	349.22	TRUE	
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	136.67	423.68	TRUE	
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	119.25	369.68	TRUE	
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	193.60	600.16	TRUE	
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	202.16	626.70	TRUE	
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	193.66	600.35	TRUE	
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	950.93	2947.88	TRUE	
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1005.35	3116.59	TRUE	
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1019.01	3158.93	TRUE	
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2868.80	8893.28	TRUE	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2996.50	9289.15	TRUE	
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2944.80	9128.88	TRUE	
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3512.70	10889.37	TRUE	
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3511.20	10884.72	TRUE	
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3514.50	10894.95	TRUE	
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3619.50	11220.45	TRUE	
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3611.60	11195.96	TRUE	
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3608.40	11186.04	TRUE	
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3618.10	11216.11	TRUE	
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3581.00	11101.10	TRUE	
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3753.40	11635.54	TRUE	
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	687.63	2131.65	FALSE	precipitate in tube
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	633.32	1963.29	FALSE	precipitate in tube
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	709.09	2198.18	FALSE	precipitate in tube
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1802.00	5586.20	TRUE	
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1534.70	4757.57	TRUE	
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1791.80	5554.58	TRUE	
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3406.20	10559.22	TRUE	
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3349.50	10383.45	TRUE	
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3461.60	10730.96	TRUE	
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3595.00	11144.50	TRUE	
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3207.70	9943.87	TRUE	
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3668.90	11373.59	TRUE	
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3674.90	11392.19	TRUE	
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3659.10	11343.21	TRUE	
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3776.90	11708.39	TRUE	
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3672.80	11385.68	TRUE	
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3426.40	10621.84	TRUE	
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3666.90	11367.39	TRUE	
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3765.20	11672.12	TRUE	
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3594.80	11143.88	TRUE	
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3588.00	11122.80	TRUE	
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3854.90	11950.19	TRUE	
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3660.70	11348.17	TRUE	
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3749.30	11622.83	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
94	1	Unknown 3	-3.01424						
95	2	Unknown 3	-3.01424						
96	3	Unknown 3	-3.01424						
97	1	Unknown 3	-4.01424	-0.8	349.215	-86.0	9860.4	-0.8	0.791509
98	2	Unknown 3	-4.01424	-0.1	423.677	-11.5	9786.0	-0.1	0.96028
99	3	Unknown 3	-4.01424	-0.6	369.675	-65.5	9840.0	-0.6	0.837882
100	1	Unknown 3	-5.01424	1.6	600.16	164.9	9609.5	1.6	1.360285
101	2	Unknown 3	-5.01424	1.9	626.696	191.5	9583.0	1.9	1.42043
102	3	Unknown 3	-5.01424	1.6	600.346	165.1	9609.3	1.6	1.360707
103	1	Unknown 3	-6.01424	24.6	2947.883	2512.7	7261.8	24.6	6.681487
104	2	Unknown 3	-6.01424	26.3	3116.585	2681.4	7093.1	26.3	7.063856
105	3	Unknown 3	-6.01424	26.7	3158.931	2723.7	7050.7	26.7	7.159835
106	1	Unknown 3	-7.01424	82.8	8893.28	8458.1	1316.4	82.8	20.15695
107	2	Unknown 3	-7.01424	86.7	9289.15	8853.9	920.5	86.7	21.0542
108	3	Unknown 3	-7.01424	85.2	9128.88	8693.7	1080.8	85.2	20.69095
109	1	Unknown 3	-8.01424	102.4	10889.37	10454.2	-679.7	102.4	24.68116
110	2	Unknown 3	-8.01424	102.3	10884.72	10449.5	-675.1	102.3	24.67062
111	3	Unknown 3	-8.01424	102.4	10894.95	10459.7	-685.3	102.4	24.69381
112	1	Unknown 3	-9.01424	105.6	11220.45	10785.2	-1010.8	105.6	25.43157
113	2	Unknown 3	-9.01424	105.4	11195.96	10760.7	-986.3	105.4	25.37606
114	3	Unknown 3	-9.01424	105.3	11186.04	10750.8	-976.4	105.3	25.35358
115	1	Unknown 3	-10.01424	105.6	11216.11	10780.9	-1006.4	105.6	25.42173
116	2	Unknown 3	-10.01424	104.5	11101.1	10665.9	-891.4	104.5	25.16106
117	3	Unknown 3	-10.01424	109.7	11635.54	11200.3	-1425.9	109.7	26.37238
118	1	Unknown 4	-3.01424						
119	2	Unknown 4	-3.01424						
120	3	Unknown 4	-3.01424						
121	1	Unknown 4	-4.01424	50.5	5586.2	5151.0	4623.5	50.5	12.66133
122	2	Unknown 4	-4.01424	42.3	4757.57	4322.4	5452.1	42.3	10.78321
123	3	Unknown 4	-4.01424	50.1	5554.58	5119.4	4655.1	50.1	12.58966
124	1	Unknown 4	-5.01424	99.2	10559.22	10124.0	-349.6	99.2	23.93287
125	2	Unknown 4	-5.01424	97.4	10383.45	9948.2	-173.8	97.4	23.53448
126	3	Unknown 4	-5.01424	100.8	10730.96	10295.7	-521.3	100.8	24.32212
127	1	Unknown 4	-6.01424	104.9	11144.5	10709.3	-934.8	104.9	25.25942
128	2	Unknown 4	-6.01424	93.1	9943.87	9508.7	265.8	93.1	22.53815
129	3	Unknown 4	-6.01424	107.1	11373.59	10938.4	-1163.9	107.1	25.77867
130	1	Unknown 4	-7.01424	107.3	11392.19	10957.0	-1182.5	107.3	25.82082
131	2	Unknown 4	-7.01424	106.8	11343.21	10908.0	-1133.5	106.8	25.70981
132	3	Unknown 4	-7.01424	110.4	11708.39	11273.2	-1498.7	110.4	26.5375
133	1	Unknown 4	-8.01424	107.3	11385.68	10950.5	-1176.0	107.3	25.80607
134	2	Unknown 4	-8.01424	99.8	10621.84	10186.6	-412.2	99.8	24.0748
135	3	Unknown 4	-8.01424	107.1	11367.39	10932.2	-1157.7	107.1	25.76461
136	1	Unknown 4	-9.01424	110.1	11672.12	11236.9	-1462.5	110.1	26.45529
137	2	Unknown 4	-9.01424	104.9	11143.88	10708.7	-934.2	104.9	25.25802
138	3	Unknown 4	-9.01424	104.7	11122.8	10687.6	-913.1	104.7	25.21024
139	1	Unknown 4	-10.01424	112.8	11950.19	11515.0	-1740.5	112.8	27.08555
140	2	Unknown 4	-10.01424	106.9	11348.17	10913.0	-1138.5	106.9	25.72105
141	3	Unknown 4	-10.01424	109.6	11622.83	11187.6	-1413.2	109.6	26.34358

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

Check the 10% rule:	24.13%	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"
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	537.22	1665.38	FALSE	precipitate in tube
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	544.07	1686.62	FALSE	precipitate in tube
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	543.54	1684.97	FALSE	precipitate in tube
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	779.74	2417.19	TRUE	
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	760.51	2357.58	TRUE	
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	797.76	2473.06	TRUE	
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2246.70	6964.77	TRUE	
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2143.50	6644.85	TRUE	
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2180.80	6760.48	TRUE	
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3288.60	10194.66	TRUE	
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3346.40	10373.84	TRUE	
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3232.90	10021.99	TRUE	
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3595.30	11145.43	TRUE	
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3531.20	10946.72	TRUE	
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3525.30	10928.43	TRUE	
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3633.40	11263.54	TRUE	
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3521.60	10916.96	TRUE	
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3527.60	10935.56	TRUE	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3617.40	11213.94	TRUE	
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3582.90	11106.99	TRUE	
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3540.10	10974.31	TRUE	
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3511.30	10885.03	TRUE	
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3461.90	10731.89	TRUE	
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3531.90	10948.89	TRUE	
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3481.50	10792.65	TRUE	
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3549.60	11003.76	TRUE	
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3535.10	10958.81	TRUE	
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	137.74	413.22	TRUE	
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	175.52	526.56	TRUE	
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	152.22	456.66	TRUE	
172	1	none	Hot		**—**	—	—	30	—	—	—	—		43627.00	43627.00	TRUE	
173	2	none	Hot		**—**	—	—	30	—	—	—	—		43876.00	43876.00	TRUE	
174	3	none	Hot		**—**	—	—	30	—	—	—	—		43995.00	43995.00	TRUE	
175	1	none	Hot		**—**	—	—	30	—	—	—	—		43719.00	43719.00	TRUE	
176	2	none	Hot		**—**	—	—	30	—	—	—	—		45342.00	45342.00	TRUE	
177	3	none	Hot		**—**	—	—	30	—	—	—	—		44162.00	44162.00	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	
142	1	Unknown 5	-3.01424							
143	2	Unknown 5	-3.01424							
144	3	Unknown 5	-3.01424							
145	1	Unknown 5	-4.01424	19.4	2417.194	1982.0	7792.5	19.4	5.47866	
146	2	Unknown 5	-4.01424	18.8	2357.581	1922.4	7852.1	18.8	5.343545	
147	3	Unknown 5	-4.01424	20.0	2473.056	2037.8	7736.6	20.0	5.605273	
148	1	Unknown 5	-5.01424	64.0	6964.77	6529.6	3244.9	64.0	15.78591	
149	2	Unknown 5	-5.01424	60.8	6644.85	6209.6	3564.8	60.8	15.0608	
150	3	Unknown 5	-5.01424	62.0	6760.48	6325.3	3449.2	62.0	15.32288	
151	1	Unknown 5	-6.01424	95.6	10194.66	9759.4	15.0	95.6	23.10658	
152	2	Unknown 5	-6.01424	97.3	10373.84	9938.6	-164.2	97.3	23.51269	
153	3	Unknown 5	-6.01424	93.9	10021.99	9586.8	187.7	93.9	22.71521	
154	1	Unknown 5	-7.01424	104.9	11145.43	10710.2	-935.8	104.9	25.26153	
155	2	Unknown 5	-7.01424	103.0	10946.72	10511.5	-737.1	103.0	24.81115	
156	3	Unknown 5	-7.01424	102.8	10928.43	10493.2	-718.8	102.8	24.76969	
157	1	Unknown 5	-8.01424	106.1	11263.54	10828.3	-1053.9	106.1	25.52923	
158	2	Unknown 5	-8.01424	102.7	10916.96	10481.7	-707.3	102.7	24.7437	
159	3	Unknown 5	-8.01424	102.8	10935.56	10500.3	-725.9	102.8	24.78585	
160	1	Unknown 5	-9.01424	105.6	11213.94	10778.7	-1004.3	105.6	25.41681	
161	2	Unknown 5	-9.01424	104.5	11106.99	10671.8	-897.3	104.5	25.17441	
162	3	Unknown 5	-9.01424	103.2	10974.31	10539.1	-764.6	103.2	24.87368	
163	1	Unknown 5	-10.01424	102.4	10885.03	10449.8	-675.4	102.4	24.67133	
164	2	Unknown 5	-10.01424	100.9	10731.89	10296.7	-522.2	100.9	24.32423	
165	3	Unknown 5	-10.01424	103.0	10948.89	10513.7	-739.2	103.0	24.81607	
166	1		—	101.4	10792.65	10357.4	-583.0	101.4	24.46194	
167	2		—	103.5	11003.76	10568.5	-794.1	103.5	24.94043	
168	3		—	103.1	10958.81	10523.6	-749.1	103.1	24.83855	
169	1		-6.00	-0.2	413.22	-22.0	9796.4	-0.2	0.936579	
170	2		-6.00	0.9	526.56	91.3	9683.1	0.9	1.193468	
171	3		-6.00	0.2	456.66	21.4	9753.0	0.2	1.035037	
172	1				43627	43191.8				
173	2				43876	43440.8				
174	3				43995	43559.8				
175	1				43719	43283.8				
176	2				45342	44906.8				
177	3				44162	43726.8				

Prism data

standard curve				weak positive				CR42400				CR42401			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-6.0	0.57406	-0.23811	-1.22511	-3	2.0823	4.5841	5.3122	-3.0				-3.0			
-6.0	-0.21548	0.89464	0.21000	-4	29.4964	30.0811	29.3612	-4.0	-0.2925	-0.2297	-0.3578	-4.0	0.1399	-0.8573	-0.2767
-7.0	1.95104	1.45825	0.89409	-5	81.0005	82.7371	82.8194	-5.0	-0.1820	1.0644	-0.3997	-5.0	-0.4443	-0.8424	-0.9957
-8.0	12.57003	13.77819	14.81267	-6	100.4174	88.3788	101.6163	-6.0	1.2834	0.6715	0.0069	-6.0	0.6752	2.3998	1.3939
-9.0	59.85555	56.23623	57.36271	-7	102.6800	105.5420	104.2755	-7.0	1.8888	1.6322	2.4933	-7.0	9.2295	7.9193	7.5242
-10.0	99.21258	91.33935	100.89774	-8	101.5223	102.5154	100.4086	-8.0	16.8655	17.1762	17.1185	-8.0	46.4076	46.7689	48.5239
-11.0	106.34797	103.97356	100.43015	-9	104.8279	102.6976	101.8837	-9.0	72.5292	65.3847	72.0282	-9.0	91.3727	93.9536	90.4527
				-10	103.2765	101.3489	105.1277	-10.0	97.4181	99.0365	97.8219	-10.0	101.7176	104.8511	102.2793

CR42402				CR42404				CR42405			
concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-3.0				-3.0				-3.0			
-4.0	-0.8424	-0.1131	-0.6420	-4.0	50.4520	42.3359	50.1423	-4.0	19.4127	18.8288	19.9599
-5.0	1.6155	1.8754	1.6174	-5.0	99.1610	97.4394	100.8431	-5.0	63.9546	60.8211	61.9537
-6.0	24.6106	26.2630	26.6778	-6.0	104.8936	93.1338	107.1374	-6.0	95.5902	97.3452	93.8990
-7.0	82.8437	86.7211	85.1513	-7.0	107.3196	106.8399	110.4167	-7.0	104.9027	102.9564	102.7772
-8.0	102.3947	102.3491	102.4493	-8.0	107.2558	99.7743	107.0767	-8.0	106.0595	102.6649	102.8471
-9.0	105.6375	105.3976	105.3004	-9.0	110.0614	104.8875	104.6810	-9.0	105.5737	104.5262	103.2266
-10.0	105.5950	104.4685	109.7031	-10.0	112.7850	106.8884	109.5786	-10.0	102.3522	100.8522	102.9776

Competitive Assay of a known Weak Positive

177 Assay Tubes

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

Provide information in all blue cells
in columns O and P, and row 45, AE through BC
If the DPM value for a tube was judged unreliable,
Include the DPM value in column O
Provide a reason in column R
The value in column Q will
automatically change to FALSE

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

They are also presented in table form in columns

AC thorugh BD

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

	Provide information in all blue cells in this column		
Laboratory Code:	**E**		
Run identification:	520		
Assay start date:	11/22/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	79.30	**Ci/mmole**	
Cytosol vial or lot identification:	051905		
Protein (cytosol):	100	**micro gram per tube**	
Standard Curve IC50:	1.77E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	4.00E-05	**M**	
RBA:	4.40809E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	1.74E-08	**CR42403**	
RBA, Unknown 1:	10.14951%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	1.33E-07	**CR42406**	
RBA, Unknown 2:	1.32907%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	2.34E-07	**CR42407**	
RBA, Unknown 3:	0.75492%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	7.85E-03	**CR42408**	
RBA, Unknown 4:	0.00002%		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	4.65E-03	**CR42409**	
RBA, Unknown 5:	0.00004%		
volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3.1		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Summary values	n	Mean	SD	
EtOH	6	11287.4	610.91	Total Binding, solvent control, tubes
Hot	6	44382.0	535.82	Total hot R1881 added to each tube
NSB	6	524.7	42.91	Nonspecific Binding
Specific EtOH	6	10762.6	610.91	

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

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

Check the 10% rule:	25.43%	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	3601.00	11163.10	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3752.90	11633.99	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3603.40	11170.54	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	169.50	508.50	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	178.66	535.98	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	161.69	485.07	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	218.47	677.26	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	213.30	661.23	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	208.37	645.95	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	685.58	2125.30	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	654.60	2029.26	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	658.62	2041.72	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2399.70	7439.07	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2289.20	7096.52	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2516.80	7802.08	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3343.30	10364.23	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3420.60	10603.86	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3553.80	11016.78	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3467.40	10748.94	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3875.00	12012.50	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3710.30	11501.93	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	374.01	1122.03	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	359.13	1077.39	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	368.68	1106.04	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1203.20	3609.60	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1249.20	3747.60	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1230.30	3690.90	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3088.20	9264.60	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2906.10	8718.30	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3080.60	9241.80	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3621.90	10865.70	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3677.40	11032.20	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3316.60	9949.80	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	2922.30	8766.90	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3687.60	11062.80	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3582.20	10746.60	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3598.40	10795.20	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3497.30	10491.90	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3475.50	10426.50	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3680.90	11042.70	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3529.90	10589.70	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3241.50	9724.50	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	2935.20	8805.60	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3677.00	11031.00	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3545.10	10635.30	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
1	1		—	98.8	11163.1	10638.4	-400.5	98.8	25.15231
2	2		—	103.2	11633.99	11109.3	-871.4	103.2	26.21331
3	3		—	98.9	11170.54	10645.8	-407.9	98.9	25.16908
4	1		-6.00	-0.2	508.5	-16.2	10254.1	-0.2	1.145735
5	2		-6.00	0.1	535.98	11.2	10226.6	0.1	1.207652
6	3		-6.00	0.0	485.07	-39.7	10277.6	-0.4	1.092943
7	1	cold R1881	-7.01	1.4	677.257	152.5	10085.4	1.4	1.525972
8	2	cold R1881	-7.01	1.3	661.23	136.5	10101.4	1.3	1.489861
9	3	cold R1881	-7.01	1.1	645.947	121.2	10116.7	1.1	1.455426
10	1	cold R1881	-8.01	14.9	2125.298	1600.6	8637.3	14.9	4.788649
11	2	cold R1881	-8.01	14.0	2029.26	1504.5	8733.4	14.0	4.572259
12	3	cold R1881	-8.01	14.1	2041.722	1517.0	8720.9	14.1	4.600338
13	1	cold R1881	-9.01	64.2	7439.07	6914.3	3323.6	64.2	16.76146
14	2	cold R1881	-9.01	61.1	7096.52	6571.8	3666.1	61.1	15.98964
15	3	cold R1881	-9.01	67.6	7802.08	7277.3	2960.5	67.6	17.57938
16	1	cold R1881	-10.01	91.4	10364.23	9839.5	398.4	91.4	23.35233
17	2	cold R1881	-10.01	93.6	10603.86	10079.1	158.8	93.6	23.89225
18	3	cold R1881	-10.01	97.5	11016.78	10492.0	-254.2	97.5	24.82263
19	1	cold R1881	-11.01	95.0	10748.94	10224.2	13.7	95.0	24.21914
20	2	cold R1881	-11.01	106.7	12012.5	11487.8	-1249.9	106.7	27.06615
21	3	cold R1881	-11.01	102.0	11501.93	10977.2	-739.3	102.0	25.91575
22	1	Weak Positive	-3	5.5	1122.03	597.3	9640.6	5.5	2.52812
23	2	Weak Positive	-3	5.1	1077.39	552.7	9685.2	5.1	2.427538
24	3	Weak Positive	-3	5.4	1106.04	581.3	9656.6	5.4	2.492091
25	1	Weak Positive	-4	28.7	3609.6	3084.9	7153.0	28.7	8.133027
26	2	Weak Positive	-4	29.9	3747.6	3222.9	7015.0	29.9	8.443964
27	3	Weak Positive	-4	29.4	3690.9	3166.2	7071.7	29.4	8.316209
28	1	Weak Positive	-5	81.2	9264.6	8739.9	1498.0	81.2	20.87468
29	2	Weak Positive	-5	76.1	8718.3	8193.6	2044.3	76.1	19.64377
30	3	Weak Positive	-5	81.0	9241.8	8717.1	1520.8	81.0	20.82331
31	1	Weak Positive	-6	96.1	10865.7	10341.0	-103.1	96.1	24.48222
32	2	Weak Positive	-6	97.6	11032.2	10507.5	-269.6	97.6	24.85737
33	3	Weak Positive	-6	87.6	9949.8	9425.1	812.8	87.6	22.41855
34	1	Weak Positive	-7	76.6	8766.9	8242.2	1995.7	76.6	19.75328
35	2	Weak Positive	-7	97.9	11062.8	10538.1	-300.2	97.9	24.92632
36	3	Weak Positive	-7	95.0	10746.6	10221.9	16.0	95.0	24.21387
37	1	Weak Positive	-8	95.4	10795.2	10270.5	-32.6	95.4	24.32337
38	2	Weak Positive	-8	92.6	10491.9	9967.2	270.7	92.6	23.63999
39	3	Weak Positive	-8	92.0	10426.5	9901.8	336.1	92.0	23.49263
40	1	Weak Positive	-9	97.7	11042.7	10518.0	-280.1	97.7	24.88103
41	2	Weak Positive	-9	93.5	10589.7	10065.0	172.9	93.5	23.86035
42	3	Weak Positive	-9	85.5	9724.5	9199.8	1038.1	85.5	21.91091
43	1	Weak Positive	-10	76.9	8805.6	8280.9	1957.0	76.9	19.84048
44	2	Weak Positive	-10	97.6	11031	10506.3	-268.4	97.6	24.85467
45	3	Weak Positive	-10	93.9	10635.3	10110.6	127.3	93.9	23.96309

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

Check the 10% rule:	25.43%	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"
46	1	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	176.31	546.56	TRUE	
47	2	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	167.34	518.75	TRUE	
48	3	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	168.14	521.23	TRUE	
49	1	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	178.07	552.02	TRUE	
50	2	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	135.24	419.24	TRUE	
51	3	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	160.11	496.34	TRUE	
52	1	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	178.54	553.47	TRUE	
53	2	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	161.63	501.05	TRUE	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	187.39	580.91	TRUE	
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	287.20	890.32	TRUE	
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	229.73	712.16	TRUE	
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	514.49	1594.92	TRUE	
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	832.20	2579.82	TRUE	
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	708.70	2196.97	TRUE	
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	772.40	2394.44	TRUE	
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2383.10	7387.61	TRUE	
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2228.90	6909.59	TRUE	
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2341.30	7258.03	TRUE	
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	3484.00	10800.40	TRUE	
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	3441.50	10668.65	TRUE	
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	3439.60	10662.76	TRUE	
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3548.40	11000.04	TRUE	
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3687.00	11429.70	TRUE	
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3555.70	11022.67	TRUE	
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	107.27	332.54	TRUE	
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	108.15	335.27	TRUE	
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	104.19	322.99	TRUE	
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	114.36	354.52	TRUE	
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	131.81	408.61	TRUE	
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	143.88	446.03	TRUE	
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	188.83	585.37	TRUE	
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	236.66	733.65	TRUE	
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	248.16	769.30	TRUE	
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	705.60	2187.36	TRUE	
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	693.85	2150.94	TRUE	
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	663.98	2058.34	TRUE	
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2230.70	6915.17	TRUE	
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	1973.20	6116.92	TRUE	
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2089.40	6477.14	TRUE	
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	3427.90	10626.49	TRUE	
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	3436.90	10654.39	TRUE	
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	3366.30	10435.53	TRUE	
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3590.40	11130.24	TRUE	
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3610.70	11193.17	TRUE	
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3777.60	11710.56	TRUE	
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3691.50	11443.65	TRUE	
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3735.10	11578.81	TRUE	
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3692.60	11447.06	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
46	1	Unknown 1	-3.01424	0.2	546.561	21.8	10216.1	0.2	1.231492
47	2	Unknown 1	-3.01424	-0.1	518.754	-6.0	10243.9	-0.1	1.168839
48	3	Unknown 1	-3.01424	0.0	521.234	-3.5	10241.4	0.0	1.174427
49	1	Unknown 1	-4.01424	0.3	552.017	27.3	10210.6	0.3	1.243786
50	2	Unknown 1	-4.01424	-1.0	419.244	-105.5	10343.4	-1.0	0.944626
51	3	Unknown 1	-4.01424	-0.3	496.341	-28.4	10266.3	-0.3	1.118339
52	1	Unknown 1	-5.01424	0.3	553.474	28.7	10209.1	0.3	1.247069
53	2	Unknown 1	-5.01424	-0.2	501.053	-23.7	10261.6	-0.2	1.128955
54	3	Unknown 1	-5.01424	0.5	580.909	56.2	10181.7	0.5	1.308884
55	1	Unknown 1	-6.01424	3.4	890.32	365.6	9872.3	3.4	2.006038
56	2	Unknown 1	-6.01424	1.7	712.163	187.4	10050.5	1.7	1.604621
57	3	Unknown 1	-6.01424	9.9	1594.919	1070.2	9167.7	9.9	3.593617
58	1	Unknown 1	-7.01424	19.1	2579.82	2055.1	8182.8	19.1	5.812762
59	2	Unknown 1	-7.01424	15.5	2196.97	1672.2	8565.7	15.5	4.950137
60	3	Unknown 1	-7.01424	17.4	2394.44	1869.7	8368.2	17.4	5.39507
61	1	Unknown 1	-8.01424	63.8	7387.61	6862.9	3375.0	63.8	16.64551
62	2	Unknown 1	-8.01424	59.3	6909.59	6384.9	3853.0	59.3	15.56845
63	3	Unknown 1	-8.01424	62.6	7258.03	6733.3	3504.6	62.6	16.35354
64	1	Unknown 1	-9.01424	95.5	10800.4	10275.7	-37.8	95.5	24.33509
65	2	Unknown 1	-9.01424	94.3	10668.65	10143.9	94.0	94.3	24.03824
66	3	Unknown 1	-9.01424	94.2	10662.76	10138.0	99.9	94.2	24.02497
67	1	Unknown 1	-10.01424	97.3	11000.04	10475.3	-237.4	97.3	24.78491
68	2	Unknown 1	-10.01424	101.3	11429.7	10905.0	-667.1	101.3	25.75301
69	3	Unknown 1	-10.01424	97.5	11022.67	10497.9	-260.0	97.5	24.8359
70	1	Unknown 2	-3.01424	-1.8	332.537	-192.2	10430.1	-1.8	0.749261
71	2	Unknown 2	-3.01424	-1.8	335.265	-189.5	10427.4	-1.8	0.755408
72	3	Unknown 2	-3.01424	-1.9	322.989	-201.7	10439.6	-1.9	0.727748
73	1	Unknown 2	-4.01424	-1.6	354.516	-170.2	10408.1	-1.6	0.798783
74	2	Unknown 2	-4.01424	-1.1	408.611	-116.1	10354.0	-1.1	0.920668
75	3	Unknown 2	-4.01424	-0.7	446.028	-78.7	10316.6	-0.7	1.004975
76	1	Unknown 2	-5.01424	0.6	585.373	60.6	10177.3	0.6	1.318942
77	2	Unknown 2	-5.01424	1.9	733.646	208.9	10029.0	1.9	1.653026
78	3	Unknown 2	-5.01424	2.3	769.296	244.6	9993.3	2.3	1.733351
79	1	Unknown 2	-6.01424	15.4	2187.36	1662.6	8575.3	15.4	4.928485
80	2	Unknown 2	-6.01424	15.1	2150.935	1626.2	8611.7	15.1	4.846413
81	3	Unknown 2	-6.01424	14.2	2058.338	1533.6	8704.3	14.2	4.637777
82	1	Unknown 2	-7.01424	59.4	6915.17	6390.4	3847.5	59.4	15.58102
83	2	Unknown 2	-7.01424	52.0	6116.92	5592.2	4645.7	52.0	13.78243
84	3	Unknown 2	-7.01424	55.3	6477.14	5952.4	4285.5	55.3	14.59407
85	1	Unknown 2	-8.01424	93.9	10626.49	10101.8	136.1	93.9	23.94324
86	2	Unknown 2	-8.01424	94.1	10654.39	10129.7	108.2	94.1	24.00611
87	3	Unknown 2	-8.01424	92.1	10435.53	9910.8	327.1	92.1	23.51298
88	1	Unknown 2	-9.01424	98.5	11130.24	10605.5	-367.6	98.5	25.07827
89	2	Unknown 2	-9.01424	99.1	11193.17	10668.4	-430.5	99.1	25.22007
90	3	Unknown 2	-9.01424	103.9	11710.56	11185.8	-947.9	103.9	26.38583
91	1	Unknown 2	-10.01424	101.5	11443.65	10918.9	-681.0	101.5	25.78444
92	2	Unknown 2	-10.01424	102.7	11578.81	11054.1	-816.2	102.7	26.08898
93	3	Unknown 2	-10.01424	101.5	11447.06	10922.3	-684.4	101.5	25.79212

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

Check the 10% rule:	25.43%	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"
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	105.76	327.86	TRUE	
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	215.32	667.49	TRUE	
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	132.85	411.84	TRUE	
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	144.52	448.01	TRUE	
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	148.84	461.40	TRUE	
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	149.16	462.40	TRUE	
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	240.41	745.27	TRUE	
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	239.37	742.05	TRUE	
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	238.74	740.09	TRUE	
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	858.05	2659.96	TRUE	
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	911.65	2826.12	TRUE	
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	852.07	2641.42	TRUE	
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2747.70	8517.87	TRUE	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2744.80	8508.88	TRUE	
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2308.80	7157.28	TRUE	
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3651.70	11320.27	TRUE	
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3005.40	9316.74	TRUE	
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3345.30	10370.43	TRUE	
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3486.40	10807.84	TRUE	
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3699.60	11468.76	TRUE	
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3469.90	10756.69	TRUE	
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3205.80	9937.98	TRUE	
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3745.90	11612.29	TRUE	
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3758.80	11652.28	TRUE	
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	3403.90	10552.09	TRUE	
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	3241.50	10048.65	TRUE	
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	3156.20	9784.22	TRUE	
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	3412.30	10578.13	TRUE	
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	3395.80	10526.98	TRUE	
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	3567.90	11060.49	TRUE	
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3489.00	10815.90	TRUE	
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3532.70	10951.37	TRUE	
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3556.90	11026.39	TRUE	
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3662.70	11354.37	TRUE	
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3575.90	11085.29	TRUE	
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3543.70	10985.47	TRUE	
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3752.10	11631.51	TRUE	
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3660.30	11346.93	TRUE	
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3542.90	10982.99	TRUE	
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3545.80	10991.98	TRUE	
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3695.80	11456.98	TRUE	
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3495.50	10836.05	TRUE	
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3768.90	11683.59	TRUE	
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3842.40	11911.44	TRUE	
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3834.30	11886.33	TRUE	
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3717.90	11525.49	TRUE	
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3670.50	11378.55	TRUE	
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3687.40	11430.94	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
94	1	Unknown 3	-3.01424	-1.8	327.856	-196.9	10434.8	-1.8	0.738714
95	2	Unknown 3	-3.01424	1.3	667.492	142.8	10095.1	1.3	1.50397
96	3	Unknown 3	-3.01424	-1.0	411.835	-112.9	10350.8	-1.0	0.927932
97	1	Unknown 3	-4.01424	-0.7	448.012	-76.7	10314.6	-0.7	1.009445
98	2	Unknown 3	-4.01424	-0.6	461.404	-63.3	10301.2	-0.6	1.03962
99	3	Unknown 3	-4.01424	-0.6	462.396	-62.3	10300.2	-0.6	1.041855
100	1	Unknown 3	-5.01424	2.0	745.271	220.5	10017.4	2.0	1.679219
101	2	Unknown 3	-5.01424	2.0	742.047	217.3	10020.6	2.0	1.671955
102	3	Unknown 3	-5.01424	2.0	740.094	215.4	10022.5	2.0	1.667554
103	1	Unknown 3	-6.01424	19.8	2659.955	2135.2	8102.7	19.8	5.993319
104	2	Unknown 3	-6.01424	21.4	2826.115	2301.4	7936.5	21.4	6.367705
105	3	Unknown 3	-6.01424	19.7	2641.417	2116.7	8121.2	19.7	5.95155
106	1	Unknown 3	-7.01424	74.3	8517.87	7993.1	2244.8	74.3	19.19217
107	2	Unknown 3	-7.01424	74.2	8508.88	7984.1	2253.7	74.2	19.17192
108	3	Unknown 3	-7.01424	61.6	7157.28	6632.5	3605.3	61.6	16.12654
109	1	Unknown 3	-8.01424	100.3	11320.27	10795.5	-557.6	100.3	25.50644
110	2	Unknown 3	-8.01424	81.7	9316.74	8792.0	1445.9	81.7	20.99216
111	3	Unknown 3	-8.01424	91.5	10370.43	9845.7	392.2	91.5	23.3663
112	1	Unknown 3	-9.01424	95.5	10807.84	10283.1	-45.2	95.5	24.35185
113	2	Unknown 3	-9.01424	101.7	11468.76	10944.0	-706.1	101.7	25.84102
114	3	Unknown 3	-9.01424	95.1	10756.69	10232.0	5.9	95.1	24.2366
115	1	Unknown 3	-10.01424	87.5	9937.98	9413.2	824.6	87.5	22.39192
116	2	Unknown 3	-10.01424	103.0	11612.29	11087.6	-849.7	103.0	26.16441
117	3	Unknown 3	-10.01424	103.4	11652.28	11127.5	-889.7	103.4	26.25452
118	1	Unknown 4	-3.01424	93.2	10552.09	10027.4	210.5	93.2	23.77561
119	2	Unknown 4	-3.01424	88.5	10048.65	9523.9	714.0	88.5	22.64127
120	3	Unknown 4	-3.01424	86.0	9784.22	9259.5	978.4	86.0	22.04547
121	1	Unknown 4	-4.01424	93.4	10578.13	10053.4	184.5	93.4	23.83428
122	2	Unknown 4	-4.01424	92.9	10526.98	10002.2	235.6	92.9	23.71903
123	3	Unknown 4	-4.01424	97.9	11060.49	10535.8	-297.9	97.9	24.92112
124	1	Unknown 4	-5.01424	95.6	10815.9	10291.2	-53.3	95.6	24.37001
125	2	Unknown 4	-5.01424	96.9	10951.37	10426.6	-188.7	96.9	24.67525
126	3	Unknown 4	-5.01424	97.6	11026.39	10501.7	-263.8	97.6	24.84428
127	1	Unknown 4	-6.01424	100.6	11354.37	10829.6	-591.7	100.6	25.58328
128	2	Unknown 4	-6.01424	98.1	11085.29	10560.6	-322.7	98.1	24.977
129	3	Unknown 4	-6.01424	97.2	10985.47	10460.7	-222.8	97.2	24.75208
130	1	Unknown 4	-7.01424	103.2	11631.51	11106.8	-868.9	103.2	26.20772
131	2	Unknown 4	-7.01424	100.6	11346.93	10822.2	-584.3	100.6	25.56651
132	3	Unknown 4	-7.01424	97.2	10982.99	10458.3	-220.4	97.2	24.7465
133	1	Unknown 4	-8.01424	97.3	10991.98	10467.2	-229.4	97.3	24.76675
134	2	Unknown 4	-8.01424	101.6	11456.98	10932.2	-694.4	101.6	25.81447
135	3	Unknown 4	-8.01424	95.8	10836.05	10311.3	-73.4	95.8	24.41542
136	1	Unknown 4	-9.01424	103.7	11683.59	11158.9	-921.0	103.7	26.32506
137	2	Unknown 4	-9.01424	105.8	11911.44	11386.7	-1148.8	105.8	26.83845
138	3	Unknown 4	-9.01424	105.6	11886.33	11361.6	-1123.7	105.6	26.78187
139	1	Unknown 4	-10.01424	102.2	11525.49	11000.8	-762.9	102.2	25.96884
140	2	Unknown 4	-10.01424	100.8	11378.55	10853.8	-615.9	100.8	25.63776
141	3	Unknown 4	-10.01424	101.3	11430.94	10906.2	-668.3	101.3	25.7558

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

Check the 10% rule:	25.43%	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"
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2589.60	8027.76	TRUE	
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	3286.00	10186.60	TRUE	
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	3185.20	9874.12	TRUE	
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	3505.60	10867.36	TRUE	
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	3571.60	11071.96	TRUE	
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	3735.10	11578.81	TRUE	
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	3791.40	11753.34	TRUE	
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	3689.00	11435.90	TRUE	
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	3685.00	11423.50	TRUE	
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3806.80	11801.08	TRUE	
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3893.40	12069.54	TRUE	
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3908.30	12115.73	TRUE	
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3764.70	11670.57	TRUE	
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3834.70	11887.57	TRUE	
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3853.70	11946.47	TRUE	
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3917.60	12144.56	TRUE	
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3415.90	10589.29	TRUE	
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3885.50	12045.05	TRUE	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3732.30	11570.13	TRUE	
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3837.60	11896.56	TRUE	
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3866.60	11986.46	TRUE	
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3820.00	11842.00	TRUE	
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3253.30	10085.23	TRUE	
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3010.40	9332.24	TRUE	
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3288.20	10193.42	TRUE	
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3841.10	11907.41	TRUE	
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3759.90	11655.69	TRUE	
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	163.74	491.22	TRUE	
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	174.89	524.67	TRUE	
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	200.99	602.97	TRUE	
172	1	none	Hot		**—**	—	—	30	—	—	—	—		43875.00	43875.00	TRUE	
173	2	none	Hot		**—**	—	—	30	—	—	—	—		44872.00	44872.00	TRUE	
174	3	none	Hot		**—**	—	—	30	—	—	—	—		44358.00	44358.00	TRUE	
175	1	none	Hot		**—**	—	—	30	—	—	—	—		44744.00	44744.00	TRUE	
176	2	none	Hot		**—**	—	—	30	—	—	—	—		44836.00	44836.00	TRUE	
177	3	none	Hot		**—**	—	—	30	—	—	—	—		43607.00	43607.00	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
142	1	Unknown 5	-3.01424	69.7	8027.76	7503.0	2734.9	69.7	18.08787
143	2	Unknown 5	-3.01424	89.8	10186.6	9661.9	576.0	89.8	22.9521
144	3	Unknown 5	-3.01424	86.9	9874.12	9349.4	888.5	86.9	22.24803
145	1	Unknown 5	-4.01424	96.1	10867.36	10342.6	-104.7	96.1	24.48596
146	2	Unknown 5	-4.01424	98.0	11071.96	10547.2	-309.3	98.0	24.94696
147	3	Unknown 5	-4.01424	102.7	11578.81	11054.1	-816.2	102.7	26.08898
148	1	Unknown 5	-5.01424	104.3	11753.34	11228.6	-990.7	104.3	26.48222
149	2	Unknown 5	-5.01424	101.4	11435.9	10911.2	-673.3	101.4	25.76698
150	3	Unknown 5	-5.01424	101.3	11423.5	10898.8	-660.9	101.3	25.73904
151	1	Unknown 5	-6.01424	104.8	11801.08	11276.3	-1038.5	104.8	26.58979
152	2	Unknown 5	-6.01424	107.3	12069.54	11544.8	-1306.9	107.3	27.19467
153	3	Unknown 5	-6.01424	107.7	12115.73	11591.0	-1353.1	107.7	27.29875
154	1	Unknown 5	-7.01424	103.6	11670.57	11145.8	-907.9	103.6	26.29573
155	2	Unknown 5	-7.01424	105.6	11887.57	11362.8	-1124.9	105.6	26.78466
156	3	Unknown 5	-7.01424	106.1	11946.47	11421.7	-1183.8	106.1	26.91738
157	1	Unknown 5	-8.01424	108.0	12144.56	11619.8	-1381.9	108.0	27.36371
158	2	Unknown 5	-8.01424	93.5	10589.29	10064.6	173.3	93.5	23.85942
159	3	Unknown 5	-8.01424	107.0	12045.05	11520.3	-1282.4	107.0	27.13949
160	1	Unknown 5	-9.01424	102.6	11570.13	11045.4	-807.5	102.6	26.06942
161	2	Unknown 5	-9.01424	105.7	11896.56	11371.8	-1133.9	105.7	26.80492
162	3	Unknown 5	-9.01424	106.5	11986.46	11461.7	-1223.8	106.5	27.00748
163	1	Unknown 5	-10.01424	105.2	11842	11317.3	-1079.4	105.2	26.68199
164	2	Unknown 5	-10.01424	88.8	10085.23	9560.5	677.4	88.8	22.72369
165	3	Unknown 5	-10.01424	81.8	9332.24	8807.5	1430.4	81.8	21.02708
166	1		—	89.8	10193.42	9668.7	569.2	89.8	22.96746
167	2		—	105.8	11907.41	11382.7	-1144.8	105.8	26.82937
168	3		—	103.4	11655.69	11131.0	-893.1	103.4	26.2622
169	1		-6.00	-0.3	491.22	-33.5	10271.4	-0.3	1.1068
170	2		-6.00	0.0	524.67	-0.1	10238.0	0.0	1.182168
171	3		-6.00	0.7	602.97	78.2	10159.7	0.7	1.358591
172	1				43875	43350.3			
173	2				44872	44347.3			
174	3				44358	43833.3			
175	1				44744	44219.3			
176	2				44836	44311.3			
177	3				43607	43082.3			

Prism data

standard curve				weak positive				CR42403				CR42406			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-6.0	-0.15085	0.10448	-0.36854	-3	5.5497	5.1349	5.4011	-3.0	0.2028	-0.0556	-0.0325	-3.0	-1.7858	-1.7604	-1.8745
-6.0	-0.31140	-0.00060	0.72691	-4	28.6628	29.9450	29.4182	-4.0	0.2535	-0.9802	-0.2638	-4.0	-1.5816	-1.0790	-0.7313
-7.0	1.41715	1.26823	1.12623	-5	81.2057	76.1298	80.9939	-5.0	0.2670	-0.2200	0.5219	-5.0	0.5634	1.9411	2.2723
-8.0	14.87150	13.97917	14.09496	-6	96.0822	97.6292	87.5722	-6.0	3.3968	1.7415	9.9435	-6.0	15.4481	15.1097	14.2493
-9.0	64.24396	61.06118	67.61683	-7	76.5814	97.9135	94.9756	-7.0	19.0946	15.5374	17.3722	-7.0	59.3762	51.9593	55.3063
-10.0	91.42283	93.64933	97.48594	-8	95.4272	92.6091	92.0014	-8.0	63.7658	59.3243	62.5618	-8.0	93.8596	94.1188	92.0853
-11.0	94.99733	106.73759	101.99367	-9	97.7268	93.5178	85.4788	-9.0	95.4755	94.2513	94.1966	-9.0	98.5401	99.1249	103.9321
				-10	76.9410	97.6181	93.9415	-10.0	97.3304	101.3226	97.5407	-10.0	101.4522	102.7080	101.4839

CR42407				CR42408				CR42409			
concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-3.0	-1.8293	1.3264	-1.0490	-3.0	93.1683	88.4906	86.0337	-3.0	69.7137	89.7724	86.8690
-4.0	-0.7129	-0.5884	-0.5792	-4.0	93.4103	92.9350	97.8921	-4.0	96.0976	97.9986	102.7080
-5.0	2.0491	2.0191	2.0010	-5.0	95.6195	96.8782	97.5752	-5.0	104.3296	101.3802	101.2649
-6.0	19.8392	21.3831	19.6670	-6.0	100.6226	98.1225	97.1950	-6.0	104.7732	107.2676	107.6967
-7.0	74.2675	74.1840	61.6257	-7.0	103.1977	100.5535	97.1720	-7.0	103.5606	105.5768	106.1241
-8.0	100.3058	81.6902	91.4804	-8.0	97.2555	101.5760	95.8067	-8.0	107.9646	93.5140	107.0400
-9.0	95.5446	101.6855	95.0693	-9.0	103.6816	105.7986	105.5653	-9.0	102.6273	105.6603	106.4956
-10.0	87.4624	103.0191	103.3906	-10.0	102.2126	100.8473	101.3341	-10.0	105.1534	88.8305	81.8342

Competitive Assay of a known Weak Positive

177 Assay Tubes

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

Provide information in all blue cells
in columns O and P, and row 45, AE through BC
If the DPM value for a tube was judged unreliable,
Include the DPM value in column O
Provide a reason in column R
The value in column Q will
automatically change to FALSE

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

They are also presented in table form in columns

AC thorugh BD

Provide information in all blue cells in this column

Laboratory Code:	**E**		
Run identification:	522		
Assay start date:	12/8/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	79.10	**Ci/mmole**	
Cytosol vial or lot identification:	062305		
Protein (cytosol):	100	**micro gram per tube**	
Standard Curve IC50:	1.90E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	2.83E-05	**M**	
RBA:	6.69492E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	1.49E-08	**CR42403**	
RBA, Unknown 1:	12.69076%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	8.99E-08	**CR42406**	
RBA, Unknown 2:	2.10854%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	2.30E-07	**CR42407**	
RBA, Unknown 3:	0.82542%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	2.14E-02	**CR42408**	
RBA, Unknown 4:	0.00001%		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	7.90E-03	**CR42409**	
RBA, Unknown 5:	0.00002%		
volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3.1		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values	n	Mean	SD	
EtOH	6	14631.6	207.51	Total Binding, solvent control, tubes
Hot	6	43885.8	1266.94	Total hot R1881 added to each tube
NSB	6	560.4	41.65	Nonspecific Binding
Specific EtOH	6	14071.2	207.51	

Assay Characterization Values		
EtOH / Hot	0.33	less than 0.1?
NSB / EtOH	0.04	around 0.25 ?

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

Check the 10% rule:	33.34%	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	4684.40	14521.64	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4660.40	14447.24	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4714.00	14613.40	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	172.89	518.67	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	183.80	551.40	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	176.36	529.08	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	251.52	779.71	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	260.08	806.25	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	283.86	879.97	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	898.74	2786.09	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	865.80	2683.98	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	835.70	2590.67	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3108.00	9634.80	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3130.70	9705.17	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3168.30	9821.73	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4774.30	14800.33	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4645.10	14399.81	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4580.90	14200.79	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4799.20	14877.52	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4864.20	15079.02	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4750.00	14725.00	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	377.01	1131.03	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	344.84	1034.52	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	413.29	1239.87	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1536.30	4608.90	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1513.30	4539.90	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1082.41	3247.23	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3149.60	9448.80	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3799.80	11399.40	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3733.60	11200.80	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	4651.60	13954.80	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	4073.30	12219.90	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	4514.50	13543.50	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	4826.70	14480.10	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	4801.10	14403.30	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	4549.90	13649.70	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	4536.30	13608.90	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	4344.00	13032.00	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	4814.40	14443.20	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	4858.80	14576.40	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	4059.30	12177.90	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	4390.80	13172.40	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3985.60	11956.80	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	4576.00	13728.00	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	4321.10	12963.30	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
1	1		—	99.2	14521.64	13961.2	-450.4	99.2	33.08958
2	2		—	98.7	14447.24	13886.8	-376.0	98.7	32.92005
3	3		—	99.9	14613.4	14053.0	-542.2	99.9	33.29867
4	1		-6.00	-0.3	518.67	-41.8	13552.5	-0.3	1.181862
5	2		-6.00	-0.1	551.4	-9.0	13519.8	-0.1	1.256442
6	3		-6.00	0.0	529.08	-31.3	13542.1	-0.2	1.205583
7	1	cold R1881	-7.01	1.6	779.712	219.3	13291.5	1.6	1.776683
8	2	cold R1881	-7.01	1.7	806.248	245.8	13265.0	1.7	1.837149
9	3	cold R1881	-7.01	2.3	879.966	319.5	13191.3	2.3	2.005125
10	1	cold R1881	-8.01	15.8	2786.094	2225.7	11285.1	15.8	6.348504
11	2	cold R1881	-8.01	15.1	2683.98	2123.6	11387.2	15.1	6.115823
12	3	cold R1881	-8.01	14.4	2590.67	2030.3	11480.5	14.4	5.903203
13	1	cold R1881	-9.01	64.5	9634.8	9074.4	4436.4	64.5	21.95424
14	2	cold R1881	-9.01	65.0	9705.17	9144.8	4366.0	65.0	22.11459
15	3	cold R1881	-9.01	65.8	9821.73	9261.3	4249.5	65.8	22.38018
16	1	cold R1881	-10.01	101.2	14800.33	14239.9	-729.1	101.2	33.72462
17	2	cold R1881	-10.01	98.4	14399.81	13839.4	-328.6	98.4	32.81198
18	3	cold R1881	-10.01	96.9	14200.79	13640.4	-129.6	96.9	32.35848
19	1	cold R1881	-11.01	101.7	14877.52	14317.1	-806.3	101.7	33.90051
20	2	cold R1881	-11.01	103.2	15079.02	14518.6	-1007.8	103.2	34.35965
21	3	cold R1881	-11.01	100.7	14725	14164.6	-653.8	100.7	33.55297
22	1	Weak Positive	-3	4.1	1131.03	570.6	12940.2	4.1	2.57721
23	2	Weak Positive	-3	3.4	1034.52	474.1	13036.7	3.4	2.357298
24	3	Weak Positive	-3	4.8	1239.87	679.5	12831.3	4.8	2.825217
25	1	Weak Positive	-4	28.8	4608.9	4048.5	9462.3	28.8	10.50202
26	2	Weak Positive	-4	28.3	4539.9	3979.5	9531.3	28.3	10.3448
27	3	Weak Positive	-4	19.1	3247.23	2686.8	10824.0	19.1	7.399267
28	1	Weak Positive	-5	63.2	9448.8	8888.4	4622.4	63.2	21.53041
29	2	Weak Positive	-5	77.0	11399.4	10839.0	2671.8	77.0	25.97512
30	3	Weak Positive	-5	75.6	11200.8	10640.4	2870.4	75.6	25.52259
31	1	Weak Positive	-6	95.2	13954.8	13394.4	116.4	95.2	31.79796
32	2	Weak Positive	-6	82.9	12219.9	11659.5	1851.3	82.9	27.84475
33	3	Weak Positive	-6	92.3	13543.5	12983.1	527.7	92.3	30.86076
34	1	Weak Positive	-7	98.9	14480.1	13919.7	-408.9	98.9	32.99493
35	2	Weak Positive	-7	98.4	14403.3	13842.9	-332.1	98.4	32.81993
36	3	Weak Positive	-7	93.0	13649.7	13089.3	421.5	93.0	31.10275
37	1	Weak Positive	-8	92.7	13608.9	13048.5	462.3	92.7	31.00978
38	2	Weak Positive	-8	88.6	13032	12471.6	1039.2	88.6	29.69523
39	3	Weak Positive	-8	98.7	14443.2	13882.8	-372.0	98.7	32.91085
40	1	Weak Positive	-9	99.6	14576.4	14016.0	-505.2	99.6	33.21436
41	2	Weak Positive	-9	82.6	12177.9	11617.5	1893.3	82.6	27.74905
42	3	Weak Positive	-9	89.6	13172.4	12612.0	898.8	89.6	30.01515
43	1	Weak Positive	-10	81.0	11956.8	11396.4	2114.4	81.0	27.24524
44	2	Weak Positive	-10	93.6	13728	13167.6	343.2	93.6	31.28117
45	3	Weak Positive	-10	88.1	12963.3	12402.9	1107.9	88.1	29.53869

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

Check the 10% rule:	33.34%	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"
46	1	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	287.91	892.52	TRUE	
47	2	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	270.67	839.08	TRUE	
48	3	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	281.49	872.62	TRUE	
49	1	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	148.74	461.09	TRUE	
50	2	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	133.00	412.30	TRUE	
51	3	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	167.10	518.01	TRUE	
52	1	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	193.19	598.89	TRUE	
53	2	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	175.65	544.52	TRUE	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	202.34	627.25	TRUE	
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	300.51	931.58	TRUE	
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	274.23	850.11	TRUE	
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	330.01	1023.03	TRUE	
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	784.80	2432.88	TRUE	
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	795.88	2467.23	TRUE	
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	869.15	2694.37	TRUE	
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	3081.10	9551.41	TRUE	
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2664.40	8259.64	TRUE	
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	3108.20	9635.42	TRUE	
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	4375.30	13563.43	TRUE	
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	4498.70	13945.97	TRUE	
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	3541.90	10979.89	TRUE	
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	4382.90	13586.99	TRUE	
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	4582.70	14206.37	TRUE	
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	4666.60	14466.46	TRUE	
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	110.65	343.02	TRUE	
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	106.69	330.74	TRUE	
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	148.55	460.51	TRUE	
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	134.11	415.74	TRUE	
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	150.88	467.73	TRUE	
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	169.38	525.08	TRUE	
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	242.17	750.73	TRUE	
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	246.57	764.37	TRUE	
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	222.63	690.15	TRUE	
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	521.58	1616.90	TRUE	
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	777.38	2409.88	TRUE	
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	637.50	1976.25	TRUE	
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2129.60	6601.76	TRUE	
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2486.10	7706.91	TRUE	
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2625.70	8139.67	TRUE	
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	4246.10	13162.91	TRUE	
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	4044.70	12538.57	TRUE	
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	3698.50	11465.35	TRUE	
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	4628.10	14347.11	TRUE	
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	4404.90	13655.19	TRUE	
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3991.40	12373.34	TRUE	
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3433.40	10643.54	FALSE	spill
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	4598.00	14253.80	TRUE	
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	4746.30	14713.53	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	
46	1	Unknown 1	-3.01424	2.4	892.521	332.1	13178.7	2.4	2.033734	
47	2	Unknown 1	-3.01424	2.0	839.077	278.7	13232.1	2.0	1.911954	
48	3	Unknown 1	-3.01424	2.2	872.619	312.2	13198.6	2.2	1.988384	
49	1	Unknown 1	-4.01424	-0.7	461.094	-99.3	13610.1	-0.7	1.050667	
50	2	Unknown 1	-4.01424	-1.1	412.3	-148.1	13658.9	-1.1	0.939483	
51	3	Unknown 1	-4.01424	-0.3	518.01	-42.4	13553.2	-0.3	1.180358	
52	1	Unknown 1	-5.01424	0.3	598.889	38.5	13472.3	0.3	1.364652	
53	2	Unknown 1	-5.01424	-0.1	544.515	-15.9	13526.7	-0.1	1.240753	
54	3	Unknown 1	-5.01424	0.5	627.254	66.8	13444.0	0.5	1.429286	
55	1	Unknown 1	-6.01424	2.6	931.581	371.2	13139.6	2.6	2.122737	
56	2	Unknown 1	-6.01424	2.1	850.113	289.7	13221.1	2.1	1.937101	
57	3	Unknown 1	-6.01424	3.3	1023.031	462.6	13048.2	3.3	2.331119	
58	1	Unknown 1	-7.01424	13.3	2432.88	1872.5	11638.3	13.3	5.543657	
59	2	Unknown 1	-7.01424	13.6	2467.228	1906.8	11604.0	13.6	5.621924	
60	3	Unknown 1	-7.01424	15.2	2694.365	2133.9	11376.9	15.2	6.139487	
61	1	Unknown 1	-8.01424	63.9	9551.41	8991.0	4519.8	63.9	21.76422	
62	2	Unknown 1	-8.01424	54.7	8259.64	7699.2	5811.6	54.7	18.82074	
63	3	Unknown 1	-8.01424	64.5	9635.42	9075.0	4435.8	64.5	21.95565	
64	1	Unknown 1	-9.01424	92.4	13563.43	13003.0	507.8	92.4	30.90617	
65	2	Unknown 1	-9.01424	95.1	13945.97	13385.6	125.2	95.1	31.77784	
66	3	Unknown 1	-9.01424	74.0	10979.89	10419.5	3091.3	74.0	25.01921	
67	1	Unknown 1	-10.01424	92.6	13586.99	13026.6	484.2	92.6	30.95985	
68	2	Unknown 1	-10.01424	97.0	14206.37	13646.0	-135.2	97.0	32.3712	
69	3	Unknown 1	-10.01424	98.8	14466.46	13906.0	-395.2	98.8	32.96385	
70	1	Unknown 2	-3.01424	-1.5	343.015	-217.4	13728.2	-1.5	0.781608	
71	2	Unknown 2	-3.01424	-1.6	330.739	-229.7	13740.5	-1.6	0.753635	
72	3	Unknown 2	-3.01424	-0.7	460.505	-99.9	13610.7	-0.7	1.049325	
73	1	Unknown 2	-4.01424	-1.0	415.741	-144.7	13655.5	-1.0	0.947324	
74	2	Unknown 2	-4.01424	-0.7	467.728	-92.7	13603.5	-0.7	1.065784	
75	3	Unknown 2	-4.01424	-0.3	525.078	-35.3	13546.1	-0.3	1.196464	
76	1	Unknown 2	-5.01424	1.4	750.727	190.3	13320.5	1.4	1.710636	
77	2	Unknown 2	-5.01424	1.4	764.367	203.9	13306.9	1.4	1.741717	
78	3	Unknown 2	-5.01424	0.9	690.153	129.7	13381.1	0.9	1.57261	
79	1	Unknown 2	-6.01424	7.5	1616.898	1056.5	12454.3	7.5	3.684328	
80	2	Unknown 2	-6.01424	13.1	2409.878	1849.5	11661.3	13.1	5.491244	
81	3	Unknown 2	-6.01424	10.1	1976.25	1415.8	12095.0	10.1	4.503162	
82	1	Unknown 2	-7.01424	42.9	6601.76	6041.3	7469.5	42.9	15.04303	
83	2	Unknown 2	-7.01424	50.8	7706.91	7146.5	6364.3	50.8	17.56127	
84	3	Unknown 2	-7.01424	53.9	8139.67	7579.3	5931.5	53.9	18.54737	
85	1	Unknown 2	-8.01424	89.6	13162.91	12602.5	908.3	89.6	29.99353	
86	2	Unknown 2	-8.01424	85.1	12538.57	11978.2	1532.6	85.1	28.57088	
87	3	Unknown 2	-8.01424	77.5	11465.35	10904.9	2605.9	77.5	26.1254	
88	1	Unknown 2	-9.01424	98.0	14347.11	13786.7	-275.9	98.0	32.69189	
89	2	Unknown 2	-9.01424	93.1	13655.19	13094.8	416.0	93.1	31.11526	
90	3	Unknown 2	-9.01424	84.0	12373.34	11812.9	1697.9	84.0	28.19438	
91	1	Unknown 2	-10.01424							
92	2	Unknown 2	-10.01424	97.3	14253.8	13693.4	-182.6	97.3	32.47927	
93	3	Unknown 2	-10.01424	100.6	14713.53	14153.1	-642.3	100.6	33.52683	

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

Check the 10% rule:	33.34%	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"
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	157.27	487.54	TRUE	
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	129.95	402.85	TRUE	
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	156.85	486.24	TRUE	
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	198.31	614.76	TRUE	
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	210.75	653.33	TRUE	
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	221.85	687.74	TRUE	
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	329.17	1020.43	TRUE	
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	313.35	971.39	TRUE	
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	296.00	917.60	TRUE	
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1162.90	3604.99	TRUE	
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1172.50	3634.75	TRUE	
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1154.20	3578.02	TRUE	
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	3143.70	9745.47	TRUE	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	3102.60	9618.06	TRUE	
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	3422.80	10610.68	TRUE	
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	4703.60	14581.16	TRUE	
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	4603.80	14271.78	TRUE	
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	4675.90	14495.29	TRUE	
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	4117.50	12764.25	TRUE	
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	4699.20	14567.52	TRUE	
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	4790.20	14849.62	TRUE	
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	4129.10	12800.21	TRUE	
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	4219.90	13081.69	TRUE	
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	4137.10	12825.01	TRUE	
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	4538.50	14069.35	TRUE	
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	4533.70	14054.47	TRUE	
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	4563.50	14146.85	TRUE	
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	4559.20	14133.52	TRUE	
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	4636.50	14373.15	TRUE	
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	4211.90	13056.89	TRUE	
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	4710.50	14602.55	TRUE	
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3988.80	12365.28	TRUE	
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	4608.80	14287.28	TRUE	
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4494.80	13933.88	TRUE	
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4805.50	14897.05	TRUE	
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4899.60	15188.76	TRUE	
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	4478.70	13883.97	TRUE	
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	4690.40	14540.24	TRUE	
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	4810.60	14912.86	TRUE	
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	4779.40	14816.14	TRUE	
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	4751.00	14728.10	TRUE	
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	4683.10	14517.61	TRUE	
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	4740.60	14695.86	TRUE	
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	4770.60	14788.86	TRUE	
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	4914.10	15233.71	TRUE	
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4754.80	14739.88	TRUE	
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4806.70	14900.77	TRUE	
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4767.50	14779.25	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
94	1	Unknown 3	-3.01424	-0.5	487.537	-72.9	13583.7	-0.5	1.110921
95	2	Unknown 3	-3.01424	-1.1	402.845	-157.6	13668.4	-1.1	0.917939
96	3	Unknown 3	-3.01424	-0.5	486.235	-74.2	13585.0	-0.5	1.107954
97	1	Unknown 3	-4.01424	0.4	614.761	54.3	13456.5	0.4	1.400819
98	2	Unknown 3	-4.01424	0.7	653.325	92.9	13417.9	0.7	1.488692
99	3	Unknown 3	-4.01424	0.9	687.735	127.3	13383.5	0.9	1.5671
100	1	Unknown 3	-5.01424	3.3	1020.427	460.0	13050.8	3.3	2.325185
101	2	Unknown 3	-5.01424	2.9	971.385	411.0	13099.8	2.9	2.213436
102	3	Unknown 3	-5.01424	2.5	917.6	357.2	13153.6	2.5	2.09088
103	1	Unknown 3	-6.01424	21.6	3604.99	3044.6	10466.2	21.6	8.214473
104	2	Unknown 3	-6.01424	21.8	3634.75	3074.3	10436.5	21.8	8.282285
105	3	Unknown 3	-6.01424	21.4	3578.02	3017.6	10493.2	21.4	8.153018
106	1	Unknown 3	-7.01424	65.3	9745.47	9185.1	4325.7	65.3	22.20641
107	2	Unknown 3	-7.01424	64.4	9618.06	9057.6	4453.2	64.4	21.91609
108	3	Unknown 3	-7.01424	71.4	10610.68	10050.3	3460.5	71.4	24.17792
109	1	Unknown 3	-8.01424	99.6	14581.16	14020.7	-509.9	99.6	33.22521
110	2	Unknown 3	-8.01424	97.4	14271.78	13711.4	-200.6	97.4	32.52024
111	3	Unknown 3	-8.01424	99.0	14495.29	13934.9	-424.1	99.0	33.02954
112	1	Unknown 3	-9.01424	86.7	12764.25	12203.8	1307.0	86.7	29.08513
113	2	Unknown 3	-9.01424	99.5	14567.52	14007.1	-496.3	99.5	33.19413
114	3	Unknown 3	-9.01424	101.5	14849.62	14289.2	-778.4	101.5	33.83693
115	1	Unknown 3	-10.01424	87.0	12800.21	12239.8	1271.0	87.0	29.16707
116	2	Unknown 3	-10.01424	89.0	13081.69	12521.3	989.5	89.0	29.80846
117	3	Unknown 3	-10.01424	87.2	12825.01	12264.6	1246.2	87.2	29.22358
118	1	Unknown 4	-3.01424	96.0	14069.35	13508.9	1.9	96.0	32.05898
119	2	Unknown 4	-3.01424	95.9	14054.47	13494.1	16.7	95.9	32.02507
120	3	Unknown 4	-3.01424	96.6	14146.85	13586.4	-75.6	96.6	32.23557
121	1	Unknown 4	-4.01424	96.5	14133.52	13573.1	-62.3	96.5	32.2052
122	2	Unknown 4	-4.01424	98.2	14373.15	13812.7	-301.9	98.2	32.75123
123	3	Unknown 4	-4.01424	88.8	13056.89	12496.5	1014.3	88.8	29.75195
124	1	Unknown 4	-5.01424	99.8	14602.55	14042.1	-531.3	99.8	33.27395
125	2	Unknown 4	-5.01424	83.9	12365.28	11804.9	1705.9	83.9	28.17602
126	3	Unknown 4	-5.01424	97.6	14287.28	13726.9	-216.1	97.6	32.55556
127	1	Unknown 4	-6.01424	95.0	13933.88	13373.5	137.3	95.0	31.75029
128	2	Unknown 4	-6.01424	101.9	14897.05	14336.6	-825.8	101.9	33.94501
129	3	Unknown 4	-6.01424	104.0	15188.76	14628.3	-1117.5	104.0	34.60971
130	1	Unknown 4	-7.01424	94.7	13883.97	13323.6	187.2	94.7	31.63656
131	2	Unknown 4	-7.01424	99.4	14540.24	13979.8	-469.0	99.4	33.13197
132	3	Unknown 4	-7.01424	102.0	14912.86	14352.4	-841.6	102.0	33.98103
133	1	Unknown 4	-8.01424	101.3	14816.14	14255.7	-744.9	101.3	33.76064
134	2	Unknown 4	-8.01424	100.7	14728.1	14167.7	-656.9	100.7	33.56003
135	3	Unknown 4	-8.01424	99.2	14517.61	13957.2	-446.4	99.2	33.0804
136	1	Unknown 4	-9.01424	100.5	14695.86	14135.4	-624.6	100.5	33.48657
137	2	Unknown 4	-9.01424	101.1	14788.86	14228.4	-717.6	101.1	33.69848
138	3	Unknown 4	-9.01424	104.3	15233.71	14673.3	-1162.5	104.3	34.71214
139	1	Unknown 4	-10.01424	100.8	14739.88	14179.5	-668.7	100.8	33.58688
140	2	Unknown 4	-10.01424	101.9	14900.77	14340.4	-829.6	101.9	33.95349
141	3	Unknown 4	-10.01424	101.0	14779.25	14218.8	-708.0	101.0	33.67659

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

Check the 10% rule:	33.34%	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"
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	4220.40	13083.24	TRUE	
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	4215.00	13066.50	TRUE	
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	4287.30	13290.63	TRUE	
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	4375.50	13564.05	TRUE	
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	4308.50	13356.35	TRUE	
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	4556.90	14126.39	TRUE	
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	4833.50	14983.85	TRUE	
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	4577.20	14189.32	TRUE	
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	4695.70	14556.67	TRUE	
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	4400.80	13642.48	TRUE	
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	4931.40	15287.34	TRUE	
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	4832.50	14980.75	TRUE	
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4625.00	14337.50	TRUE	
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4836.60	14993.46	TRUE	
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4766.60	14776.46	TRUE	
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	4753.40	14735.54	TRUE	
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	4882.80	15136.68	TRUE	
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	4864.20	15079.02	TRUE	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3958.40	12271.04	TRUE	
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	4770.40	14788.24	TRUE	
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	4840.70	15006.17	TRUE	
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	4865.30	15082.43	TRUE	
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	4859.20	15063.52	TRUE	
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	4728.20	14657.42	TRUE	
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4750.00	14725.00	TRUE	
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4839.40	15002.14	TRUE	
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4671.10	14480.41	TRUE	
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	204.33	612.99	TRUE	
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	179.56	538.68	TRUE	
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	203.90	611.70	TRUE	
172	1	none	Hot		**—**	—	—	30	—	—	—	—		42175.00	42175.00	TRUE	
173	2	none	Hot		**—**	—	—	30	—	—	—	—		44596.00	44596.00	TRUE	
174	3	none	Hot		**—**	—	—	30	—	—	—	—		44361.00	44361.00	TRUE	
175	1	none	Hot		**—**	—	—	30	—	—	—	—		44693.00	44693.00	TRUE	
176	2	none	Hot		**—**	—	—	30	—	—	—	—		42389.00	42389.00	TRUE	
177	3	none	Hot		**—**	—	—	30	—	—	—	—		45101.00	45101.00	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
142	1	Unknown 5	-3.01424	89.0	13083.24	12522.8	988.0	89.0	29.81199
143	2	Unknown 5	-3.01424	88.9	13066.5	12506.1	1004.7	88.9	29.77385
144	3	Unknown 5	-3.01424	90.5	13290.63	12730.2	780.6	90.5	30.28456
145	1	Unknown 5	-4.01424	92.4	13564.05	13003.6	507.2	92.4	30.90758
146	2	Unknown 5	-4.01424	90.9	13356.35	12795.9	714.9	90.9	30.43431
147	3	Unknown 5	-4.01424	96.4	14126.39	13566.0	-55.2	96.4	32.18895
148	1	Unknown 5	-5.01424	102.5	14983.85	14423.4	-912.6	102.5	34.14279
149	2	Unknown 5	-5.01424	96.9	14189.32	13628.9	-118.1	96.9	32.33235
150	3	Unknown 5	-5.01424	99.5	14556.67	13996.3	-485.5	99.5	33.16941
151	1	Unknown 5	-6.01424	93.0	13642.48	13082.1	428.7	93.0	31.0863
152	2	Unknown 5	-6.01424	104.7	15287.34	14726.9	-1216.1	104.7	34.83434
153	3	Unknown 5	-6.01424	102.5	14980.75	14420.3	-909.5	102.5	34.13573
154	1	Unknown 5	-7.01424	97.9	14337.5	13777.1	-266.3	97.9	32.67
155	2	Unknown 5	-7.01424	102.6	14993.46	14433.0	-922.2	102.6	34.16469
156	3	Unknown 5	-7.01424	101.0	14776.46	14216.0	-705.2	101.0	33.67023
157	1	Unknown 5	-8.01424	100.7	14735.54	14175.1	-664.3	100.7	33.57699
158	2	Unknown 5	-8.01424	103.6	15136.68	14576.3	-1065.5	103.6	34.49104
159	3	Unknown 5	-8.01424	103.2	15079.02	14518.6	-1007.8	103.2	34.35965
160	1	Unknown 5	-9.01424	83.2	12271.04	11710.6	1800.2	83.2	27.96128
161	2	Unknown 5	-9.01424	101.1	14788.24	14227.8	-717.0	101.1	33.69707
162	3	Unknown 5	-9.01424	102.7	15006.17	14445.8	-935.0	102.7	34.19365
163	1	Unknown 5	-10.01424	103.2	15082.43	14522.0	-1011.2	103.2	34.36742
164	2	Unknown 5	-10.01424	103.1	15063.52	14503.1	-992.3	103.1	34.32433
165	3	Unknown 5	-10.01424	100.2	14657.42	14097.0	-586.2	100.2	33.39898
166	1		—	100.7	14725	14164.6	-653.8	100.7	33.55297
167	2		—	102.6	15002.14	14441.7	-930.9	102.6	34.18447
168	3		—	98.9	14480.41	13920.0	-409.2	98.9	32.99564
169	1		-6.00	0.4	612.99	52.6	13458.2	0.4	1.396783
170	2		-6.00	-0.2	538.68	-21.7	13532.5	-0.2	1.227458
171	3		-6.00	0.4	611.7	51.3	13459.5	0.4	1.393844
172	1				42175	41614.6			
173	2				44596	44035.6			
174	3				44361	43800.6			
175	1				44693	44132.6			
176	2				42389	41828.6			
177	3				45101	44540.6			

Prism data

standard curve				weak positive				CR42403				CR42406			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-6.0	-0.29670	-0.06410	-0.22272	-3	4.0552	3.3693	4.8287	-3.0	2.3601	1.9803	2.2187	-3.0	-1.5450	-1.6323	-0.7101
-6.0	0.37360	-0.15450	0.36443	-4	28.7714	28.2810	19.0944	-4.0	-0.7059	-1.0526	-0.3014	-4.0	-1.0282	-0.6587	-0.2512
-7.0	1.55844	1.74703	2.27092	-5	63.1671	77.0294	75.6180	-5.0	0.2734	-0.1130	0.4750	-5.0	1.3525	1.4494	0.9220
-8.0	15.81721	15.09151	14.42839	-6	95.1899	82.8605	92.2669	-6.0	2.6377	2.0588	3.2876	-6.0	7.5081	13.1436	10.0619
-9.0	64.48894	64.98904	65.81740	-7	98.9231	98.3773	93.0217	-7.0	13.3070	13.5511	15.1653	-7.0	42.9340	50.7880	53.8635
-10.0	101.19884	98.35246	96.93809	-8	92.7317	88.6318	98.6608	-8.0	63.8963	54.7161	64.4933	-8.0	89.5622	85.1252	77.4981
-11.0	101.74741	103.17941	100.66349	-9	99.6074	82.5620	89.6296	-9.0	92.4086	95.1272	74.0481	-9.0	97.9779	93.0607	83.9509
				-10	80.9907	93.5781	88.1436	-10.0	92.5760	96.9777	98.8261	-10.0		97.3148	100.5820

CR42407				CR42408				CR42409			
concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-3.0	-0.5180	-1.1198	-0.5272	-3.0	96.0040	95.8982	96.5548	-3.0	88.9960	88.8770	90.4698
-4.0	0.3862	0.6602	0.9048	-4.0	96.4600	98.1630	88.8087	-4.0	92.4130	90.9369	96.4093
-5.0	3.2691	2.9206	2.5384	-5.0	99.7933	83.8937	97.5527	-5.0	102.5031	96.8566	99.4672
-6.0	21.6369	21.8484	21.4452	-6.0	95.0412	101.8862	103.9593	-6.0	92.9703	104.6599	102.4810
-7.0	65.2754	64.3700	71.4242	-7.0	94.6865	99.3505	101.9986	-7.0	97.9096	102.5714	101.0292
-8.0	99.6413	97.4426	99.0310	-8.0	101.3112	100.6855	99.1896	-8.0	100.7384	103.5892	103.1794
-9.0	86.7290	99.5443	101.5491	-9.0	100.4564	101.1173	104.2787	-9.0	83.2239	101.1129	102.6617
-10.0	86.9846	88.9850	87.1608	-10.0	100.7692	101.9126	101.0490	-10.0	103.2036	103.0693	100.1832

Competitive Assay of a known Weak Positive

177 Assay Tubes

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

Provide information in all blue cells
in columns O and P, and row 45, AE through BC
If the DPM value for a tube was judged unreliable,
Include the DPM value in column O
Provide a reason in column R
The value in column Q will
automatically change to FALSE

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

They are also presented in table form in columns

AC thorugh BD

Provide information in all blue cells in this column

Laboratory Code:	**E**		
Run identification:	523		
Assay start date:	12/20/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	78.96	**Ci/mmole**	
Cytosol vial or lot identification:	062305		
Protein (cytosol):	100	**micro gram per tube**	
Standard Curve IC50:	1.69E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	3.36E-05	**M**	
RBA:	5.0342E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	1.68E-08	**CR42403**	
RBA, Unknown 1:	10.10746%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	1.20E-07	**CR42406**	
RBA, Unknown 2:	1.40615%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	2.51E-07	**CR42407**	
RBA, Unknown 3:	0.67343%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	1.46E-02	**CR42408**	
RBA, Unknown 4:	0.00001%		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	6.17E-03	**CR42409**	
RBA, Unknown 5:	0.00003%		
volume of ethanol counted:	2	**mL**	protcol calls for counting decanted EtOH supernate
mulitply DPM in sample by :	3.1		reflects 100ul of reaction mixture processed
working volume	**3.1E+02**	uL	

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

Summary values				
	n	Mean	SD	
EtOH	6	15095.3	236.79	Total Binding, solvent control, tubes
Hot	6	44568.3	1065.99	Total hot R1881 added to each tube
NSB	6	608.5	38.86	Nonspecific Binding
Specific EtOH	6	14486.8	236.79	

Assay Characterization Values		
EtOH / Hot	0.34	less than 0.1?
NSB / EtOH	0.04	around 0.25 ?

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

Check the 10% rule:	33.87%	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	4874.80	15111.88	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4769.30	14784.83	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4933.40	15293.54	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	204.27	612.81	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	192.46	577.38	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	226.88	680.64	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	291.19	902.69	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	272.36	844.32	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	271.85	842.74	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	888.26	2753.61	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	863.91	2678.12	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	909.97	2820.91	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3225.90	10000.29	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3151.40	9769.34	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3025.40	9378.74	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4628.40	14348.04	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4546.60	14094.46	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4553.20	14114.92	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4763.10	14765.61	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4888.00	15152.80	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4801.20	14883.72	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	392.12	1176.36	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	489.97	1469.91	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	439.38	1318.14	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1485.30	4455.90	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1585.80	4757.40	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1548.10	4644.30	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3821.20	11463.60	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3757.30	11271.90	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3905.90	11717.70	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	4554.40	13663.20	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	4698.40	14095.20	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	4634.40	13903.20	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	4741.90	14225.70	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	4786.90	14360.70	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	4639.30	13917.90	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	4663.10	13989.30	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	4775.30	14325.90	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	4595.70	13787.10	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	4861.90	14585.70	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	4588.50	13765.50	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	4645.70	13937.10	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	4663.90	13991.70	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	4297.00	12891.00	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	4670.30	14010.90	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
1	1		—	100.1	15111.88	14503.4	-625.1	100.1	33.90721
2	2		—	97.9	14784.83	14176.3	-298.1	97.9	33.1734
3	3		—	101.4	15293.54	14685.0	-806.8	101.4	34.31481
4	1		-6.00	0.0	612.81	4.3	13874.0	0.0	1.37499
5	2		-6.00	-0.2	577.38	-31.1	13909.4	-0.2	1.295494
6	3		-6.00	0.0	680.64	72.1	13806.1	0.5	1.527183
7	1	cold R1881	-7.01	2.0	902.689	294.2	13584.1	2.0	2.025404
8	2	cold R1881	-7.01	1.6	844.316	235.8	13642.5	1.6	1.89443
9	3	cold R1881	-7.01	1.6	842.735	234.2	13644.0	1.6	1.890883
10	1	cold R1881	-8.01	14.8	2753.606	2145.1	11733.2	14.8	6.178391
11	2	cold R1881	-8.01	14.3	2678.121	2069.6	11808.6	14.3	6.009022
12	3	cold R1881	-8.01	15.3	2820.907	2212.4	11665.9	15.3	6.329398
13	1	cold R1881	-9.01	64.8	10000.29	9391.8	4486.5	64.8	22.43811
14	2	cold R1881	-9.01	63.2	9769.34	9160.8	4717.4	63.2	21.91991
15	3	cold R1881	-9.01	60.5	9378.74	8770.2	5108.0	60.5	21.04351
16	1	cold R1881	-10.01	94.8	14348.04	13739.5	138.7	94.8	32.19335
17	2	cold R1881	-10.01	93.1	14094.46	13485.9	392.3	93.1	31.62438
18	3	cold R1881	-10.01	93.2	14114.92	13506.4	371.9	93.2	31.67029
19	1	cold R1881	-11.01	97.7	14765.61	14157.1	-278.8	97.7	33.13027
20	2	cold R1881	-11.01	100.4	15152.8	14544.3	-666.0	100.4	33.99903
21	3	cold R1881	-11.01	98.5	14883.72	14275.2	-396.9	98.5	33.39528
22	1	Weak Positive	-3	3.9	1176.36	567.8	13310.4	3.9	2.639453
23	2	Weak Positive	-3	5.9	1469.91	861.4	13016.9	5.9	3.298104
24	3	Weak Positive	-3	4.9	1318.14	709.6	13168.6	4.9	2.957571
25	1	Weak Positive	-4	26.6	4455.9	3847.4	10030.9	26.6	9.997906
26	2	Weak Positive	-4	28.6	4757.4	4148.9	9729.4	28.6	10.6744
27	3	Weak Positive	-4	27.9	4644.3	4035.8	9842.5	27.9	10.42063
28	1	Weak Positive	-5	74.9	11463.6	10855.1	3023.2	74.9	25.7214
29	2	Weak Positive	-5	73.6	11271.9	10663.4	3214.9	73.6	25.29128
30	3	Weak Positive	-5	76.7	11717.7	11109.2	2769.1	76.7	26.29154
31	1	Weak Positive	-6	90.1	13663.2	13054.7	823.6	90.1	30.65674
32	2	Weak Positive	-6	93.1	14095.2	13486.7	391.6	93.1	31.62604
33	3	Weak Positive	-6	91.8	13903.2	13294.7	583.6	91.8	31.19524
34	1	Weak Positive	-7	94.0	14225.7	13617.2	261.1	94.0	31.91885
35	2	Weak Positive	-7	94.9	14360.7	13752.2	126.1	94.9	32.22176
36	3	Weak Positive	-7	91.9	13917.9	13309.4	568.9	91.9	31.22823
37	1	Weak Positive	-8	92.4	13989.3	13380.8	497.5	92.4	31.38843
38	2	Weak Positive	-8	94.7	14325.9	13717.4	160.9	94.7	32.14367
39	3	Weak Positive	-8	91.0	13787.1	13178.6	699.7	91.0	30.93474
40	1	Weak Positive	-9	96.5	14585.7	13977.2	-98.9	96.5	32.7266
41	2	Weak Positive	-9	90.8	13765.5	13157.0	721.3	90.8	30.88628
42	3	Weak Positive	-9	92.0	13937.1	13328.6	549.7	92.0	31.27131
43	1	Weak Positive	-10	92.4	13991.7	13383.2	495.1	92.4	31.39381
44	2	Weak Positive	-10	84.8	12891	12282.5	1595.8	84.8	28.92412
45	3	Weak Positive	-10	92.5	14010.9	13402.4	475.9	92.5	31.43689

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

Check the 10% rule:	33.87%	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"
46	1	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	242.28	751.07	TRUE	
47	2	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	269.46	835.33	TRUE	
48	3	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	234.97	728.41	TRUE	
49	1	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	201.96	626.08	TRUE	
50	2	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	140.43	435.33	TRUE	
51	3	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	183.48	568.79	TRUE	
52	1	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	255.01	790.53	TRUE	
53	2	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	200.12	620.37	TRUE	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	199.86	619.57	TRUE	
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	296.93	920.48	TRUE	
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	330.15	1023.47	TRUE	
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	313.00	970.30	TRUE	
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	1033.91	3205.12	TRUE	
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	964.63	2990.35	TRUE	
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	982.54	3045.87	TRUE	
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	3095.40	9595.74	TRUE	
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	3088.60	9574.66	TRUE	
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	3075.10	9532.81	TRUE	
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	4524.20	14025.02	TRUE	
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	4532.50	14050.75	TRUE	
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	4374.70	13561.57	TRUE	
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	4744.00	14706.40	TRUE	
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	4665.00	14461.50	TRUE	
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	4667.70	14469.87	TRUE	
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	79.20	245.52	TRUE	
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	107.32	332.69	TRUE	
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	109.43	339.23	TRUE	
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	189.66	587.95	TRUE	
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	172.78	535.62	TRUE	
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	169.29	524.80	TRUE	
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	265.18	822.06	TRUE	
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	276.37	856.75	TRUE	
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	259.75	805.23	TRUE	
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	813.07	2520.52	TRUE	
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	840.92	2606.85	TRUE	
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	874.13	2709.80	TRUE	
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2680.60	8309.86	TRUE	
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2777.90	8611.49	TRUE	
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2815.90	8729.29	TRUE	
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	4273.30	13247.23	TRUE	
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	4384.80	13592.88	TRUE	
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	4247.00	13165.70	TRUE	
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	4608.00	14284.80	TRUE	
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	4690.20	14539.62	TRUE	
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	4702.60	14578.06	TRUE	
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	4834.20	14986.02	TRUE	
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	4632.30	14360.13	TRUE	
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	4580.00	14198.00	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
46	1	Unknown 1	-3.01424	1.0	751.068	142.5	13735.7	1.0	1.685205
47	2	Unknown 1	-3.01424	1.6	835.326	226.8	13651.4	1.6	1.874259
48	3	Unknown 1	-3.01424	0.8	728.407	119.9	13758.4	0.8	1.63436
49	1	Unknown 1	-4.01424	0.1	626.076	17.6	13860.7	0.1	1.404755
50	2	Unknown 1	-4.01424	-1.2	435.333	-173.2	14051.4	-1.2	0.976776
51	3	Unknown 1	-4.01424	-0.3	568.788	-39.7	13918.0	-0.3	1.276216
52	1	Unknown 1	-5.01424	1.3	790.531	182.0	13696.2	1.3	1.77375
53	2	Unknown 1	-5.01424	0.1	620.372	11.8	13866.4	0.1	1.391957
54	3	Unknown 1	-5.01424	0.1	619.566	11.0	13867.2	0.1	1.390148
55	1	Unknown 1	-6.01424	2.2	920.483	312.0	13566.3	2.2	2.06533
56	2	Unknown 1	-6.01424	2.9	1023.465	414.9	13463.3	2.9	2.296395
57	3	Unknown 1	-6.01424	2.5	970.3	361.8	13516.5	2.5	2.177106
58	1	Unknown 1	-7.01424	17.9	3205.121	2596.6	11281.6	17.9	7.191476
59	2	Unknown 1	-7.01424	16.4	2990.353	2381.8	11496.4	16.4	6.709591
60	3	Unknown 1	-7.01424	16.8	3045.874	2437.3	11440.9	16.8	6.834166
61	1	Unknown 1	-8.01424	62.0	9595.74	8987.2	4891.0	62.0	21.5304
62	2	Unknown 1	-8.01424	61.9	9574.66	8966.1	4912.1	61.9	21.4831
63	3	Unknown 1	-8.01424	61.6	9532.81	8924.3	4954.0	61.6	21.3892
64	1	Unknown 1	-9.01424	92.6	14025.02	13416.5	461.8	92.6	31.46858
65	2	Unknown 1	-9.01424	92.8	14050.75	13442.2	436.0	92.8	31.52631
66	3	Unknown 1	-9.01424	89.4	13561.57	12953.0	925.2	89.4	30.42871
67	1	Unknown 1	-10.01424	97.3	14706.4	14097.9	-219.6	97.3	32.99742
68	2	Unknown 1	-10.01424	95.6	14461.5	13853.0	25.3	95.6	32.44793
69	3	Unknown 1	-10.01424	95.7	14469.87	13861.3	16.9	95.7	32.46671
70	1	Unknown 2	-3.01424	-2.5	245.52	-363.0	14241.3	-2.5	0.550884
71	2	Unknown 2	-3.01424	-1.9	332.692	-275.8	14154.1	-1.9	0.746476
72	3	Unknown 2	-3.01424	-1.9	339.233	-269.3	14147.5	-1.9	0.761153
73	1	Unknown 2	-4.01424	-0.1	587.946	-20.6	13898.8	-0.1	1.319201
74	2	Unknown 2	-4.01424	-0.5	535.618	-72.9	13951.2	-0.5	1.201791
75	3	Unknown 2	-4.01424	-0.6	524.799	-83.7	13962.0	-0.6	1.177515
76	1	Unknown 2	-5.01424	1.5	822.058	213.5	13664.7	1.5	1.844489
77	2	Unknown 2	-5.01424	1.7	856.747	248.2	13630.0	1.7	1.922322
78	3	Unknown 2	-5.01424	1.4	805.225	196.7	13681.5	1.4	1.80672
79	1	Unknown 2	-6.01424	13.2	2520.517	1912.0	11966.3	13.2	5.655399
80	2	Unknown 2	-6.01424	13.8	2606.852	1998.3	11879.9	13.8	5.849113
81	3	Unknown 2	-6.01424	14.5	2709.803	2101.3	11777.0	14.5	6.080108
82	1	Unknown 2	-7.01424	53.2	8309.86	7701.3	6176.9	53.2	18.64521
83	2	Unknown 2	-7.01424	55.2	8611.49	8003.0	5875.3	55.2	19.32199
84	3	Unknown 2	-7.01424	56.1	8729.29	8120.8	5757.5	56.1	19.58631
85	1	Unknown 2	-8.01424	87.2	13247.23	12638.7	1239.5	87.2	29.72341
86	2	Unknown 2	-8.01424	89.6	13592.88	12984.4	893.9	89.6	30.49896
87	3	Unknown 2	-8.01424	86.7	13165.7	12557.2	1321.1	86.7	29.54048
88	1	Unknown 2	-9.01424	94.4	14284.8	13676.3	202.0	94.4	32.05146
89	2	Unknown 2	-9.01424	96.2	14539.62	13931.1	-52.8	96.2	32.62321
90	3	Unknown 2	-9.01424	96.4	14578.06	13969.5	-91.3	96.4	32.70946
91	1	Unknown 2	-10.01424	99.2	14986.02	14377.5	-499.2	99.2	33.62482
92	2	Unknown 2	-10.01424	94.9	14360.13	13751.6	126.6	94.9	32.22048
93	3	Unknown 2	-10.01424	93.8	14198	13589.5	288.8	93.8	31.8567

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

Check the 10% rule:	33.87%	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"
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	145.30	450.43	TRUE	
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	144.12	446.77	TRUE	
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	127.02	393.76	TRUE	
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	191.22	592.78	TRUE	
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	182.27	565.04	TRUE	
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	181.08	561.35	TRUE	
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	322.13	998.60	TRUE	
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	356.09	1103.88	TRUE	
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	315.94	979.41	TRUE	
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1169.60	3625.76	TRUE	
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1251.30	3879.03	TRUE	
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1169.10	3624.21	TRUE	
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	3490.40	10820.24	TRUE	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	3510.10	10881.31	TRUE	
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	3527.70	10935.87	TRUE	
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	4694.00	14551.40	TRUE	
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	4440.70	13766.17	TRUE	
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	4612.60	14299.06	TRUE	
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	4864.40	15079.64	TRUE	
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	4791.40	14853.34	TRUE	
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	4808.70	14906.97	TRUE	
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	4802.60	14888.06	TRUE	
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	4781.40	14822.34	TRUE	
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	4900.10	15190.31	TRUE	
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	4675.70	14494.67	TRUE	
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	4641.40	14388.34	TRUE	
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	4457.60	13818.56	TRUE	
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	4666.30	14465.53	TRUE	
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	4706.50	14590.15	TRUE	
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	4591.00	14232.10	TRUE	
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	4701.20	14573.72	TRUE	
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	4722.20	14638.82	TRUE	
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	4635.50	14370.05	TRUE	
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4902.00	15196.20	TRUE	
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4938.40	15309.04	TRUE	
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4987.10	15460.01	TRUE	
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	4850.40	15036.24	TRUE	
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	4851.00	15038.10	TRUE	
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	4926.50	15272.15	TRUE	
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	4789.30	14846.83	TRUE	
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	4987.00	15459.70	TRUE	
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	4787.70	14841.87	TRUE	
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	4829.80	14972.38	TRUE	
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	4881.40	15132.34	TRUE	
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	4849.90	15034.69	TRUE	
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4956.30	15364.53	TRUE	
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4919.40	15250.14	TRUE	
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	5049.90	15654.69	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
94	1	Unknown 3	-3.01424	-1.1	450.43	-158.1	14036.3	-1.1	1.01065
95	2	Unknown 3	-3.01424	-1.1	446.772	-161.8	14040.0	-1.1	1.002443
96	3	Unknown 3	-3.01424	-1.5	393.762	-214.8	14093.0	-1.5	0.883502
97	1	Unknown 3	-4.01424	-0.1	592.782	-15.7	13894.0	-0.1	1.330052
98	2	Unknown 3	-4.01424	-0.3	565.037	-43.5	13921.7	-0.3	1.267799
99	3	Unknown 3	-4.01424	-0.3	561.348	-47.2	13925.4	-0.3	1.259522
100	1	Unknown 3	-5.01424	2.7	998.603	390.1	13488.2	2.7	2.240611
101	2	Unknown 3	-5.01424	3.4	1103.879	495.4	13382.9	3.4	2.476824
102	3	Unknown 3	-5.01424	2.6	979.414	370.9	13507.4	2.6	2.197556
103	1	Unknown 3	-6.01424	20.8	3625.76	3017.2	10861.0	20.8	8.135283
104	2	Unknown 3	-6.01424	22.6	3879.03	3270.5	10607.7	22.6	8.703556
105	3	Unknown 3	-6.01424	20.8	3624.21	3015.7	10862.6	20.8	8.131805
106	1	Unknown 3	-7.01424	70.5	10820.24	10211.7	3666.5	70.5	24.27787
107	2	Unknown 3	-7.01424	70.9	10881.31	10272.8	3605.5	70.9	24.41489
108	3	Unknown 3	-7.01424	71.3	10935.87	10327.3	3550.9	71.3	24.53731
109	1	Unknown 3	-8.01424	96.2	14551.4	13942.9	-64.6	96.2	32.64964
110	2	Unknown 3	-8.01424	90.8	13766.17	13157.6	720.6	90.8	30.88778
111	3	Unknown 3	-8.01424	94.5	14299.06	13690.5	187.7	94.5	32.08345
112	1	Unknown 3	-9.01424	99.9	15079.64	14471.1	-592.9	99.9	33.83488
113	2	Unknown 3	-9.01424	98.3	14853.34	14244.8	-366.6	98.3	33.32712
114	3	Unknown 3	-9.01424	98.7	14906.97	14298.4	-420.2	98.7	33.44745
115	1	Unknown 3	-10.01424	98.6	14888.06	14279.5	-401.3	98.6	33.40502
116	2	Unknown 3	-10.01424	98.1	14822.34	14213.8	-335.6	98.1	33.25756
117	3	Unknown 3	-10.01424	100.7	15190.31	14581.8	-703.5	100.7	34.08319
118	1	Unknown 4	-3.01424	95.9	14494.67	13886.1	-7.9	95.9	32.52235
119	2	Unknown 4	-3.01424	95.1	14388.34	13779.8	98.4	95.1	32.28377
120	3	Unknown 4	-3.01424	91.2	13818.56	13210.0	668.2	91.2	31.00533
121	1	Unknown 4	-4.01424	95.7	14465.53	13857.0	21.2	95.7	32.45697
122	2	Unknown 4	-4.01424	96.5	14590.15	13981.6	-103.4	96.5	32.73658
123	3	Unknown 4	-4.01424	94.0	14232.1	13623.6	254.7	94.0	31.93321
124	1	Unknown 4	-5.01424	96.4	14573.72	13965.2	-86.9	96.4	32.69972
125	2	Unknown 4	-5.01424	96.8	14638.82	14030.3	-152.0	96.8	32.84579
126	3	Unknown 4	-5.01424	95.0	14370.05	13761.5	116.7	95.0	32.24274
127	1	Unknown 4	-6.01424	100.7	15196.2	14587.7	-709.4	100.7	34.09641
128	2	Unknown 4	-6.01424	101.5	15309.04	14700.5	-822.3	101.5	34.34959
129	3	Unknown 4	-6.01424	102.5	15460.01	14851.5	-973.2	102.5	34.68833
130	1	Unknown 4	-7.01424	99.6	15036.24	14427.7	-549.5	99.6	33.7375
131	2	Unknown 4	-7.01424	99.6	15038.1	14429.6	-551.3	99.6	33.74167
132	3	Unknown 4	-7.01424	101.2	15272.15	14663.6	-785.4	101.2	34.26682
133	1	Unknown 4	-8.01424	98.3	14846.83	14238.3	-360.1	98.3	33.31251
134	2	Unknown 4	-8.01424	102.5	15459.7	14851.2	-972.9	102.5	34.68763
135	3	Unknown 4	-8.01424	98.3	14841.87	14233.3	-355.1	98.3	33.30138
136	1	Unknown 4	-9.01424	99.2	14972.38	14363.9	-485.6	99.2	33.59421
137	2	Unknown 4	-9.01424	100.3	15132.34	14523.8	-645.6	100.3	33.95312
138	3	Unknown 4	-9.01424	99.6	15034.69	14426.2	-547.9	99.6	33.73402
139	1	Unknown 4	-10.01424	101.9	15364.53	14756.0	-877.8	101.9	34.4741
140	2	Unknown 4	-10.01424	101.1	15250.14	14641.6	-763.4	101.1	34.21743
141	3	Unknown 4	-10.01424	103.9	15654.69	15046.2	-1167.9	103.9	35.12514

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

Check the 10% rule:	33.87%	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"
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	4165.80	12913.98	TRUE	
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	4275.70	13254.67	TRUE	
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	4241.10	13147.41	TRUE	
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	4691.70	14544.27	TRUE	
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	4704.50	14583.95	TRUE	
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	4778.50	14813.35	TRUE	
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	4821.30	14946.03	TRUE	
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	4802.20	14886.82	TRUE	
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	4887.30	15150.63	TRUE	
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	4908.30	15215.73	TRUE	
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	4911.90	15226.89	TRUE	
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	4826.30	14961.53	TRUE	
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4772.60	14795.06	TRUE	
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4897.70	15182.87	TRUE	
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4797.60	14872.56	TRUE	
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	4893.80	15170.78	TRUE	
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	4702.60	14578.06	TRUE	
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	4822.80	14950.68	TRUE	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	4768.50	14782.35	TRUE	
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	4972.90	15415.99	TRUE	
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	4782.00	14824.20	TRUE	
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	4757.80	14749.18	TRUE	
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	4719.70	14631.07	TRUE	
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	4907.00	15211.70	TRUE	
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4790.80	14851.48	TRUE	
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4961.90	15381.89	TRUE	
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4886.50	15148.15	TRUE	
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	200.11	600.33	TRUE	
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	190.85	572.55	TRUE	
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	202.48	607.44	TRUE	
172	1	none	Hot		**—**	—	—	30	—	—	—	—		42607.00	42607.00	TRUE	
173	2	none	Hot		**—**	—	—	30	—	—	—	—		45054.00	45054.00	TRUE	
174	3	none	Hot		**—**	—	—	30	—	—	—	—		44749.00	44749.00	TRUE	
175	1	none	Hot		**—**	—	—	30	—	—	—	—		45434.00	45434.00	TRUE	
176	2	none	Hot		**—**	—	—	30	—	—	—	—		45385.00	45385.00	TRUE	
177	3	none	Hot		**—**	—	—	30	—	—	—	—		44181.00	44181.00	TRUE	

Position	Replicate		Values for analysis by nonlinear regression: concentration (log)	Values for analysis by nonlinear regression: percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
142	1	Unknown 5	-3.01424	84.9	12913.98	12305.5	1572.8	84.9	28.97569
143	2	Unknown 5	-3.01424	87.3	13254.67	12646.1	1232.1	87.3	29.74011
144	3	Unknown 5	-3.01424	86.6	13147.41	12538.9	1339.4	86.6	29.49944
145	1	Unknown 5	-4.01424	96.2	14544.27	13935.7	-57.5	96.2	32.63364
146	2	Unknown 5	-4.01424	96.5	14583.95	13975.4	-97.2	96.5	32.72267
147	3	Unknown 5	-4.01424	98.1	14813.35	14204.8	-326.6	98.1	33.23739
148	1	Unknown 5	-5.01424	99.0	14946.03	14337.5	-459.3	99.0	33.53509
149	2	Unknown 5	-5.01424	98.6	14886.82	14278.3	-400.0	98.6	33.40224
150	3	Unknown 5	-5.01424	100.4	15150.63	14542.1	-663.9	100.4	33.99416
151	1	Unknown 5	-6.01424	100.8	15215.73	14607.2	-729.0	100.8	34.14023
152	2	Unknown 5	-6.01424	100.9	15226.89	14618.4	-740.1	100.9	34.16527
153	3	Unknown 5	-6.01424	99.1	14961.53	14353.0	-474.8	99.1	33.56987
154	1	Unknown 5	-7.01424	97.9	14795.06	14186.5	-308.3	97.9	33.19635
155	2	Unknown 5	-7.01424	100.6	15182.87	14574.3	-696.1	100.6	34.0665
156	3	Unknown 5	-7.01424	98.5	14872.56	14264.0	-385.8	98.5	33.37024
157	1	Unknown 5	-8.01424	100.5	15170.78	14562.3	-684.0	100.5	34.03937
158	2	Unknown 5	-8.01424	96.4	14578.06	13969.5	-91.3	96.4	32.70946
159	3	Unknown 5	-8.01424	99.0	14950.68	14342.2	-463.9	99.0	33.54552
160	1	Unknown 5	-9.01424	97.8	14782.35	14173.8	-295.6	97.8	33.16783
161	2	Unknown 5	-9.01424	102.2	15415.99	14807.5	-929.2	102.2	34.58956
162	3	Unknown 5	-9.01424	98.1	14824.2	14215.7	-337.4	98.1	33.26173
163	1	Unknown 5	-10.01424	97.6	14749.18	14140.7	-262.4	97.6	33.09341
164	2	Unknown 5	-10.01424	96.8	14631.07	14022.5	-144.3	96.8	32.8284
165	3	Unknown 5	-10.01424	100.8	15211.7	14603.2	-724.9	100.8	34.13118
166	1		—	98.3	14851.48	14243.0	-364.7	98.3	33.32294
167	2		—	102.0	15381.89	14773.4	-895.1	102.0	34.51305
168	3		—	100.4	15148.15	14539.6	-661.4	100.4	33.98859
169	1		-6.00	-0.1	600.33	-8.2	13886.4	-0.1	1.346988
170	2		-6.00	-0.2	572.55	-36.0	13914.2	-0.2	1.284657
171	3		-6.00	0.0	607.44	-1.1	13879.3	0.0	1.362941
172	1				42607	41998.5			
173	2				45054	44445.5			
174	3				44749	44140.5			
175	1				45434	44825.5			
176	2				45385	44776.5			
177	3				44181	43572.5			

Prism data

standard curve				weak positive				CR42403				CR42406			
concentration (log)	Y1-SC	Y2-SC	Y3-SC	concentration (log)	y1-PC	y2-PC	y3-PC	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-6.0	0.02958	-0.21499	0.49780	-3	3.9197	5.9460	4.8984	-3.0	0.9840	1.5656	0.8275	-3.0	-2.5058	-1.9040	-1.8589
-6.0	-0.05657	-0.24833	-0.00749	-4	26.5579	28.6391	27.8583	-4.0	0.1212	-1.1955	-0.2743	-4.0	-0.1421	-0.5033	-0.5779
-7.0	2.03057	1.62763	1.61672	-5	74.9310	73.6077	76.6850	-5.0	1.2564	0.0818	0.0762	-5.0	1.4740	1.7134	1.3578
-8.0	14.80717	14.28611	15.27174	-6	90.1145	93.0965	91.7711	-6.0	2.1534	2.8643	2.4973	-6.0	13.1982	13.7942	14.5048
-9.0	64.82994	63.23573	60.53948	-7	93.9973	94.9292	91.8726	-7.0	17.9239	16.4414	16.8247	-7.0	53.1612	55.2433	56.0564
-10.0	94.84181	93.09139	93.23262	-8	92.3655	94.6890	90.9697	-8.0	62.0374	61.8919	61.6030	-8.0	87.2431	89.6291	86.6803
-11.0	97.72423	100.39695	98.53953	-9	96.4823	90.8206	92.0052	-9.0	92.6121	92.7897	89.4129	-9.0	94.4053	96.1643	96.4296
				-10	92.3820	84.7841	92.5146	-10.0	97.3155	95.6250	95.6828	-10.0	99.2457	94.9253	93.8061

CR42407				CR42408				CR42409			
concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1	concentration (log)	y1-U1	y2-U1	y3-U1
-3.0	-1.0913	-1.1166	-1.4825	-3.0	95.8540	95.1200	91.1869	-3.0	84.9427	87.2944	86.5540
-4.0	-0.1087	-0.3002	-0.3257	-4.0	95.6528	96.5131	94.0415	-4.0	96.1964	96.4703	98.0538
-5.0	2.6926	3.4194	2.5602	-5.0	96.3996	96.8490	94.9937	-5.0	98.9696	98.5609	100.3820
-6.0	20.8275	22.5758	20.8168	-6.0	100.6965	101.4754	102.5176	-6.0	100.8313	100.9084	99.0766
-7.0	70.4899	70.9115	71.2881	-7.0	99.5924	99.6052	101.2208	-7.0	97.9275	100.6045	98.4625
-8.0	96.2456	90.8252	94.5037	-8.0	98.2849	102.5154	98.2506	-8.0	100.5211	96.4296	99.0017
-9.0	99.8919	98.3298	98.7000	-9.0	99.1515	100.2557	99.5817	-9.0	97.8398	102.2137	98.1287
-10.0	98.5695	98.1158	100.6559	-10.0	101.8585	101.0689	103.8614	-10.0	97.6108	96.7955	100.8035