DRAFT REPORT

Evaluation of the Binding Potential of Test Articles to Androgen Receptor in Rat Ventral Prostate Cytosol (WA 4-11–Task 4)

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EPA Contract Number:	68-W-01-023 (Battelle Prime Contractor)
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This report contains 152 pages, including five appendices.

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Participation

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

Study Director:	Aruna Koganti, Ph.D.
Scientists:	LaShawn Brown, B.Sc.
	Parveen Kaushal, Ph.D.
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	Steven Sorrow, B.Sc.

Study Dates and Data Retention

Study initiation date:	22 September 2005
Date protocol signed by Sponsor:	16 September 2005
Date test articles received:	07 October 2005, 07 November 2005
Experimental start date:	07 November 2005
Experimental end date:	20 December 2005
Study completion date:	

In Vitro Technologies will retain all supporting documentation, including raw data and written records, for a period of up to five years following submission of the final report to Battelle Memorial Institute. At the end of this period, Battelle will be notified to determine whether the data will be transferred, retained, or destroyed.

Statement of Compliance

This study was conducted to the standards of U.S. FDA 21 CFR Part 58 with the exceptions stated below. This study was conducted under my scientific guidance and management.

Exceptions: 1) The computer systems at In Vitro Technologies, Inc. are not validated. 2) The dosing solutions used in the study were not analyzed and the stability has not been determined. However, the stability of the methyltrienolone and dexamethasone in ethanol were evaluated and reported separately by Battelle. 3) The graphing and data analysis to determine B_{max}, K_d, IC_{50}, and RBA were conducted by Battelle and reported to In Vitro Technologies, but a signed report was not provided. However, this information was audited by Battelle QAU and a QA statement is provided in Appendix 5.

Aruna Koganti, Ph.D.		
Study Director	Signature	Date

Quality Assurance Statement

This study was inspected in accordance with In Vitro Technologies 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 In Vitro Technologies Management.

Inspection/Audit Dates:	Study Phase Audited:	Date(s) reported to Study Director and Management:
11 November 2005	Competition assay	11 November 2005
15 November 2005	Competition assay	15 November 2005
22 November 2005	Competition assay	22 November 2005
30 November 2005	Competition assay	01 December 2005
06–08 February 2006	Data and report	09 February 2006

Quality Assurance	Date

Glossary of Abbreviations

AR ..... androgen receptor
B_{max} ..... binding maximum
DMSO ..... dimethyl sulfoxide
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
NSB ..... non-specific binding
RBA ..... relative binding affinity
TEDG + PMSF buffer ..... buffer with Tris, ethylene diamine tetraacetic acid, dithiothreitol, glycerol, and phenylmethylsulfonyl fluoride

Summary

The objective of this study was to evaluate binding potential of 10 test articles to the androgen receptor, by looking at the inhibition of androgen receptor binding of [^{3}H]-R1881. This study was part of a multi-laboratory effort for the evaluation of the androgen receptor-binding assay. In Vitro Technologies prepared rat ventral prostate cytosol and characterized it by conducting saturation and competitive binding receptor assays in a previous study (270-1147-10, WA4-11, Task 3). The cytosol prepared in the previous study was used in the experiments for the current study. This *in vitro* test method involved combining cytosol, substrate, and test articles in a common reaction vessel. The inhibitory effect of the test articles on androgen receptor binding of R1881 was evaluated by measuring the amount of bound [^{3}H]-R1881 (substrate).

The cytosol (Lot #: 0725-06-01) used in these experiments was prepared and characterized at In Vitro Technologies. The protein concentration was determined to be 4.005 mg protein/mL.

A total of nine competitive binding experiments were conducted, with each experiment evaluating the inhibitory potential of R1881, dexamethasone, and five of the 10 test chemicals (CR42400, CR42401, CR42402, CR42403, CR42404, CR42405, CR42406, CR42407, CR42408, and CR42409). The data from three experiments for each set of five chemicals is reported. The concentrations at which 50% of the binding of [^{3}H]-R1881 to AR was inhibited by these chemicals (IC_{50} values), the relative binding affinity (RBA) values of the chemicals relative to R1881, and the inhibitory constant (K_i) values for the chemicals were determined.

The IC_{50} values for R1881 were determined to be 4.51, 5.16, 6.54, 2.10, 2.86, and 1.83 nM. The RBA value for R1881 was set at 100%. The K_i values for R1881 were 2.17, 2.49, 3.15, 1.01, 1.38, and 0.882 nM.

The IC_{50} values for dexamethasone were determined to be 81,600, 109,000, 131,000, 69,400, 71,200, and 69,400 nM. The RBA values for norethynodrel in comparison to R1881 were 0.0055, 0.0048, 0.0050, 0.0030, 0.0040, and 0.0026%. The K_i values for norethynodrel were 39,320, 52,523, 63,124, 33,441, 34,309, and 33,441 nM.

The IC_{50} values for CR42400 were determined to be 2.36, 2.86, and 4.94 nM. The RBA values for CR42400 in comparison to R1881 were 191, 181, and 132%. The K_i values for CR42400 were 1.14, 1.38, and 2.38 nM.

The IC_{50} values for CR42401 were determined to be 19.7, 14.0, and 30.7 nM. The RBA values for CR42401 in comparison to R1881 were 22.9, 36.8, and 21.3%. The K_i values for CR42401 were 9.49, 6.75, and 14.8 nM.

The IC_{50} values for CR42402 were determined to be 454, 413, and 526 nM. The RBA values for CR42402 in comparison to R1881 were 0.993, 1.25, and 1.24%. The K_i values for CR42402 were 219, 199, and 253 nM.

The IC_{50} values for CR42403 were determined to be 43.7, 36.9, and 50.9 nM. The RBA values for CR42403 in comparison to R1881 were 10.3, 14.0, and 12.9%. The K_i values for CR42403 were 21.1, 17.8, and 24.5 nM.

The IC_{50} values for CR42404 were determined to be 130,000, 111,000, and 496,000 nM. The RBA values for CR42404 in comparison to R1881 were 0.0035, 0.0047, and 0.0132%. The K_i values for CR42404 were 62,642, 53,487, and 239,005 nM.

The IC_{50} values for CR42405 were determined to be 11,100, 18,400, and 14,300 nM. The RBA values for CR42405 in comparison to R1881 were 0.0188, 0.0156, and 0.0128%. The K_i values for CR42405 were 5,349, 8,866, and 6,891 nM.

The IC_{50} values for CR42406 were determined to be 212, 167, and 311 nM. The RBA values for CR42406 in comparison to R1881 were 0.988, 1.72, and 0.591%. The K_i values for CR42406 were 102, 80.5, and 150 nM.

The IC_{50} values for CR42407 were determined to be 359, 294, and 469 nM. The RBA values for CR42407 in comparison to R1881 were 0.584, 0.974, and 0.391%. The K_i values for CR42407 were 173, 142, 226 nM.

The IC_{50} values for CR42408 could not be calculated for two of the three replicate experiments, since the inhibition was very low or not observed. The IC_{50} as determined by one experiment was 26,300,000 nM. The RBA value for CR42408 in comparison to R1881 in this experiment was 0.000%. The K_i value for CR42408 in this experiment was 12,673,057 nM.

The IC_{50} values for CR42409 were determined to be 5,580,000, 4,840,000, and 12,800,000 nM. The RBA values for CR42409 in comparison to R1881 were 0.000, 0.000, and 0.000%. The K_i values for CR42409 were 2,688,808, 2,332,228, 6,167,876 nM.

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 and Reference Article Information and Preparation

Battelle provided the following test materials:

- CR42400
- CR42401
- CR42402
- CR42403
- CR42404
- R1881
- CR42405
- CR42406
- CR42407
- CR42408
- CR42409
- Dexamethasone

The numbers listed above starting with "CR" are code numbers assigned by Battelle to unknown test articles, such that In Vitro Technologies is blinded to the test articles to be evaluated in this study. R1881, the reference article identified as Radioinert Methyltrienolone (CAS #965-93-5, Perkin-Elmer, NEN, catalog number NLP 005005MG) was prepared by and supplied by the Chemical Repository, Battelle, Sequim, Washington. Dexamethasone (CAS #50-02-2) (also, identified as weak positive test article) was prepared by and supplied by the Chemical Repository, Battelle, Sequim, Washington.

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

The following were prepared at In Vitro Technologies or were supplied by In Vitro Technologies:

- Buffer with Tris, sodium molybdate, ethylene diamine tetraacetic acid, dithiothreitol, glycerol, and phenylmethylsulfonyl fluoride (Low Salt TEDG-PMSF Buffer) (pH 7.4) (Refer to In Vitro Technologies method B065.A)
- Absolute ethanol (Sigma, lot 07945HD, 02257JD)
- Hydroxylapatite (HAP; lot 83862B)
- Liquid scintillation cocktail (Formula 989, Perkin Elmer; lot 138-050201)
- Rat ventral prostate cytosol (lot 0725-06-01)

R1881 Preparation

Battelle provided the R1881 stock solutions prepared in absolute ethanol. Battelle was responsible for the preparation and analysis of the R1881 stocks.

Fresh dilutions of the R1881 stock solutions were prepared in assay buffer on the day of use. The final concentrations for R1881 were 3.3×10^{-7} (NSB), 10^{-7}, 3.3×10^{-8}, 10^{-8}, 10^{-9}, 10^{-10}, and 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

Battelle provided the dexamethasone stock solutions prepared in absolute ethanol. Battelle was responsible for the preparation and analysis of the dexamethasone stocks.

Fresh dilutions of the dexamethasone stock solutions were prepared in assay buffer on the day of use. The final concentrations for dexamethasone 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.

Test Article Preparation

Battelle provided the test article stock solutions prepared in absolute ethanol. Battelle was responsible for the preparation and analysis of the test article stocks.

Fresh dilutions of the test article stock solutions were prepared in assay buffer on the day of use. The final concentrations for test articles 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.

Substrate Preparation

The substrate solution was prepared from the radiolabeled R1881 received from Battelle and stored at 4 ± 3°C in the original container. The [^{3}H]-R1881 was diluted with TEDG + PMSF buffer 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 assay.

Rat Ventral Prostate Cytosol

Rat ventral prostate cytosol (Lot 0725-06-01) prepared previously at In Vitro Technologies for study 270-1147-10 (WA 4-11, Task 3) was used in this study.

Assays

Protein Assay

The protein concentration of the cytosol as determined during study 270-1147-10 (WA 4-11, Task 3) was used in this study. No additional protein assays were conducted.

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

An androgen receptor competitive binding assay measured the binding of a single concentration of [^{3}H]-R1881 in the presence of increasing concentrations of a test article. The experimental setup and details of the competitive binding assay are specified in the In Vitro Technologies biological method B065.A.

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

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

- Vehicle or ethanol control (substrate, buffer, vehicle [used for preparation of test article 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) are run at the beginning and three at the end of each replicate set.

Description of Data Calculations

In Vitro Technologies supplied all raw data to Battelle in electronic format using Microsoft Excel® spreadsheets and Prism templates (developed and provided by Battelle).

IC_{50} Calculation

Data for the non-radiolabeled R1881 standard curve and each test or reference article 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 article and expressing the value as a percent (e.g., RBA for R1881 = 100%).

K_i Calculation

An estimate of the K_i was calculated using the Cheng-Prusoff equation if the competitive binding curve reflects a pure competition for a single binding site: $K_i = IC_{50}/(1 + (L/K_d))$ where L = radioligand concentration and K_d = equilibrium dissociation constant of radioligand.

Reporting of Ambiguities

Evaluation of test article CR42400–CR42404 (Assay runs 3, 4, and 5):

1. The stock solution of CR42403 was cloudy even after sonication during runs 3, 4, and 5.
2. An error occurred in the preparation of triamcinolone acetonide which resulted in a final concentration about 10% higher than the target in run 3.
3. The 30 µM R1881 stock, instead of the 10 µM stock, was used in preparing R1881 dilutions resulting in R1881 concentrations that were 3-fold higher for run 3. The actual concentrations were calculated and reflected in the spreadsheets.
4. The highest concentration samples for CR42400–CR42404, and the three highest concentrations for CR42402 were observed to be cloudy after adding cytosol in run 4.
5. The three highest concentrations for CR42402 were observed to be cloudy after adding cytosol in run 5.
6. Some of the cytosol splashed out while it was being added to sample 150 in run 5.
7. Samples 13 and 160 received a double shot of ethanol in run 5.
8. A splatter was observed in sample vials 172–173 after adding ethanol in run 5

Evaluation of test article CR42405–CR42409 (Assay runs 6, 8, and 9):

1. The highest concentration samples for all test articles appeared cloudy after addition of the cytosol in run 6, 8, and 9.
2. Sample 80 appeared cloudy after addition of scintillation fluid in run 6.
3. Sample 174 in run 9 got a double shot of cytosol.
4. Samples 67 and 86 spilled a little while capping the scintillations vials.

Criteria for Data Acceptance

Examination of the data from the competitive binding assay runs 1, 2, and 3, which evaluated R1881, dexamethasone, and test articles CR42400–CR42404, showed the data for the test articles to be highly variable. The IC_{50} values observed for R1881 and dexamethasone were consistent, but the IC_{50} values observed for the test articles varied by 10 fold or more. In discussing the data with the technician and going over the laboratory procedure, it was realized that the test article stocks that were received and stored at room temperature were put on ice along with some other reagents prior to making dilutions of the stock solutions. This likely caused precipitation of the test chemical in the stocks, resulting in the preparation of varied and incorrect dilutions. Additional assay runs (run 4 and run 5) for test articles CR42400–CR42404, were conducted with the test article stocks and dilutions maintained at room temperature. The data obtained for the test articles in these two runs were consistent. The data obtained in run 3 were also similar to the data obtained in runs 4 and 5. Thus, the data obtained from runs 3, 4, and 5 for test articles CR42400–CR42404 are accepted and reported. Battelle identifications for these runs are run 3 (C2), run 4 (C3), run 5 (C4).

The test articles CR42405–CR42409 were evaluated in runs 6–9. In comparing the data from runs 6, 7, and 8, the data from run 7 appeared very different from runs 6 and 8. The observations made during run 7 indicated that several of the sample tubes did not appear to have the correct amount of HAP, and for some other samples part of the ethanol extract was lost while decanting in to scintillation vials. Therefore, run 7 data were considered not acceptable and the test articles were reevaluated in run 9. The data from runs 6, 8, and 9 are reported. Battelle identifications for these runs are run 6 (C5), run 8 (C7), run 9 (C8).

Results

The cytosol (Lot 0725-06-01) used in these experiments was prepared and characterized at In Vitro Technologies. The protein concentration was determined to be 4.005 mg protein/mL.

A total of nine competitive binding experiments were conducted, with each experiment evaluating the inhibitory potential of R1881, dexamethasone, and five of the 10 test chemicals (CR42400, CR42401, CR42402, CR42403, CR42404, CR42405, CR42406, CR42407, CR42408, and CR42409). The data from three experiments for each set of five chemicals is reported. The concentrations at which 50% of the binding of [^{3}H]-R1881 to AR was inhibited by

these chemicals (IC_{50} values), the relative binding affinity (RBA) values of the chemicals relative to R1881, and the inhibitory constant (K_i) values for the chemicals were determined.

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The IC_{50} values for CR42400 were determined to be 2.36, 2.86, and 4.94 nM. The RBA values for CR42400 in comparison to R1881 were 191, 181, and 132%. The K_i values for CR42400 were 1.14, 1.38, and 2.38 nM.

The IC_{50} values for CR42401 were determined to be 19.7, 14.0, and 30.7 nM. The RBA values for CR42401 in comparison to R1881 were 22.9, 36.8, and 21.3%. The K_i values for CR42401 were 9.49, 6.75, and 14.8 nM.

The IC_{50} values for CR42402 were determined to be 454, 413, and 526 nM. The RBA values for CR42402 in comparison to R1881 were 0.993, 1.25, and 1.24%. The K_i values for CR42402 were 219, 199, and 253 nM.

The IC_{50} values for CR42403 were determined to be 43.7, 36.9, and 50.9 nM. The RBA values for CR42403 in comparison to R1881 were 10.3, 14.0, and 12.9%. The K_i values for CR42403 were 21.1, 17.8, and 24.5 nM.

The IC_{50} values for CR42404 were determined to be 130,000, 111,000, and 496,000 nM. The RBA values for CR42404 in comparison to R1881 were 0.0035, 0.0047, and 0.0132%. The K_i values for CR42404 were 62,642, 53,487, and 239,005 nM.

The IC_{50} values for CR42405 were determined to be 11,100, 18,400, and 14,300 nM. The RBA values for CR42405 in comparison to R1881 were 0.0188, 0.0156, and 0.0128%. The K_i values for CR42405 were 5,349, 8,866, and 6,891 nM.

The IC_{50} values for CR42406 were determined to be 212, 167, and 311 nM. The RBA values for CR42406 in comparison to R1881 were 0.988, 1.72, and 0.591%. The K_i values for CR42406 were 102, 80.5, and 150 nM.

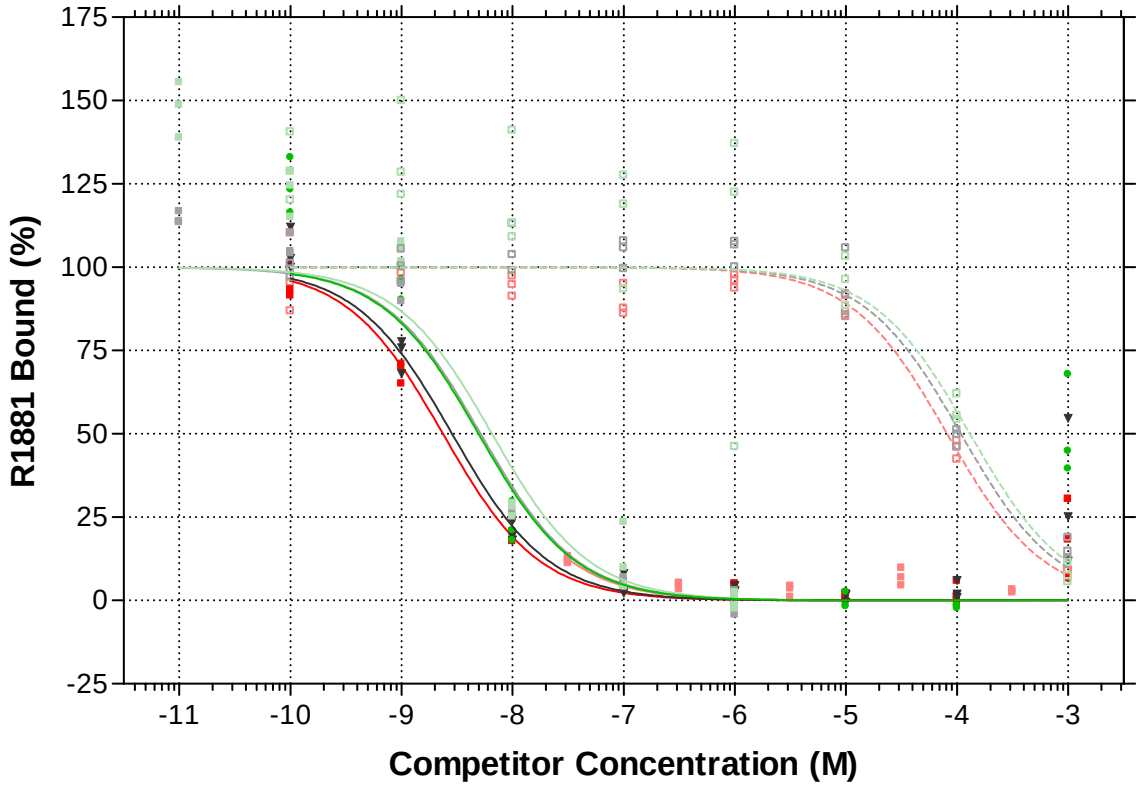
The IC_{50} values for CR42407 were determined to be 359, 294, and 469 nM. The RBA values for CR42407 in comparison to R1881 were 0.584, 0.974, and 0.391%. The K_i values for CR42407 were 173, 142, 226 nM.

The IC_{50} values for CR42408 could not be calculated for two of the three replicate experiments, since the inhibition was very low or not observed. The IC_{50} as determined by one experiment

was 26,300,000 nM. The RBA value for CR42408 in comparison to R1881 in this experiment was 0.000%. The K_i value for CR42408 in this experiment was 12,673,057 nM.

The IC_{50} values for CR42409 were determined to be 5,580,000, 4,840,000, and 12,800,000 nM. The RBA values for CR42409 in comparison to R1881 were 0.000, 0.000, and 0.000%. The K_i values for CR42409 were 2,688,808, 2,332,228, 6,167,876 nM.

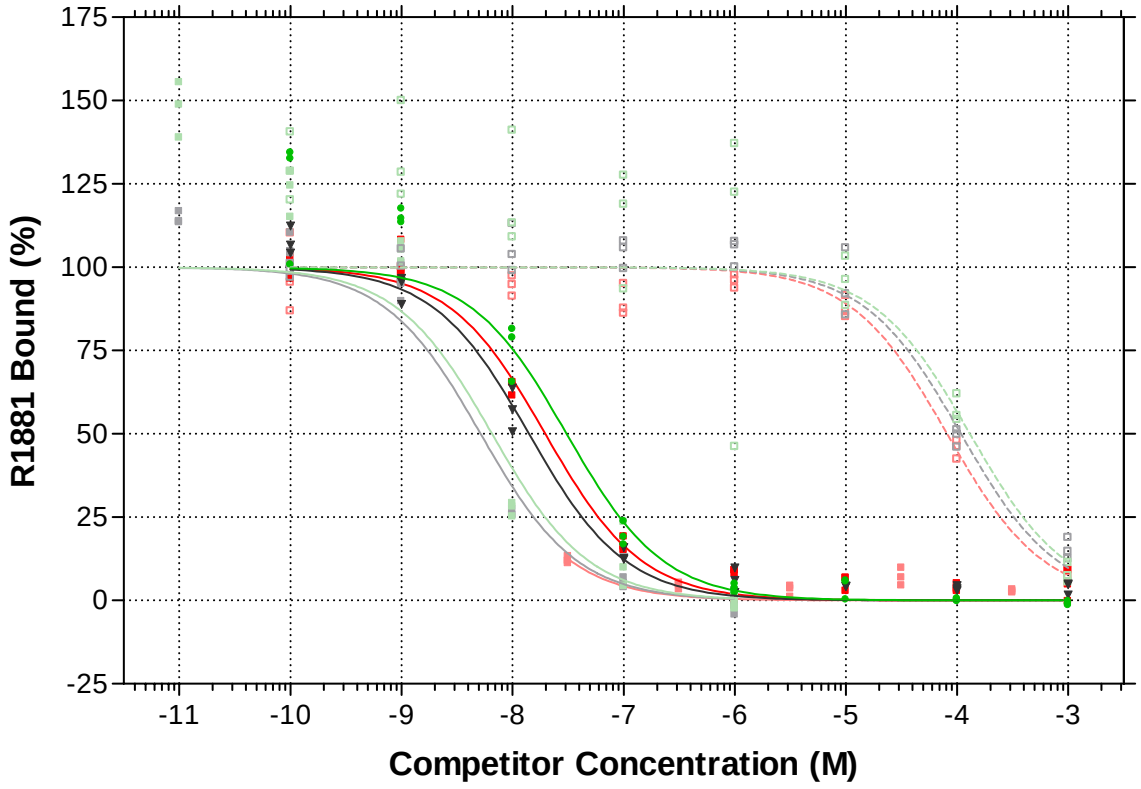
Figure 1: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42400 to the Androgen Receptor



- C2-11/15/05
- C3-11/28/05
- C4-11/29/05

CR42400	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-8.626	0.078
C3-11/28/05	0.000	0.000	100.000	0.000	-8.544	0.130
C4-11/29/05	0.000	0.000	100.000	0.000	-8.306	0.208

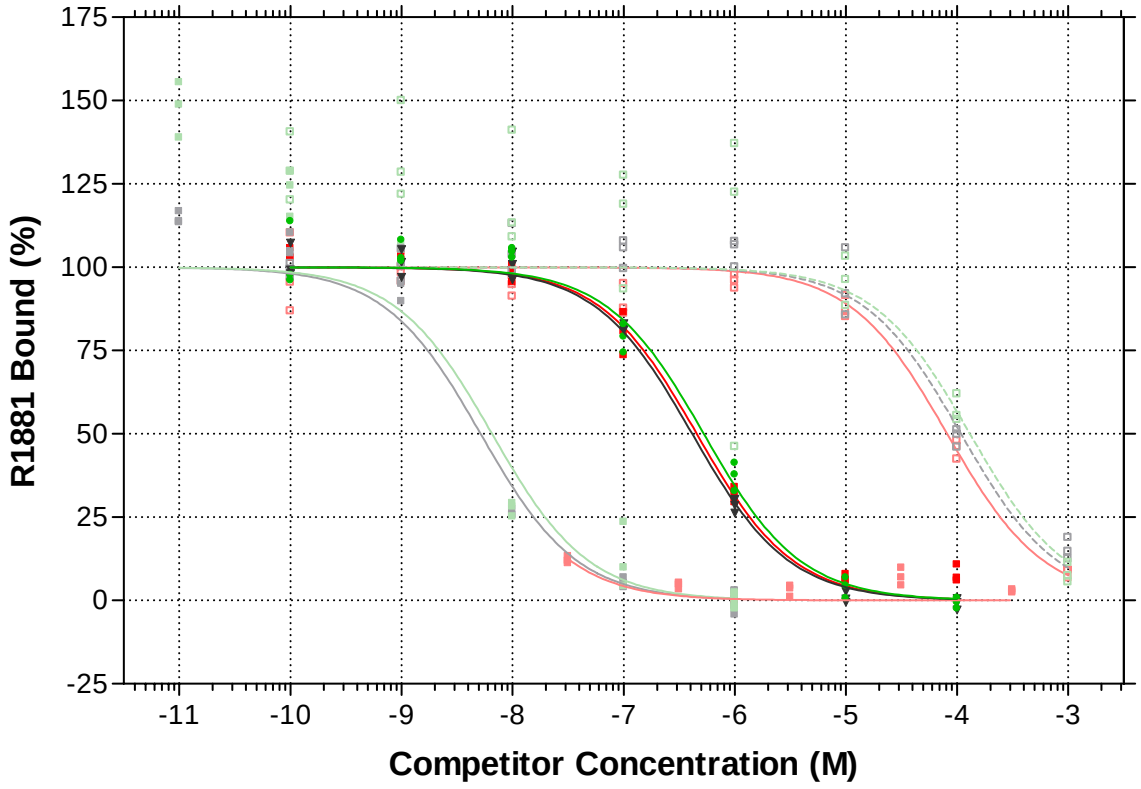
Figure 2: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42401 to the Androgen Receptor



C2-11/15/05
C3-11/28/05
C4-11/29/05

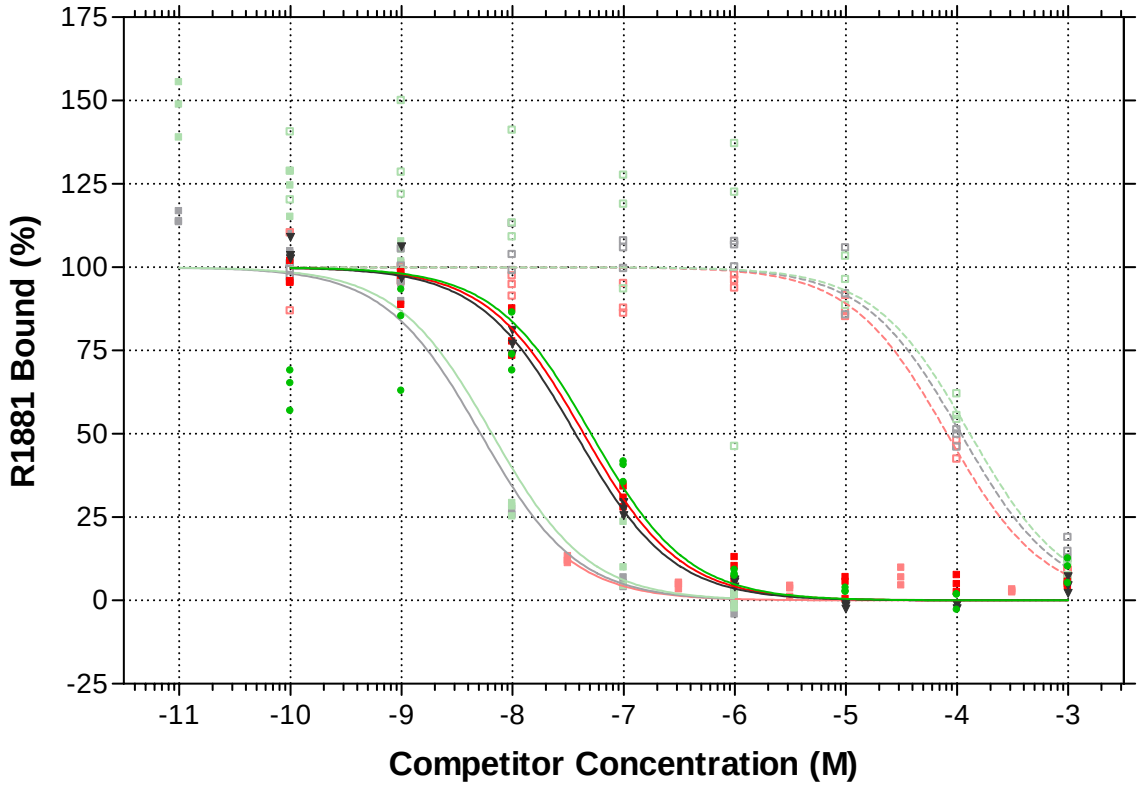
CR42401	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-7.706	0.050
C3-11/28/05	0.000	0.000	100.000	0.000	-7.853	0.048
C4-11/29/05	0.000	0.000	100.000	0.000	-7.513	0.120

Figure 3: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42402 to the Androgen Receptor



CR42402	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-6.343	0.042
C3-11/28/05	0.000	0.000	100.000	0.000	-6.384	0.032
C4-11/29/05	0.000	0.000	100.000	0.000	-6.279	0.052

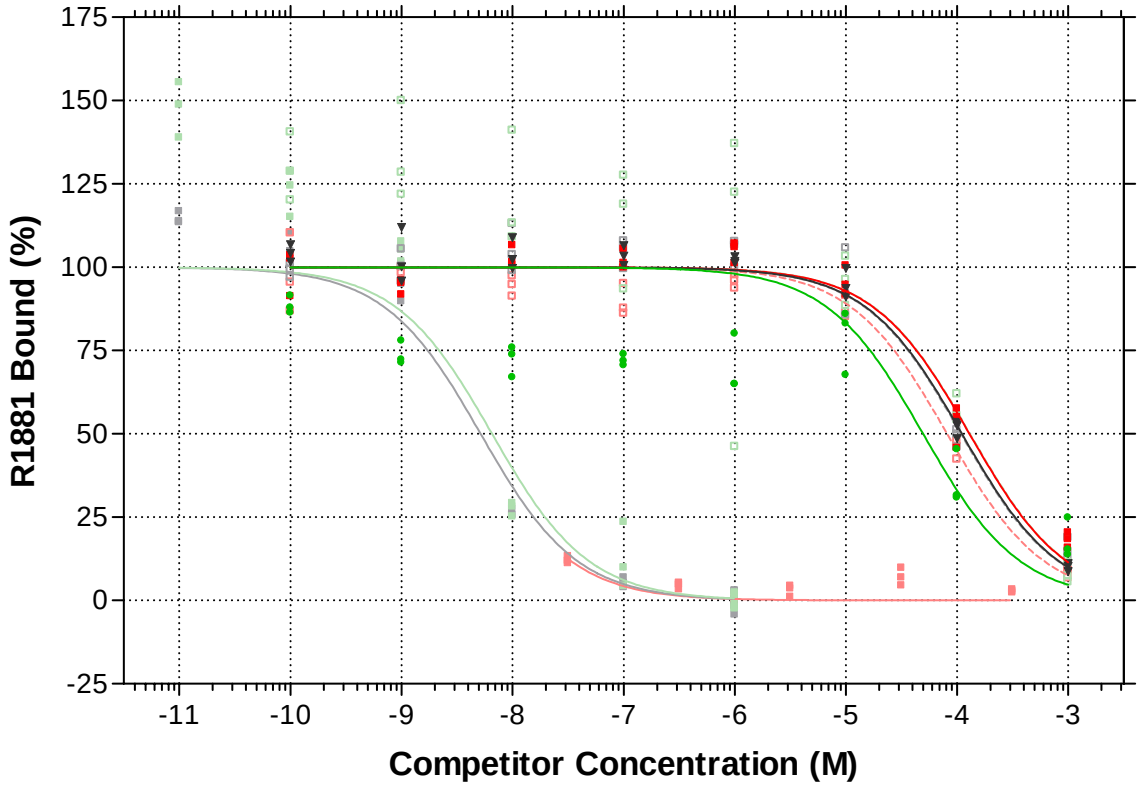
Figure 4: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42403 to the Androgen Receptor



C2-11/15/05
C3-11/28/05
C4-11/29/05

CR42403	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-7.359	0.049
C3-11/28/05	0.000	0.000	100.000	0.000	-7.433	0.036
C4-11/29/05	0.000	0.000	100.000	0.000	-7.293	0.154

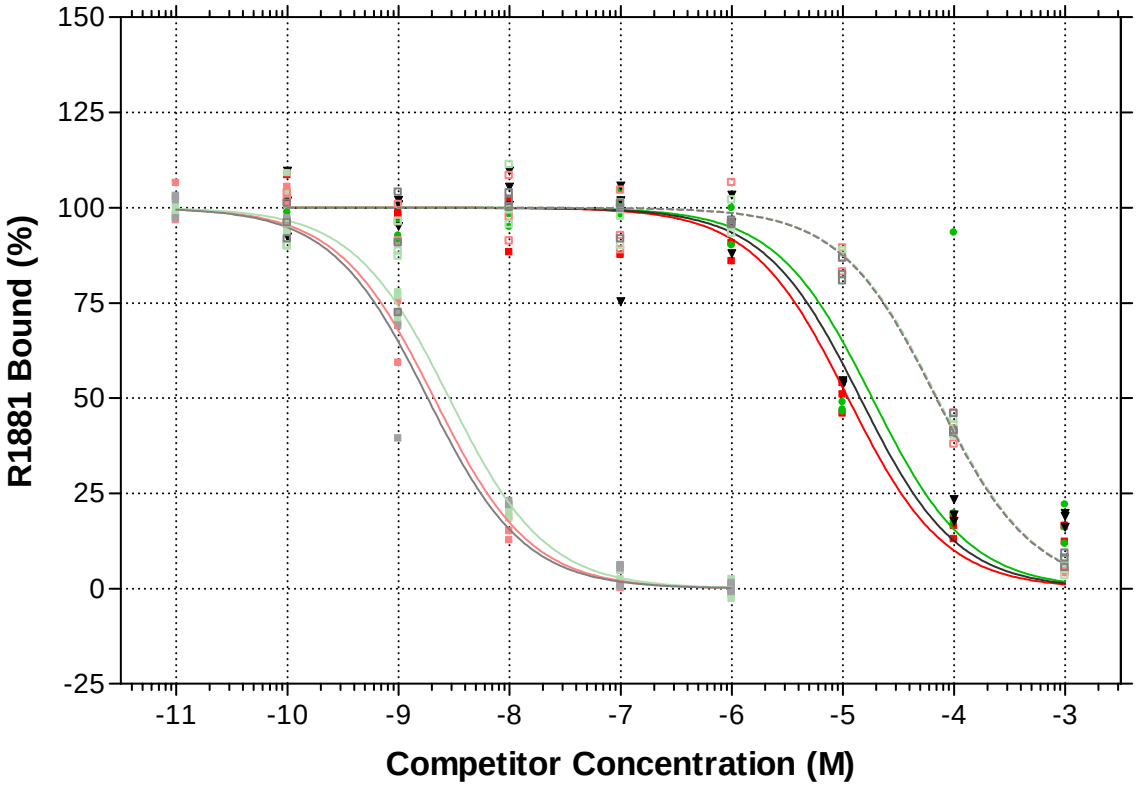
Figure 5: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42404 to the Androgen Receptor



C2-11/15/05
C3-11/28/05
C4-11/29/05

CR42404	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-3.887	0.051
C3-11/28/05	0.000	0.000	100.000	0.000	-3.956	0.041
C4-11/29/05	0.000	0.000	100.000	0.000	-4.305	0.203

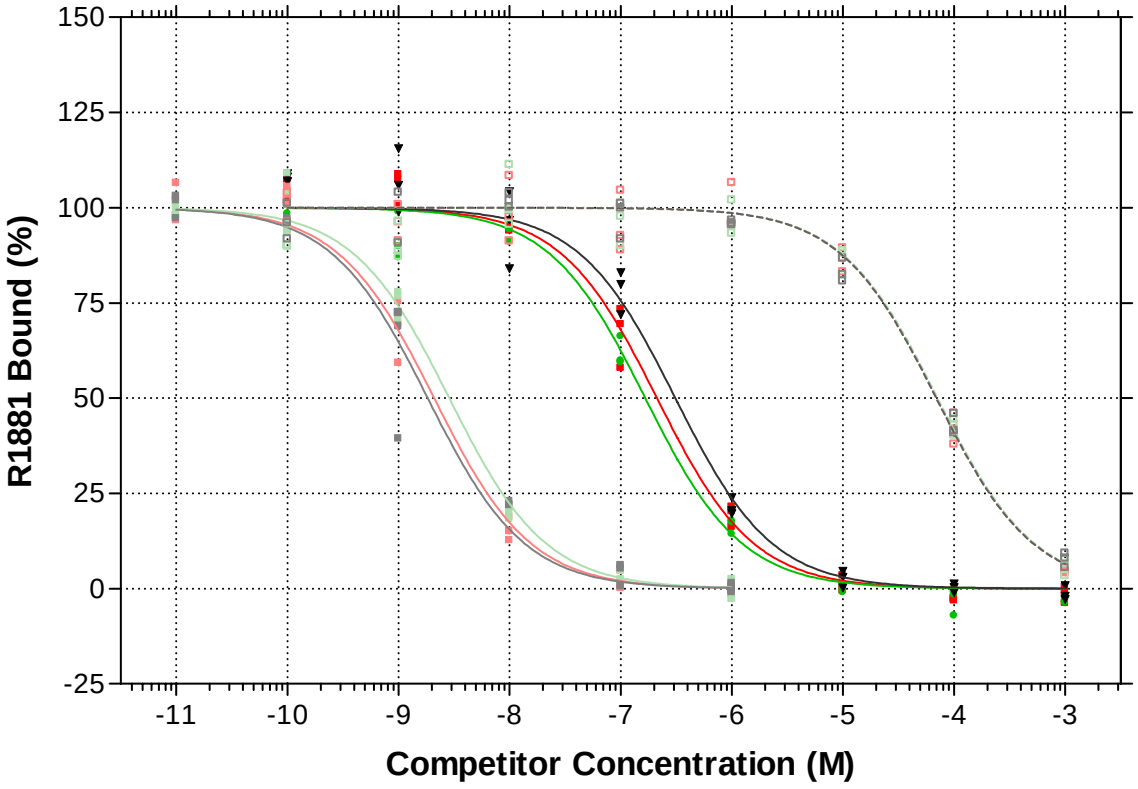
Figure 6: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42405 to the Androgen Receptor



- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42405	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-4.954	0.066
C7-11/22/05	0.000	0.000	100.000	0.000	-4.736	0.174
C8-12/19/05	0.000	0.000	100.000	0.000	-4.844	0.090

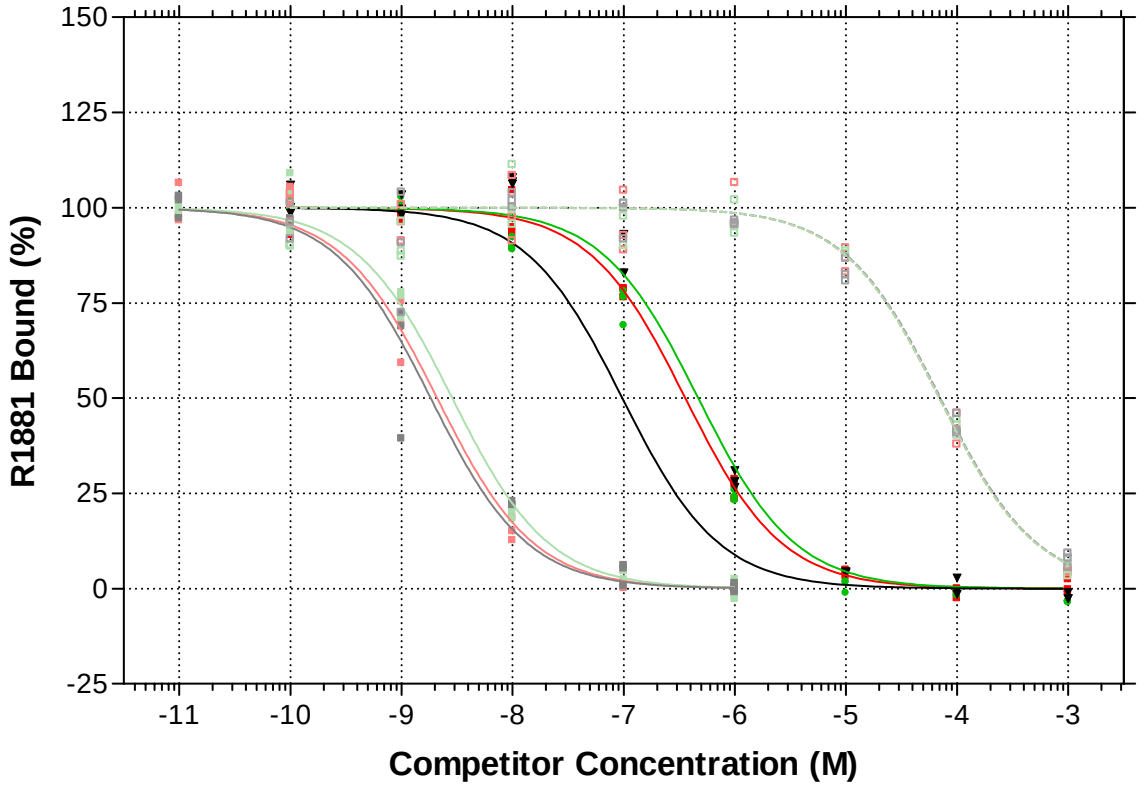
Figure 7: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42406 to the Androgen Receptor



- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42406	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-6.673	0.041
C7-11/22/05	0.000	0.000	100.000	0.000	-6.779	0.041
C8-12/19/05	0.000	0.000	100.000	0.000	-6.508	0.058

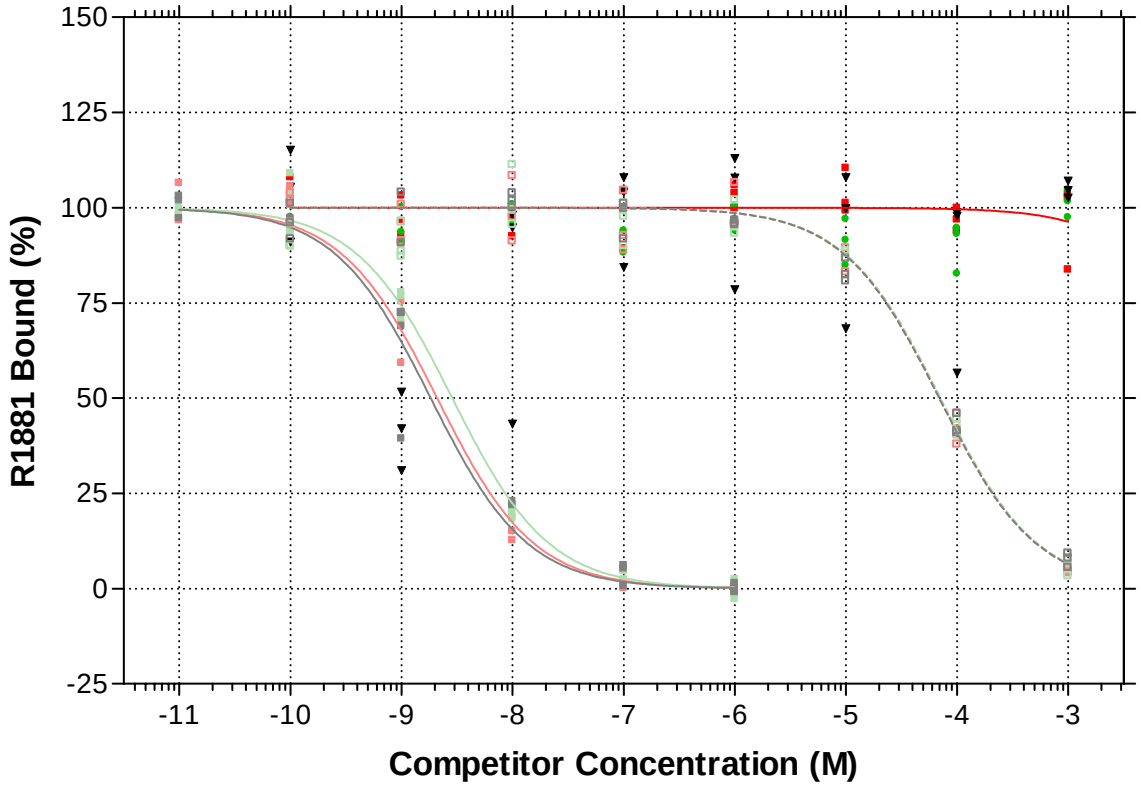
Figure 8: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42407 to the Androgen Receptor



- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42407	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-6.445	0.030
C7-11/22/05	0.000	0.000	100.000	0.000	-6.531	0.033
C8-12/19/05	0.000	0.000	100.000	0.000	-6.329	0.043

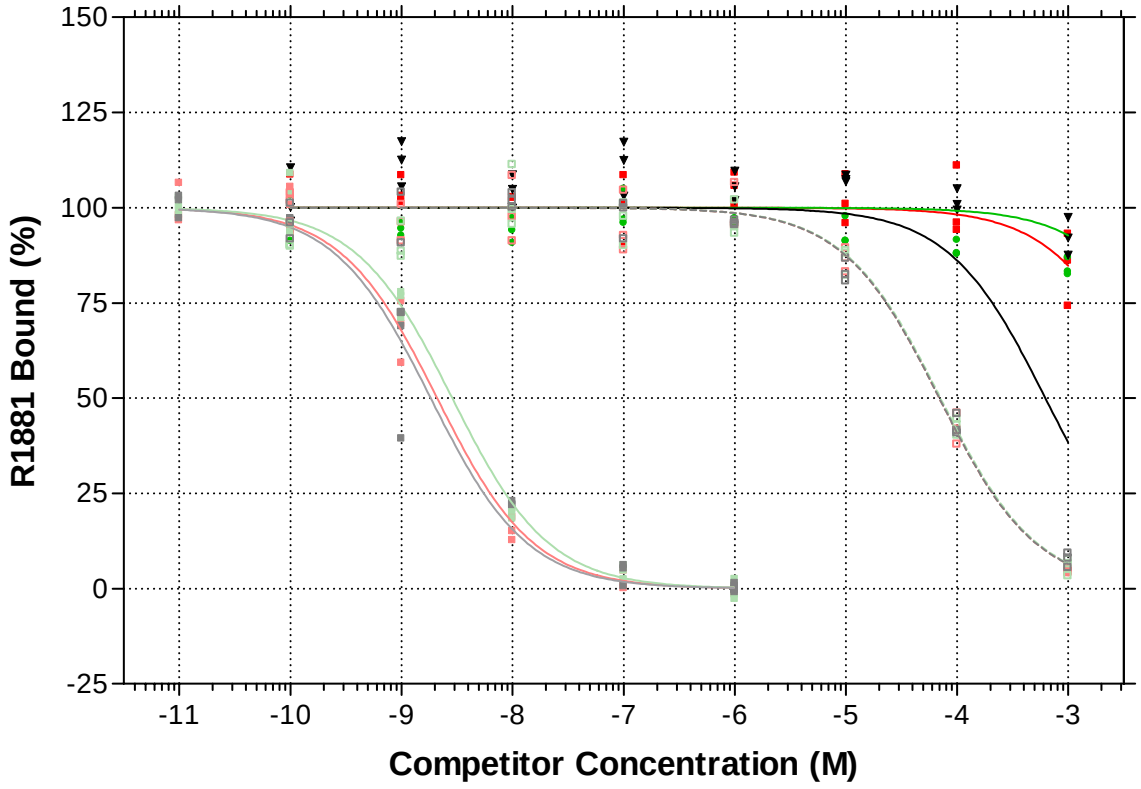
Figure 9: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42408 to the Androgen Receptor



C5-11/16/05
C7-11/22/05
C8-12/19/05

CR42408	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-1.580	0.416
C7-11/22/05						
C8-12/19/05						

Figure 10: Competitive Binding of R1881, Dexamethasone (Weak Positive), and CR42409 to the Androgen Receptor



- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42409	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-2.254	0.128
C7-11/22/05	0.000	0.000	100.000	0.000	-2.315	0.105
C8-12/19/05	0.000	0.000	100.000	0.000	-1.892	0.306

Table 1: Competitive Binding Experiments (CR42400–CR42404)

Run Number	Assay Date	Technician	IC_{50} (nM)	RBA (%)	Ki (nM)
R1881					
3	11/15/2005	LSB/BMF	4.51	100	2.17
4	11/28/2005	LSB/BMF	5.16	100	2.49
5	11/29/2005	LSB/BMF	6.54	100	3.15
Dexamethasone					
3	11/15/2005	LSB/BMF	81600	0.0055	39320
4	11/28/2005	LSB/BMF	109000	0.0048	52523
5	11/29/2005	LSB/BMF	131000	0.0050	63124
CR42400					
3	11/15/2005	LSB/BMF	2.36	191	1.14
4	11/28/2005	LSB/BMF	2.86	181	1.38
5	11/29/2005	LSB/BMF	4.94	132	2.38
CR42401					
3	11/15/2005	LSB/BMF	19.7	22.9	9.49
4	11/28/2005	LSB/BMF	14.0	36.8	6.75
5	11/29/2005	LSB/BMF	30.7	21.3	14.8
CR42402					
3	11/15/2005	LSB/BMF	454	0.993	219
4	11/28/2005	LSB/BMF	413	1.25	199
5	11/29/2005	LSB/BMF	526	1.24	253
CR42403					
3	11/15/2005	LSB/BMF	43.7	10.3	21.1
4	11/28/2005	LSB/BMF	36.9	14.0	17.8
5	11/29/2005	LSB/BMF	50.9	12.9	24.5
CR42404					
3	11/15/2005	LSB/BMF	130,000	0.0035	62,642
4	11/28/2005	LSB/BMF	111,000	0.0047	53,487
5	11/29/2005	LSB/BMF	496,000	0.0132	239,005

Abbreviations: IC_{50}, concentration at which 50% of activity is inhibited; RBA, relative binding affinity; K_i, inhibitory constant

RBA of dexamethasone = (IC_{50} of R1881 / IC_{50} of dexamethasone) × 100

$K_i = IC_{50} /(1 + (L/K_d))$; L = 1 nM; mean K_d = 0.93 nM

Table 2: Competitive Binding Experiments (CR42405–CR42409)

Run Number	Assay Date	Technician	IC_{50} (nM)	RBA (%)	K_i (nM)
R1881					
6	11/16/2005	LSB/BMF	2.10	100	1.01
8	11/22/2005	LSB/BMF	2.86	100	1.38
9	12/19/2005	LSB/BMF	1.83	100	0.882
Dexamethasone					
6	11/16/2005	LSB/BMF	69,400	0.00300	33,441
8	11/22/2005	LSB/BMF	71,200	0.00400	34,309
9	12/19/2005	LSB/BMF	69,400	0.0026	33,441
CR42405					
6	11/16/2005	LSB/BMF	11,100	0.0188	5,349
8	11/22/2005	LSB/BMF	18,400	0.0156	8,866
9	12/19/2005	LSB/BMF	14,300	0.0128	6,891
CR42406					
6	11/16/2005	LSB/BMF	212	0.988	102
8	11/22/2005	LSB/BMF	167	1.72	80.5
9	12/19/2005	LSB/BMF	311	0.591	150
CR42407					
6	11/16/2005	LSB/BMF	359	0.584	173
8	11/22/2005	LSB/BMF	294	0.974	142
9	12/19/2005	LSB/BMF	469	0.391	226
CR42408					
6	11/16/2005	LSB/BMF	26,300,000	0.00	12,673,057
8	11/22/2005	LSB/BMF	n/a	n/a	n/a
9	12/19/2005	LSB/BMF	n/a	n/a	n/a
CR42409					
6	11/16/2005	LSB/BMF	5,580,000	0.00	2,688,808
8	11/22/2005	LSB/BMF	4,840,000	0.00	2,332,228
9	12/19/2005	LSB/BMF	12,800,000	0.00	6,167,876

Abbreviations: IC_{50}, concentration at which 50% of activity is inhibited; RBA, relative binding affinity; K_i, inhibitory constant

RBA of dexamethasone = (IC_{50} of R1881 / IC_{50} of dexamethasone) × 100

$K_i = IC_{50} /(1 + (L/K_d))$; L = 1 nM; mean K_d = 0.93 nM.

Appendix 1: Copy of In Vitro Technologies Protocol No. 1161

In Vitro Technologies, Inc.
Protocol No. 1161
Version: Final (16 September 2005)

Evaluation of the Binding Potential of Test Articles to Androgen Receptor in Rat Ventral Prostate Cytosol (WA 4-11–Task 4)

Sponsor:	Battelle Memorial Institute 505 King Avenue Columbus, OH 43201-2639
Sponsor Representative:	David P. Houchens, Ph.D. E-mail: houchensd@battelle.org Tel.: (614) 424-3564 Fax: (614) 458-3564
Testing Facility:	In Vitro Technologies, Inc. 1450 South Rolling Road Baltimore, Maryland 21227 Tel.: (410) 455-1242 Fax: (410) 455-1245 E-mail: contractservices@invitrotech.com
Study Director:	Aruna Koganti, Ph.D.
EPA Contract Number:	68-W-01-023 (Battelle Prime Contractor)
In Vitro Technologies Study Number:	270-1161-14
Proposed Experimental Start Date:	17 October 2005
Proposed Experimental End Date:	18 November 2005

Objective

The objective of this study is to evaluate the binding potential of 10 test articles to androgen receptor (AR). This study is part of a multi-laboratory effort for the evaluation of the androgen receptor binding assay. This protocol is specific to the study to be conducted at In Vitro Technologies, Inc.

Test / Reference Article Identification

- CR42400
- CR42401
- CR42402
- CR42403
- CR42404
- R1881
- CR42405
- CR42406
- CR42407
- CR42408
- CR42409
- Dexamethasone

The numbers listed above starting with "CR" are code numbers assigned by Battelle to unknown test articles, such that In Vitro Technologies is blinded to the test articles to be evaluated in this study. R1881, the reference article identified as 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. Dexamethasone (CAS # 50-02-2) (also, identified as weak positive test article) will be prepared by and supplied by the Chemical Repository, Battelle, Sequim, Washington.

Test System Identification

The test system for this study is rat ventral prostate cytosol prepared by In Vitro Technologies. The lot or batch number of the cytosol preparation and any other unique identifier assigned by In Vitro Technologies will be recorded in study documentation and will be reported in the study report. Each incubation tube will be labeled with a unique identifier.

Test System Justification

This test system was selected because it provides a biological source of the androgen receptor. Since the assay is being evaluated for its potential to serve as a screening assay, the use of rat tissue enhances its availability.

Description of Study

In Vitro Technologies will evaluate the binding potential of test articles by conducting competitive receptor binding assays, using radiolabeled and inert R1881, dexamethasone, and coded test articles, protocol, and reporting criteria provided by Battelle. This *in vitro* test method

involves combining cytosol, substrate, and test/reference articles in a common reaction vessel. The inhibitory effect of the test articles on androgen receptor binding of R1881 will be evaluated by measuring the amount of bound [^{3}H]-R1881 (substrate). In Vitro Technologies will conduct three separate experiments to evaluate the inhibition of androgen receptor binding of [^{3}H]-R1881 by the test articles.

There is no applicable route of administration since this is an *in vitro* study.

Experimental Methods

Materials

Battelle will provide the following materials:

- [^{3}H]-R1881
- R1881 stock solutions
- Dexamethasone stock solutions
- Test article stock solutions

The following will be prepared at In Vitro Technologies or will be supplied by In Vitro Technologies:

- Buffer with Tris, sodium molybdate, ethylene diamine tetraacetic acid, dithiothreitol, glycerol, and phenylmethylsulfonyl fluoride (low salt TEDG-PMSF buffer; pH 7.4) (Refer to In Vitro Technologies method B065.A)
- Absolute ethanol
- Dimethyl sulfoxide (DMSO)
- Hydroxylapatite (HAP)
- Liquid scintillation cocktail (Formula 989, Perkin Elmer)
- Rat ventral prostate cytosol

The supplier, lot numbers, and purity of the materials received and used in this study will be included in the study report.

R1881 Preparation

Battelle will provide the R1881 stock solutions prepared in absolute ethanol. Battelle will be responsible for the preparation and analysis of the R1881 stocks.

Fresh dilutions of the R1881 stock solutions will be prepared in assay buffer on the day of use. The final target concentrations for R1881 are 3.3×10^{-7} (non-specific binding; NSB), 10^{-7}, 3.3×10^{-8}, 10^{-8}, 10^{-9}, 10^{-10}, and 10^{-11} M. The total volume of solvent used in each assay will be no more than 3.33% of the total assay volume.

Dexamethasone Preparation

Battelle will provide the dexamethasone stock solutions prepared in absolute ethanol. Battelle will be responsible for the preparation and analysis of the dexamethasone stocks.

Fresh dilutions of the dexamethasone stock solutions will be prepared in assay buffer on the day of use. The final target concentrations for dexamethasone are 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 will be no more than 3.33% of the total assay volume.

Test Article Preparation

Battelle will provide the test article stock solutions prepared in absolute ethanol. Battelle will be responsible for the preparation and analysis of the test article stocks.

Fresh dilutions of the test article stock solutions will be prepared in assay buffer on the day of use. The final target concentrations for test articles are 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 will be no more than 3.33% of the total assay volume.

Substrate Preparation

The substrate solution will be prepared from the radiolabeled R1881 received from Battelle and will be stored at 4 ± 3°C in the original container. The [^{3}H]-R1881 will be diluted with TEDG + PMSF buffer to achieve a substrate solution at a concentration of 10 nM. The substrate solution (30 µL) will be added to the incubation mixtures to achieve a final concentration of 1 nM [^{3}H]-R1881 in the assay.

Rat Ventral Prostate Cytosol

Rat ventral prostate cytosol previously prepared at In Vitro Technologies will be used in these studies.

Assays

Protein Assay

The protein concentration of the cytosol preparation will be determined as described in In Vitro Technologies method B020.A. In brief, a six-point standard curve will be prepared, ranging from 0.1 to 1.0 mg protein/mL. The protein standards will be made from bovine serum albumin. Protein will be determined using a BioRad® Protein Assay Kit II (Hercules, CA). To a 10 µL aliquot of standard or unknown, 200 µL of dye reagent will be added and mixed. The samples will be placed at room temperature for at least 5 minutes and up to an hour to allow for color development. The absorbance (600 nm) will be measured using a plate reader. The protein

concentration of the cytosol sample will be determined by extrapolation of the absorbance value using the standard curve developed using the protein standards.

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

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/reference article. The experimental setup and details of the competitive binding assay are specified in the In Vitro Technologies biological method B065.A.

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

Control samples will be included for each replicate experiment. These include:

- Vehicle or ethanol control (substrate, buffer, vehicle [used for preparation of test article solutions], and cytosol)
- Non-specific background control (substrate, buffer, R1881, and cytosol).

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

Description of Data Calculations

In Vitro Technologies will supply all raw data to Battelle in electronic format using Microsoft Excel® spreadsheets and Prism templates (to be developed and provided by Battelle).

IC_{50} Calculation

Data for the non-radiolabeled R1881 standard curve and each test/reference article will be plotted as the percentage of [^{3}H]-R1881 bound versus the molar concentration (log) of competitor. Estimates of IC_{50} values will be determined using appropriate nonlinear curve fitting software to fit a one-site competitive binding model. The model will be 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 is calculated by dividing the IC_{50} value for R1881 by the IC_{50} of the test article and expressing the value as a percent (e.g., RBA for R1881 = 100%).

K_i Calculation

An estimate of the K_i can be calculated using the Cheng-Prusoff equation if the competitive binding curve reflects a pure competition for a single binding site: $K_i = IC_{50}/(1 + (L/K_d))$ where L = radioligand concentration and K_d = equilibrium dissociation constant of radioligand. Alternatively, the K_i can be determined experimentally.

Reporting of Ambiguities

Ambiguities or unclear directions in the written protocol and a list of all problems that are encountered will be reported to Battelle.

Criteria for Data Acceptance

All data obtained will be reported.

Study Report

At completion of Task 4, tabular and graphical summaries of data will be prepared using the Excel spreadsheet and Prism document templates provided by Battelle. These electronic files will be submitted to Battelle within 7 days after completion of the task. Data to be reported will include the following information: assay date and run number, technician, chemical and log chemical concentration, total DPM – background DPM, RBA, IC_{50}, and K_i.

Data Retention

In Vitro Technologies will retain all supporting documentation, including raw data and written records, for a period of up to five years following issuance of the final report. At the end of this period, Battelle will be notified to determine whether the data (excluding proprietary information) will be transferred, retained, or destroyed. Study records to be maintained will include:

- All records that document the conduct of the laboratory experiments and results obtained, as well as the equipment and chemicals used.
- Protocol and any amendments
- List of any protocol deviations
- List of standard operating procedures

Protocol Approval

This protocol has been reviewed and approved by the following:

Sponsor Representatives

David P. Houchens, Ph.D.
Program Manager
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

Signature: David P. Houchens Date: 9/16/05

James E. Morris, Ph.D.
Principal Investigator
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

Signature: James E. Morris Date: 9/20/05

Study Director

The study will be conducted to the standards of U.S. FDA 21 CFR Part 58. The study will be conducted under my scientific guidance and management. I have reviewed the procedures outlined in this protocol.

Aruna Koganti, Ph.D.
Study Director
In Vitro Technologies

Signature: K. Aruna Koganti Date: 22 Sep 2005

Review

Terri L. Pollock, B.A.
Quality Assurance Manager
Battelle Memorial Institute

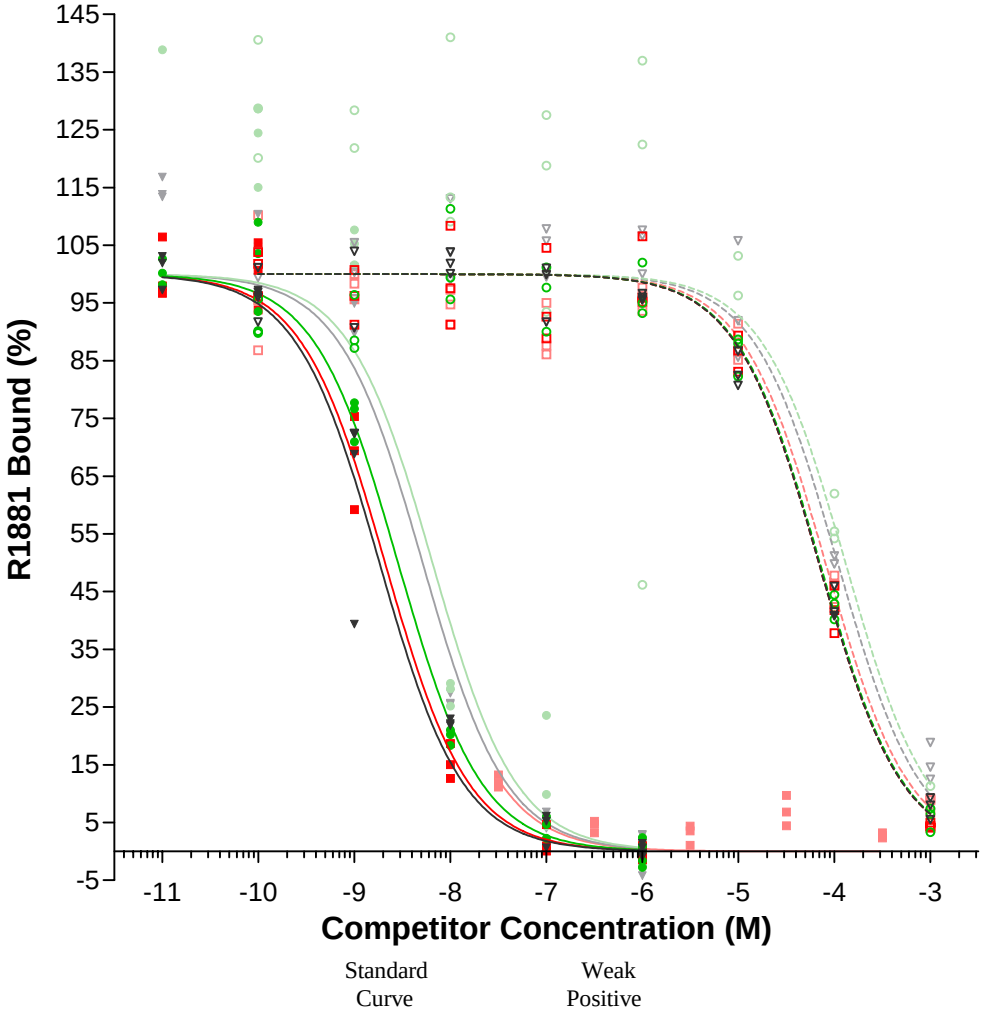
Signature: Terri L Pollock Date: 9-16-05

Sharon Isbell
Director, Quality Systems
In Vitro Technologies

Signature: Sharon Isbell Date: 23 Sept 2005

Appendix 2: Prism Files for Competitive Binding Experiments

Standard Curve and 'Weak Positive' Protein
1.0 mg per tube



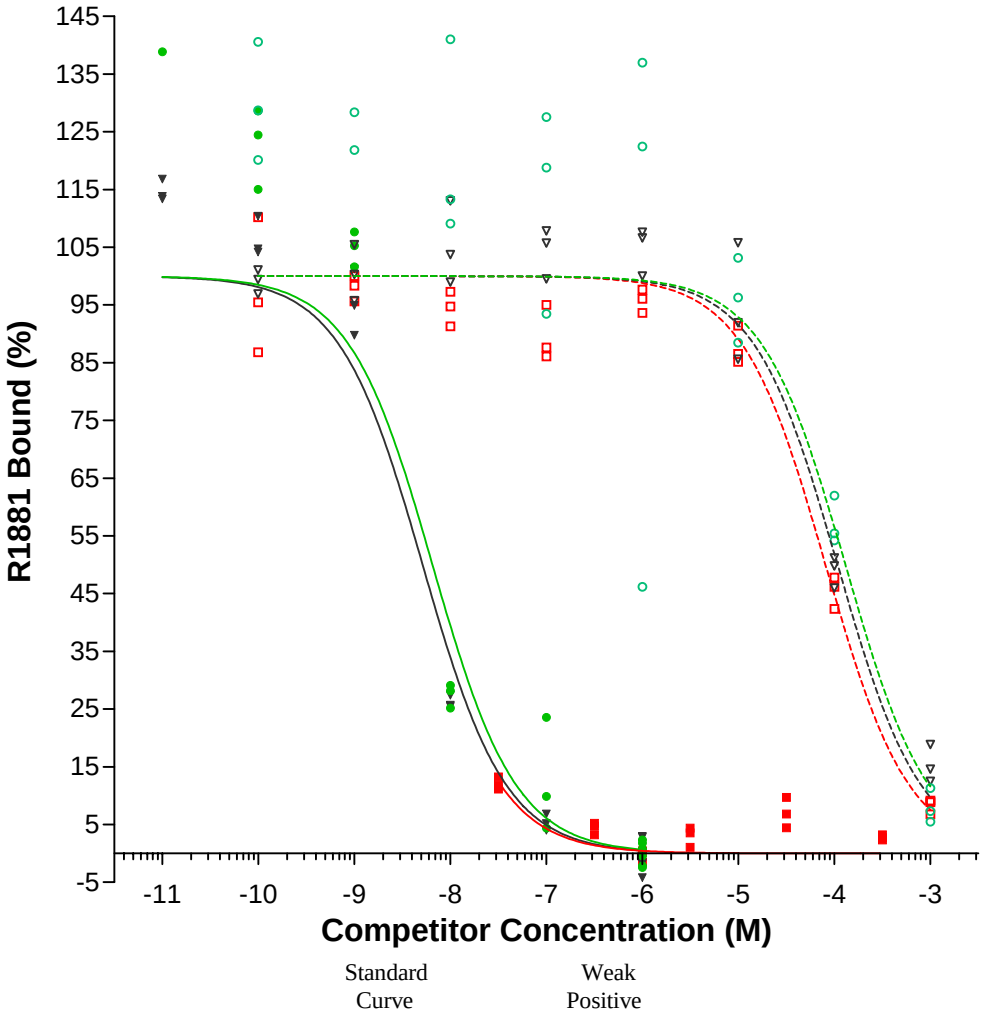
Standard Curve

C2-11/15/05
C3-11/28/05
C4-11/29/05
C5-11/16/05
C7-11/22/05
C8-12/19/05

Weak Positive

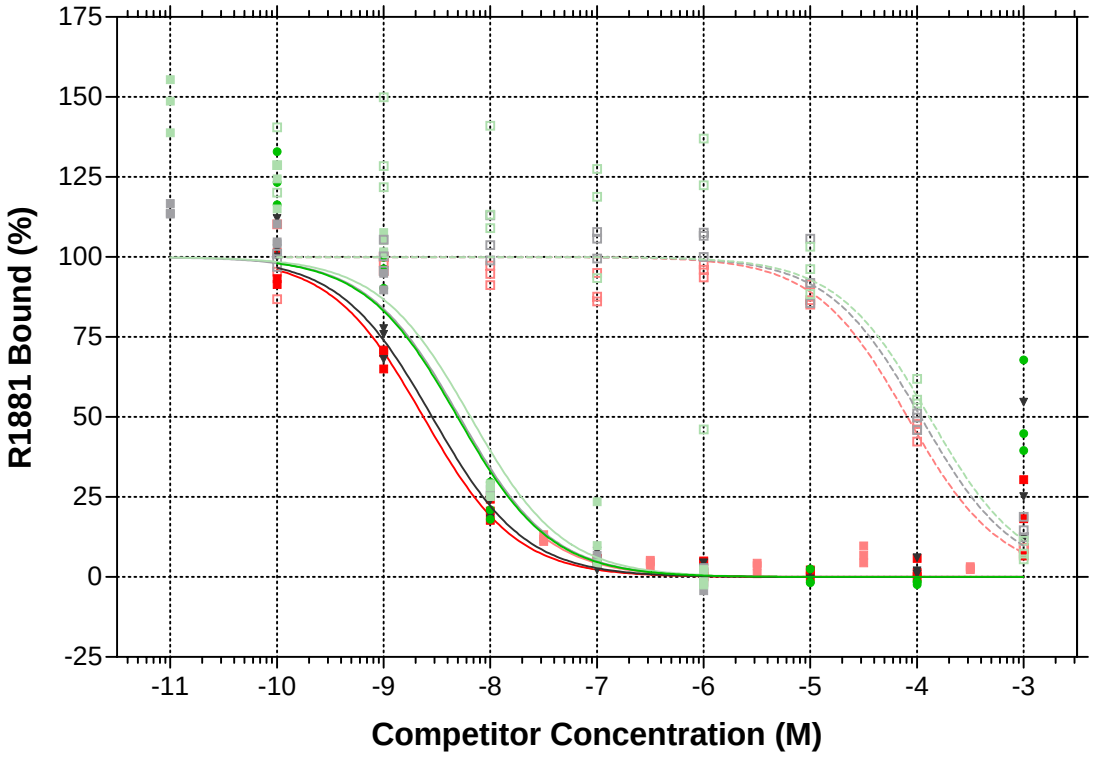
C2-11/15/05
C3-11/28/05
C4-11/29/05
C5-11/16/05
C7-11/22/05
C8-12/19/05

Standard Curve and 'Weak Positive'
Runs C2, C3, and C4
Protein
1.0 mg per tube



WA 4-11 Competitive

Lab C CR42400
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

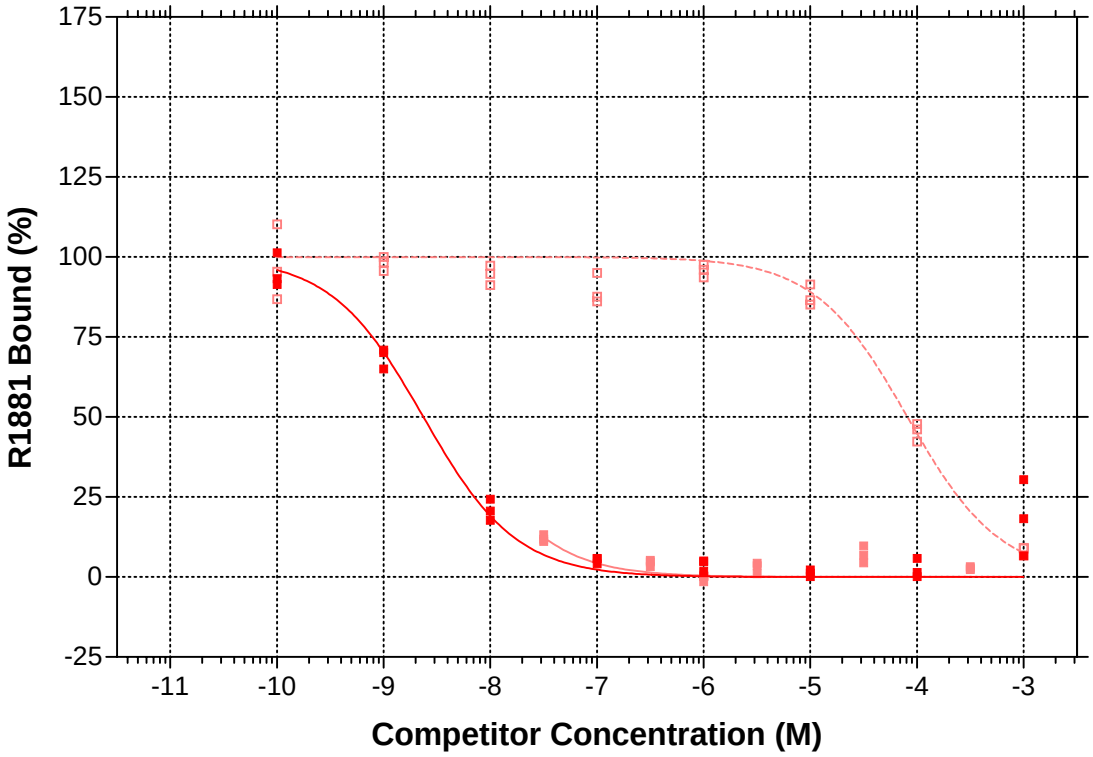


- C2-11/15/05
- C3-11/28/05
- C4-11/29/05

CR42400	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-8.626	0.078
C3-11/28/05	0.000	0.000	100.000	0.000	-8.544	0.130
C4-11/29/05	0.000	0.000	100.000	0.000	-8.306	0.208

WA 4-11 Competitive

Lab C CR42400 Run C2
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

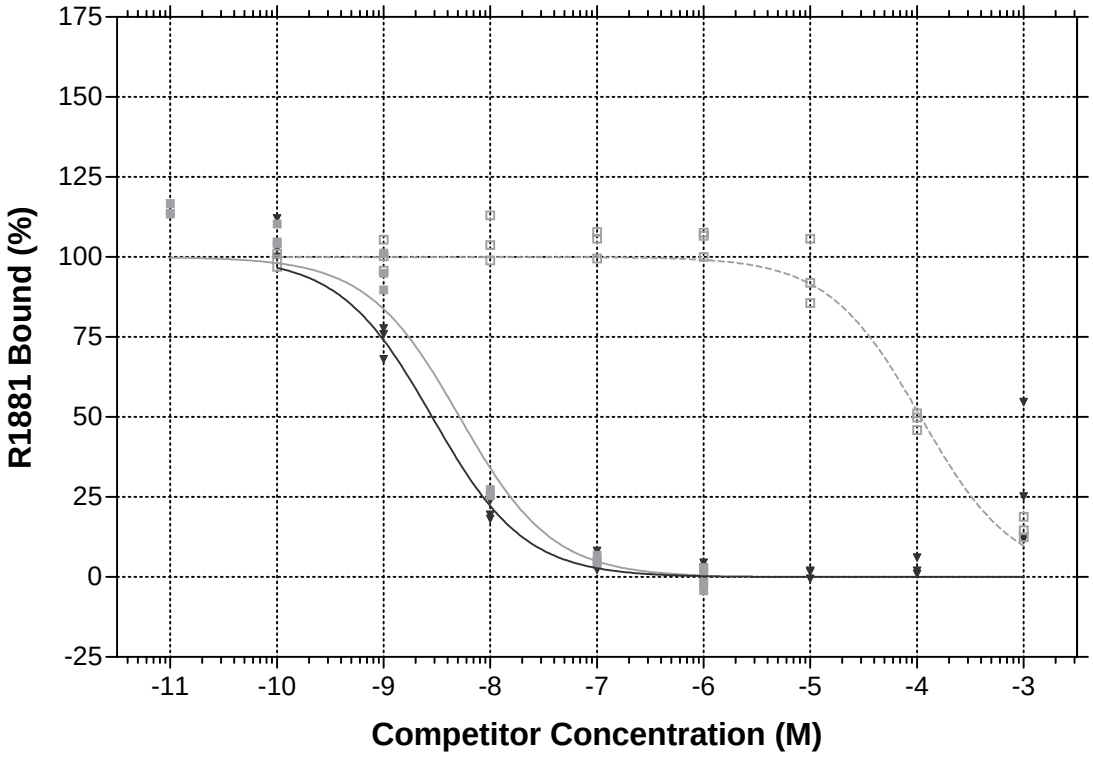


CR42400	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-8.626	0.078

■ C2-11/15/05

WA 4-11 Competitive

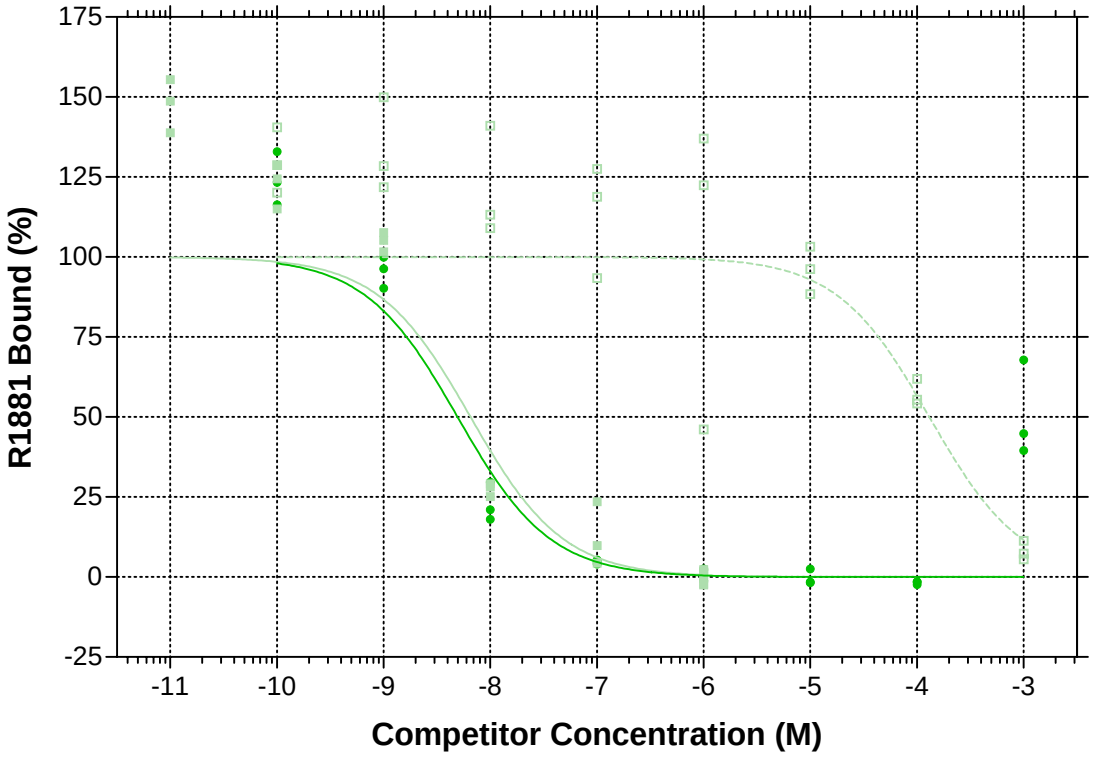
Lab C CR42400 Run C3
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42400	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C3-11/28/05	0.000	0.000	100.000	0.000	-8.544	0.130

WA 4-11 Competitive

Lab C CR42400 Run C4
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

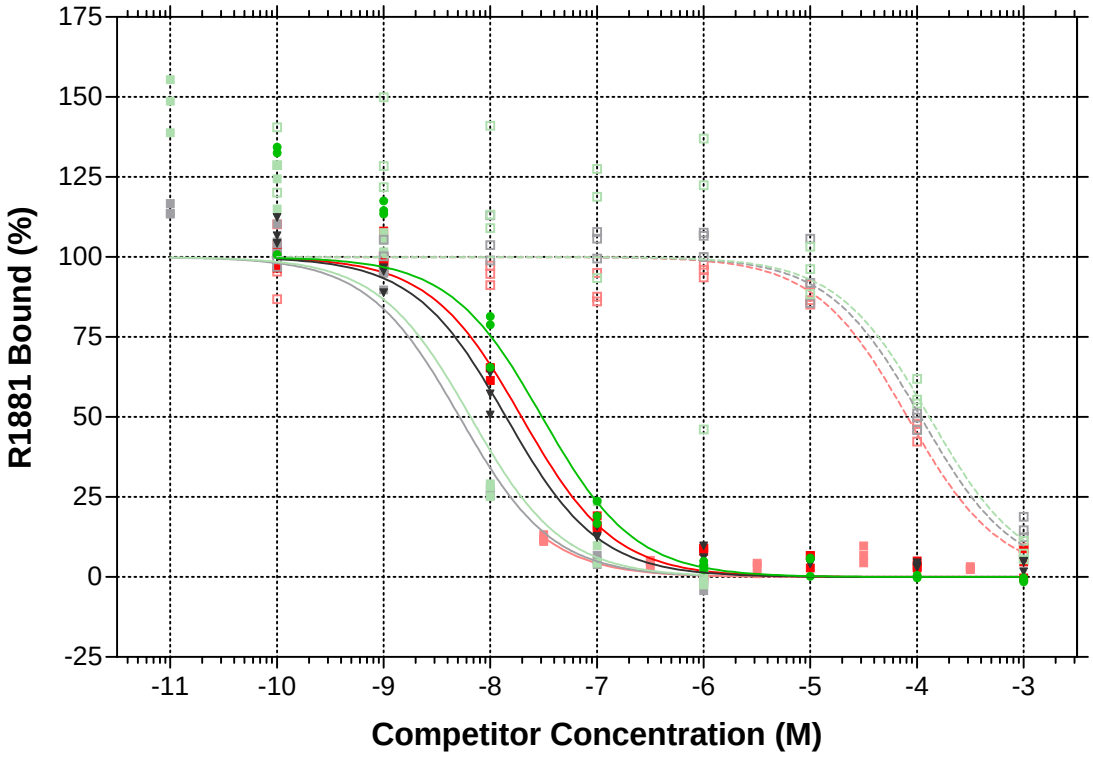


CR42400	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C4-11/29/05	0.000	0.000	100.000	0.000	-8.306	0.208

• C4-11/29/05

WA 4-11 Competitive

Lab C CR42401
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

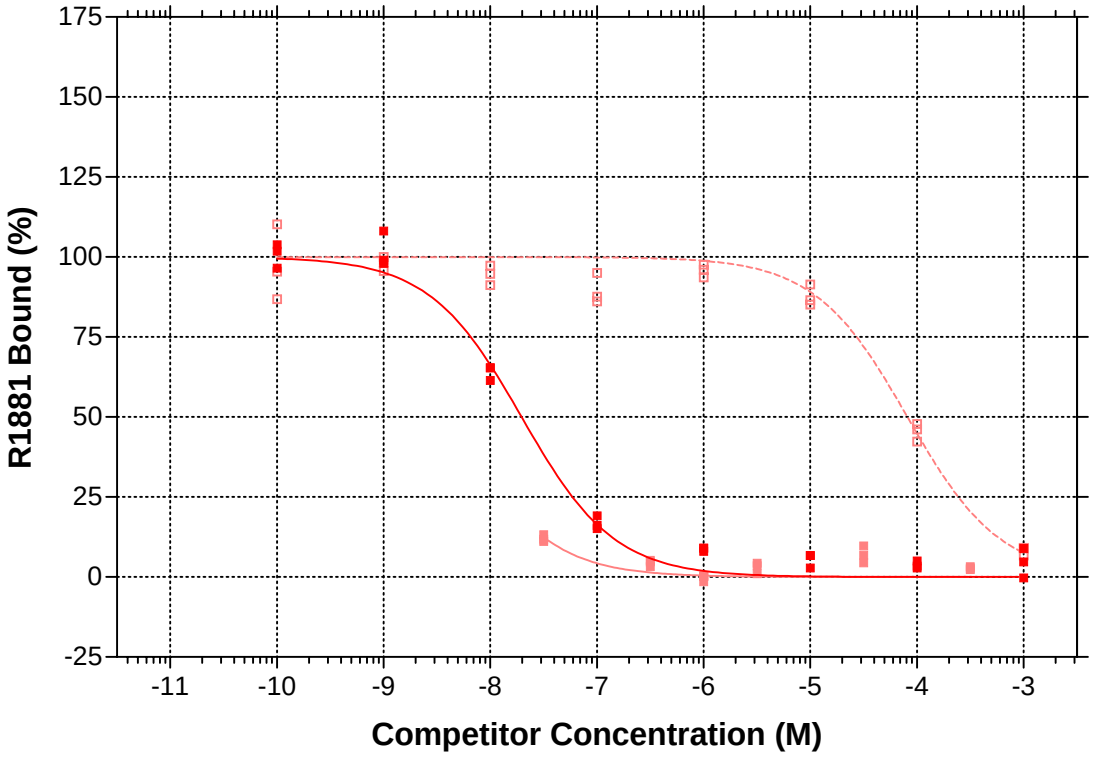


- C2-11/15/05
- C3-11/28/05
- C4-11/29/05

CR42401	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-7.706	0.050
C3-11/28/05	0.000	0.000	100.000	0.000	-7.853	0.048
C4-11/29/05	0.000	0.000	100.000	0.000	-7.513	0.120

WA 4-11 Competitive

Lab C CR42401 Run C2
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

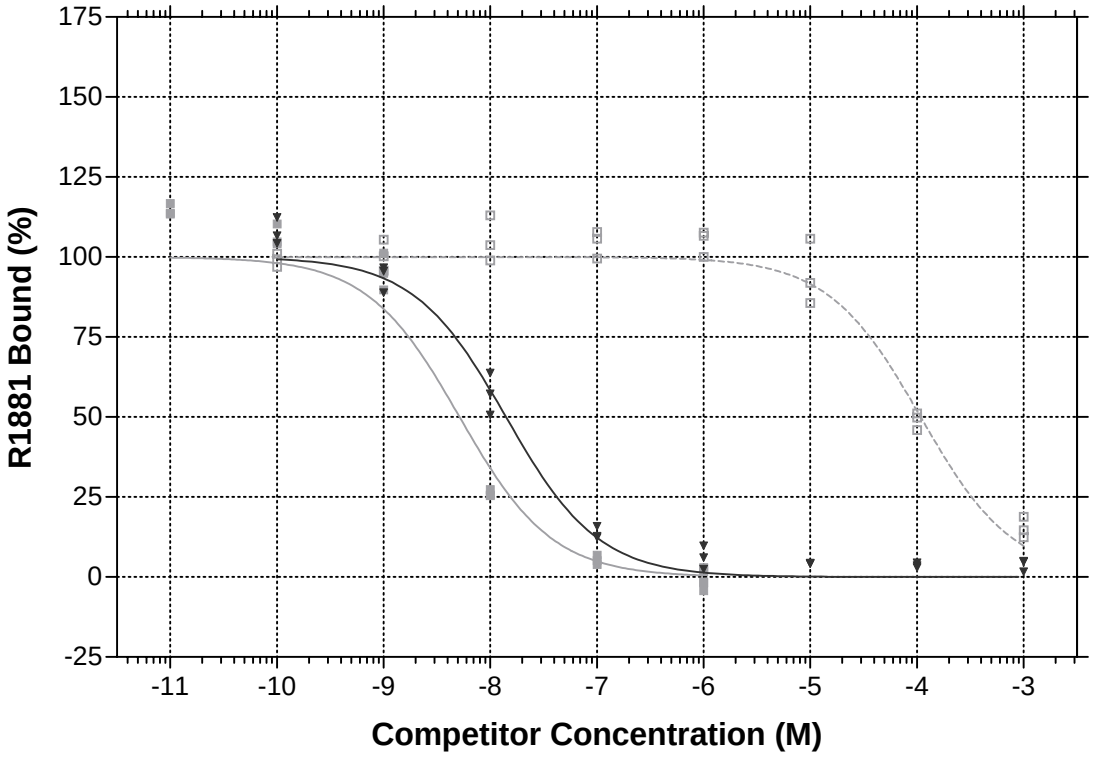


CR42401	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-7.706	0.050

■ C2-11/15/05

WA 4-11 Competitive

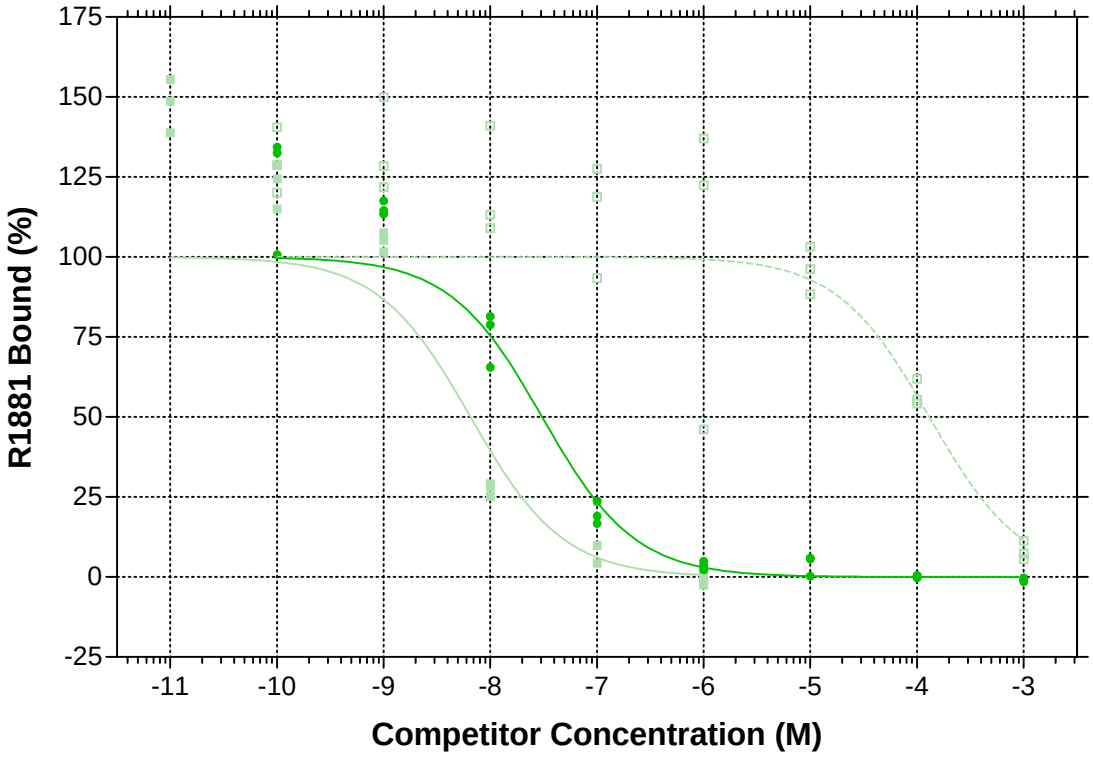
Lab C CR42401 Run C3
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42401	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C3-11/28/05	0.000	0.000	100.000	0.000	-7.853	0.048

WA 4-11 Competitive

Lab C CR42401 Run C4
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

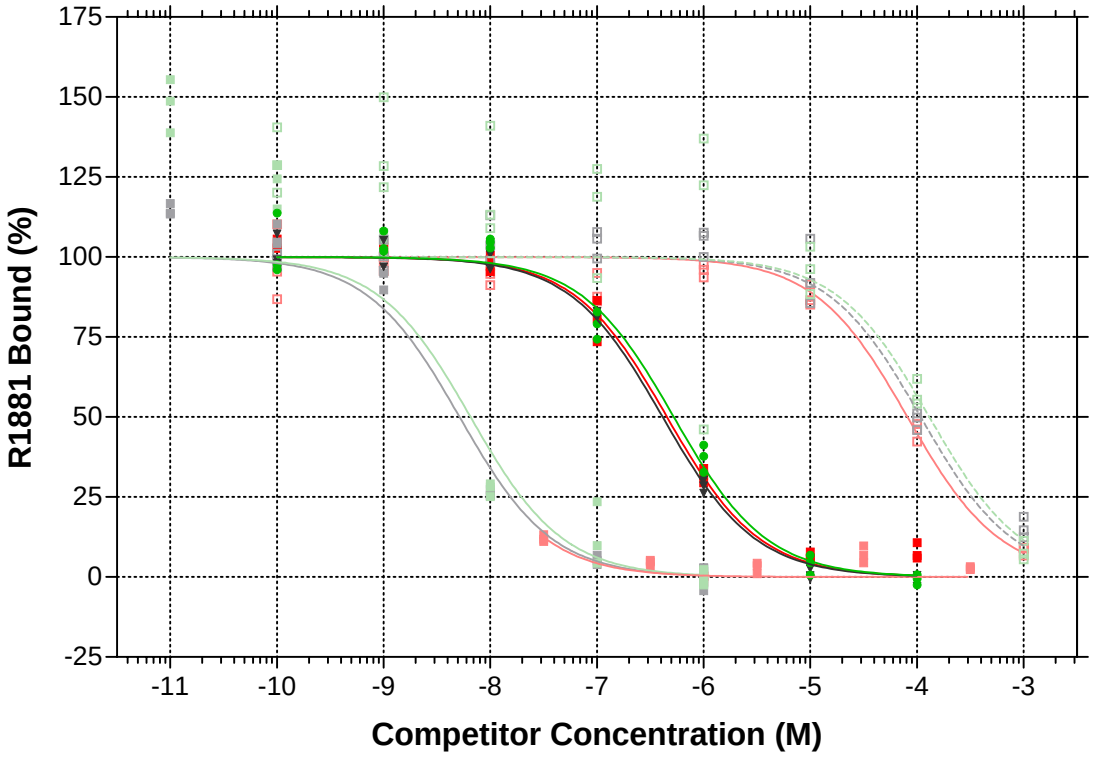


CR42401	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C4-11/29/05	0.000	0.000	100.000	0.000	-7.513	0.120

• C4-11/29/05

WA 4-11 Competitive

Lab C CR42402
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

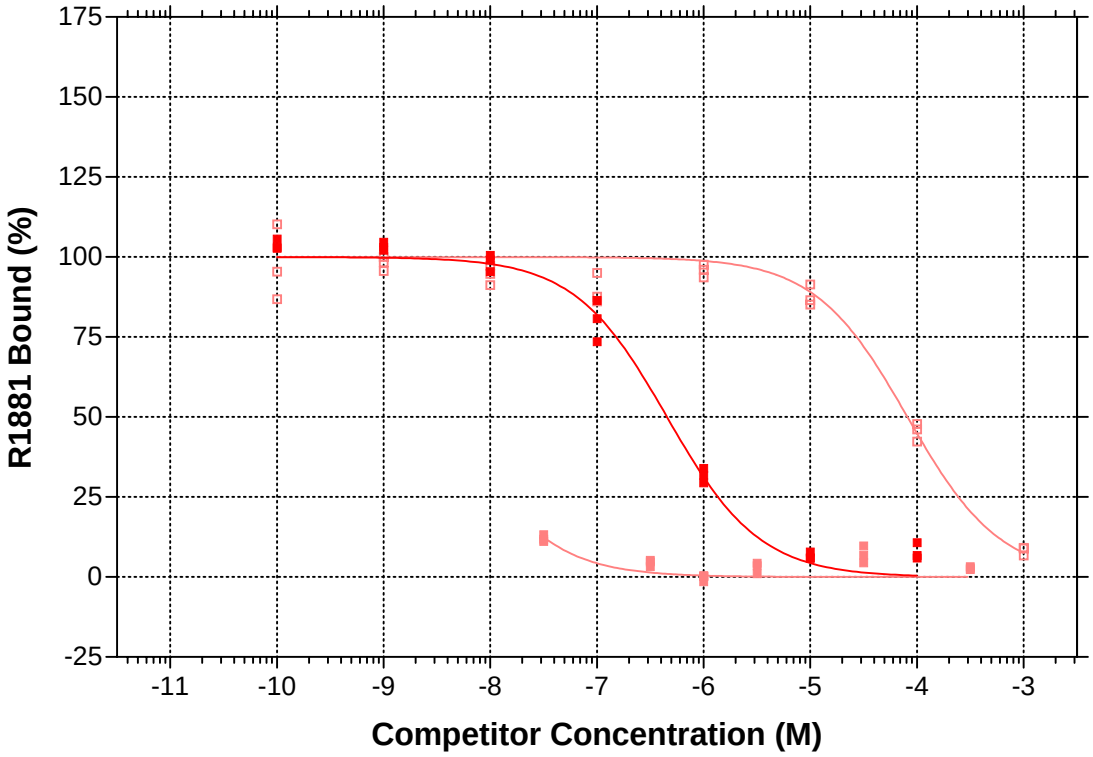


- C2-11/15/05
- C3-11/28/05
- C4-11/29/05

CR42402	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-6.343	0.042
C3-11/28/05	0.000	0.000	100.000	0.000	-6.384	0.032
C4-11/29/05	0.000	0.000	100.000	0.000	-6.279	0.052

WA 4-11 Competitive

Lab C CR42402 Run C2
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

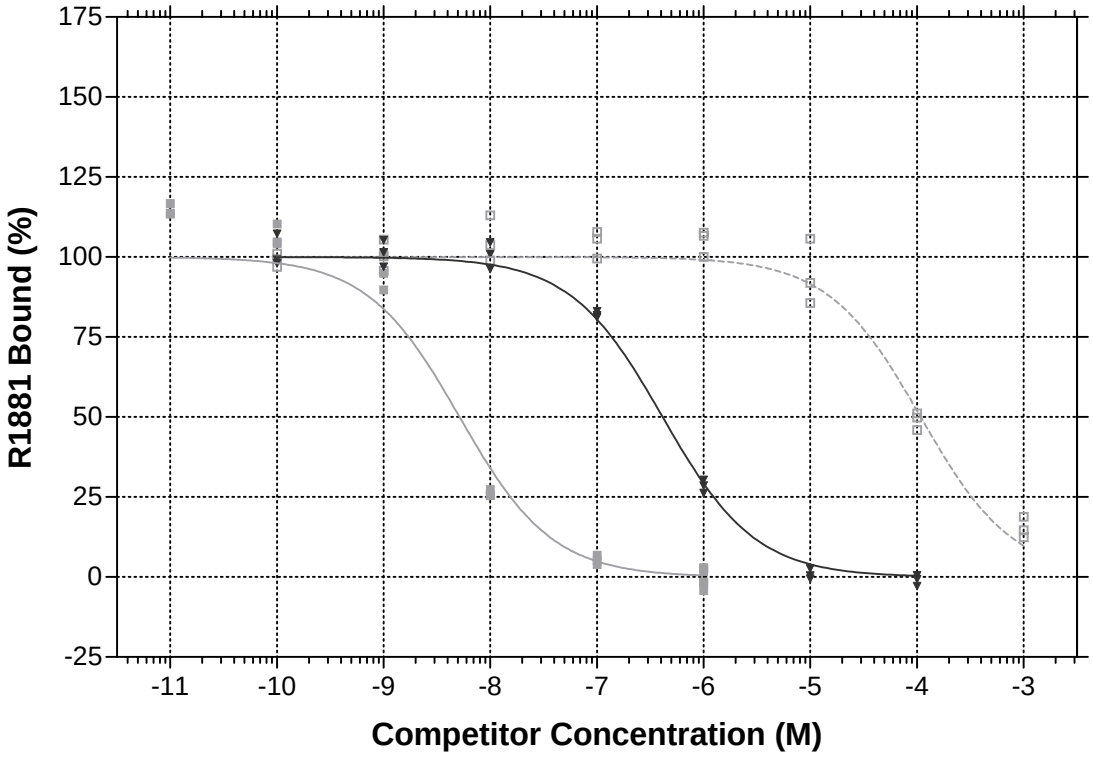


CR42402	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-6.343	0.042

■ C2-11/15/05

WA 4-11 Competitive

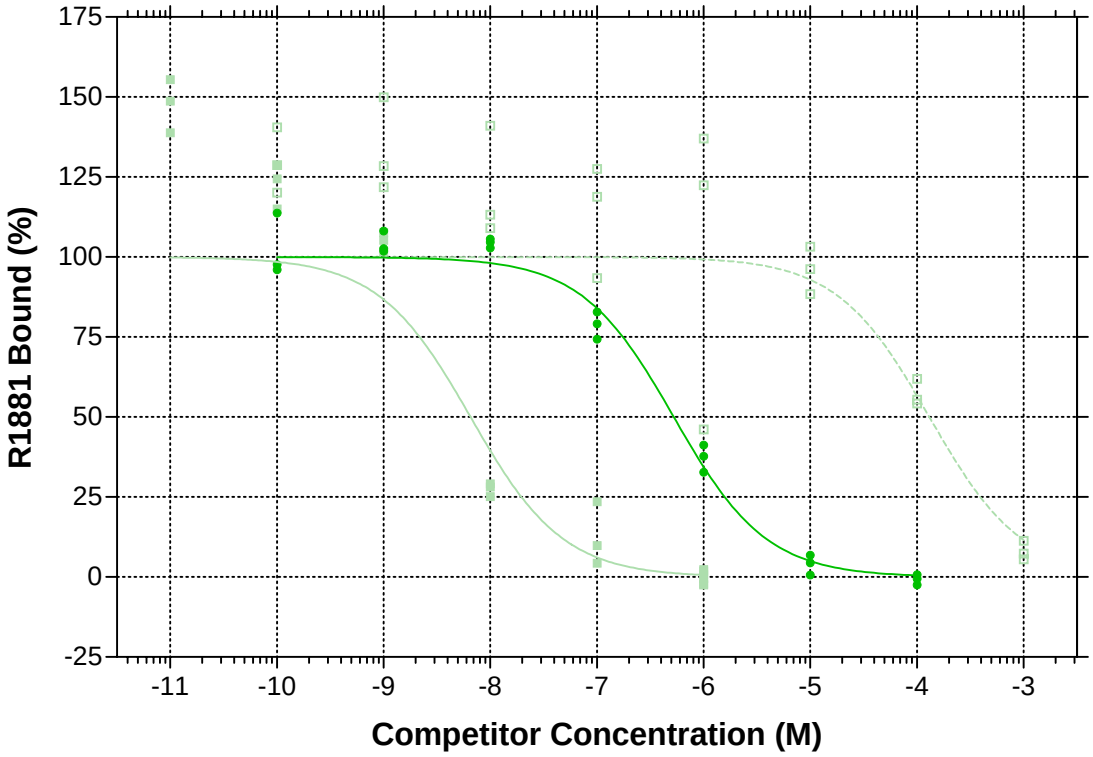
Lab C CR42402 Run C3
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42402	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C3-11/28/05	0.000	0.000	100.000	0.000	-6.384	0.032

WA 4-11 Competitive

Lab C CR42402 Run C4
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

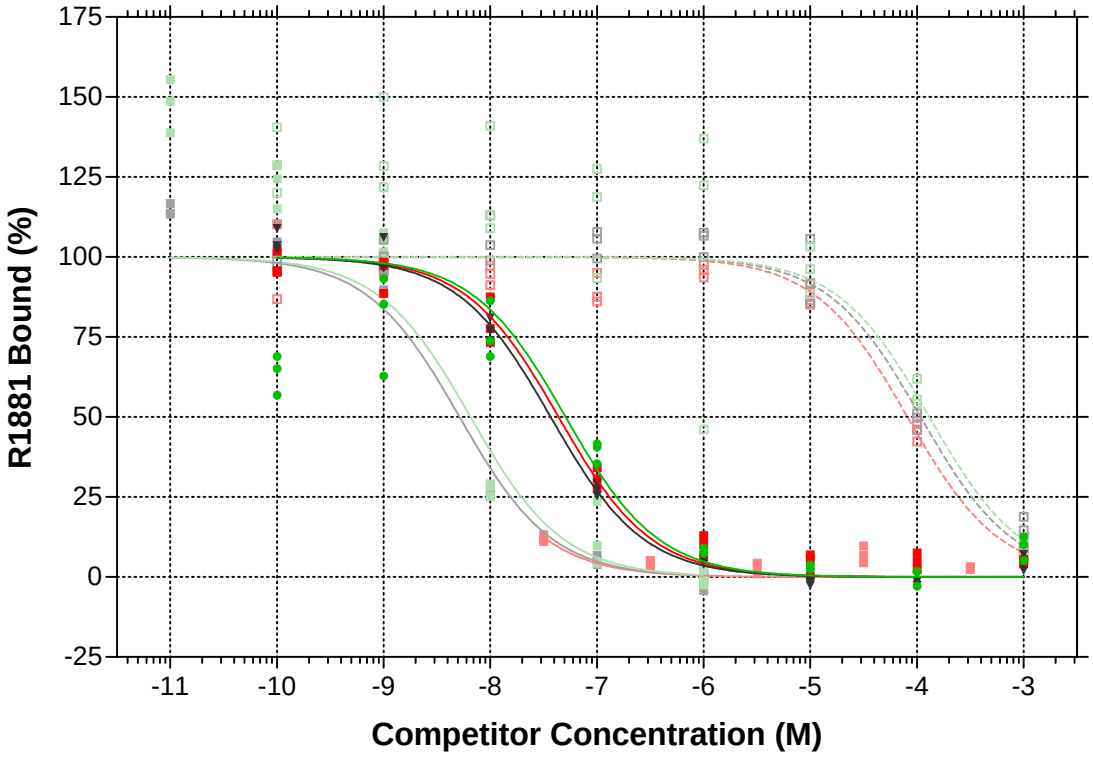


CR42402	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C4-11/29/05	0.000	0.000	100.000	0.000	-6.279	0.052

• C4-11/29/05

WA 4-11 Competitive

Lab C CR42403
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

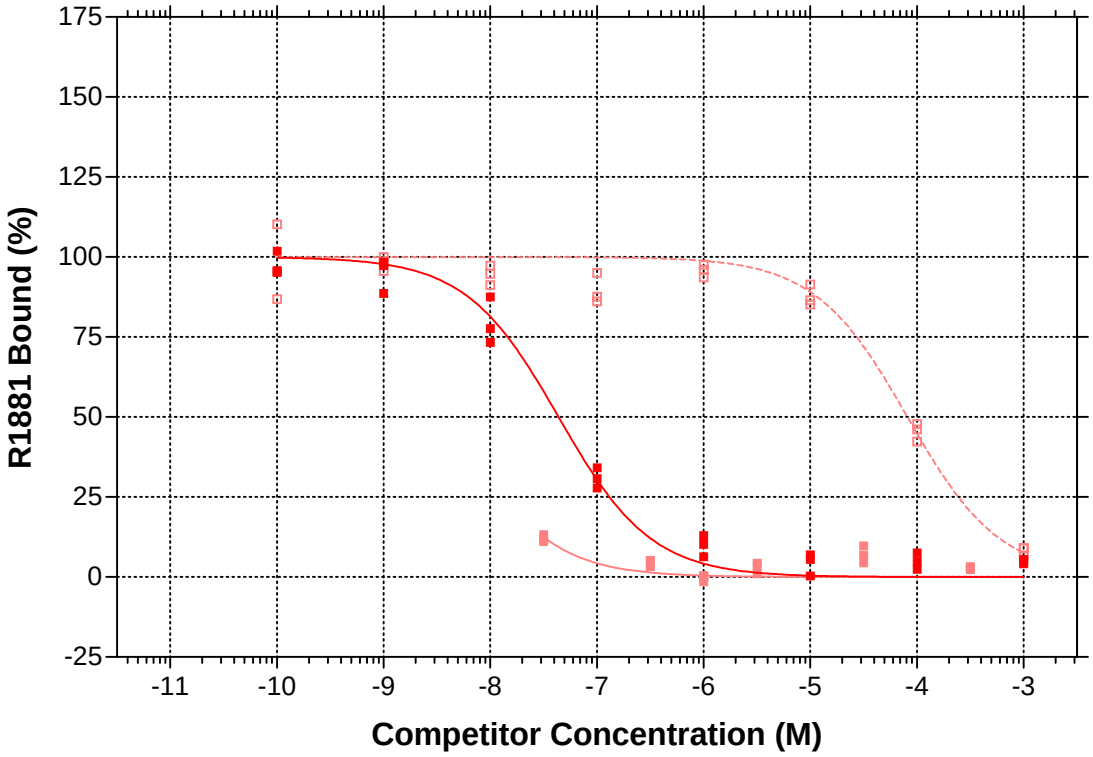


- C2-11/15/05
- C3-11/28/05
- C4-11/29/05

CR42403	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-7.359	0.049
C3-11/28/05	0.000	0.000	100.000	0.000	-7.433	0.036
C4-11/29/05	0.000	0.000	100.000	0.000	-7.293	0.154

WA 4-11 Competitive

Lab C CR42403 Run C2
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

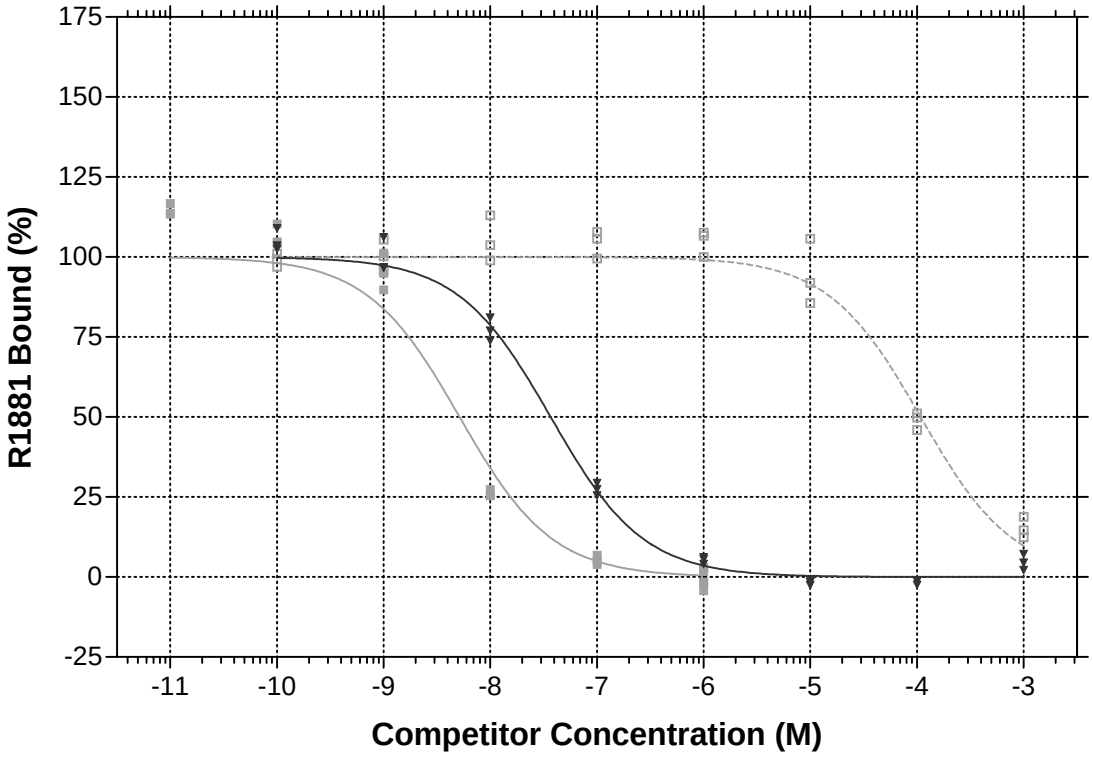


CR42403	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-7.359	0.049

■ C2-11/15/05

WA 4-11 Competitive

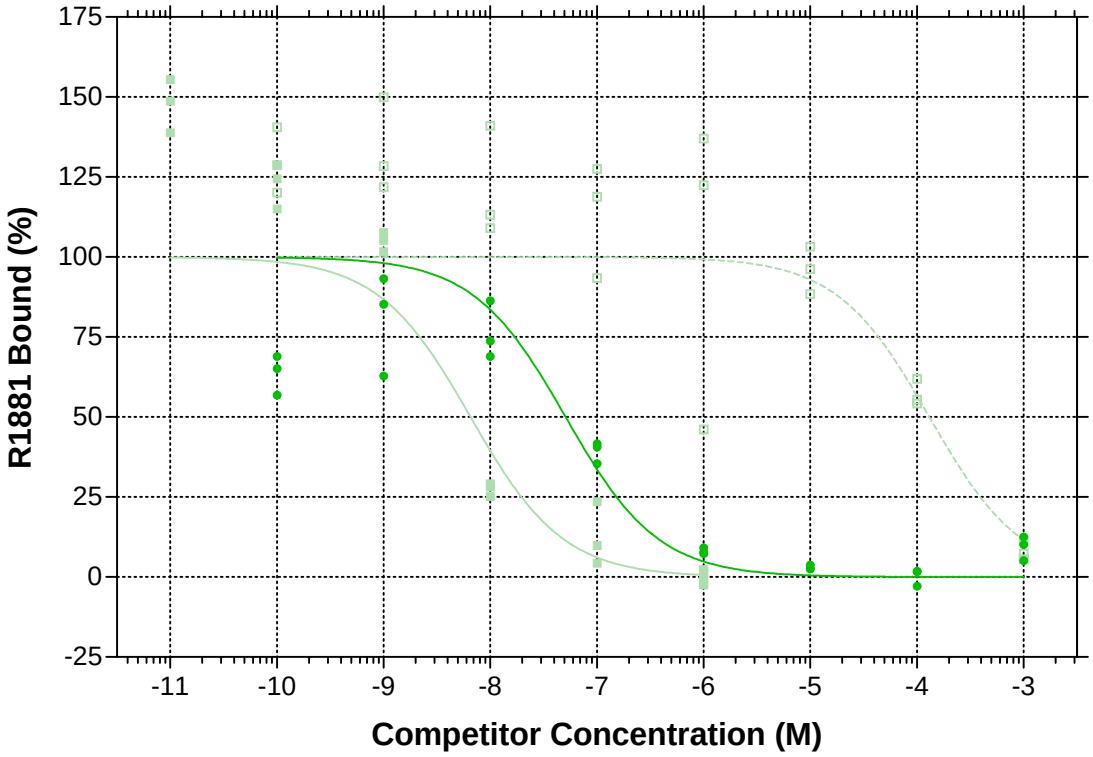
Lab C CR42403 Run C3
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42403	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C3-11/28/05	0.000	0.000	100.000	0.000	-7.433	0.036

WA 4-11 Competitive

Lab C CR42403 Run C4
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

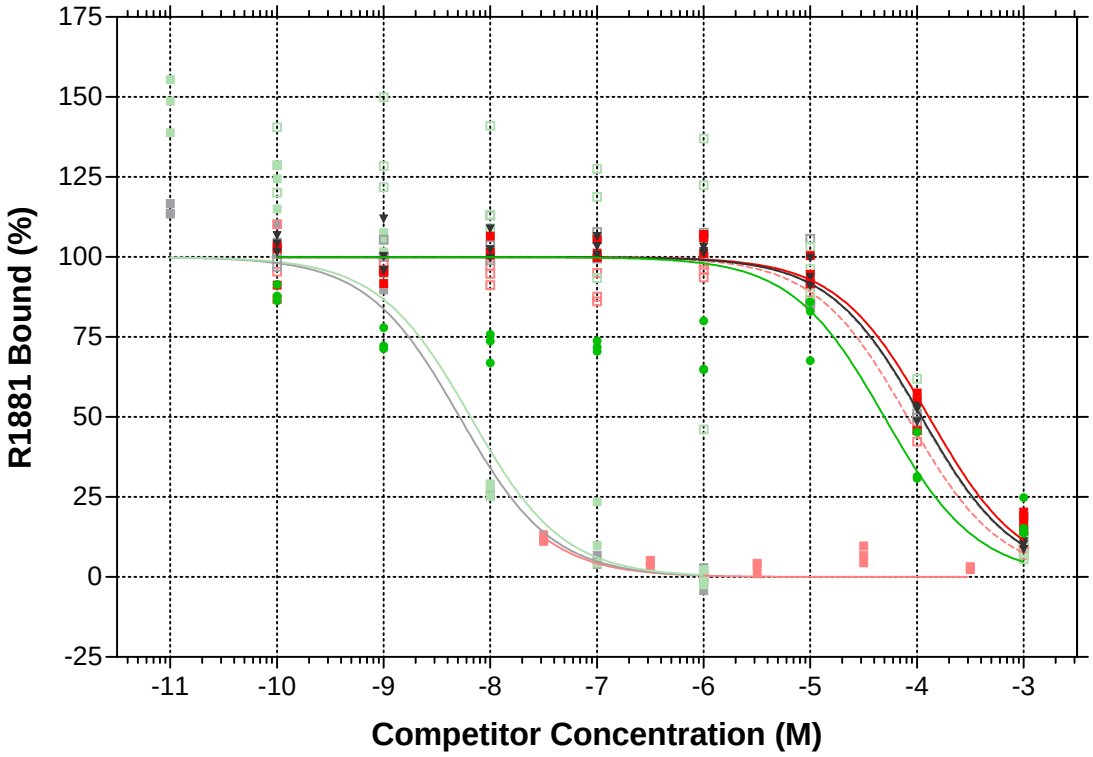


CR42403	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C4-11/29/05	0.000	0.000	100.000	0.000	-7.293	0.154

• C4-11/29/05

WA 4-11 Competitive

Lab C CR42404
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

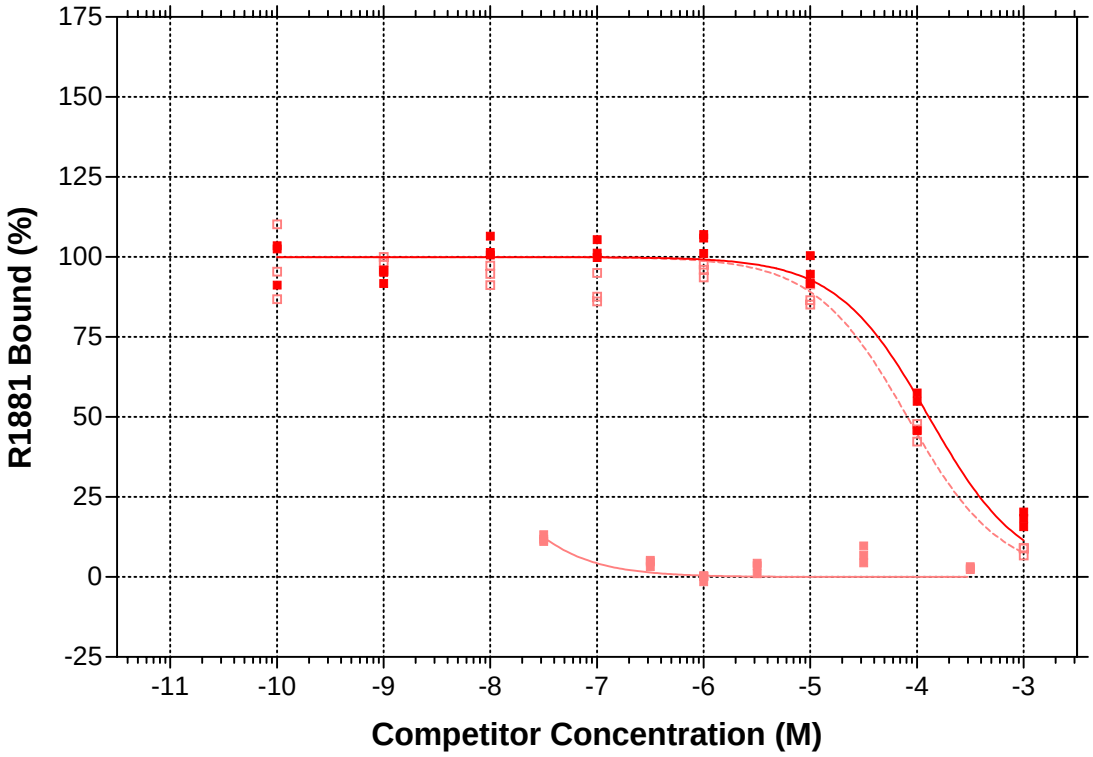


- C2-11/15/05
- C3-11/28/05
- C4-11/29/05

CR42404	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-3.887	0.051
C3-11/28/05	0.000	0.000	100.000	0.000	-3.956	0.041
C4-11/29/05	0.000	0.000	100.000	0.000	-4.305	0.203

WA 4-11 Competitive

Lab C CR42404 Run C2
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

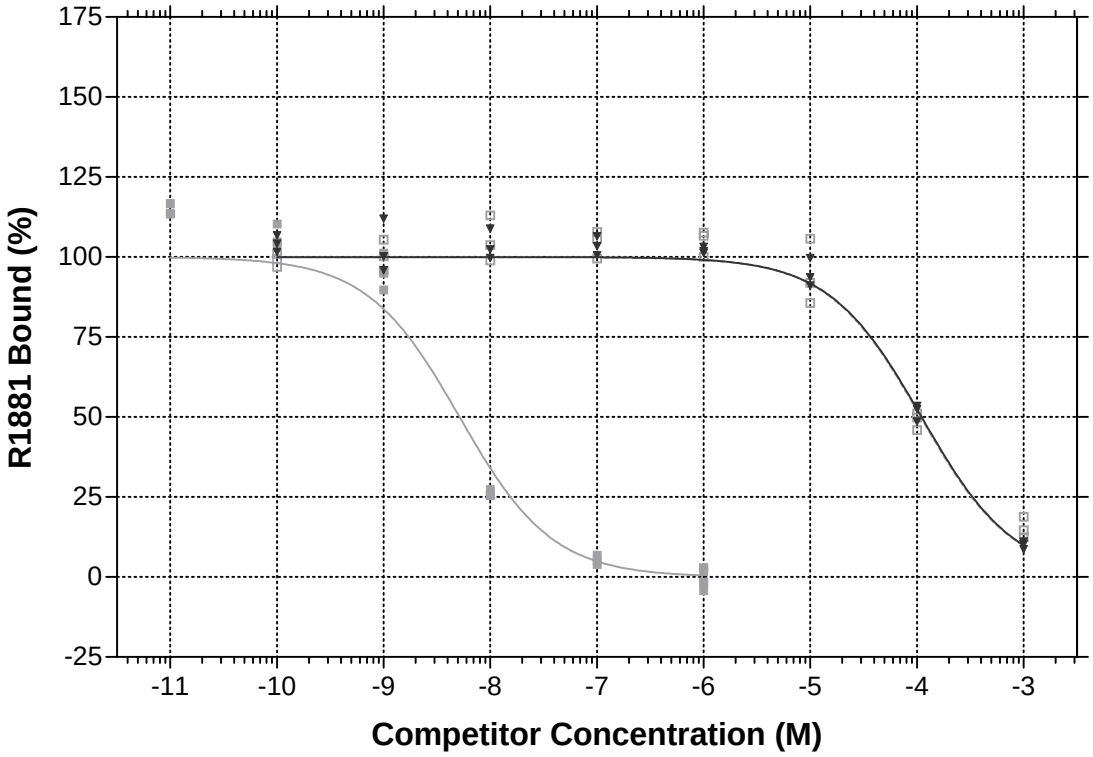


CR42404	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C2-11/15/05	0.000	0.000	100.000	0.000	-3.887	0.051

■ C2-11/15/05

WA 4-11 Competitive

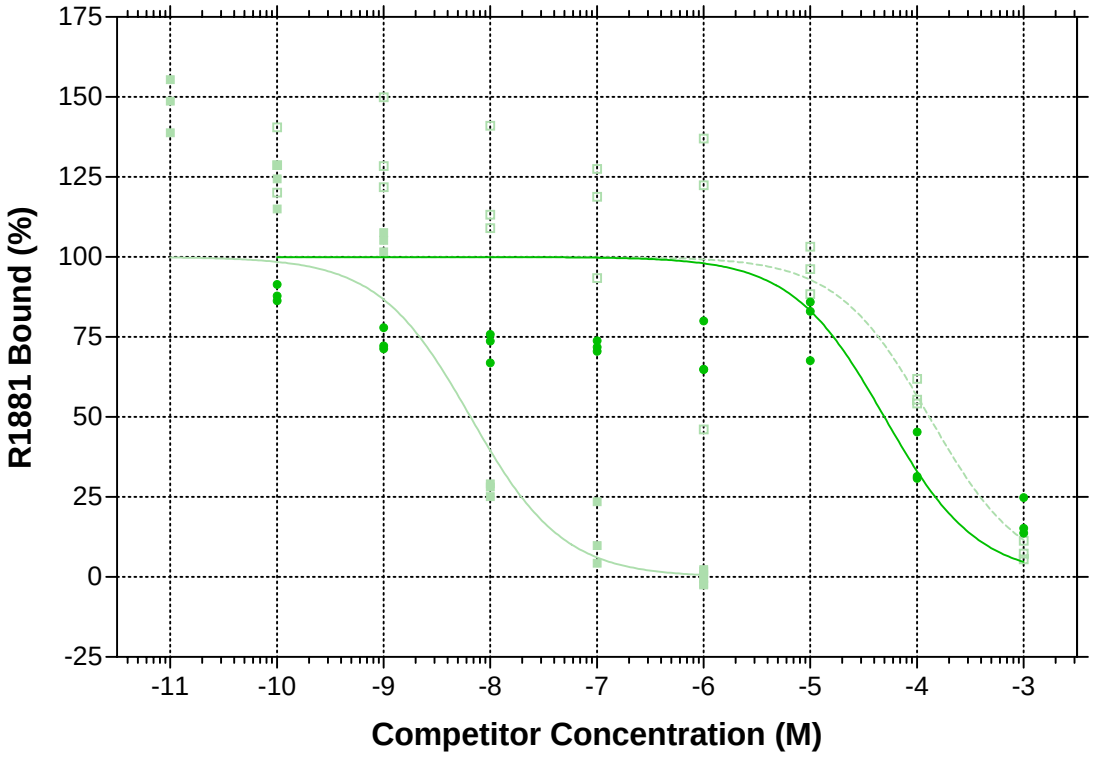
Lab C CR42404 Run C3
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42404	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C3-11/28/05	0.000	0.000	100.000	0.000	-3.956	0.041

WA 4-11 Competitive

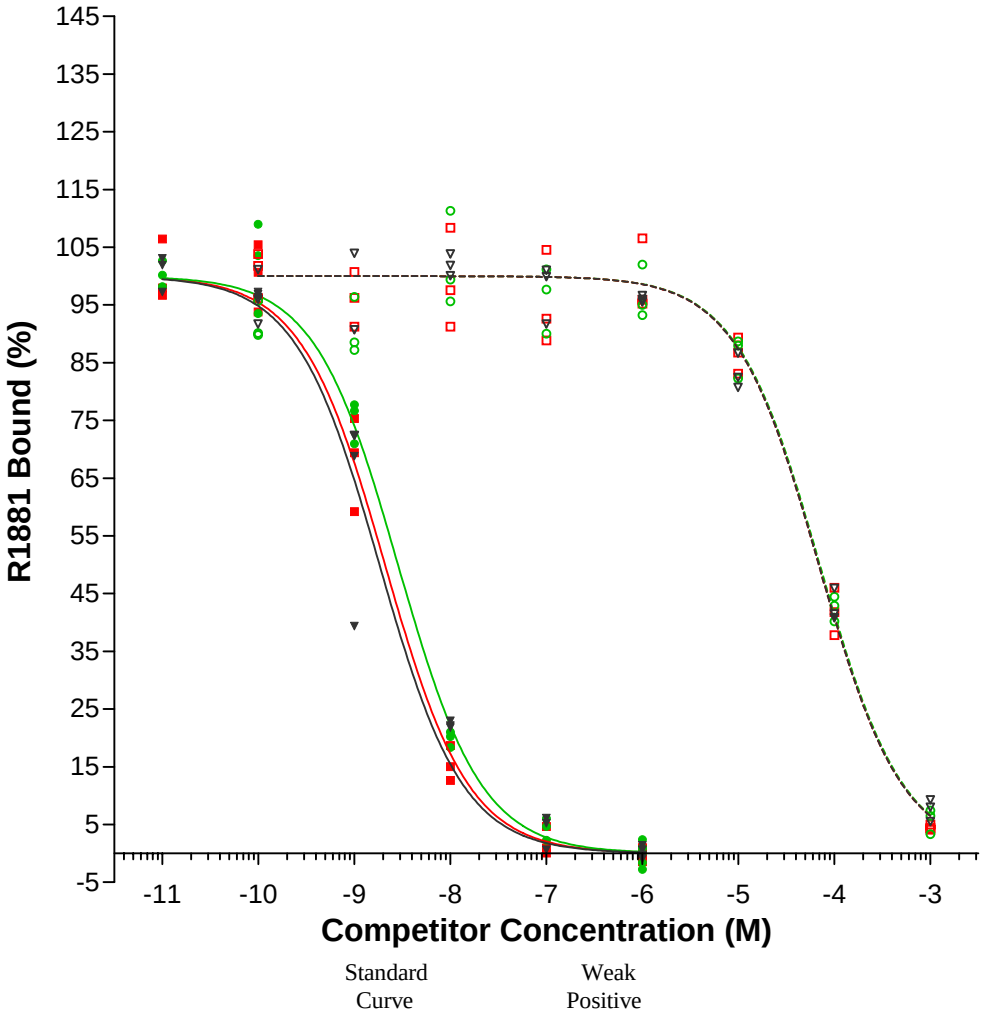
Lab C CR42404 Run C4
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42404	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C4-11/29/05	0.000	0.000	100.000	0.000	-4.305	0.203

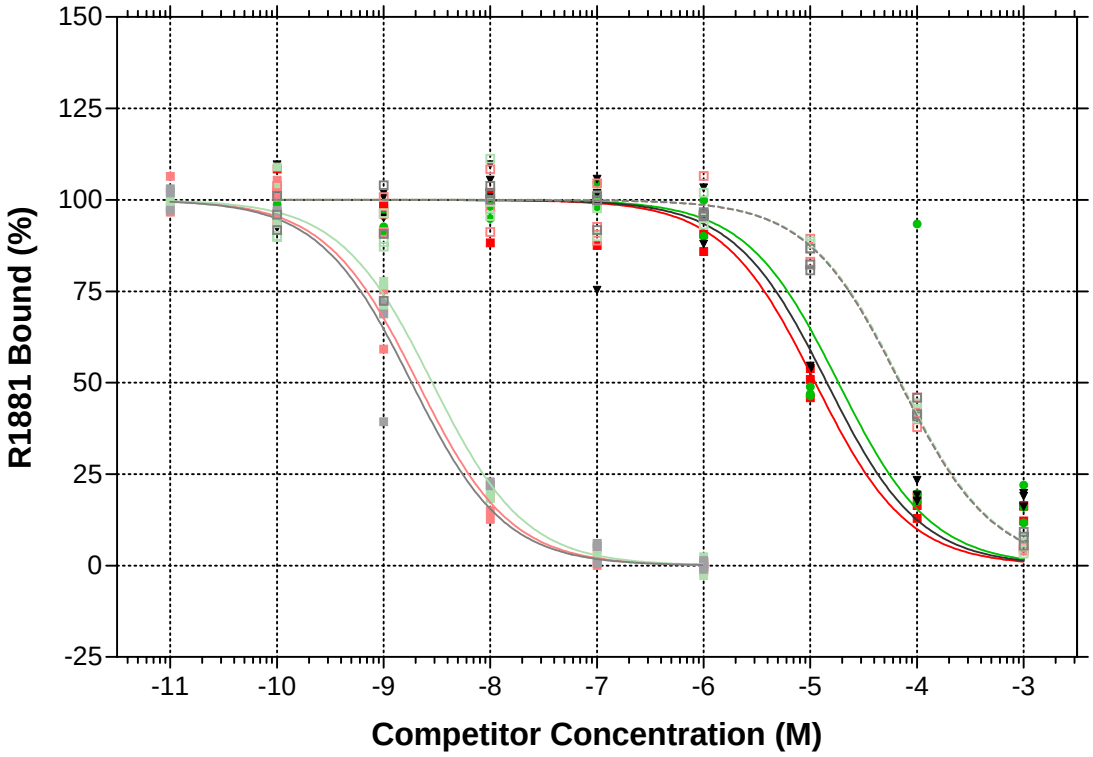
• C4-11/29/05

Standard Curve and 'Weak Positive'
Runs 5,7, and 8
Protein
1.0 mg per tube



WA 4-11 Competitive

Lab C CR42405
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

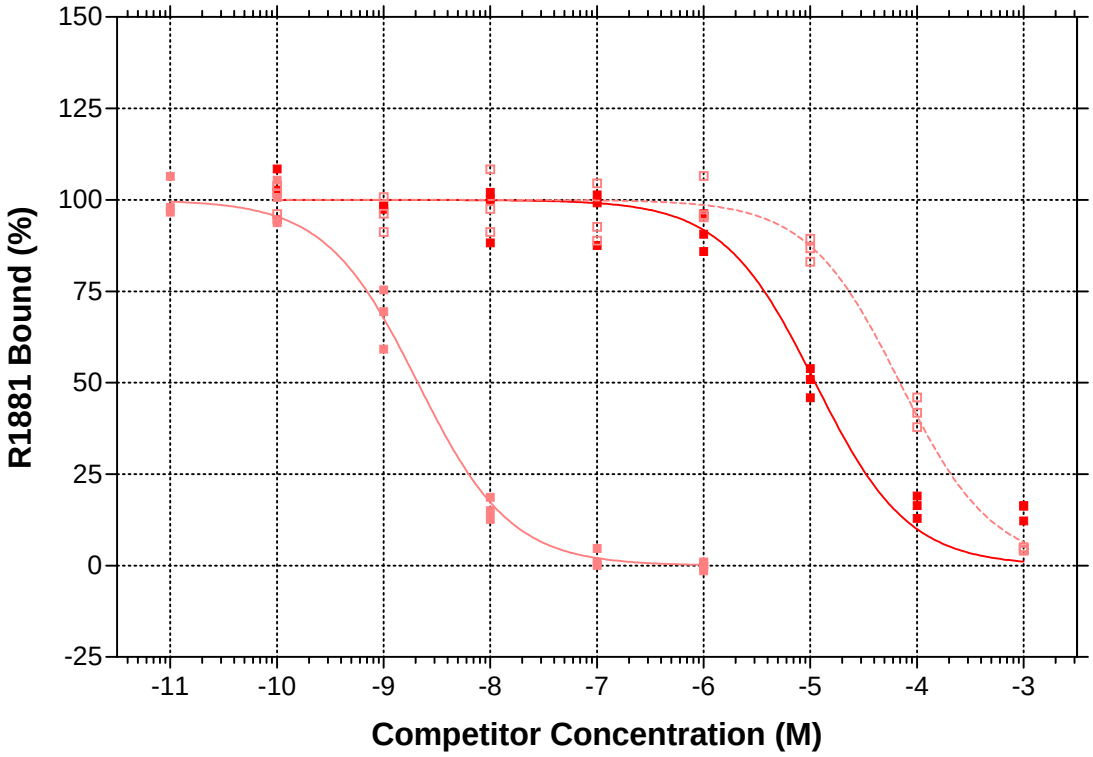


- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42405	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-4.954	0.066
C7-11/22/05	0.000	0.000	100.000	0.000	-4.736	0.174
C8-12/19/05	0.000	0.000	100.000	0.000	-4.844	0.090

WA 4-11 Competitive

Lab C CR42405 Run C5
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

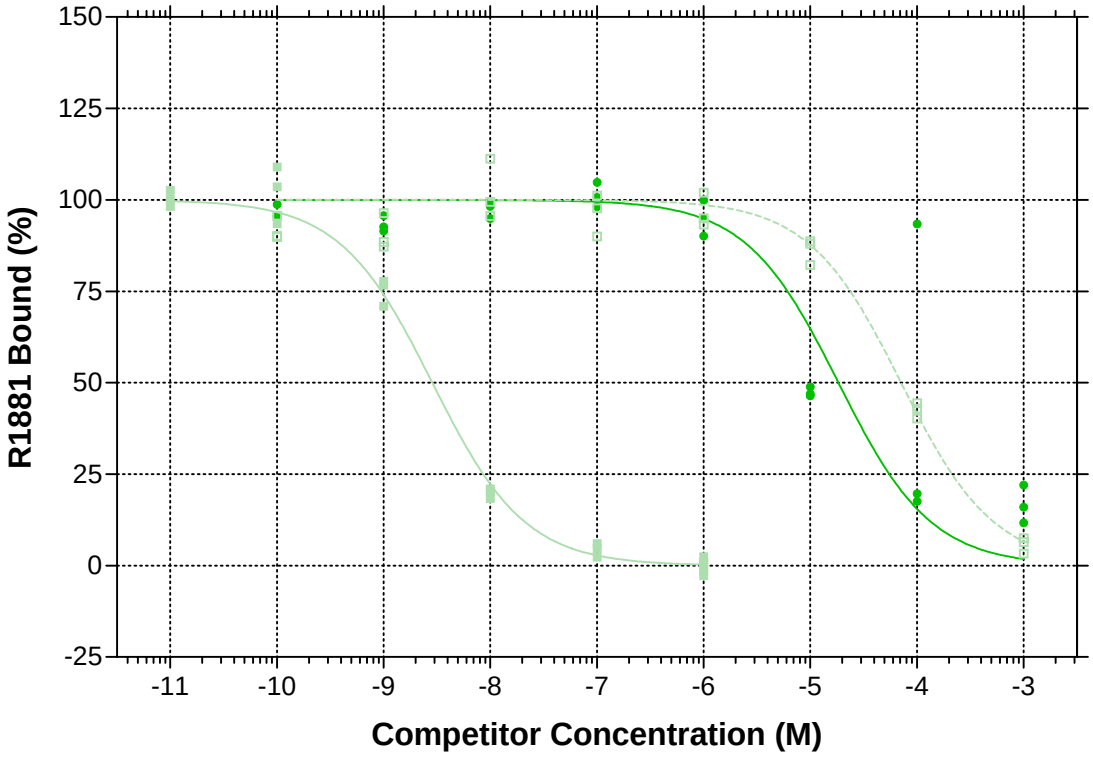


CR42405	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-4.954	0.066

■ C5-11/16/05

WA 4-11 Competitive

Lab C CR42405 Run C7
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

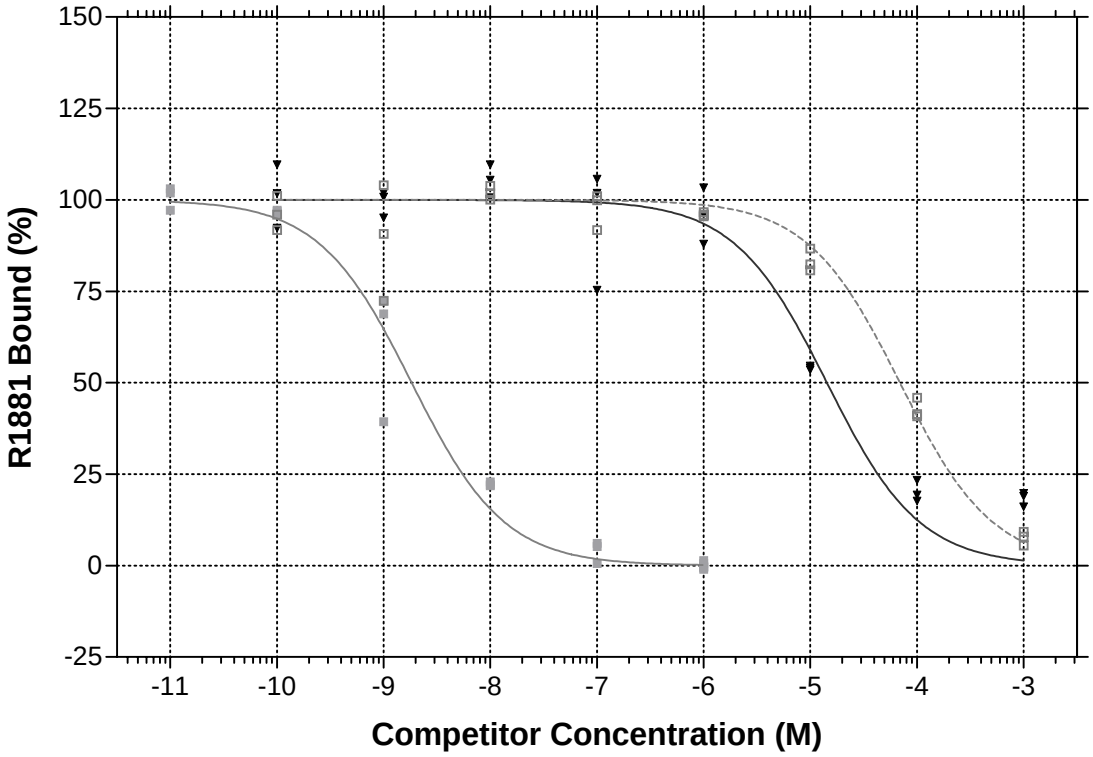


CR42405	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C7-11/22/05	0.000	0.000	100.000	0.000	-4.736	0.174

• C7-11/22/05

WA 4-11 Competitive

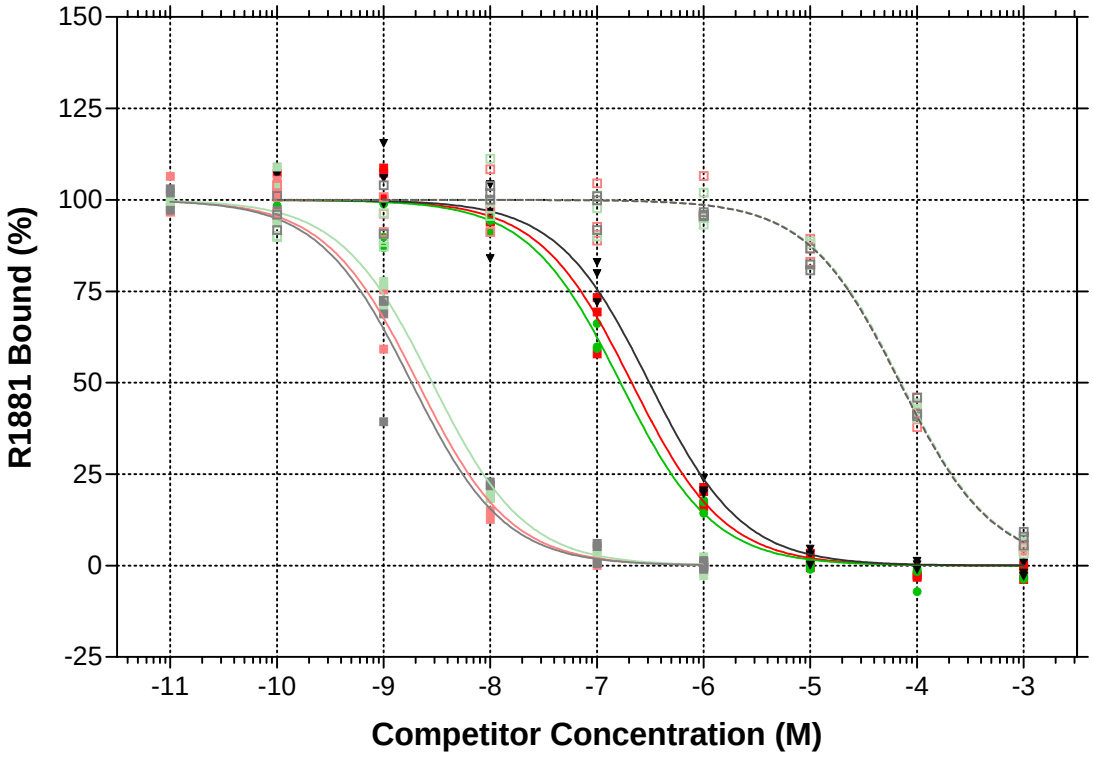
Lab C CR42405 Run C8
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42405	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C8-12/19/05	0.000	0.000	100.000	0.000	-4.844	0.090

WA 4-11 Competitive

Lab C CR42406
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

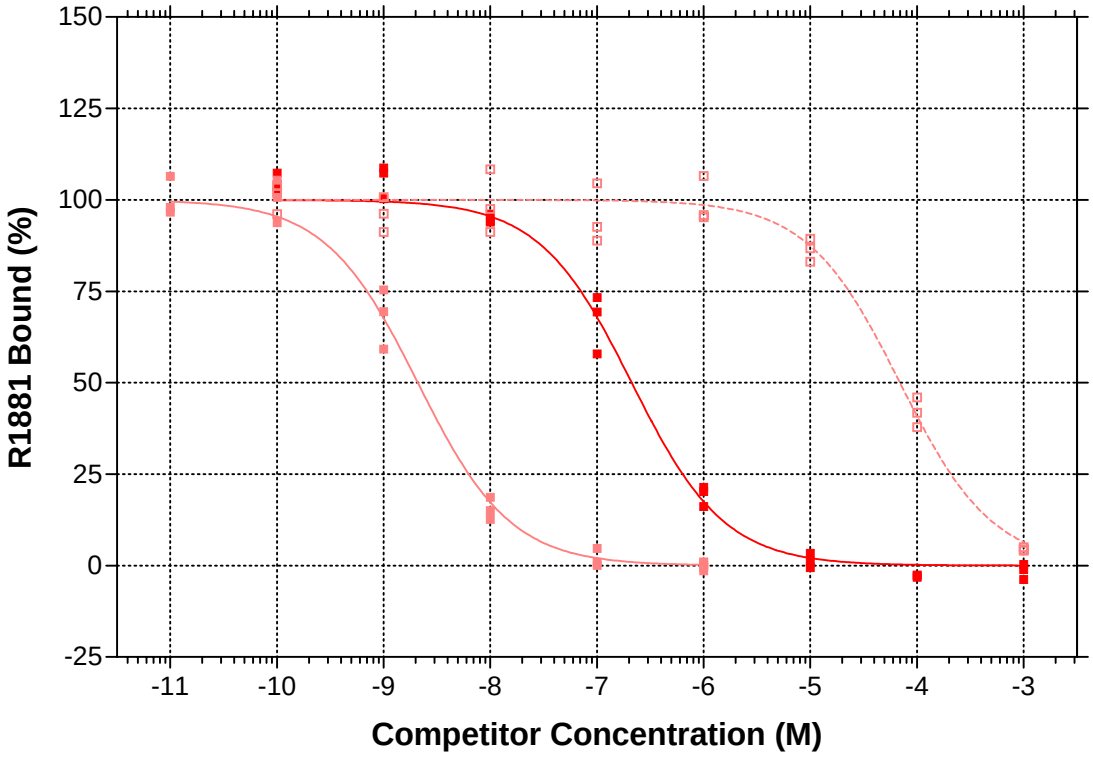


- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42406	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-6.673	0.041
C7-11/22/05	0.000	0.000	100.000	0.000	-6.779	0.041
C8-12/19/05	0.000	0.000	100.000	0.000	-6.508	0.058

WA 4-11 Competitive

Lab C CR42406 Run C5
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

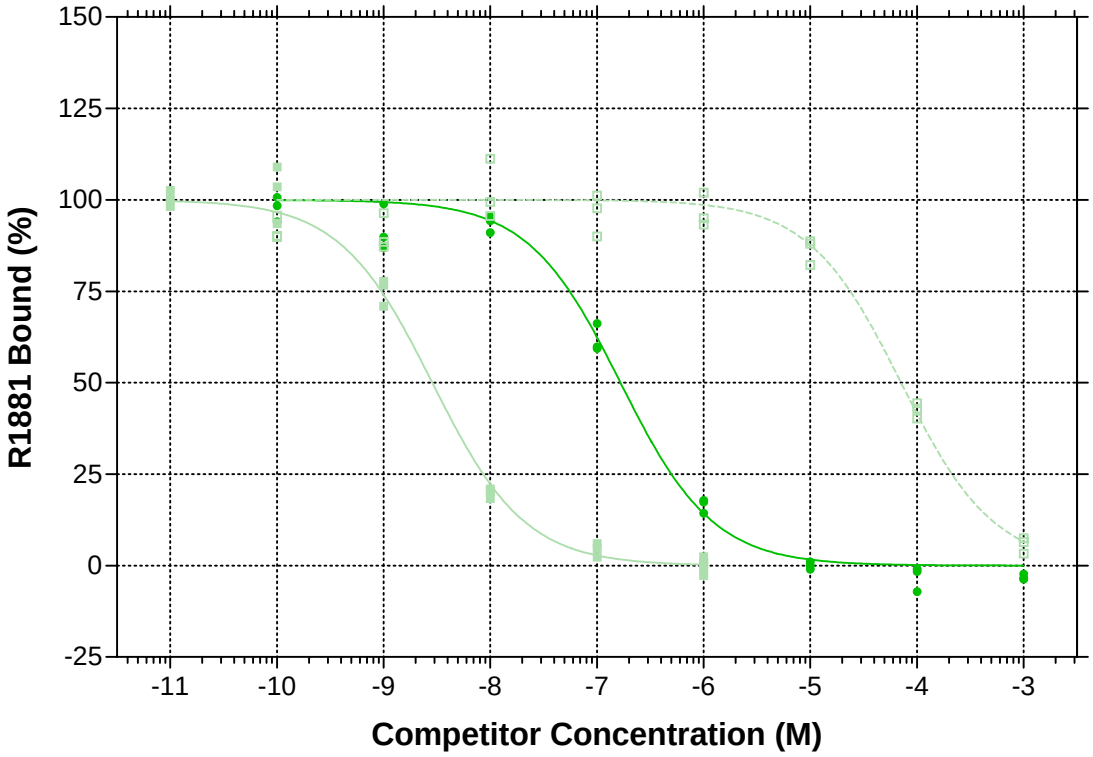


CR42406	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-6.673	0.041

■ C5-11/16/05

WA 4-11 Competitive

Lab C CR42406 Run C7
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

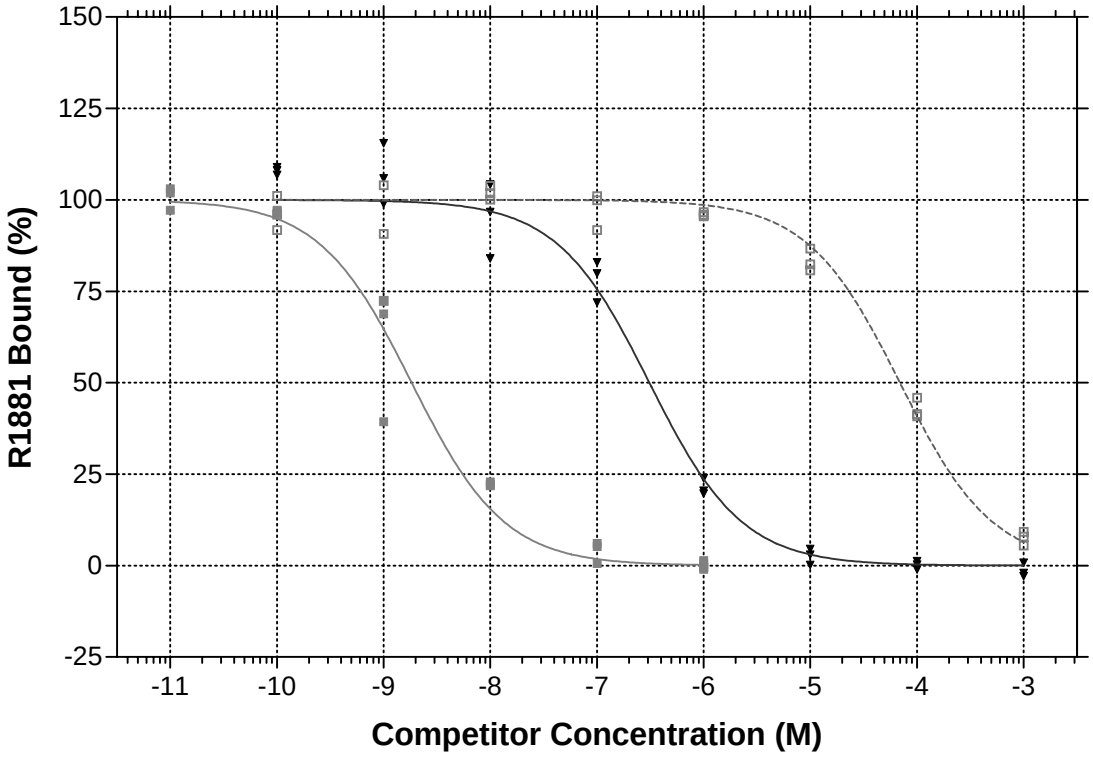


CR42406	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C7-11/22/05	0.000	0.000	100.000	0.000	-6.779	0.041

• C7-11/22/05

WA 4-11 Competitive

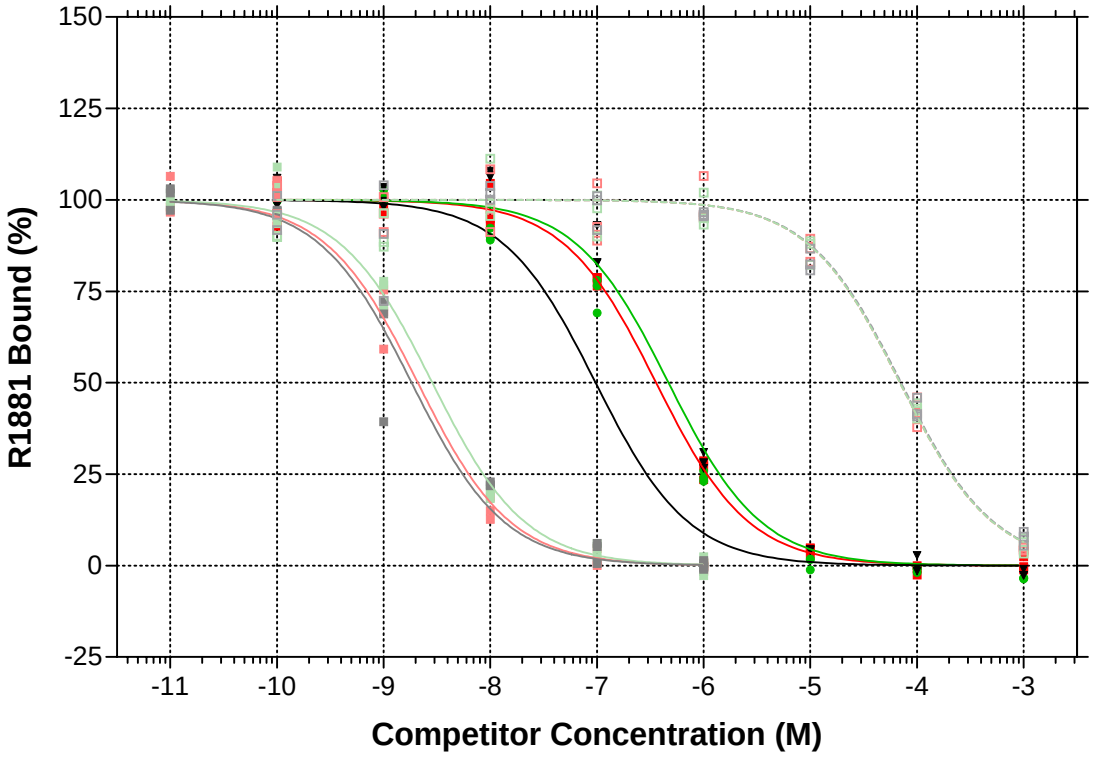
Lab C CR42406 Run C8
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42406	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C8-12/19/05	0.000	0.000	100.000	0.000	-6.508	0.058

WA 4-11 Competitive

Lab C CR42407
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

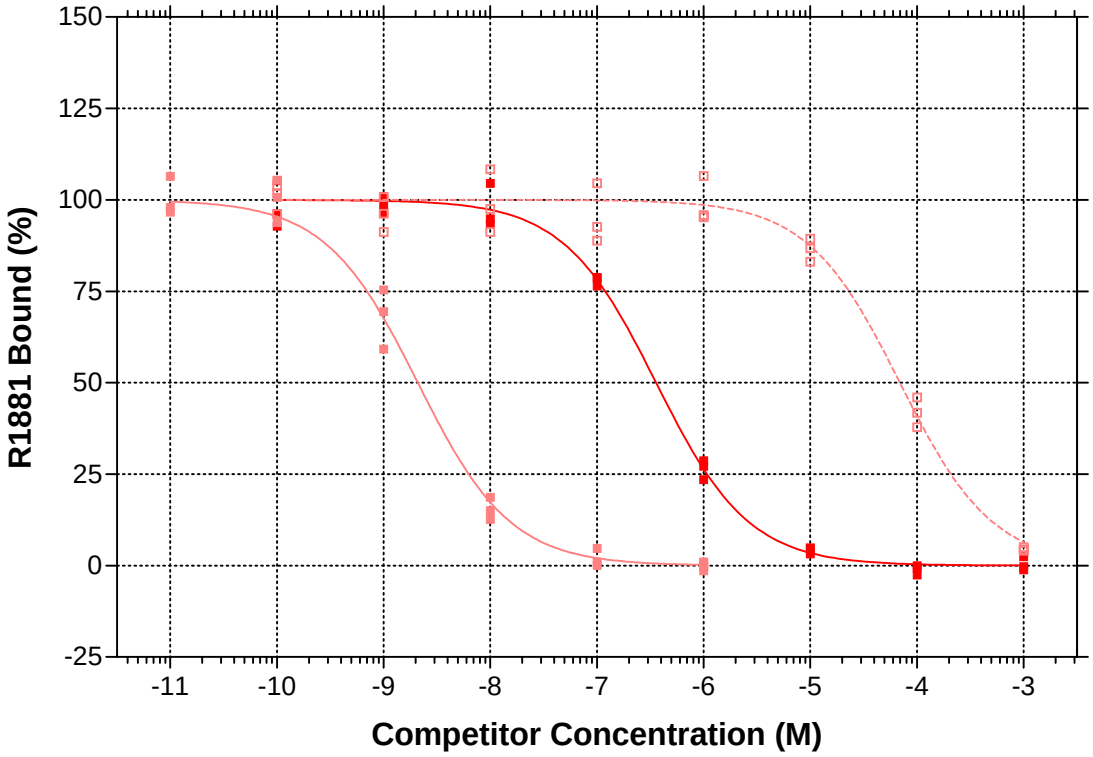


- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42407	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-6.445	0.030
C7-11/22/05	0.000	0.000	100.000	0.000	-6.531	0.033
C8-12/19/05	0.000	0.000	100.000	0.000	-6.329	0.043

WA 4-11 Competitive

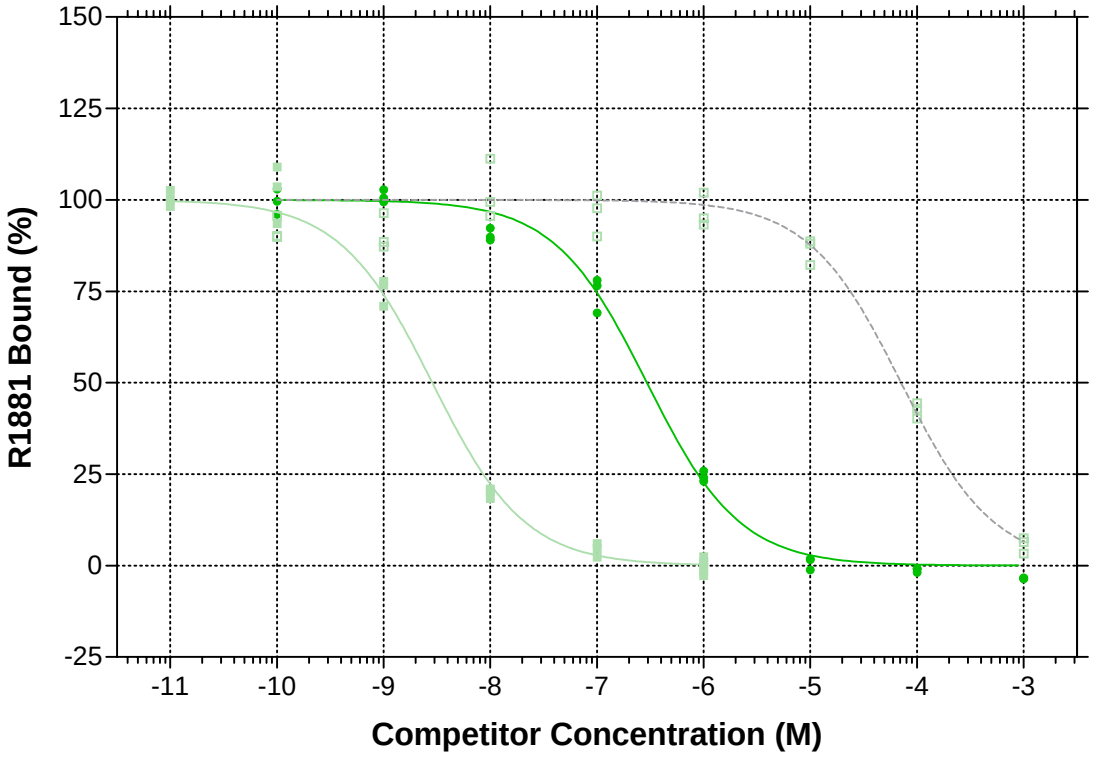
Lab C CR42407 Run C5
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42407	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
■ C5-11/16/05	0.000	0.000	100.000	0.000	-6.445	0.030

WA 4-11 Competitive

Lab C CR42407 Run C7
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

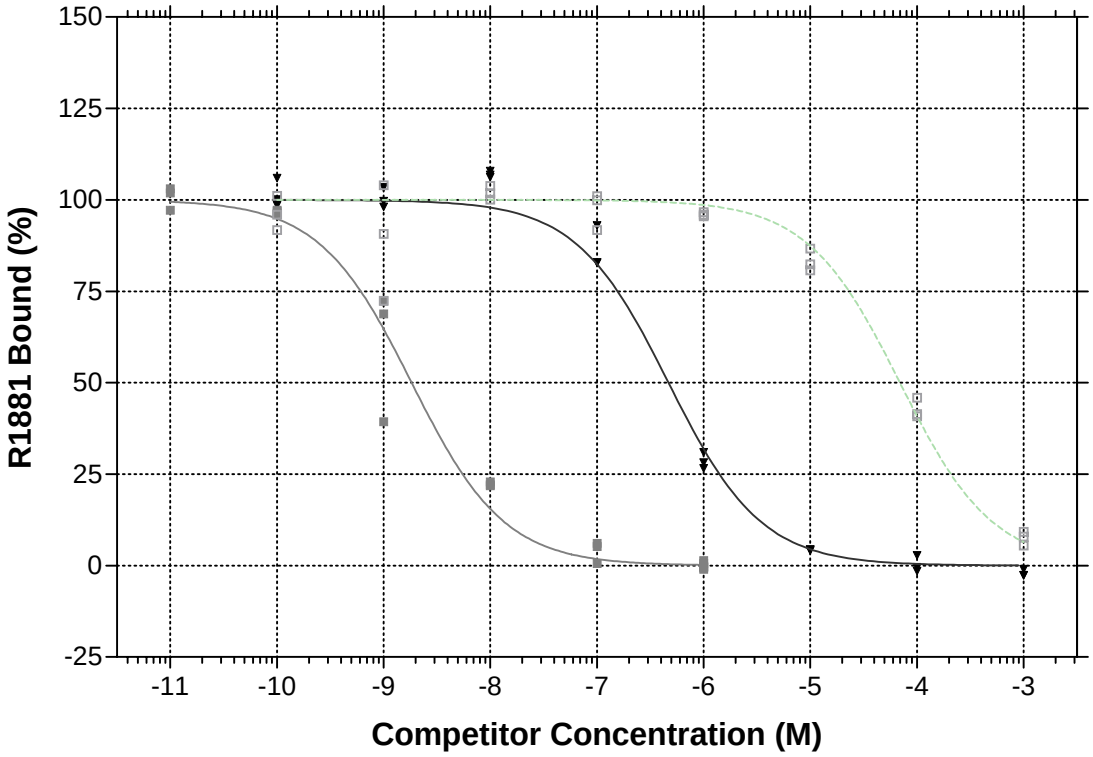


CR42407	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C7-11/22/05	0.000	0.000	100.000	0.000	-6.531	0.033

• C7-11/22/05

WA 4-11 Competitive

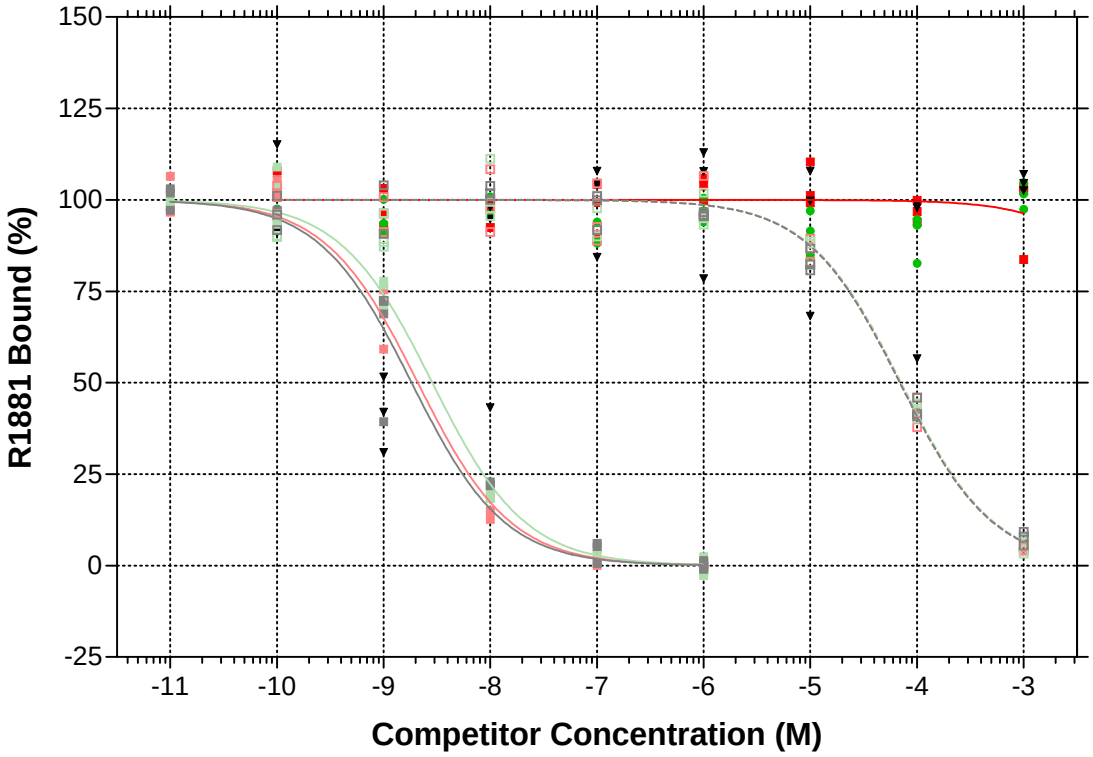
Lab C CR42407 Run C8
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42407	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C8-12/19/05	0.000	0.000	100.000	0.000	-6.329	0.043

WA 4-11 Competitive

Lab C CR42408
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

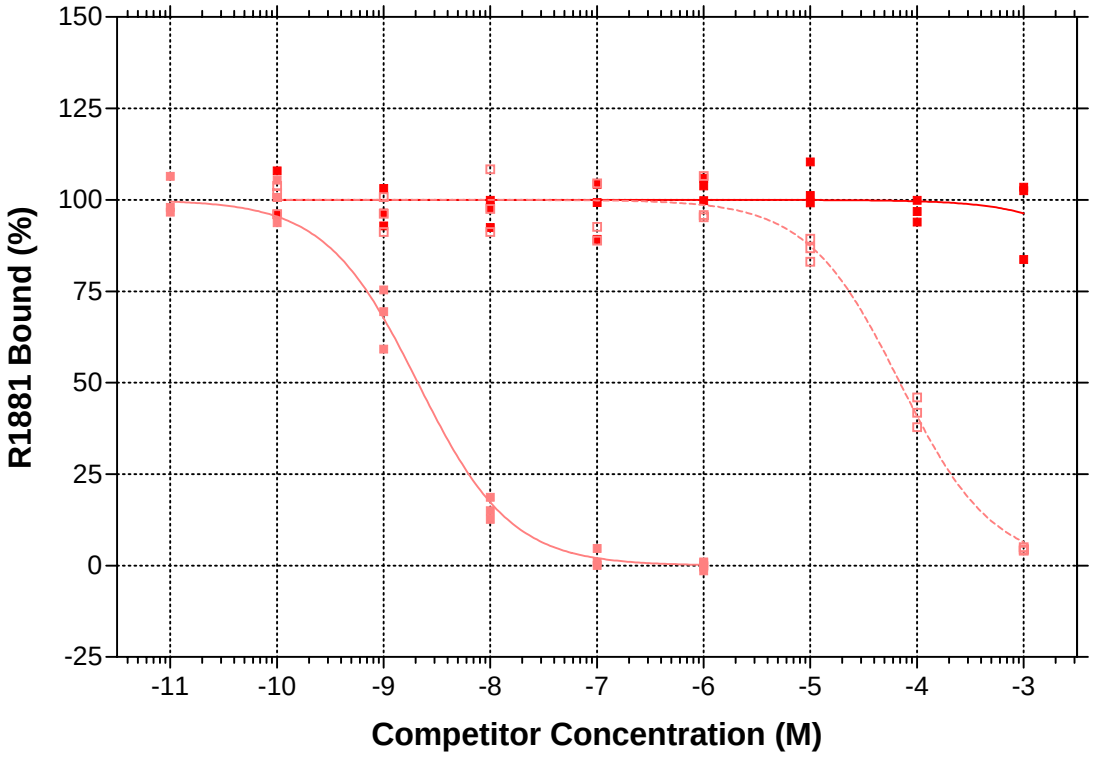


- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42408	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-1.580	0.416
C7-11/22/05						
C8-12/19/05						

WA 4-11 Competitive

Lab C CR42408 Run C5
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

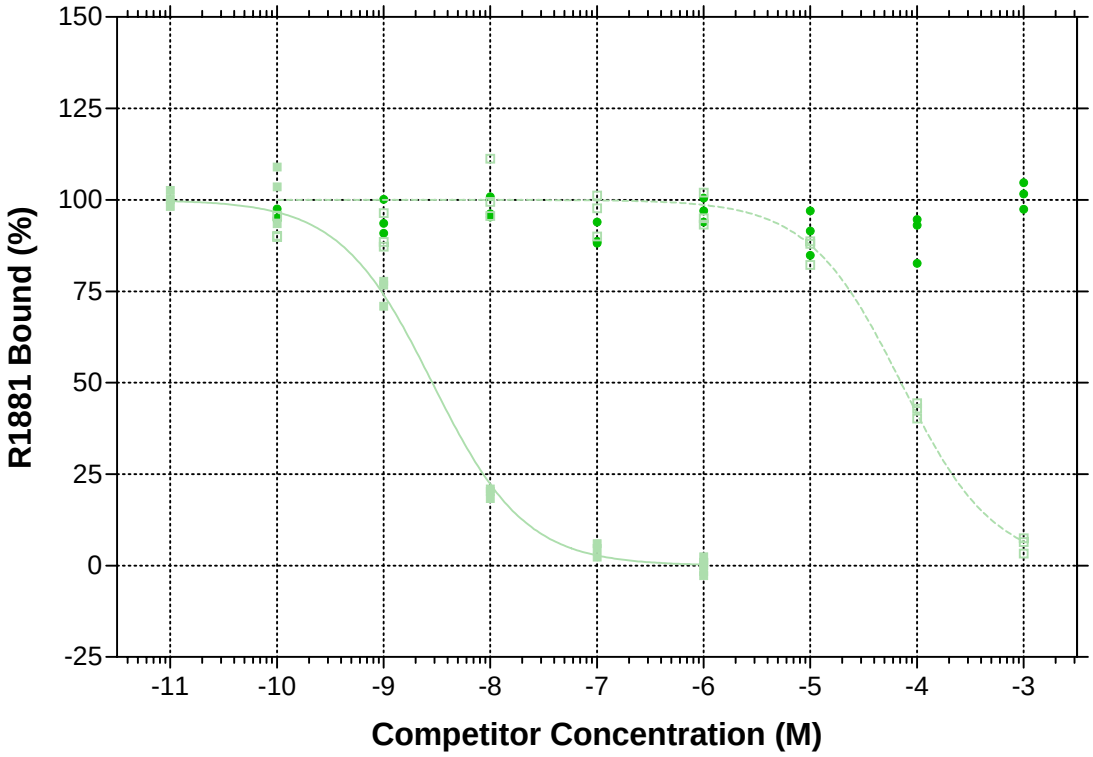


CR42408	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-1.580	0.416

■ C5-11/16/05

WA 4-11 Competitive

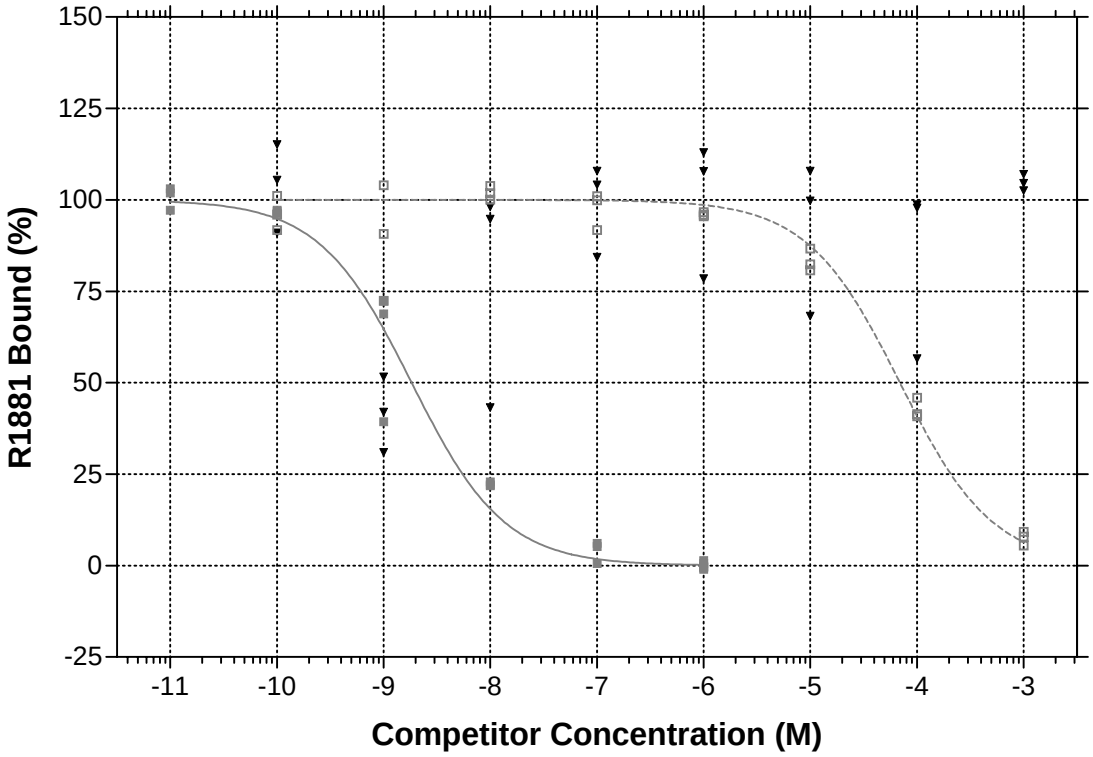
Lab C CR42408 Run C7
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



- C7-11/22/05 Does not converge

WA 4-11 Competitive

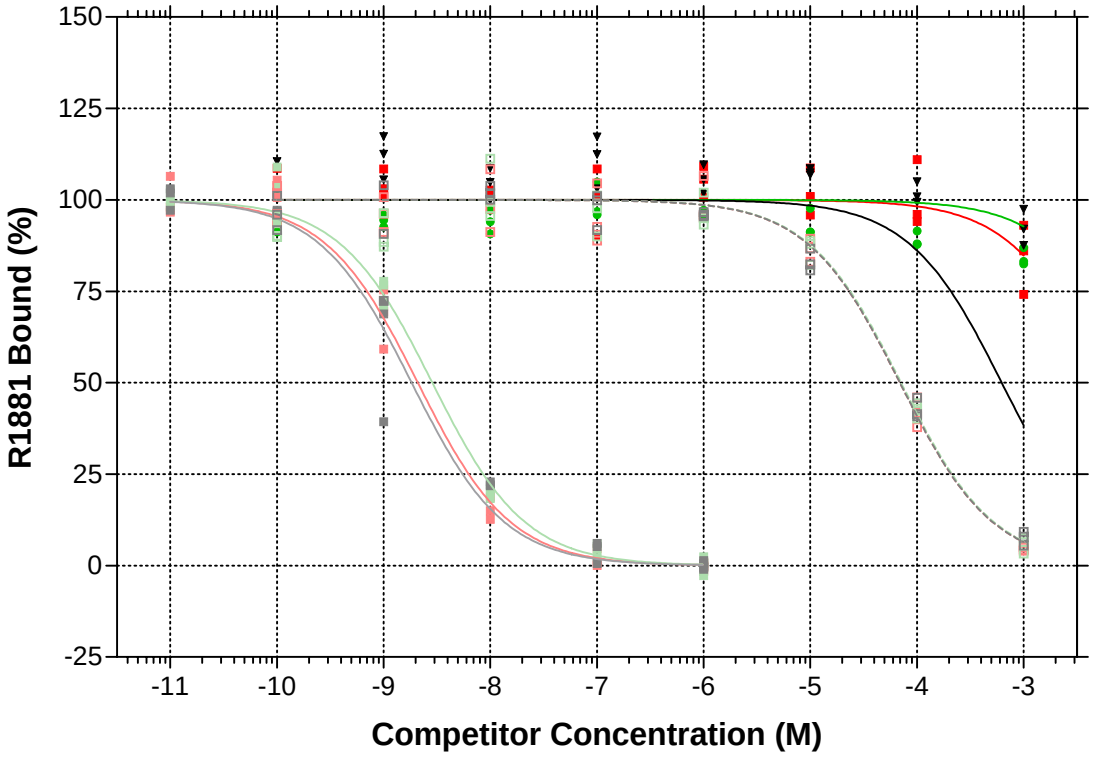
Lab C CR42408 Run C8
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



▼ C8-12/19/05 Does not converge

WA 4-11 Competitive

Lab C CR42409
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

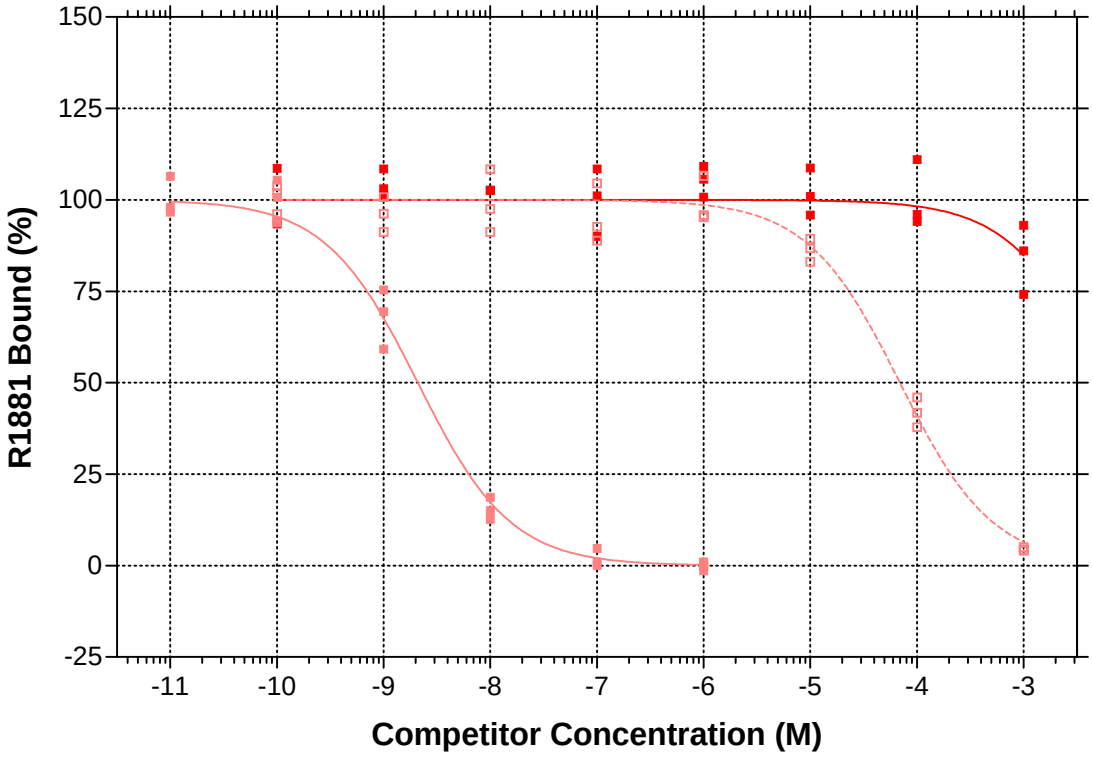


- C5-11/16/05
- C7-11/22/05
- C8-12/19/05

CR42409	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-2.254	0.128
C7-11/22/05	0.000	0.000	100.000	0.000	-2.315	0.105
C8-12/19/05	0.000	0.000	100.000	0.000	-1.892	0.306

WA 4-11 Competitive

Lab C CR42409 Run C5
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

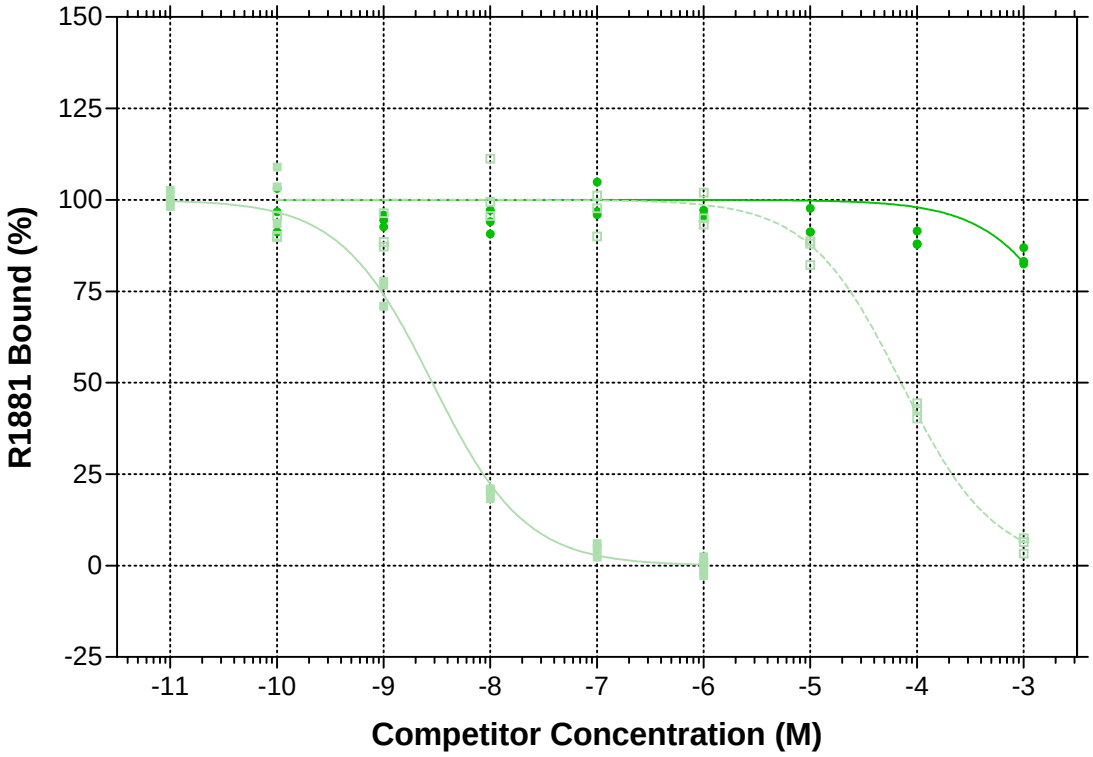


CR42409	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C5-11/16/05	0.000	0.000	100.000	0.000	-2.254	0.128

■ C5-11/16/05

WA 4-11 Competitive

Lab C CR42409 Run C7
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive

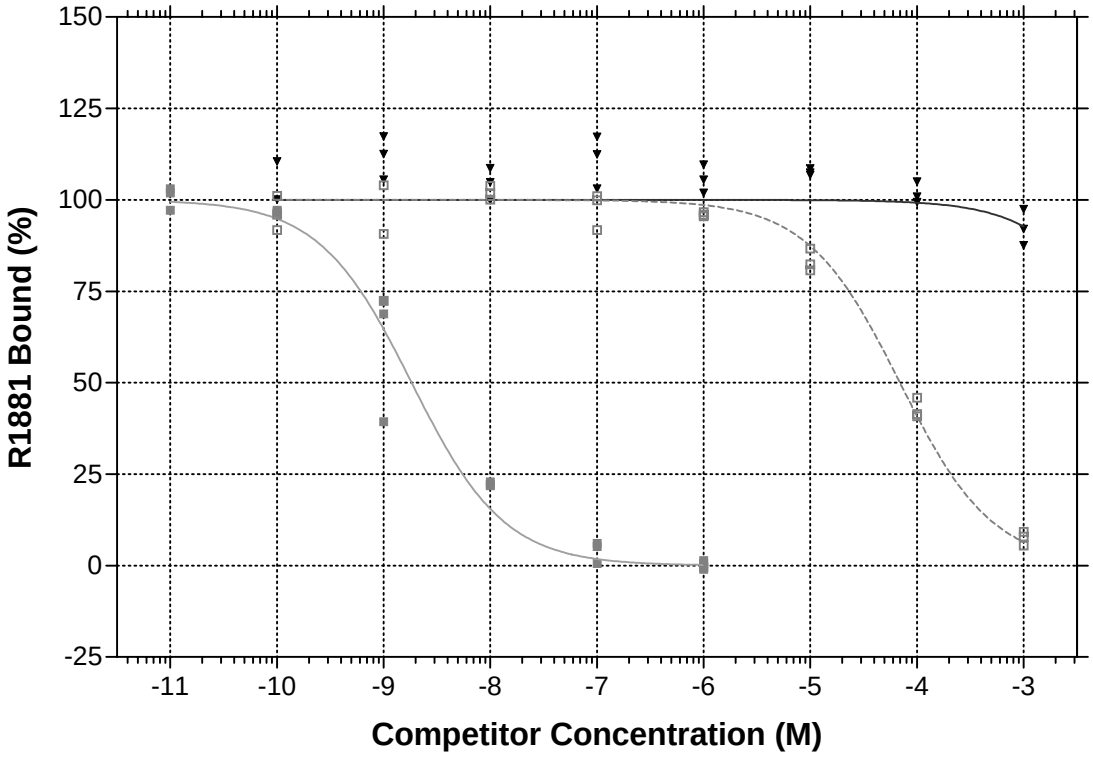


CR42409	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
C7-11/22/05	0.000	0.000	100.000	0.000	-2.315	0.105

• C7-11/22/05

WA 4-11 Competitive

Lab C CR42409 Run C8
Protein: 100 ug per tube
Solid squares = Standard Curve, Open squares = Weak Positive



CR42409	BOTTOM		TOP		LOGEC50	
	Y	SEM	Y	SEM	Y	SEM
▼ C8-12/19/05	0.000	0.000	100.000	0.000	-1.892	0.306

Appendix 3: 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:	**C**		
Run identification:	3(Seq#6862)		
Assay start date:	11/15/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	79.38	**Ci/mmole**	
Cytosol vial or lot identification:	0725-06-01		
Protein (cytosol):	600	**micro gram per tube**	
Standard Curve IC50:	4.51E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	8.16E-05	**M**	
RBA:	5.52899E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	2.36E-09	**CR42400**	
RBA, Unknown 1:	190.77834%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	1.97E-08	**CR42401**	
RBA, Unknown 2:	22.91667%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	4.54E-07	**CR42402**	
RBA, Unknown 3:	0.99295%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	4.37E-08	**CR42403**	
RBA, Unknown 4:	10.31801%		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	1.30E-04	**CR42404**	
RBA, Unknown 5:	0.00348%		
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

Summary values				
	n	Mean	SD	
EtOH	6	9212.6	439.82	Total Binding, solvent control, tubes
Hot	6	50230.7	452.04	Total hot R1881 added to each tube
NSB	6	622.5	61.93	Nonspecific Binding
Specific EtOH	6	8590.2	439.82	

Assay Characterization Values		
EtOH / Hot	0.18	less than 0.1?
NSB / EtOH	0.07	around 0.25 ?

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 18.34% 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	3089.05	9576.07	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3023.15	9371.75	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3143.14	9743.74	TRUE	
4	1	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	212.27	636.82	TRUE	
5	2	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	165.78	497.33	TRUE	
6	3	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	215.94	647.82	TRUE	
7	1	Inert R1881	S	1	E-1-S1	9.00E-03	300	30	10	50	310	2.9E-04	100	265.33	822.52	TRUE	
8	2	Inert R1881	S	1	E-1-S1	9.00E-03	300	30	10	50	310	2.9E-04	100	290.11	899.34	TRUE	
9	3	Inert R1881	S	1	E-1-S1	9.00E-03	300	30	10	50	310	2.9E-04	100	276.29	856.51	TRUE	
10	1	Inert R1881	S	2	E-1-S2	9.00E-04	300	30	10	50	310	2.9E-05	100	323.63	1003.26	TRUE	
11	2	Inert R1881	S	2	E-1-S2	9.00E-04	300	30	10	50	310	2.9E-05	100	389.75	1208.23	TRUE	
12	3	Inert R1881	S	2	E-1-S2	9.00E-04	300	30	10	50	310	2.9E-05	100	469.87	1456.60	TRUE	
13	1	Inert R1881	S	3	E-1-S3	9.00E-05	300	30	10	50	310	2.9E-06	100	299.27	927.74	TRUE	
14	2	Inert R1881	S	3	E-1-S3	9.00E-05	300	30	10	50	310	2.9E-06	100	229.67	711.99	TRUE	
15	3	Inert R1881	S	3	E-1-S3	9.00E-05	300	30	10	50	310	2.9E-06	100	322.20	998.81	TRUE	
16	1	Inert R1881	S	4	E-1-S4	9.00E-06	300	30	10	50	310	2.9E-07	100	289.70	898.06	TRUE	
17	2	Inert R1881	S	4	E-1-S4	9.00E-06	300	30	10	50	310	2.9E-07	100	345.37	1070.65	TRUE	
18	3	Inert R1881	S	4	E-1-S4	9.00E-06	300	30	10	50	310	2.9E-07	100	331.56	1027.82	TRUE	
19	1	Inert R1881	S	5	E-1-S5	9.00E-07	300	30	10	50	310	2.9E-08	100	510.72	1583.22	TRUE	
20	2	Inert R1881	S	5	E-1-S5	9.00E-07	300	30	10	50	310	2.9E-08	100	528.95	1639.74	TRUE	
21	3	Inert R1881	S	5	E-1-S5	9.00E-07	300	30	10	50	310	2.9E-08	100	568.08	1761.06	TRUE	
22	1	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	469.79	1409.37	TRUE	
23	2	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	462.63	1387.89	TRUE	
24	3	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	401.03	1203.10	TRUE	
25	1	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1576.92	4730.76	TRUE	
26	2	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1529.99	4589.96	TRUE	
27	3	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1420.06	4260.18	TRUE	
28	1	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2645.53	7936.60	TRUE	
29	2	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2826.10	8478.29	TRUE	
30	3	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2685.84	8057.53	TRUE	
31	1	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	3004.92	9014.76	TRUE	
32	2	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2957.94	8873.82	TRUE	
33	3	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2888.34	8665.03	TRUE	
34	1	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2928.86	8786.57	TRUE	
35	2	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2717.95	8153.85	TRUE	
36	3	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2673.71	8021.14	TRUE	
37	1	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2821.67	8465.02	TRUE	
38	2	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2920.33	8761.00	TRUE	
39	3	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2994.36	8983.07	TRUE	
40	1	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	2946.01	8838.02	TRUE	
41	2	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	3024.06	9072.18	TRUE	
42	3	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	3071.73	9215.18	TRUE	
43	1	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2693.89	8081.66	TRUE	
44	2	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2940.90	8822.70	TRUE	
45	3	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	3363.31	10089.93	TRUE	
46	1	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	707.55	2193.41	TRUE	
47	2	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	1044.32	3237.39	TRUE	
48	3	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	383.89	1190.07	TRUE	
49	1	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	363.33	1126.32	TRUE	
50	2	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	241.76	749.46	TRUE	
51	3	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	205.66	637.54	TRUE	
52	1	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	234.92	728.27	TRUE	
53	2	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	203.77	631.68	TRUE	

Values for analysis by nonlinear regression

Standard Curve

unknown

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		—	104.2	9576.067	8953.6	-985.9	104.2	19.06419
2	2		—	101.9	9371.753	8749.3	-781.6	101.9	18.65743
3	3		—	106.2	9743.74	9121.3	-1153.6	106.2	19.39799
4	1		-6.00	0.2	636.8238	14.4	7953.3	0.2	1.267799
5	2		-6.00	-1.5	497.3301	-125.1	8092.8	-1.5	0.990093
6	3		-6.00	0.0	647.817	25.4	7942.3	0.3	1.289684
7	1	cold R1881	-3.54	2.3	822.5246	200.1	7767.6	2.3	1.637495
8	2	cold R1881	-3.54	3.2	899.3382	276.9	7690.8	3.2	1.790417
9	3	cold R1881	-3.54	2.7	856.5126	234.0	7733.6	2.7	1.705159
10	1	cold R1881	-4.54	4.4	1003.258	380.8	7586.9	4.4	1.997303
11	2	cold R1881	-4.54	6.8	1208.225	585.8	7381.9	6.8	2.405354
12	3	cold R1881	-4.54	9.7	1456.602	834.1	7133.6	9.7	2.899827
13	1	cold R1881	-5.54	3.6	927.7444	305.3	7662.4	3.6	1.846968
14	2	cold R1881	-5.54	1.0	711.9897	89.5	7878.2	1.0	1.41744
15	3	cold R1881	-5.54	4.4	998.8092	376.3	7591.3	4.4	1.988445
16	1	cold R1881	-6.54	3.2	898.0604	275.6	7692.1	3.2	1.787873
17	2	cold R1881	-6.54	5.2	1070.646	448.2	7519.5	5.2	2.13146
18	3	cold R1881	-6.54	4.7	1027.821	405.4	7562.3	4.7	2.046202
19	1	cold R1881	-7.54	11.2	1583.219	960.8	7006.9	11.2	3.151897
20	2	cold R1881	-7.54	11.8	1639.743	1017.3	6950.4	11.8	3.264426
21	3	cold R1881	-7.54	13.3	1761.056	1138.6	6829.1	13.3	3.505938
22	1	Weak Positive	-3	9.2	1409.372	786.9	7180.8	9.2	2.805799
23	2	Weak Positive	-3	8.9	1387.89	765.4	7202.3	8.9	2.763034
24	3	Weak Positive	-3	6.8	1203.095	580.6	7387.1	6.8	2.395142
25	1	Weak Positive	-4	47.8	4730.757	4108.3	3859.4	47.8	9.418067
26	2	Weak Positive	-4	46.2	4589.964	3967.5	4000.2	46.2	9.137774
27	3	Weak Positive	-4	42.3	4260.183	3637.7	4330.0	42.3	8.48124
28	1	Weak Positive	-5	85.1	7936.599	7314.1	653.6	85.1	15.80031
29	2	Weak Positive	-5	91.5	8478.288	7855.8	111.9	91.5	16.87871
30	3	Weak Positive	-5	86.6	8057.532	7435.1	532.6	86.6	16.04106
31	1	Weak Positive	-6	97.7	9014.757	8392.3	-424.6	97.7	17.94672
32	2	Weak Positive	-6	96.1	8873.82	8251.4	-283.7	96.1	17.66614
33	3	Weak Positive	-6	93.6	8665.026	8042.6	-74.9	93.6	17.25047
34	1	Weak Positive	-7	95.0	8786.568	8164.1	-196.4	95.0	17.49244
35	2	Weak Positive	-7	87.7	8153.853	7531.4	436.3	87.7	16.23282
36	3	Weak Positive	-7	86.1	8021.139	7398.7	569.0	86.1	15.96861
37	1	Weak Positive	-8	91.3	8465.016	7842.6	125.1	91.3	16.85229
38	2	Weak Positive	-8	94.7	8761.002	8138.5	-170.8	94.7	17.44154
39	3	Weak Positive	-8	97.3	8983.071	8360.6	-392.9	97.3	17.88364
40	1	Weak Positive	-9	95.6	8838.015	8215.6	-247.9	95.6	17.59486
41	2	Weak Positive	-9	98.4	9072.177	8449.7	-482.0	98.4	18.06103
42	3	Weak Positive	-9	100.0	9215.181	8592.7	-625.0	100.0	18.34573
43	1	Weak Positive	-10	86.8	8081.655	7459.2	508.5	86.8	16.08909
44	2	Weak Positive	-10	95.5	8822.7	8200.2	-232.5	95.5	17.56437
45	3	Weak Positive	-10	110.2	10089.93	9467.5	-1499.8	110.2	20.08719
46	1	Unknown 1	-3.01424	18.3	2193.411	1570.9	6396.7	18.3	4.366677
47	2	Unknown 1	-3.01424	30.4	3237.389	2614.9	5352.8	30.4	6.445046
48	3	Unknown 1	-3.01424	6.6	1190.07	567.6	7400.1	6.6	2.369211
49	1	Unknown 1	-4.01424	5.9	1126.323	503.9	7463.8	5.9	2.242301
50	2	Unknown 1	-4.01424	1.5	749.4566	127.0	7840.7	1.5	1.49203
51	3	Unknown 1	-4.01424	0.2	637.541	15.1	7952.6	0.2	1.269227
52	1	Unknown 1	-5.01424	1.2	728.2672	105.8	7861.9	1.2	1.449846
53	2	Unknown 1	-5.01424	0.1	631.6848	9.2	7958.5	0.1	1.257568

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	18.34%	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"	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	262.48	813.70	TRUE		Unknown 1
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	249.82	774.44	TRUE		Unknown 1
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	332.48	1030.69	TRUE		Unknown 1
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	340.17	1054.53	TRUE		Unknown 1
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	362.64	1124.18	TRUE		Unknown 1
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	316.68	981.69	TRUE		Unknown 1
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	361.15	1119.57	TRUE		Unknown 1
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	692.51	2146.78	TRUE		Unknown 1
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	773.63	2398.26	TRUE		Unknown 1
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	874.24	2710.14	TRUE		Unknown 1
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2166.27	6715.43	TRUE		Unknown 1
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2003.16	6209.78	TRUE		Unknown 1
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2143.65	6645.31	TRUE		Unknown 1
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2735.34	8479.57	TRUE		Unknown 1
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2783.70	8629.46	TRUE		Unknown 1
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3008.44	9326.17	TRUE		Unknown 1
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	194.88	604.12	TRUE		Unknown 2
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	331.56	1027.83	TRUE		Unknown 2
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	448.91	1391.63	TRUE		Unknown 2
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	341.92	1059.95	TRUE		Unknown 2
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	278.40	863.03	TRUE		Unknown 2
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	297.02	920.76	TRUE		Unknown 2
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	279.75	867.23	TRUE		Unknown 2
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	388.23	1203.51	TRUE		Unknown 2
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	386.82	1199.13	TRUE		Unknown 2
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	452.43	1402.52	TRUE		Unknown 2
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	424.81	1316.90	TRUE		Unknown 2
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	452.45	1402.59	TRUE		Unknown 2
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	730.09	2263.27	TRUE		Unknown 2
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	649.30	2012.84	TRUE		Unknown 2
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	621.67	1927.18	TRUE		Unknown 2
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	1902.35	5897.29	TRUE		Unknown 2
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2010.42	6232.31	TRUE		Unknown 2
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2014.90	6246.19	TRUE		Unknown 2
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2918.68	9047.91	TRUE		Unknown 2
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2946.04	9132.71	TRUE		Unknown 2
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3198.16	9914.29	TRUE		Unknown 2
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3024.09	9374.68	TRUE		Unknown 2
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3078.04	9541.91	TRUE		Unknown 2
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2876.96	8918.58	TRUE		Unknown 2
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	4012.40	12438.44	TRUE		Unknown 3
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	3038.32	9418.78	TRUE		Unknown 3
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	3267.69	10129.83	TRUE		Unknown 3
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	366.10	1134.90	TRUE		Unknown 3
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	387.33	1200.71	TRUE		Unknown 3
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	498.14	1544.24	TRUE		Unknown 3
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	417.60	1294.55	TRUE		Unknown 3
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	369.55	1145.60	TRUE		Unknown 3
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	362.64	1124.19	TRUE		Unknown 3
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1016.15	3150.07	TRUE		Unknown 3
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1141.41	3538.37	TRUE		Unknown 3
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1082.27	3355.04	TRUE		Unknown 3
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2439.04	7561.02	TRUE		Unknown 3

Values for analysis by nonlinear regression
Standard Curve
unknown

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
54	3	-5.01424	2.2	813.6958	191.2	7776.5	2.2	1.619918
55	1	-6.01424	1.8	774.4355	152.0	7815.7	1.8	1.541759
56	2	-6.01424	4.8	1030.691	408.2	7559.5	4.8	2.051917
57	3	-6.01424	5.0	1054.53	432.1	7535.6	5.0	2.099375
58	1	-7.01424	5.8	1124.185	501.7	7466.0	5.8	2.238045
59	2	-7.01424	4.2	981.6934	359.2	7608.5	4.2	1.954371
60	3	-7.01424	5.8	1119.575	497.1	7470.6	5.8	2.228868
61	1	-8.01424	17.7	2146.78	1524.3	6443.4	17.7	4.273843
62	2	-8.01424	20.7	2398.261	1775.8	6191.9	20.7	4.774496
63	3	-8.01424	24.3	2710.142	2087.7	5880.0	24.3	5.395394
64	1	-9.01424	70.9	6715.434	6093.0	1874.7	70.9	13.36919
65	2	-9.01424	65.0	6209.784	5587.3	2380.4	65.0	12.36254
66	3	-9.01424	70.1	6645.309	6022.8	1944.8	70.1	13.22959
67	1	-10.01424	91.5	8479.566	7857.1	110.6	91.5	16.88126
68	2	-10.01424	93.2	8629.458	8007.0	-39.3	93.2	17.17966
69	3	-10.01424	101.3	9326.167	8703.7	-736.0	101.3	18.56668
70	1	-3.01424	-0.2	604.119	-18.3	7986.0	-0.2	1.20269
71	2	-3.01424	4.7	1027.826	405.4	7562.3	4.7	2.046213
72	3	-3.01424	9.0	1391.631	769.2	7198.5	9.0	2.770482
73	1	-4.01424	5.1	1059.946	437.5	7530.2	5.1	2.110157
74	2	-4.01424	2.8	863.027	240.6	7727.1	2.8	1.718128
75	3	-4.01424	3.5	920.7611	298.3	7669.4	3.5	1.833066
76	1	-5.01424	2.8	867.2284	244.8	7722.9	2.8	1.726492
77	2	-5.01424	6.8	1203.515	581.1	7386.6	6.8	2.395976
78	3	-5.01424	6.7	1199.131	576.7	7391.0	6.7	2.387248
79	1	-6.01424	9.1	1402.525	780.1	7187.6	9.1	2.792168
80	2	-6.01424	8.1	1316.902	694.4	7273.3	8.1	2.62171
81	3	-6.01424	9.1	1402.59	780.1	7187.6	9.1	2.792299
82	1	-7.01424	19.1	2263.271	1640.8	6326.9	19.1	4.505756
83	2	-7.01424	16.2	2012.837	1390.4	6577.3	16.2	4.007188
84	3	-7.01424	15.2	1927.184	1304.7	6663.0	15.2	3.836668
85	1	-8.01424	61.4	5897.285	5274.8	2692.9	61.4	11.74041
86	2	-8.01424	65.3	6232.305	5609.8	2357.9	65.3	12.40737
87	3	-8.01424	65.5	6246.193	5623.7	2344.0	65.5	12.43502
88	1	-9.01424	98.1	9047.914	8425.5	-457.8	98.1	18.01273
89	2	-9.01424	99.1	9132.712	8510.2	-542.6	99.1	18.18155
90	3	-9.01424	108.2	9914.293	9291.8	-1324.1	108.2	19.73753
91	1	-10.01424	101.9	9374.682	8752.2	-784.5	101.9	18.66327
92	2	-10.01424	103.8	9541.915	8919.5	-951.8	103.8	18.9962
93	3	-10.01424	96.6	8918.579	8296.1	-328.4	96.6	17.75525
94	1	-3.01424	137.6	12438.44	11816.0	-3848.3	137.6	24.76265
95	2	-3.01424	102.4	9418.78	8796.3	-828.6	102.4	18.75106
96	3	-3.01424	110.7	10129.83	9507.4	-1539.7	110.7	20.16663
97	1	-4.01424	6.0	1134.897	512.4	7455.3	6.0	2.259372
98	2	-4.01424	6.7	1200.709	578.2	7389.4	6.7	2.39039
99	3	-4.01424	10.7	1544.242	921.8	7045.9	10.7	3.074302
100	1	-5.01424	7.8	1294.547	672.1	7295.6	7.8	2.577205
101	2	-5.01424	6.1	1145.604	523.1	7444.6	6.1	2.280687
102	3	-5.01424	5.8	1124.191	501.7	7466.0	5.8	2.238057
103	1	-6.01424	29.4	3150.065	2527.6	5440.1	29.4	6.2712
104	2	-6.01424	33.9	3538.371	2915.9	5051.8	33.9	7.044245
105	3	-6.01424	31.8	3355.037	2732.6	5235.1	31.8	6.679261
106	1	-7.01424	80.8	7561.018	6938.6	1029.1	80.8	15.05259

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	18.34%	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"	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2238.25	6938.56	TRUE		Unknown 3
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2596.06	8047.77	TRUE		Unknown 3
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2987.48	9261.19	TRUE		Unknown 3
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2949.30	9142.82	TRUE		Unknown 3
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2846.61	8824.50	TRUE		Unknown 3
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3056.56	9475.32	TRUE		Unknown 3
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3101.45	9614.51	TRUE		Unknown 3
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3032.38	9400.37	TRUE		Unknown 3
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3047.19	9446.28	TRUE		Unknown 3
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3129.08	9700.16	TRUE		Unknown 3
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3055.43	9471.82	TRUE		Unknown 3
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	335.01	1038.54	TRUE		Unknown 4
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	355.74	1102.78	TRUE		Unknown 4
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	316.68	981.70	TRUE		Unknown 4
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	267.36	828.81	TRUE		Unknown 4
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	411.32	1275.10	TRUE		Unknown 4
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	335.91	1041.33	TRUE		Unknown 4
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	394.18	1221.97	TRUE		Unknown 4
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	354.48	1098.87	TRUE		Unknown 4
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	210.68	653.10	TRUE		Unknown 4
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	483.88	1500.03	TRUE		Unknown 4
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	376.46	1167.02	TRUE		Unknown 4
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	560.28	1736.86	TRUE		Unknown 4
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	1146.65	3554.60	TRUE		Unknown 4
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	1053.40	3265.52	TRUE		Unknown 4
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	972.27	3014.05	TRUE		Unknown 4
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2626.28	8141.46	TRUE		Unknown 4
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2352.47	7292.66	TRUE		Unknown 4
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2234.58	6927.19	TRUE		Unknown 4
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2898.29	8984.69	TRUE		Unknown 4
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2927.83	9076.26	TRUE		Unknown 4
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2656.45	8234.99	TRUE		Unknown 4
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3024.15	9374.87	TRUE		Unknown 4
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2840.46	8805.42	TRUE		Unknown 4
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2854.62	8849.32	TRUE		Unknown 4
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	638.16	1978.30	TRUE		Unknown 5
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	765.60	2373.35	TRUE		Unknown 5
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	709.92	2200.74	TRUE		Unknown 5
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	1795.27	5565.32	TRUE		Unknown 5
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	1469.17	4554.44	TRUE		Unknown 5
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	1723.42	5342.61	TRUE		Unknown 5
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2983.93	9250.18	TRUE		Unknown 5
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2741.99	8500.17	TRUE		Unknown 5
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2822.27	8749.03	TRUE		Unknown 5
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3004.98	9315.45	TRUE		Unknown 5
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3166.28	9815.46	TRUE		Unknown 5
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3136.03	9721.68	TRUE		Unknown 5
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3121.56	9676.85	TRUE		Unknown 5
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3006.09	9318.87	TRUE		Unknown 5
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2966.41	9195.88	TRUE		Unknown 5
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2990.15	9269.47	TRUE		Unknown 5
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3011.69	9336.24	TRUE		Unknown 5
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3153.29	9775.21	TRUE		Unknown 5

Values for analysis by nonlinear regression
Standard Curve
unknown

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
107	2	-7.01424	73.5	6938.56	6316.1	1651.6	73.5	13.8134
108	3	-7.01424	86.4	8047.771	7425.3	542.4	86.4	16.02163
109	1	-8.01424	100.6	9261.191	8638.7	-671.0	100.6	18.43733
110	2	-8.01424	99.2	9142.824	8520.4	-552.7	99.2	18.20168
111	3	-8.01424	95.5	8824.5	8202.0	-234.3	95.5	17.56796
112	1	-9.01424	103.1	9475.321	8852.9	-885.2	103.1	18.86362
113	2	-9.01424	104.7	9614.507	8992.0	-1024.4	104.7	19.14071
114	3	-9.01424	102.2	9400.375	8777.9	-810.2	102.2	18.71442
115	1	-10.01424	102.7	9446.283	8823.8	-856.1	102.7	18.80581
116	2	-10.01424	105.7	9700.16	9077.7	-1110.0	105.7	19.31123
117	3	-10.01424	103.0	9471.824	8849.4	-881.7	103.0	18.85666
118	1	-3.01424	4.8	1038.544	416.1	7551.6	4.8	2.067549
119	2	-3.01424	5.6	1102.783	480.3	7487.4	5.6	2.195439
120	3	-3.01424	4.2	981.7037	359.2	7608.5	4.2	1.954391
121	1	-4.01424	2.4	828.812	206.3	7761.3	2.4	1.650012
122	2	-4.01424	7.6	1275.096	652.6	7315.1	7.6	2.538481
123	3	-4.01424	4.9	1041.328	418.9	7548.8	4.9	2.073093
124	1	-5.01424	7.0	1221.967	599.5	7368.2	7.0	2.432711
125	2	-5.01424	5.5	1098.873	476.4	7491.3	5.5	2.187654
126	3	-5.01424	0.4	653.1049	30.6	7937.1	0.4	1.300212
127	1	-6.01424	10.2	1500.029	877.6	7090.1	10.2	2.986282
128	2	-6.01424	6.3	1167.023	544.6	7423.1	6.3	2.323328
129	3	-6.01424	13.0	1736.86	1114.4	6853.3	13.0	3.457769
130	1	-7.01424	34.1	3554.603	2932.1	5035.6	34.1	7.07656
131	2	-7.01424	30.8	3265.525	2643.1	5324.6	30.8	6.501058
132	3	-7.01424	27.8	3014.051	2391.6	5576.1	27.8	6.000421
133	1	-8.01424	87.5	8141.462	7519.0	448.7	87.5	16.20815
134	2	-8.01424	77.6	7292.657	6670.2	1297.5	77.6	14.51834
135	3	-8.01424	73.4	6927.192	6304.7	1663.0	73.4	13.79076
136	1	-9.01424	97.3	8984.69	8362.2	-394.5	97.3	17.88686
137	2	-9.01424	98.4	9076.261	8453.8	-486.1	98.4	18.06916
138	3	-9.01424	88.6	8234.992	7612.5	355.2	88.6	16.39435
139	1	-10.01424	101.9	9374.871	8752.4	-784.7	101.9	18.66364
140	2	-10.01424	95.3	8805.417	8183.0	-215.3	95.3	17.52996
141	3	-10.01424	95.8	8849.319	8226.9	-259.2	95.8	17.61737
142	1	-3.01424	15.8	1978.299	1355.8	6611.9	15.8	3.938429
143	2	-3.01424	20.4	2373.349	1750.9	6216.8	20.4	4.724902
144	3	-3.01424	18.4	2200.742	1578.3	6389.4	18.4	4.381273
145	1	-4.01424	57.5	5565.325	4942.9	3024.8	57.5	11.07954
146	2	-4.01424	45.8	4554.436	3932.0	4035.7	45.8	9.067045
147	3	-4.01424	54.9	5342.611	4720.1	3247.5	54.9	10.63616
148	1	-5.01424	100.4	9250.177	8627.7	-660.0	100.4	18.4154
149	2	-5.01424	91.7	8500.166	7877.7	90.0	91.7	16.92227
150	3	-5.01424	94.6	8749.031	8126.6	-158.9	94.6	17.41771
151	1	-6.01424	101.2	9315.447	8693.0	-725.3	101.2	18.54534
152	2	-6.01424	107.0	9815.459	9193.0	-1225.3	107.0	19.54077
153	3	-6.01424	105.9	9721.678	9099.2	-1131.5	105.9	19.35407
154	1	-7.01424	105.4	9676.845	9054.4	-1086.7	105.4	19.26482
155	2	-7.01424	101.2	9318.873	8696.4	-728.7	101.2	18.55216
156	3	-7.01424	99.8	9195.877	8573.4	-605.7	99.8	18.3073
157	1	-8.01424	100.7	9269.465	8647.0	-679.3	100.7	18.4538
158	2	-8.01424	101.4	9336.236	8713.8	-746.1	101.4	18.58673
159	3	-8.01424	106.5	9775.208	9152.7	-1185.1	106.5	19.46064

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	18.34%	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"	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2742.16	8500.68	TRUE		Unknown 5
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2860.56	8867.74	TRUE		Unknown 5
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2839.68	8803.02	TRUE		Unknown 5
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2728.21	8457.46	TRUE		Unknown 5
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3042.77	9432.60	TRUE		Unknown 5
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3070.15	9517.45	TRUE		Unknown 5
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	2772.44	8594.55	TRUE		
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	2952.98	9154.22	TRUE		
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	2850.12	8835.38	TRUE		
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	221.04	663.12	TRUE		
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	215.76	647.28	TRUE		
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	214.13	642.40	TRUE		
172	1	none	Hot		**—**	—	—	30	—	—	—	—		50980.37	50980.37	TRUE		
173	2	none	Hot		**—**	—	—	30	—	—	—	—		50282.68	50282.68	TRUE		
174	3	none	Hot		**—**	—	—	30	—	—	—	—		49914.60	49914.60	TRUE		
175	1	none	Hot		**—**	—	—	30	—	—	—	—		49809.57	49809.57	TRUE		
176	2	none	Hot		**—**	—	—	30	—	—	—	—		50496.41	50496.41	TRUE		
177	3	none	Hot		**—**	—	—	30	—	—	—	—		49900.33	49900.33	TRUE		

Values for analysis by nonlinear regression
Standard Curve
unknown

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
160	1	-9.01424	91.7	8500.684	7878.2	89.5	91.7	16.9233
161	2	-9.01424	96.0	8867.742	8245.3	-277.6	96.0	17.65404
162	3	-9.01424	95.2	8803.017	8180.6	-212.9	95.2	17.52519
163	1	-10.01424	91.2	8457.463	7835.0	132.7	91.2	16.83725
164	2	-10.01424	102.6	9432.596	8810.1	-842.4	102.6	18.77856
165	3	-10.01424	103.5	9517.453	8895.0	-927.3	103.5	18.9475
166	1	—	92.8	8594.555	7972.1	-4.4	92.8	17.11018
167	2	—	99.3	9154.223	8531.8	-564.1	99.3	18.22437
168	3	—	95.6	8835.381	8212.9	-245.2	95.6	17.58962
169	1	-6.00	0.5	663.1242	40.7	7927.0	0.5	1.320158
170	2	-6.00	0.3	647.2806	24.8	7942.9	0.3	1.288617
171	3	-6.00	0.2	642.4014	19.9	7947.8	0.2	1.278903
172	1			50980.37	50357.9			
173	2			50282.68	49660.2			
174	3			49914.6	49292.1			
175	1			49809.57	49187.1			
176	2			50496.41	49873.9			
177	3			49900.33	49277.9			

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:	**C**			
Run identification:	4(Seq#6952)			
Assay start date:	11/28/2005			
Tracer lot number:	3559-507			
Specific activity on day of assay:	79.23	**Ci/mmole**		
Cytosol vial or lot identification:	0725-06-01			
Protein (cytosol):	600	**micro gram per tube**		
Standard Curve IC50:	5.16E-09	**M**		
Weak Positive, Max Concentration:	**3.00E-02**	**M**		
Weak Positive IC50:	1.09E-04	**M**		
RBA:	4.7523E-05			
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)	
IC50, Unknown 1:	2.86E-09	**CR42400**		
RBA, Unknown 1:	180.77058%			
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 2:	1.40E-08	**CR42401**		
RBA, Unknown 2:	36.75926%			
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 3:	4.13E-07	**CR42402**		
RBA, Unknown 3:	1.24843%			
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 4:	3.69E-08	**CR42403**		
RBA, Unknown 4:	14.00163%			
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 5:	1.11E-04	**CR42404**		
RBA, Unknown 5:	0.00466%			
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	

Summary values				
	n	Mean	SD	
EtOH	6	8990.6	884.60	Total Binding, solvent control, tubes
Hot	6	48781.3	575.79	Total hot R1881 added to each tube
NSB	6	810.9	239.28	Nonspecific Binding
Specific EtOH	6	8179.7	884.60	

Assay Characterization Values		
EtOH / Hot	0.18	less than 0.1?
NSB / EtOH	0.09	around 0.25 ?

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 18.43% 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	2466.75	7646.91	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3203.59	9931.13	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3031.54	9397.78	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	350.63	1051.89	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	346.19	1038.56	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	318.77	956.30	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	441.95	1370.04	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	366.75	1136.94	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	398.35	1234.90	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	932.68	2891.30	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	938.86	2910.47	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	984.30	3051.33	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2766.53	8576.24	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2933.14	9092.73	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2629.74	8152.20	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3010.14	9331.43	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3025.44	9378.86	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3173.70	9838.46	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3264.19	10118.98	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3343.18	10363.86	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3253.79	10086.75	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	610.12	1830.35	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	668.39	2005.16	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	784.30	2352.90	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1665.82	4997.47	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1524.21	4572.63	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1628.61	4885.82	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	3154.87	9464.61	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2776.85	8330.55	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2604.99	7814.98	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3177.40	9532.21	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3204.83	9614.48	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	2997.21	8991.64	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3153.09	9459.27	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	2984.00	8951.99	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3210.79	9632.38	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3098.97	9296.91	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	2968.32	8904.96	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3352.05	10056.14	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3004.92	9014.76	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	3145.86	9437.57	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	2881.38	8644.15	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	2912.70	8738.10	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	3025.44	9076.32	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	2980.38	8941.13	TRUE	
46	1	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	924.27	2865.23	TRUE	
47	2	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	573.31	1777.27	TRUE	
48	3	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	1702.68	5278.31	TRUE	
49	1	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	285.89	886.27	TRUE	
50	2	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	314.30	974.34	TRUE	
51	3	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	421.07	1305.33	TRUE	
52	1	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	303.93	942.17	TRUE	
53	2	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	311.92	966.94	TRUE	

Values for analysis by nonlinear regression

Standard Curve

unknown

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		—	83.6	7646.91	6836.1	532.8	83.6	15.67589
2	2		—	111.5	9931.132	9120.3	-1751.4	111.5	20.35846
3	3		—	105.0	9397.78	8586.9	-1218.1	105.0	19.26511
4	1		-6.00	2.9	1051.892	241.0	7127.8	2.9	2.15634
5	2		-6.00	2.8	1038.564	227.7	7141.2	2.8	2.129019
6	3		-6.00	0.0	956.3016	145.4	7223.4	1.8	1.960384
7	1	cold R1881	-7.01	6.8	1370.041	559.2	6809.7	6.8	2.808535
8	2	cold R1881	-7.01	4.0	1136.936	326.1	7042.8	4.0	2.330678
9	3	cold R1881	-7.01	5.2	1234.898	424.0	6944.8	5.2	2.531496
10	1	cold R1881	-8.01	25.4	2891.299	2080.4	5288.4	25.4	5.927059
11	2	cold R1881	-8.01	25.7	2910.472	2099.6	5269.3	25.7	5.966362
12	3	cold R1881	-8.01	27.4	3051.327	2240.5	5128.4	27.4	6.25511
13	1	cold R1881	-9.01	94.9	8576.24	7765.4	-396.5	94.9	17.58098
14	2	cold R1881	-9.01	101.2	9092.734	8281.9	-913.0	101.2	18.63978
15	3	cold R1881	-9.01	89.8	8152.2	7341.3	27.5	89.8	16.71172
16	1	cold R1881	-10.01	104.2	9331.431	8520.6	-1151.7	104.2	19.1291
17	2	cold R1881	-10.01	104.7	9378.864	8568.0	-1199.1	104.7	19.22633
18	3	cold R1881	-10.01	110.4	9838.458	9027.6	-1658.7	110.4	20.16848
19	1	cold R1881	-11.01	113.8	10118.98	9308.1	-1939.2	113.8	20.74354
20	2	cold R1881	-11.01	116.8	10363.86	9553.0	-2184.1	116.8	21.24554
21	3	cold R1881	-11.01	113.4	10086.75	9275.9	-1907.0	113.4	20.67748
22	1	Weak Positive	-3	12.5	1830.35	1019.5	6349.4	12.5	3.752152
23	2	Weak Positive	-3	14.6	2005.159	1194.3	6174.6	14.6	4.110504
24	3	Weak Positive	-3	18.9	2352.904	1542.1	5826.8	18.9	4.823368
25	1	Weak Positive	-4	51.2	4997.472	4186.6	3182.3	51.2	10.24464
26	2	Weak Positive	-4	46.0	4572.627	3761.8	3607.1	46.0	9.373721
27	3	Weak Positive	-4	49.8	4885.821	4075.0	3293.9	49.8	10.01576
28	1	Weak Positive	-5	105.8	9464.61	8653.8	-1284.9	105.8	19.40211
29	2	Weak Positive	-5	91.9	8330.553	7519.7	-150.8	91.9	17.07733
30	3	Weak Positive	-5	85.6	7814.979	7004.1	364.7	85.6	16.02043
31	1	Weak Positive	-6	106.6	9532.209	8721.4	-1352.5	106.6	19.54069
32	2	Weak Positive	-6	107.6	9614.481	8803.6	-1434.8	107.6	19.70934
33	3	Weak Positive	-6	100.0	8991.639	8180.8	-811.9	100.0	18.43254
34	1	Weak Positive	-7	105.7	9459.273	8648.4	-1279.5	105.7	19.39117
35	2	Weak Positive	-7	99.5	8951.988	8141.1	-772.3	99.5	18.35125
36	3	Weak Positive	-7	107.8	9632.376	8821.5	-1452.6	107.8	19.74602
37	1	Weak Positive	-8	103.7	9296.913	8486.1	-1117.2	103.7	19.05834
38	2	Weak Positive	-8	99.0	8904.963	8094.1	-725.2	99.0	18.25485
39	3	Weak Positive	-8	113.0	10056.14	9245.3	-1876.4	113.0	20.61471
40	1	Weak Positive	-9	100.3	9014.757	8203.9	-835.0	100.3	18.47993
41	2	Weak Positive	-9	105.5	9437.568	8626.7	-1257.8	105.5	19.34667
42	3	Weak Positive	-9	95.8	8644.146	7833.3	-464.4	95.8	17.72019
43	1	Weak Positive	-10	96.9	8738.103	7927.3	-558.4	96.9	17.9128
44	2	Weak Positive	-10	101.0	9076.32	8265.5	-896.6	101.0	18.60613
45	3	Weak Positive	-10	99.4	8941.125	8130.3	-761.4	99.4	18.32898
46	1	Unknown 1	-3.01424	25.1	2865.23	2054.4	5314.5	25.1	5.873618
47	2	Unknown 1	-3.01424	11.8	1777.273	966.4	6402.5	11.8	3.643346
48	3	Unknown 1	-3.01424	54.6	5278.314	4467.5	2901.4	54.6	10.82035
49	1	Unknown 1	-4.01424	0.9	886.2736	75.4	7293.5	0.9	1.816829
50	2	Unknown 1	-4.01424	2.0	974.3424	163.5	7205.4	2.0	1.997367
51	3	Unknown 1	-4.01424	6.0	1305.328	494.5	6874.4	6.0	2.675876
52	1	Unknown 1	-5.01424	1.6	942.174	131.3	7237.6	1.6	1.931423
53	2	Unknown 1	-5.01424	1.9	966.9374	156.1	7212.8	1.9	1.982187

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule: 18.43% 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"	
54	3	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	246.79	765.05	TRUE		Unknown 1
55	1	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	378.68	1173.92	TRUE		Unknown 1
56	2	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	338.46	1049.24	TRUE		Unknown 1
57	3	Unknown 1	U1	4	E-1-U1	3.00E-05	300	30	10	50	310	9.7E-07	100	337.56	1046.42	TRUE		Unknown 1
58	1	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	320.16	992.48	TRUE		Unknown 1
59	2	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	347.99	1078.78	TRUE		Unknown 1
60	3	Unknown 1	U1	5	E-1-U1	3.00E-06	300	30	10	50	310	9.7E-08	100	475.74	1474.78	TRUE		Unknown 1
61	1	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	774.90	2402.19	TRUE		Unknown 1
62	2	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	893.83	2770.86	TRUE		Unknown 1
63	3	Unknown 1	U1	6	E-1-U1	3.00E-07	300	30	10	50	310	9.7E-09	100	741.41	2298.38	TRUE		Unknown 1
64	1	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2261.97	7012.09	TRUE		Unknown 1
65	2	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2310.54	7162.66	TRUE		Unknown 1
66	3	Unknown 1	U1	7	E-1-U1	3.00E-08	300	30	10	50	310	9.7E-10	100	2058.22	6380.49	TRUE		Unknown 1
67	1	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	2902.28	8997.06	TRUE		Unknown 1
68	2	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	3218.86	9978.48	TRUE		Unknown 1
69	3	Unknown 1	U1	8	E-1-U1	3.00E-09	300	30	10	50	310	9.7E-11	100	2971.18	9210.64	TRUE		Unknown 1
70	1	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	386.82	1199.13	TRUE		Unknown 2
71	2	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	392.71	1217.40	TRUE		Unknown 2
72	3	Unknown 2	U2	1	E-1-U2	3.00E-02	300	30	10	50	310	9.7E-04	100	306.24	949.33	TRUE		Unknown 2
73	1	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	354.14	1097.84	TRUE		Unknown 2
74	2	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	338.46	1049.24	TRUE		Unknown 2
75	3	Unknown 2	U2	2	E-1-U2	3.00E-03	300	30	10	50	310	9.7E-05	100	379.31	1175.87	TRUE		Unknown 2
76	1	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	377.04	1168.83	TRUE		Unknown 2
77	2	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	370.19	1147.57	TRUE		Unknown 2
78	3	Unknown 2	U2	3	E-1-U2	3.00E-04	300	30	10	50	310	9.7E-06	100	373.61	1158.20	TRUE		Unknown 2
79	1	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	518.94	1608.71	TRUE		Unknown 2
80	2	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	421.07	1305.33	TRUE		Unknown 2
81	3	Unknown 2	U2	4	E-1-U2	3.00E-05	300	30	10	50	310	9.7E-07	100	327.12	1014.06	TRUE		Unknown 2
82	1	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	597.50	1852.24	TRUE		Unknown 2
83	2	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	587.13	1820.12	TRUE		Unknown 2
84	3	Unknown 2	U2	5	E-1-U2	3.00E-06	300	30	10	50	310	9.7E-08	100	680.39	2109.20	TRUE		Unknown 2
85	1	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1772.10	5493.51	TRUE		Unknown 2
86	2	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1597.30	4951.64	TRUE		Unknown 2
87	3	Unknown 2	U2	6	E-1-U2	3.00E-07	300	30	10	50	310	9.7E-09	100	1942.52	6021.82	TRUE		Unknown 2
88	1	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	2605.55	8077.22	TRUE		Unknown 2
89	2	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	2783.26	8628.10	TRUE		Unknown 2
90	3	Unknown 2	U2	7	E-1-U2	3.00E-08	300	30	10	50	310	9.7E-10	100	2812.21	8717.85	TRUE		Unknown 2
91	1	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3011.66	9336.14	TRUE		Unknown 2
92	2	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3075.08	9532.74	TRUE		Unknown 2
93	3	Unknown 2	U2	8	E-1-U2	3.00E-09	300	30	10	50	310	9.7E-11	100	3225.93	10000.38	TRUE		Unknown 2
94	1	Unknown 3	U3	1	E-1-U3	3.00E-02	300	30	10	50	310	9.7E-04	100	3145.21	9750.14	TRUE		Unknown 3
95	2	Unknown 3	U3	1	E-1-U3	3.00E-02	300	30	10	50	310	9.7E-04	100	2432.24	7539.93	TRUE		Unknown 3
96	3	Unknown 3	U3	1	E-1-U3	3.00E-02	300	30	10	50	310	9.7E-04	100	3229.41	10011.17	TRUE		Unknown 3
97	1	Unknown 3	U3	2	E-1-U3	3.00E-03	300	30	10	50	310	9.7E-05	100	186.50	578.16	TRUE		Unknown 3
98	2	Unknown 3	U3	2	E-1-U3	3.00E-03	300	30	10	50	310	9.7E-05	100	277.00	858.71	TRUE		Unknown 3
99	3	Unknown 3	U3	2	E-1-U3	3.00E-03	300	30	10	50	310	9.7E-05	100	245.45	760.88	TRUE		Unknown 3
100	1	Unknown 3	U3	3	E-1-U3	3.00E-04	300	30	10	50	310	9.7E-06	100	274.21	850.06	TRUE		Unknown 3
101	2	Unknown 3	U3	3	E-1-U3	3.00E-04	300	30	10	50	310	9.7E-06	100	248.33	769.81	TRUE		Unknown 3
102	3	Unknown 3	U3	3	E-1-U3	3.00E-04	300	30	10	50	310	9.7E-06	100	330.60	1024.85	TRUE		Unknown 3
103	1	Unknown 3	U3	4	E-1-U3	3.00E-05	300	30	10	50	310	9.7E-07	100	1061.39	3290.31	TRUE		Unknown 3
104	2	Unknown 3	U3	4	E-1-U3	3.00E-05	300	30	10	50	310	9.7E-07	100	953.23	2955.02	TRUE		Unknown 3
105	3	Unknown 3	U3	4	E-1-U3	3.00E-05	300	30	10	50	310	9.7E-07	100	1016.15	3150.07	TRUE		Unknown 3
106	1	Unknown 3	U3	5	E-1-U3	3.00E-06	300	30	10	50	310	9.7E-08	100	2402.79	7448.64	TRUE		Unknown 3

Values for analysis by nonlinear regression
Standard Curve
unknown

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
54	3	-5.01424	-0.6	765.0493	-45.8	7414.7	-0.6	1.568324
55	1	-6.01424	4.4	1173.923	363.1	7005.8	4.4	2.4065
56	2	-6.01424	2.9	1049.239	238.4	7130.5	2.9	2.150903
57	3	-6.01424	2.9	1046.421	235.6	7133.3	2.9	2.145124
58	1	-7.01424	2.2	992.4811	181.6	7187.2	2.2	2.034551
59	2	-7.01424	3.3	1078.784	267.9	7100.9	3.3	2.211468
60	3	-7.01424	8.1	1474.783	663.9	6704.9	8.1	3.023252
61	1	-8.01424	19.5	2402.195	1591.3	5777.5	19.5	4.924413
62	2	-8.01424	24.0	2770.859	1960.0	5408.9	24.0	5.680162
63	3	-8.01424	18.2	2298.38	1487.5	5881.3	18.2	4.711597
64	1	-9.01424	75.8	7012.095	6201.2	1167.6	75.8	14.37454
65	2	-9.01424	77.7	7162.662	6351.8	1017.1	77.7	14.6832
66	3	-9.01424	68.1	6380.491	5569.6	1799.2	68.1	13.07978
67	1	-10.01424	100.1	8997.059	8186.2	-817.3	100.1	18.44365
68	2	-10.01424	112.1	9978.478	9167.6	-1798.8	112.1	20.45552
69	3	-10.01424	102.7	9210.643	8399.8	-1030.9	102.7	18.88149
70	1	-3.01424	4.7	1199.131	388.3	6980.6	4.7	2.458174
71	2	-3.01424	5.0	1217.402	406.5	6962.3	5.0	2.49563
72	3	-3.01424	1.7	949.3297	138.5	7230.4	1.7	1.946092
73	1	-4.01424	3.5	1097.836	287.0	7081.9	3.5	2.250523
74	2	-4.01424	2.9	1049.239	238.4	7130.5	2.9	2.150903
75	3	-4.01424	4.5	1175.874	365.0	7003.9	4.5	2.4105
76	1	-5.01424	4.4	1168.825	358.0	7010.9	4.4	2.39605
77	2	-5.01424	4.1	1147.574	336.7	7032.2	4.1	2.352486
78	3	-5.01424	4.2	1158.2	347.3	7021.5	4.2	2.374268
79	1	-6.01424	9.8	1608.71	797.9	6571.0	9.8	3.297797
80	2	-6.01424	6.0	1305.328	494.5	6874.4	6.0	2.675876
81	3	-6.01424	2.5	1014.057	203.2	7165.7	2.5	2.07878
82	1	-7.01424	12.7	1852.238	1041.4	6327.5	12.7	3.797022
83	2	-7.01424	12.3	1820.118	1009.3	6359.6	12.3	3.731177
84	3	-7.01424	15.9	2109.196	1298.3	6070.5	15.9	4.323776
85	1	-8.01424	57.2	5493.507	4682.7	2686.2	57.2	11.26149
86	2	-8.01424	50.6	4951.642	4140.8	3228.1	50.6	10.15069
87	3	-8.01424	63.7	6021.824	5211.0	2157.9	63.7	12.34452
88	1	-9.01424	88.8	8077.217	7266.4	102.5	88.8	16.558
89	2	-9.01424	95.6	8628.103	7817.3	-448.4	95.6	17.6873
90	3	-9.01424	96.7	8717.848	7907.0	-538.1	96.7	17.87127
91	1	-10.01424	104.2	9336.137	8525.3	-1156.4	104.2	19.13874
92	2	-10.01424	106.6	9532.742	8721.9	-1353.0	106.6	19.54178
93	3	-10.01424	112.3	10000.38	9189.5	-1820.7	112.3	20.50042
94	1	-3.01424	109.3	9750.136	8939.3	-1570.4	109.3	19.98743
95	2	-3.01424	82.3	7539.929	6729.1	639.8	82.3	15.45658
96	3	-3.01424	112.5	10011.17	9200.3	-1831.4	112.5	20.52253
97	1	-4.01424	-2.8	578.1553	-232.7	7601.6	-2.8	1.185197
98	2	-4.01424	0.6	858.7078	47.9	7321.0	0.6	1.76032
99	3	-4.01424	-0.6	760.8801	-50.0	7418.8	-0.6	1.559777
100	1	-5.01424	0.5	850.0591	39.2	7329.7	0.5	1.74259
101	2	-5.01424	-0.5	769.8109	-41.0	7409.9	-0.5	1.578085
102	3	-5.01424	2.6	1024.85	214.0	7154.9	2.6	2.100906
103	1	-6.01424	30.3	3290.309	2479.5	4889.4	30.3	6.745015
104	2	-6.01424	26.2	2955.016	2144.2	5224.7	26.2	6.057676
105	3	-6.01424	28.6	3150.065	2339.2	5029.7	28.6	6.45752
106	1	-7.01424	81.1	7448.643	6637.8	731.1	81.1	15.26945

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	18.43%	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"	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2454.20	7608.03	TRUE		Unknown 3
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2419.39	7500.11	TRUE		Unknown 3
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2801.61	8684.99	TRUE		Unknown 3
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3020.65	9364.02	TRUE		Unknown 3
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2921.73	9057.36	TRUE		Unknown 3
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2937.50	9106.26	TRUE		Unknown 3
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3038.32	9418.78	TRUE		Unknown 3
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2819.11	8739.25	TRUE		Unknown 3
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3091.31	9583.06	TRUE		Unknown 3
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2849.35	8832.97	TRUE		Unknown 3
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2876.98	8918.63	TRUE		Unknown 3
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	317.75	985.01	TRUE		Unknown 4
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	448.99	1391.86	TRUE		Unknown 4
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	379.32	1175.89	TRUE		Unknown 4
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	229.68	712.00	TRUE		Unknown 4
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	196.86	610.28	TRUE		Unknown 4
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	233.08	722.55	TRUE		Unknown 4
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	219.24	679.64	TRUE		Unknown 4
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	236.64	733.58	TRUE		Unknown 4
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	193.41	599.57	TRUE		Unknown 4
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	407.54	1263.38	TRUE		Unknown 4
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	368.17	1141.33	TRUE		Unknown 4
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	424.56	1316.13	TRUE		Unknown 4
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	986.51	3058.17	TRUE		Unknown 4
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	932.51	2890.79	TRUE		Unknown 4
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	1036.13	3211.99	TRUE		Unknown 4
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2211.14	6854.52	TRUE		Unknown 4
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2401.19	7443.69	TRUE		Unknown 4
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2293.17	7108.83	TRUE		Unknown 4
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2814.81	8725.91	TRUE		Unknown 4
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2811.83	8716.67	TRUE		Unknown 4
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3061.34	9490.14	TRUE		Unknown 4
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2994.40	9282.65	TRUE		Unknown 4
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3135.47	9719.94	TRUE		Unknown 4
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2966.77	9197.00	TRUE		Unknown 4
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	532.97	1652.21	TRUE		Unknown 5
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	490.89	1521.77	TRUE		Unknown 5
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	554.01	1717.42	TRUE		Unknown 5
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	1541.63	4779.06	TRUE		Unknown 5
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	1672.71	5185.39	TRUE		Unknown 5
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	1644.58	5098.19	TRUE		Unknown 5
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2662.84	8254.82	TRUE		Unknown 5
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2891.87	8964.79	TRUE		Unknown 5
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2731.80	8468.59	TRUE		Unknown 5
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2982.36	9245.33	TRUE		Unknown 5
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2932.36	9090.32	TRUE		Unknown 5
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2947.56	9137.45	TRUE		Unknown 5
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2989.32	9266.90	TRUE		Unknown 5
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2914.70	9035.55	TRUE		Unknown 5
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3070.15	9517.45	TRUE		Unknown 5
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2891.88	8964.84	TRUE		Unknown 5
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3133.16	9712.79	TRUE		Unknown 5
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2961.53	9180.74	TRUE		Unknown 5

Values for analysis by nonlinear regression
Standard Curve
unknown

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
107	2	-7.01424	83.1	7608.029	6797.2	571.7	83.1	15.59619
108	3	-7.01424	81.8	7500.106	6689.3	679.6	81.8	15.37495
109	1	-8.01424	96.3	8684.991	7874.1	-505.3	96.3	17.80392
110	2	-8.01424	104.6	9364.021	8553.2	-1184.3	104.6	19.19591
111	3	-8.01424	100.8	9057.363	8246.5	-877.6	100.8	18.56727
112	1	-9.01424	101.4	9106.259	8295.4	-926.5	101.4	18.6675
113	2	-9.01424	105.2	9418.78	8607.9	-1239.1	105.2	19.30816
114	3	-9.01424	96.9	8739.253	7928.4	-559.5	96.9	17.91515
115	1	-10.01424	107.2	9583.064	8772.2	-1403.3	107.2	19.64494
116	2	-10.01424	98.1	8832.973	8022.1	-653.2	98.1	18.10728
117	3	-10.01424	99.1	8918.626	8107.8	-738.9	99.1	18.28286
118	1	-3.01424	2.1	985.0104	174.2	7194.7	2.1	2.019236
119	2	-3.01424	7.1	1391.862	581.0	6787.9	7.1	2.853268
120	3	-3.01424	4.5	1175.887	365.0	7003.8	4.5	2.410525
121	1	-4.01424	-1.2	712.0049	-98.8	7467.7	-1.2	1.459584
122	2	-4.01424	-2.5	610.2781	-200.6	7569.5	-2.5	1.251048
123	3	-4.01424	-1.1	722.5542	-88.3	7457.2	-1.1	1.48121
124	1	-5.01424	-1.6	679.6409	-131.2	7500.1	-1.6	1.393239
125	2	-5.01424	-0.9	733.5809	-77.3	7446.1	-0.9	1.503814
126	3	-5.01424	-2.6	599.5716	-211.3	7580.2	-2.6	1.2291
127	1	-6.01424	5.5	1263.383	452.5	6916.3	5.5	2.58989
128	2	-6.01424	4.0	1141.326	330.5	7038.4	4.0	2.339678
129	3	-6.01424	6.2	1316.13	505.3	6863.6	6.2	2.698019
130	1	-7.01424	27.5	3058.169	2247.3	5121.6	27.5	6.269136
131	2	-7.01424	25.4	2890.792	2079.9	5288.9	25.4	5.926019
132	3	-7.01424	29.4	3211.991	2401.1	4967.7	29.4	6.584465
133	1	-8.01424	73.9	6854.519	6043.7	1325.2	73.9	14.05152
134	2	-8.01424	81.1	7443.686	6632.8	736.0	81.1	15.25929
135	3	-8.01424	77.0	7108.833	6298.0	1070.9	77.0	14.57285
136	1	-9.01424	96.8	8725.908	7915.1	-546.2	96.8	17.8878
137	2	-9.01424	96.7	8716.667	7905.8	-536.9	96.7	17.86885
138	3	-9.01424	106.1	9490.139	8679.3	-1310.4	106.1	19.45444
139	1	-10.01424	103.6	9282.652	8471.8	-1102.9	103.6	19.0291
140	2	-10.01424	108.9	9719.945	8909.1	-1540.2	108.9	19.92554
141	3	-10.01424	102.5	9196.999	8386.1	-1017.3	102.5	18.85352
142	1	-3.01424	10.3	1652.206	841.4	6527.5	10.3	3.386962
143	2	-3.01424	8.7	1521.768	710.9	6658.0	8.7	3.11957
144	3	-3.01424	11.1	1717.424	906.6	6462.3	11.1	3.520658
145	1	-4.01424	48.5	4779.062	3968.2	3400.7	48.5	9.796906
146	2	-4.01424	53.5	5185.389	4374.5	2994.3	53.5	10.62986
147	3	-4.01424	52.4	5098.192	4287.3	3081.5	52.4	10.45111
148	1	-5.01424	91.0	8254.816	7444.0	-75.1	91.0	16.92208
149	2	-5.01424	99.7	8964.788	8153.9	-785.1	99.7	18.37749
150	3	-5.01424	93.6	8468.586	7657.7	-288.9	93.6	17.3603
151	1	-6.01424	103.1	9245.325	8434.5	-1065.6	103.1	18.95258
152	2	-6.01424	101.2	9090.316	8279.5	-910.6	101.2	18.63482
153	3	-6.01424	101.8	9137.445	8326.6	-957.7	101.8	18.73143
154	1	-7.01424	103.4	9266.901	8456.0	-1087.2	103.4	18.99681
155	2	-7.01424	100.5	9035.555	8224.7	-855.8	100.5	18.52256
156	3	-7.01424	106.4	9517.453	8706.6	-1337.7	106.4	19.51043
157	1	-8.01424	99.7	8964.837	8154.0	-785.1	99.7	18.37759
158	2	-8.01424	108.8	9712.79	8901.9	-1533.1	108.8	19.91087
159	3	-8.01424	102.3	9180.737	8369.9	-1001.0	102.3	18.82018

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	18.43%	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"	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2903.30	9000.22	TRUE		Unknown 5
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3215.37	9967.64	TRUE		Unknown 5
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2790.65	8651.01	TRUE		Unknown 5
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3009.52	9329.50	TRUE		Unknown 5
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3077.21	9539.35	TRUE		Unknown 5
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2940.60	9115.87	TRUE		Unknown 5
166	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2956.43	9164.93	TRUE		
167	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3100.68	9612.12	TRUE		
168	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2642.14	8190.62	TRUE		
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	222.72	668.16	TRUE		
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	227.95	683.85	TRUE		
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	155.45	466.35	TRUE		
172	1	none	Hot		—	—	—	30	—	—	—	—		49200.54	49200.54	TRUE		
173	2	none	Hot		—	—	—	30	—	—	—	—		47958.16	47958.16	TRUE		
174	3	none	Hot		—	—	—	30	—	—	—	—		48394.30	48394.30	TRUE		
175	1	none	Hot		—	—	—	30	—	—	—	—		49043.73	49043.73	TRUE		
176	2	none	Hot		—	—	—	30	—	—	—	—		48573.48	48573.48	TRUE		
177	3	none	Hot		—	—	—	30	—	—	—	—		49517.86	49517.86	TRUE		

Values for analysis by nonlinear regression
Standard Curve
unknown

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
160	1	-9.01424	100.1	9000.221	8189.4	-820.5	100.1	18.45013
161	2	-9.01424	111.9	9967.638	9156.8	-1787.9	111.9	20.4333
162	3	-9.01424	95.8	8651.009	7840.2	-471.3	95.8	17.73426
163	1	-10.01424	104.1	9329.5	8518.6	-1149.8	104.1	19.12514
164	2	-10.01424	106.7	9539.354	8728.5	-1359.6	106.7	19.55533
165	3	-10.01424	101.5	9115.869	8305.0	-936.1	101.5	18.6872
166	1	—	102.1	9164.927	8354.1	-985.2	102.1	18.78777
167	2	—	107.6	9612.117	8801.3	-1432.4	107.6	19.70449
168	3	—	90.2	8190.619	7379.8	-10.9	90.2	16.79047
169	1	-6.00	-1.7	668.1606	-142.7	7511.6	-1.7	1.369705
170	2	-6.00	-1.6	683.8467	-127.0	7495.9	-1.6	1.401861
171	3	-6.00	-4.2	466.3512	-344.5	7713.4	-4.2	0.956003
172	1			49200.54	48389.7			
173	2			47958.16	47147.3			
174	3			48394.3	47583.4			
175	1			49043.73	48232.9			
176	2			48573.48	47762.6			
177	3			49517.86	48707.0			

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:	**C**			
Run identification:	5 (Seq#6953)			
Assay start date:	11/29/2005			
Tracer lot number:	3559-507			
Specific activity on day of assay:	79.21	**Ci/mmole**		
Cytosol vial or lot identification:	0725-06-01			
Protein (cytosol):	600	**micro gram per tube**		
Standard Curve IC50:	6.54E-09	**M**		
Weak Positive, Max Concentration:	**3.00E-02**	**M**		
Weak Positive IC50:	1.31E-04	**M**		
RBA:	4.99084E-05			
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)	
IC50, Unknown 1:	4.94E-09	**CR42400**		
RBA, Unknown 1:	132.37497%			
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 2:	3.07E-08	**CR42401**		
RBA, Unknown 2:	21.32420%			
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 3:	5.26E-07	**CR42402**		
RBA, Unknown 3:	1.24391%			
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 4:	5.09E-08	**CR42403**		
RBA, Unknown 4:	12.84984%			
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 5:	4.96E-05	**CR42404**		
RBA, Unknown 5:	0.01319%			
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

Summary values				
	n	Mean	SD	
EtOH	6	5655.7	1736.76	Total Binding, solvent control, tubes
Hot	6	47517.7	735.44	Total hot R1881 added to each tube
NSB	6	623.6	100.30	Nonspecific Binding
Specific EtOH	6	5032.1	1736.76	

Assay Characterization Values		
EtOH / Hot	0.12	less than 0.1?
NSB / EtOH	0.11	around 0.25 ?

Column O, Rows 10 through 28 will contain output parameters

working volume **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 11.90% 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-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	1478.64	4583.77	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2585.57	8015.28	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2503.92	7762.15	TRUE	
4	1	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	221.11	663.33	TRUE	
5	2	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	247.07	741.22	TRUE	
6	3	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	238.30	714.91	TRUE	
7	1	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	583.67	1809.38	TRUE	
8	2	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	272.13	843.62	TRUE	
9	3	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	361.15	1119.56	TRUE	
10	1	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	673.53	2087.95	TRUE	
11	2	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	610.11	1891.35	TRUE	
12	3	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	658.10	2040.11	TRUE	
13	1	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1909.89	5920.64	TRUE	
14	2	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1948.76	6041.16	TRUE	
15	3	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1851.18	5738.66	TRUE	
16	1	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2289.34	7096.95	TRUE	
17	2	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2068.77	6413.17	TRUE	
18	3	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2221.10	6885.41	TRUE	
19	1	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2615.90	8109.29	TRUE	
20	2	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2724.79	8446.83	TRUE	
21	3	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2455.58	7612.30	TRUE	
22	1	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	300.45	901.36	TRUE	
23	2	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	397.60	1192.81	TRUE	
24	3	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	330.84	992.51	TRUE	
25	1	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1117.42	3352.25	TRUE	
26	2	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1247.65	3742.94	TRUE	
27	3	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1137.67	3413.02	TRUE	
28	1	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	1938.95	5816.86	TRUE	
29	2	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	1692.30	5076.91	TRUE	
30	3	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	1822.98	5468.93	TRUE	
31	1	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2262.22	6786.66	TRUE	
32	2	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	982.38	2947.15	TRUE	
33	3	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2505.97	7517.90	TRUE	
34	1	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2347.23	7041.69	TRUE	
35	2	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2201.08	6603.23	TRUE	
36	3	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	1775.71	5327.14	TRUE	
37	1	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2108.23	6324.70	TRUE	
38	2	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2037.61	6112.84	TRUE	
39	3	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2573.44	7720.33	TRUE	
40	1	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	2251.81	6755.44	TRUE	
41	2	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	2361.63	7084.90	TRUE	
42	3	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	2722.93	8168.80	TRUE	
43	1	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2223.04	6669.11	TRUE	
44	2	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2367.19	7101.57	TRUE	
45	3	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2565.68	7697.05	TRUE	
46	1	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	928.89	2879.55	TRUE	
47	2	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	842.70	2612.38	TRUE	
48	3	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	1302.05	4036.36	TRUE	
49	1	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	178.24	552.54	TRUE	
50	2	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	162.32	503.21	TRUE	
51	3	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	176.14	546.03	TRUE	
52	1	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	241.76	749.46	TRUE	
53	2	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	176.14	546.03	TRUE	

Values for analysis by nonlinear regression

Standard Curve

unknown

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		—	78.7	4583.772	3960.2	448.4	78.7	9.646459
2	2		—	146.9	8015.279	7391.7	-2983.2	146.9	16.868
3	3		—	141.9	7762.146	7138.6	-2730.0	141.9	16.33529
4	1		-6.00	0.8	663.3264	39.7	4368.8	0.8	1.395958
5	2		-6.00	2.3	741.2211	117.6	4290.9	2.3	1.559885
6	3		-6.00	0.0	714.9117	91.3	4317.2	1.8	1.504518
7	1	cold R1881	-7.01	23.6	1809.383	1185.8	3222.7	23.6	3.807811
8	2	cold R1881	-7.01	4.4	843.6151	220.0	4188.5	4.4	1.775372
9	3	cold R1881	-7.01	9.9	1119.563	496.0	3912.6	9.9	2.356099
10	1	cold R1881	-8.01	29.1	2087.947	1464.4	2944.2	29.1	4.394044
11	2	cold R1881	-8.01	25.2	1891.352	1267.8	3140.8	25.2	3.980313
12	3	cold R1881	-8.01	28.1	2040.11	1416.5	2992.0	28.1	4.293371
13	1	cold R1881	-9.01	105.3	5920.644	5297.1	-888.5	105.3	12.45988
14	2	cold R1881	-9.01	107.7	6041.159	5417.6	-1009.0	107.7	12.7135
15	3	cold R1881	-9.01	101.6	5738.664	5115.1	-706.5	101.6	12.07691
16	1	cold R1881	-10.01	128.6	7096.948	6473.4	-2064.8	128.6	14.93539
17	2	cold R1881	-10.01	115.1	6413.172	5789.6	-1381.0	115.1	13.4964
18	3	cold R1881	-10.01	124.4	6885.407	6261.8	-1853.3	124.4	14.49021
19	1	cold R1881	-11.01	148.8	8109.293	7485.7	-3077.2	148.8	17.06585
20	2	cold R1881	-11.01	155.5	8446.834	7823.2	-3414.7	155.5	17.7762
21	3	cold R1881	-11.01	138.9	7612.298	6988.7	-2580.2	138.9	16.01993
22	1	Weak Positive	-3	5.5	901.3605	277.8	4130.8	5.5	1.896896
23	2	Weak Positive	-3	11.3	1192.806	569.2	3839.3	11.3	2.510238
24	3	Weak Positive	-3	7.3	992.5092	368.9	4039.6	7.3	2.088716
25	1	Weak Positive	-4	54.2	3352.251	2728.7	1679.9	54.2	7.054747
26	2	Weak Positive	-4	62.0	3742.944	3119.4	1289.2	62.0	7.876953
27	3	Weak Positive	-4	55.4	3413.016	2789.4	1619.1	55.4	7.182626
28	1	Weak Positive	-5	103.2	5816.862	5193.3	-784.7	103.2	12.24147
29	2	Weak Positive	-5	88.5	5076.909	4453.3	-44.8	88.5	10.68426
30	3	Weak Positive	-5	96.3	5468.928	4845.3	-436.8	96.3	11.50925
31	1	Weak Positive	-6	122.5	6786.657	6163.1	-1754.5	122.5	14.28239
32	2	Weak Positive	-6	46.2	2947.145	2323.6	2085.0	46.2	6.20221
33	3	Weak Positive	-6	137.0	7517.898	6894.3	-2485.8	137.0	15.82127
34	1	Weak Positive	-7	127.5	7041.687	6418.1	-2009.6	127.5	14.81909
35	2	Weak Positive	-7	118.8	6603.225	5979.6	-1571.1	118.8	13.89636
36	3	Weak Positive	-7	93.5	5327.142	4703.6	-295.0	93.5	11.21087
37	1	Weak Positive	-8	113.3	6324.699	5701.1	-1292.6	113.3	13.31021
38	2	Weak Positive	-8	109.1	6112.839	5489.2	-1080.7	109.1	12.86435
39	3	Weak Positive	-8	141.0	7720.332	7096.7	-2688.2	141.0	16.24729
40	1	Weak Positive	-9	121.9	6755.436	6131.8	-1723.3	121.9	14.21668
41	2	Weak Positive	-9	128.4	7084.896	6461.3	-2052.8	128.4	14.91003
42	3	Weak Positive	-9	149.9	8168.799	7545.2	-3136.7	149.9	17.19108
43	1	Weak Positive	-10	120.1	6669.105	6045.5	-1637.0	120.1	14.035
44	2	Weak Positive	-10	128.7	7101.567	6478.0	-2069.4	128.7	14.94511
45	3	Weak Positive	-10	140.6	7697.052	7073.5	-2664.9	140.6	16.1983
46	1	Unknown 1	-3.01424	44.8	2879.545	2256.0	2152.6	44.8	6.059948
47	2	Unknown 1	-3.01424	39.5	2612.378	1988.8	2419.7	39.5	5.497698
48	3	Unknown 1	-3.01424	67.8	4036.358	3412.8	995.8	67.8	8.494438
49	1	Unknown 1	-4.01424	-1.4	552.5356	-71.1	4479.6	-1.4	1.162801
50	2	Unknown 1	-4.01424	-2.4	503.2066	-120.4	4528.9	-2.4	1.058988
51	3	Unknown 1	-4.01424	-1.5	546.0328	-77.6	4486.1	-1.5	1.149115
52	1	Unknown 1	-5.01424	2.5	749.4566	125.9	4282.7	2.5	1.577217
53	2	Unknown 1	-5.01424	-1.5	546.0328	-77.6	4486.1	-1.5	1.149115

Column O, Rows 10 through 28 will contain output parameters

working volume **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule:	11.90%	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"	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	173.49	537.81	TRUE		Unknown 1
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	182.30	565.13	TRUE		Unknown 1
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	241.76	749.46	TRUE		Unknown 1
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	236.64	733.57	TRUE		Unknown 1
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	276.83	858.16	TRUE		Unknown 1
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	287.92	892.56	TRUE		Unknown 1
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	267.36	828.80	TRUE		Unknown 1
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	543.52	1684.92	TRUE		Unknown 1
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	682.10	2114.51	TRUE		Unknown 1
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	493.88	1531.03	TRUE		Unknown 1
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	1765.61	5473.38	TRUE		Unknown 1
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	1823.51	5652.87	TRUE		Unknown 1
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	1666.84	5167.20	TRUE		Unknown 1
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2203.47	6830.76	TRUE		Unknown 1
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2089.50	6477.45	TRUE		Unknown 1
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2359.77	7315.28	TRUE		Unknown 1
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	178.24	552.54	TRUE		Unknown 2
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	185.09	573.79	TRUE		Unknown 2
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	196.36	608.70	TRUE		Unknown 2
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	210.68	653.10	TRUE		Unknown 2
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	196.86	610.27	TRUE		Unknown 2
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	202.23	626.92	TRUE		Unknown 2
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	298.04	923.92	TRUE		Unknown 2
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	292.55	906.90	TRUE		Unknown 2
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	205.32	636.48	TRUE		Unknown 2
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	260.50	807.55	TRUE		Unknown 2
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	238.43	739.14	TRUE		Unknown 2
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	281.88	873.82	TRUE		Unknown 2
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	473.16	1466.80	TRUE		Unknown 2
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	587.13	1820.12	TRUE		Unknown 2
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	511.15	1584.57	TRUE		Unknown 2
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	1264.81	3920.90	TRUE		Unknown 2
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	1523.54	4722.97	TRUE		Unknown 2
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	1481.65	4593.12	TRUE		Unknown 2
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2060.14	6386.43	TRUE		Unknown 2
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2042.88	6332.94	TRUE		Unknown 2
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2108.86	6537.47	TRUE		Unknown 2
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2352.77	7293.58	TRUE		Unknown 2
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	1837.12	5695.08	TRUE		Unknown 2
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2382.22	7384.89	TRUE		Unknown 2
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2034.25	6306.18	TRUE		Unknown 3
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	2224.21	6895.04	TRUE		Unknown 3
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	1752.11	5431.54	TRUE		Unknown 3
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	214.13	663.81	TRUE		Unknown 3
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	191.95	595.04	TRUE		Unknown 3
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	161.10	499.41	TRUE		Unknown 3
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	210.91	653.81	TRUE		Unknown 3
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	272.85	845.82	TRUE		Unknown 3
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	311.92	966.94	TRUE		Unknown 3
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	733.52	2273.91	TRUE		Unknown 3
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	870.34	2698.06	TRUE		Unknown 3
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	813.48	2521.77	TRUE		Unknown 3
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	1408.77	4367.18	TRUE		Unknown 3

Values for analysis by nonlinear regression

Standard Curve

unknown

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
54	3	-5.01424	-1.7	537.8103	-85.8	4494.3	-1.7	1.131811
55	1	-6.01424	-1.2	565.1288	-58.5	4467.0	-1.2	1.189303
56	2	-6.01424	2.5	749.4566	125.9	4282.7	2.5	1.577217
57	3	-6.01424	2.2	733.5732	110.0	4298.6	2.2	1.543791
58	1	-7.01424	4.7	858.1584	234.6	4174.0	4.7	1.805978
59	2	-7.01424	5.3	892.5576	269.0	4139.6	5.3	1.87837
60	3	-7.01424	4.1	828.8033	205.2	4203.3	4.1	1.7442
61	1	-8.01424	21.1	1684.92	1061.3	3347.2	21.1	3.545882
62	2	-8.01424	29.6	2114.511	1490.9	2917.6	29.6	4.449948
63	3	-8.01424	18.0	1531.033	907.4	3501.1	18.0	3.222029
64	1	-9.01424	96.4	5473.376	4849.8	-441.3	96.4	11.51861
65	2	-9.01424	99.9	5652.866	5029.3	-620.7	99.9	11.89635
66	3	-9.01424	90.3	5167.198	4543.6	-135.1	90.3	10.87427
67	1	-10.01424	123.4	6830.76	6207.2	-1798.6	123.4	14.3752
68	2	-10.01424	116.3	6477.447	5853.9	-1445.3	116.3	13.63166
69	3	-10.01424	133.0	7315.281	6691.7	-2283.2	133.0	15.39487
70	1	-3.01424	-1.4	552.5356	-71.1	4479.6	-1.4	1.162801
71	2	-3.01424	-1.0	573.7871	-49.8	4458.3	-1.0	1.207524
72	3	-3.01424	-0.3	608.7008	-14.9	4423.4	-0.3	1.280999
73	1	-4.01424	0.6	653.0978	29.5	4379.0	0.6	1.374432
74	2	-4.01424	-0.3	610.2719	-13.3	4421.9	-0.3	1.284305
75	3	-4.01424	0.1	626.9155	3.3	4405.2	0.1	1.319332
76	1	-5.01424	6.0	923.9212	300.3	4108.2	6.0	1.944374
77	2	-5.01424	5.6	906.8957	283.3	4125.2	5.6	1.908544
78	3	-5.01424	0.3	636.4824	12.9	4395.6	0.3	1.339465
79	1	-6.01424	3.7	807.5522	184.0	4224.6	3.7	1.699478
80	2	-6.01424	2.3	739.137	115.5	4293.0	2.3	1.5555
81	3	-6.01424	5.0	873.8196	250.2	4158.3	5.0	1.838937
82	1	-7.01424	16.8	1466.801	843.2	3565.3	16.8	3.086855
83	2	-7.01424	23.8	1820.118	1196.5	3212.0	23.8	3.830404
84	3	-7.01424	19.1	1584.574	961.0	3447.6	19.1	3.334705
85	1	-8.01424	65.5	3920.899	3297.3	1111.2	65.5	8.251455
86	2	-8.01424	81.5	4722.974	4099.4	309.2	81.5	9.939407
87	3	-8.01424	78.9	4593.121	3969.5	439.0	78.9	9.666135
88	1	-9.01424	114.5	6386.434	5762.8	-1354.3	114.5	13.44013
89	2	-9.01424	113.5	6332.94	5709.3	-1300.8	113.5	13.32755
90	3	-9.01424	117.5	6537.466	5913.9	-1505.3	117.5	13.75797
91	1	-10.01424	132.5	7293.581	6670.0	-2261.5	132.5	15.3492
92	2	-10.01424	100.8	5695.078	5071.5	-663.0	100.8	11.98518
93	3	-10.01424	134.4	7384.888	6761.3	-2352.8	134.4	15.54135
94	1	-3.01424	112.9	6306.175	5682.6	-1274.0	112.9	13.27122
95	2	-3.01424	124.6	6895.036	6271.4	-1862.9	124.6	14.51047
96	3	-3.01424	95.5	5431.544	4808.0	-399.4	95.5	11.43058
97	1	-4.01424	0.8	663.808	40.2	4368.3	0.8	1.396971
98	2	-4.01424	-0.6	595.0416	-28.6	4437.1	-0.6	1.252254
99	3	-4.01424	-2.5	499.4097	-124.2	4532.7	-2.5	1.050998
100	1	-5.01424	0.6	653.812	30.2	4378.3	0.6	1.375935
101	2	-5.01424	4.4	845.8198	222.2	4186.3	4.4	1.780011
102	3	-5.01424	6.8	966.9424	343.4	4065.2	6.8	2.034912
103	1	-6.01424	32.8	2273.909	1650.3	2758.2	32.8	4.785397
104	2	-6.01424	41.2	2698.058	2074.5	2334.1	41.2	5.67801
105	3	-6.01424	37.7	2521.774	1898.2	2510.4	37.7	5.307025
106	1	-7.01424	74.4	4367.181	3743.6	664.9	74.4	9.190648

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule:	11.90%	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"	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	1486.56	4608.32	TRUE		Unknown 3
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	1546.18	4793.15	TRUE		Unknown 3
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	1916.82	5942.15	TRUE		Unknown 3
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	1899.55	5888.62	TRUE		Unknown 3
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	1870.95	5799.95	TRUE		Unknown 3
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	1867.55	5789.40	TRUE		Unknown 3
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	1957.19	6067.30	TRUE		Unknown 3
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	1853.94	5747.22	TRUE		Unknown 3
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2047.85	6348.34	TRUE		Unknown 3
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	1785.92	5536.34	TRUE		Unknown 3
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	1760.87	5458.70	TRUE		Unknown 3
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	285.36	884.61	TRUE		Unknown 4
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	366.10	1134.90	TRUE		Unknown 4
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	404.09	1252.68	TRUE		Unknown 4
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	155.42	481.80	TRUE		Unknown 4
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	229.68	712.00	TRUE		Unknown 4
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	229.65	711.93	TRUE		Unknown 4
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	241.94	750.01	TRUE		Unknown 4
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	243.60	755.16	TRUE		Unknown 4
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	262.49	813.70	TRUE		Unknown 4
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	321.20	995.72	TRUE		Unknown 4
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	328.11	1017.13	TRUE		Unknown 4
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	350.38	1086.18	TRUE		Unknown 4
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	775.93	2405.38	TRUE		Unknown 4
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	861.11	2669.44	TRUE		Unknown 4
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	877.76	2721.05	TRUE		Unknown 4
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	1319.90	4091.69	TRUE		Unknown 4
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	1602.19	4966.78	TRUE		Unknown 4
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	1398.09	4334.07	TRUE		Unknown 4
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	1584.66	4912.43	TRUE		Unknown 4
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	1222.07	3788.41	TRUE		Unknown 4
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	1714.44	5314.77	TRUE		Unknown 4
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	1123.74	3483.61	TRUE		Unknown 4
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	1258.62	3901.72	TRUE		Unknown 4
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	1319.97	4091.91	TRUE		Unknown 4
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	425.02	1317.57	TRUE		Unknown 5
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	449.02	1391.96	TRUE		Unknown 5
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	604.28	1873.28	TRUE		Unknown 5
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	937.56	2906.44	TRUE		Unknown 5
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	712.31	2208.18	TRUE		Unknown 5
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	702.19	2176.78	TRUE		Unknown 5
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	1299.72	4029.14	TRUE		Unknown 5
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	1595.86	4947.16	TRUE		Unknown 5
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	1549.29	4802.78	TRUE		Unknown 5
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	1500.91	4652.81	TRUE		Unknown 5
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	1255.84	3893.09	TRUE		Unknown 5
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	1253.73	3886.56	TRUE		Unknown 5
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	1400.40	4341.24	TRUE		Unknown 5
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	1346.51	4174.17	TRUE		Unknown 5
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	1367.48	4239.19	TRUE		Unknown 5
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	1397.62	4332.63	TRUE		Unknown 5
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	1432.11	4439.55	TRUE		Unknown 5
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	1288.23	3993.51	TRUE		Unknown 5

Values for analysis by nonlinear regression

Standard Curve

unknown

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
107	2	-7.01424	79.2	4608.321	3984.7	423.8	79.2	9.698121
108	3	-7.01424	82.9	4793.152	4169.6	239.0	82.9	10.0871
109	1	-8.01424	105.7	5942.151	5318.6	-910.0	105.7	12.50514
110	2	-8.01424	104.6	5888.617	5265.0	-856.5	104.6	12.39248
111	3	-8.01424	102.9	5799.945	5176.4	-767.8	102.9	12.20587
112	1	-9.01424	102.7	5789.399	5165.8	-757.3	102.7	12.18368
113	2	-9.01424	108.2	6067.298	5443.7	-1035.2	108.2	12.76851
114	3	-9.01424	101.8	5747.217	5123.6	-715.1	101.8	12.09491
115	1	-10.01424	113.8	6348.338	5724.7	-1316.2	113.8	13.35995
116	2	-10.01424	97.6	5536.34	4912.7	-504.2	97.6	11.65112
117	3	-10.01424	96.1	5458.703	4835.1	-426.6	96.1	11.48774
118	1	-3.01424	5.2	884.6123	261.0	4147.5	5.2	1.861649
119	2	-3.01424	10.2	1134.903	511.3	3897.2	10.2	2.388382
120	3	-3.01424	12.5	1252.676	629.1	3779.4	12.5	2.636233
121	1	-4.01424	-2.8	481.7986	-141.8	4550.3	-2.8	1.013936
122	2	-4.01424	1.8	712.0049	88.4	4320.1	1.8	1.498401
123	3	-4.01424	1.8	711.9283	88.3	4320.2	1.8	1.498239
124	1	-5.01424	2.5	750.0146	126.4	4282.1	2.5	1.578391
125	2	-5.01424	2.6	755.1566	131.6	4277.0	2.6	1.589212
126	3	-5.01424	3.8	813.7044	190.1	4218.4	3.8	1.712425
127	1	-6.01424	7.4	995.7172	372.1	4036.4	7.4	2.095468
128	2	-6.01424	7.8	1017.13	393.5	4015.0	7.8	2.140531
129	3	-6.01424	9.2	1086.177	462.6	3945.9	9.2	2.285839
130	1	-7.01424	35.4	2405.383	1781.8	2626.7	35.4	5.062081
131	2	-7.01424	40.7	2669.439	2045.8	2362.7	40.7	5.617783
132	3	-7.01424	41.7	2721.05	2097.5	2311.1	41.7	5.726397
133	1	-8.01424	68.9	4091.693	3468.1	940.4	68.9	8.610889
134	2	-8.01424	86.3	4966.783	4343.2	65.3	86.3	10.4525
135	3	-8.01424	73.7	4334.073	3710.5	698.1	73.7	9.120972
136	1	-9.01424	85.2	4912.431	4288.8	119.7	85.2	10.33811
137	2	-9.01424	62.9	3788.414	3164.8	1243.7	62.9	7.972644
138	3	-9.01424	93.2	5314.773	4691.2	-282.6	93.2	11.18484
139	1	-10.01424	56.8	3483.606	2860.0	1548.5	56.8	7.331182
140	2	-10.01424	65.1	3901.719	3278.1	1130.4	65.1	8.211092
141	3	-10.01424	68.9	4091.907	3468.3	940.2	68.9	8.611339
142	1	-3.01424	13.8	1317.571	694.0	3714.6	13.8	2.772803
143	2	-3.01424	15.3	1391.965	768.4	3640.2	15.3	2.929363
144	3	-3.01424	24.8	1873.277	1249.7	3158.8	24.8	3.942275
145	1	-4.01424	45.4	2906.441	2282.8	2125.7	45.4	6.116549
146	2	-4.01424	31.5	2208.176	1584.6	2823.9	31.5	4.647064
147	3	-4.01424	30.9	2176.78	1553.2	2855.3	30.9	4.580991
148	1	-5.01424	67.7	4029.135	3405.5	1003.0	67.7	8.479237
149	2	-5.01424	85.9	4947.16	4323.6	85.0	85.9	10.4112
150	3	-5.01424	83.1	4802.784	4179.2	229.3	83.1	10.10736
151	1	-6.01424	80.1	4652.812	4029.2	379.3	80.1	9.791752
152	2	-6.01424	65.0	3893.089	3269.5	1139.0	65.0	8.192929
153	3	-6.01424	64.8	3886.56	3263.0	1145.6	64.8	8.17919
154	1	-7.01424	73.9	4341.24	3717.6	690.9	73.9	9.136056
155	2	-7.01424	70.6	4174.166	3550.6	858.0	70.6	8.784451
156	3	-7.01424	71.9	4239.188	3615.6	792.9	71.9	8.921289
157	1	-8.01424	73.7	4332.631	3709.0	699.5	73.7	9.117939
158	2	-8.01424	75.8	4439.547	3816.0	592.6	75.8	9.342941
159	3	-8.01424	67.0	3993.513	3369.9	1038.6	67.0	8.404271

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 11.90% 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"	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	1358.70	4211.98	TRUE		Unknown 5
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	1373.60	4258.15	TRUE		Unknown 5
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	1467.02	4547.77	TRUE		Unknown 5
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	1627.18	5044.27	TRUE		Unknown 5
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	1602.52	4967.80	TRUE		Unknown 5
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	1686.39	5227.81	TRUE		Unknown 5
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	1517.15	4703.18	TRUE		
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	1384.93	4293.29	TRUE		
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	1476.33	4576.64	TRUE		
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	201.01	603.04	TRUE		
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	167.51	502.54	TRUE		
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	172.17	516.51	TRUE		
172	1	none	Hot		**—**	—	—	30	—	—	—	—		47200.20	47200.20	TRUE		
173	2	none	Hot		**—**	—	—	30	—	—	—	—		47060.86	47060.86	TRUE		
174	3	none	Hot		**—**	—	—	30	—	—	—	—		47817.04	47817.04	TRUE		
175	1	none	Hot		**—**	—	—	30	—	—	—	—		46629.05	46629.05	TRUE		
176	2	none	Hot		**—**	—	—	30	—	—	—	—		47658.09	47658.09	TRUE		
177	3	none	Hot		**—**	—	—	30	—	—	—	—		48740.73	48740.73	TRUE		

Values for analysis by nonlinear regression
Standard Curve
unknown

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
160	1	-9.01424	71.3	4211.979	3588.4	820.1	71.3	8.864029
161	2	-9.01424	72.2	4258.154	3634.6	774.0	72.2	8.961202
162	3	-9.01424	78.0	4547.768	3924.2	484.4	78.0	9.57069
163	1	-10.01424	87.8	5044.27	4420.7	-12.1	87.8	10.61557
164	2	-10.01424	86.3	4967.8	4344.2	64.3	86.3	10.45464
165	3	-10.01424	91.5	5227.809	4604.2	-195.7	91.5	11.00182
166	1	—	81.1	4703.177	4079.6	328.9	81.1	9.897746
167	2	—	72.9	4293.292	3669.7	738.8	72.9	9.035151
168	3	—	78.6	4576.635	3953.0	455.5	78.6	9.631441
169	1	-6.00	-0.4	603.0432	-20.5	4429.1	-0.4	1.269093
170	2	-6.00	-2.4	502.536	-121.1	4529.6	-2.4	1.057577
171	3	-6.00	-2.1	516.5127	-107.1	4515.6	-2.1	1.086991
172	1			47200.2	46576.6			
173	2			47060.86	46437.3			
174	3			47817.04	47193.4			
175	1			46629.05	46005.5			
176	2			47658.09	47034.5			
177	3			48740.73	48117.1			

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:	**C**			
Run identification:	1b(Seq#6863)			
Assay start date:	11/16/2005			
Tracer lot number:	3559-507			
Specific activity on day of assay:	79.37	**Ci/mmole**		
Cytosol vial or lot identification:	0725-06-01			
Protein (cytosol):	600	**micro gram per tube**		
Standard Curve IC50:	2.10E-09	**M**		
Weak Positive, Max Concentration:	**3.00E-02**	**M**		
Weak Positive IC50:	6.94E-05	**M**		
RBA:	3.0196E-05			
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)	
IC50, Unknown 1:	1.11E-05	**CR42405**		
RBA, Unknown 1:	0.01882%			
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 2:	2.12E-07	**CR42406**		
RBA, Unknown 2:	0.98774%			
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 3:	3.59E-07	**CR42407**		
RBA, Unknown 3:	0.58389%			
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 4:	2.63E-02	**CR42408**		
RBA, Unknown 4:	0.00001%			
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 5:	5.58E-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

Summary values				
	n	Mean	SD	
EtOH	6	9323.4	89.50	Total Binding, solvent control, tubes
Hot	6	51203.0	736.45	Total hot R1881 added to each tube
NSB	6	859.0	72.81	Nonspecific Binding
Specific EtOH	6	8464.4	89.50	

Assay Characterization Values		
EtOH / Hot	0.18	less than 0.1?
NSB / EtOH	0.09	around 0.25 ?

Column O, Rows 10 through 28 will contain output parameters

working volume **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 18.21% 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-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	3042.69	9432.35	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3040.29	9424.88	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3000.76	9302.35	TRUE	
4	1	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	314.43	943.28	TRUE	
5	2	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	286.66	859.97	TRUE	
6	3	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	285.35	856.06	TRUE	
7	1	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	278.94	864.71	TRUE	
8	2	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	302.75	938.53	TRUE	
9	3	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	404.46	1253.82	TRUE	
10	1	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	622.93	1931.09	TRUE	
11	2	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	787.83	2442.27	TRUE	
12	3	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	687.24	2130.43	TRUE	
13	1	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2172.72	6735.42	TRUE	
14	2	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2335.21	7239.15	TRUE	
15	3	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1893.63	5870.25	TRUE	
16	1	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3025.96	9380.47	TRUE	
17	2	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3156.68	9785.71	TRUE	
18	3	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2838.94	8800.71	TRUE	
19	1	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3184.14	9870.82	TRUE	
20	2	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2952.32	9152.20	TRUE	
21	3	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2917.26	9043.51	TRUE	
22	1	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	410.63	1231.90	TRUE	
23	2	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	428.03	1284.09	TRUE	
24	3	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	402.75	1208.25	TRUE	
25	1	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1584.86	4754.57	TRUE	
26	2	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1353.69	4061.08	TRUE	
27	3	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1466.15	4398.45	TRUE	
28	1	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2807.86	8423.57	TRUE	
29	2	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2735.23	8205.68	TRUE	
30	3	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2631.72	7895.15	TRUE	
31	1	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2992.31	8976.94	TRUE	
32	2	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2973.64	8920.91	TRUE	
33	3	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	3292.44	9877.31	TRUE	
34	1	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2794.38	8383.15	TRUE	
35	2	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2899.77	8699.30	TRUE	
36	3	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	3236.34	9709.01	TRUE	
37	1	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2860.50	8581.51	TRUE	
38	2	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	3345.03	10035.10	TRUE	
39	3	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	3039.26	9117.77	TRUE	
40	1	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	2861.64	8584.92	TRUE	
41	2	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	3129.05	9387.15	TRUE	
42	3	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	3001.26	9003.79	TRUE	
43	1	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	3158.40	9475.21	TRUE	
44	2	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	3215.46	9646.37	TRUE	
45	3	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2999.70	8999.10	TRUE	
46	1	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	611.30	1895.04	TRUE	
47	2	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	720.35	2233.07	TRUE	
48	3	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	725.28	2248.37	TRUE	
49	1	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	629.87	1952.60	TRUE	
50	2	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	725.81	2250.02	TRUE	
51	3	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	796.91	2470.42	TRUE	
52	1	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	1748.79	5421.25	TRUE	
53	2	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	1665.92	5164.35	TRUE	

Values for analysis by nonlinear regression

Standard Curve

unknown

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		—	101.3	9432.345	8573.3	-967.9	101.3	18.42147
2	2		—	101.2	9424.884	8565.9	-960.5	101.2	18.4069
3	3		—	99.8	9302.347	8443.3	-837.9	99.8	18.16759
4	1		-6.00	1.0	943.2762	84.3	7521.1	1.0	1.842229
5	2		-6.00	0.0	859.9665	1.0	7604.4	0.0	1.679524
6	3		-6.00	0.0	856.0581	-3.0	7608.4	0.0	1.671891
7	1	cold R1881	-7.01	0.1	864.7053	5.7	7599.7	0.1	1.688779
8	2	cold R1881	-7.01	0.9	938.5321	79.5	7525.9	0.9	1.832964
9	3	cold R1881	-7.01	4.7	1253.817	394.8	7210.6	4.7	2.448719
10	1	cold R1881	-8.01	12.7	1931.092	1072.1	6533.3	12.7	3.771445
11	2	cold R1881	-8.01	18.7	2442.273	1583.3	6022.1	18.7	4.769786
12	3	cold R1881	-8.01	15.0	2130.431	1271.4	6334.0	15.0	4.160755
13	1	cold R1881	-9.01	69.4	6735.417	5876.4	1729.0	69.4	13.15434
14	2	cold R1881	-9.01	75.4	7239.154	6380.1	1225.3	75.4	14.13815
15	3	cold R1881	-9.01	59.2	5870.247	5011.2	2594.2	59.2	11.46466
16	1	cold R1881	-10.01	100.7	9380.467	8521.5	-916.1	100.7	18.32016
17	2	cold R1881	-10.01	105.5	9785.711	8926.7	-1321.3	105.5	19.1116
18	3	cold R1881	-10.01	93.8	8800.714	7941.7	-336.3	93.8	17.18789
19	1	cold R1881	-11.01	106.5	9870.819	9011.8	-1406.4	106.5	19.27782
20	2	cold R1881	-11.01	98.0	9152.204	8293.2	-687.8	98.0	17.87436
21	3	cold R1881	-11.01	96.7	9043.509	8184.5	-579.1	96.7	17.66207
22	1	Weak Positive	-3	4.4	1231.895	372.9	7232.5	4.4	2.405905
23	2	Weak Positive	-3	5.0	1284.094	425.1	7180.3	5.0	2.50785
24	3	Weak Positive	-3	4.1	1208.248	349.2	7256.2	4.1	2.359722
25	1	Weak Positive	-4	46.0	4754.574	3895.6	3709.8	46.0	9.285736
26	2	Weak Positive	-4	37.8	4061.079	3202.1	4403.3	37.8	7.931332
27	3	Weak Positive	-4	41.8	4398.447	3539.4	4066.0	41.8	8.590216
28	1	Weak Positive	-5	89.4	8423.571	7564.6	40.8	89.4	16.45133
29	2	Weak Positive	-5	86.8	8205.675	7346.7	258.7	86.8	16.02577
30	3	Weak Positive	-5	83.1	7895.154	7036.1	569.3	83.1	15.41932
31	1	Weak Positive	-6	95.9	8976.942	8117.9	-512.5	95.9	17.53207
32	2	Weak Positive	-6	95.2	8920.905	8061.9	-456.5	95.2	17.42263
33	3	Weak Positive	-6	106.5	9877.314	9018.3	-1412.9	106.5	19.2905
34	1	Weak Positive	-7	88.9	8383.152	7524.1	81.3	88.9	16.37239
35	2	Weak Positive	-7	92.6	8699.304	7840.3	-234.9	92.6	16.98984
36	3	Weak Positive	-7	104.6	9709.008	8850.0	-1244.6	104.6	18.9618
37	1	Weak Positive	-8	91.2	8581.506	7722.5	-117.1	91.2	16.75978
38	2	Weak Positive	-8	108.4	10035.1	9176.1	-1570.7	108.4	19.59865
39	3	Weak Positive	-8	97.6	9117.765	8258.8	-653.4	97.6	17.8071
40	1	Weak Positive	-9	91.3	8584.92	7725.9	-120.5	91.3	16.76644
41	2	Weak Positive	-9	100.8	9387.153	8528.1	-922.7	100.8	18.33321
42	3	Weak Positive	-9	96.2	9003.792	8144.8	-539.4	96.2	17.58451
43	1	Weak Positive	-10	101.8	9475.209	8616.2	-1010.8	101.8	18.50519
44	2	Weak Positive	-10	103.8	9646.365	8787.4	-1182.0	103.8	18.83946
45	3	Weak Positive	-10	96.2	8999.097	8140.1	-534.7	96.2	17.57534
46	1	Unknown 1	-3.01424	12.2	1895.044	1036.0	6569.4	12.2	3.701042
47	2	Unknown 1	-3.01424	16.2	2233.071	1374.1	6231.3	16.2	4.361212
48	3	Unknown 1	-3.01424	16.4	2248.37	1389.4	6216.0	16.4	4.391091
49	1	Unknown 1	-4.01424	12.9	1952.599	1093.6	6511.8	12.9	3.813447
50	2	Unknown 1	-4.01424	16.4	2250.019	1391.0	6214.4	16.4	4.394313
51	3	Unknown 1	-4.01424	19.0	2470.415	1611.4	5994.0	19.0	4.824748
52	1	Unknown 1	-5.01424	53.9	5421.246	4562.2	3043.2	53.9	10.58775
53	2	Unknown 1	-5.01424	50.9	5164.346	4305.3	3300.1	50.9	10.08602

Column O, Rows 10 through 28 will contain output parameters

working volume **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule:	18.21%	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"	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	1531.18	4746.65	TRUE		Unknown 1
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2622.15	8128.65	TRUE		Unknown 1
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2752.48	8532.68	TRUE		Unknown 1
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2904.05	9002.56	TRUE		Unknown 1
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	2669.12	8274.27	TRUE		Unknown 1
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	2985.80	9255.96	TRUE		Unknown 1
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	3045.37	9440.63	TRUE		Unknown 1
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	3064.31	9499.36	TRUE		Unknown 1
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	3012.90	9339.98	TRUE		Unknown 1
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2686.52	8328.21	TRUE		Unknown 1
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2957.96	9169.66	TRUE		Unknown 1
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2938.32	9108.78	TRUE		Unknown 1
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2975.36	9223.60	TRUE		Unknown 1
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2858.65	8861.82	TRUE		Unknown 1
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3239.86	10043.57	TRUE		Unknown 1
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	3081.45	9552.49	TRUE		Unknown 1
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	172.69	535.33	TRUE		Unknown 2
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	248.67	770.87	TRUE		Unknown 2
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	286.66	888.64	TRUE		Unknown 2
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	189.95	588.86	TRUE		Unknown 2
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	202.56	627.92	TRUE		Unknown 2
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	207.50	643.26	TRUE		Unknown 2
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	370.19	1147.57	TRUE		Unknown 2
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	262.48	813.70	TRUE		Unknown 2
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	307.38	952.88	TRUE		Unknown 2
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	718.37	2226.96	TRUE		Unknown 2
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	830.02	2573.05	TRUE		Unknown 2
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	863.03	2675.38	TRUE		Unknown 2
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2171.50	6731.65	TRUE		Unknown 2
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2279.47	7066.34	TRUE		Unknown 2
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	1858.37	5760.95	TRUE		Unknown 2
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2842.42	8811.51	TRUE		Unknown 2
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2850.09	8835.29	TRUE		Unknown 2
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2903.27	9000.12	TRUE		Unknown 2
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3018.09	9356.07	TRUE		Unknown 2
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3208.53	9946.44	TRUE		Unknown 2
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	3246.51	10064.18	TRUE		Unknown 2
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3208.52	9946.41	TRUE		Unknown 2
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3125.01	9687.53	TRUE		Unknown 2
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3069.33	9514.92	TRUE		Unknown 2
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	344.52	1068.00	TRUE		Unknown 3
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	269.39	835.11	TRUE		Unknown 3
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	245.45	760.88	TRUE		Unknown 3
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	276.30	856.53	TRUE		Unknown 3
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	207.22	642.39	TRUE		Unknown 3
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	257.07	796.93	TRUE		Unknown 3
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	410.99	1274.08	TRUE		Unknown 3
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	404.09	1252.67	TRUE		Unknown 3
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	364.66	1130.45	TRUE		Unknown 3
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	918.67	2847.87	TRUE		Unknown 3
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1019.63	3160.85	TRUE		Unknown 3
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	1061.39	3290.31	TRUE		Unknown 3
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2423.35	7512.40	TRUE		Unknown 3

Values for analysis by nonlinear regression

Standard Curve

unknown

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
54	3	-5.01424	45.9	4746.649	3887.6	3717.8	45.9	9.270257
55	1	-6.01424	85.9	8128.65	7269.6	335.8	85.9	15.87534
56	2	-6.01424	90.7	8532.685	7673.7	-68.3	90.7	16.66443
57	3	-6.01424	96.2	9002.555	8143.5	-538.1	96.2	17.58209
58	1	-7.01424	87.6	8274.272	7415.3	190.1	87.6	16.15974
59	2	-7.01424	99.2	9255.965	8397.0	-791.6	99.2	18.077
60	3	-7.01424	101.4	9440.635	8581.6	-976.2	101.4	18.43766
61	1	-8.01424	102.1	9499.361	8640.4	-1035.0	102.1	18.55236
62	2	-8.01424	100.2	9339.978	8481.0	-875.6	100.2	18.24108
63	3	-8.01424	88.2	8328.212	7469.2	136.2	88.2	16.26509
64	1	-9.01424	98.2	9169.664	8310.7	-705.3	98.2	17.90845
65	2	-9.01424	97.5	9108.777	8249.8	-644.4	97.5	17.78954
66	3	-9.01424	98.8	9223.601	8364.6	-759.2	98.8	18.01379
67	1	-10.01424	94.5	8861.821	8002.8	-397.4	94.5	17.30723
68	2	-10.01424	108.5	10043.57	9184.6	-1579.2	108.5	19.6152
69	3	-10.01424	102.7	9552.489	8693.5	-1088.1	102.7	18.65612
70	1	-3.01424	-3.8	535.326	-323.7	7929.1	-3.8	1.045498
71	2	-3.01424	-1.0	770.8696	-88.1	7693.5	-1.0	1.505517
72	3	-3.01424	0.4	888.6414	29.6	7575.8	0.4	1.735526
73	1	-4.01424	-3.2	588.8586	-270.2	7875.6	-3.2	1.150047
74	2	-4.01424	-2.7	627.9208	-231.1	7836.5	-2.7	1.226336
75	3	-4.01424	-2.5	643.2633	-215.7	7821.1	-2.5	1.2563
76	1	-5.01424	3.4	1147.574	288.6	7316.8	3.4	2.241225
77	2	-5.01424	-0.5	813.6958	-45.3	7650.7	-0.5	1.589157
78	3	-5.01424	1.1	952.8805	93.9	7511.5	1.1	1.860986
79	1	-6.01424	16.2	2226.957	1367.9	6237.5	16.2	4.349271
80	2	-6.01424	20.2	2573.053	1714.0	5891.4	20.2	5.025201
81	3	-6.01424	21.5	2675.384	1816.4	5789.0	21.5	5.225055
82	1	-7.01424	69.4	6731.647	5872.6	1732.8	69.4	13.14698
83	2	-7.01424	73.3	7066.342	6207.3	1398.1	73.3	13.80064
84	3	-7.01424	57.9	5760.95	4901.9	2703.5	57.9	11.2512
85	1	-8.01424	94.0	8811.514	7952.5	-347.1	94.0	17.20899
86	2	-8.01424	94.2	8835.285	7976.3	-370.9	94.2	17.25541
87	3	-8.01424	96.2	9000.125	8141.1	-535.7	96.2	17.57734
88	1	-9.01424	100.4	9356.067	8497.1	-891.7	100.4	18.2725
89	2	-9.01424	107.4	9946.44	9087.4	-1482.0	107.4	19.42551
90	3	-9.01424	108.8	10064.18	9205.2	-1599.8	108.8	19.65546
91	1	-10.01424	107.4	9946.412	9087.4	-1482.0	107.4	19.42545
92	2	-10.01424	104.3	9687.531	8828.5	-1223.1	104.3	18.91986
93	3	-10.01424	102.3	9514.923	8655.9	-1050.5	102.3	18.58275
94	1	-3.01424	2.5	1068.002	209.0	7396.4	2.5	2.085819
95	2	-3.01424	-0.3	835.113	-23.9	7629.3	-0.3	1.630985
96	3	-3.01424	-1.2	760.8801	-98.1	7703.5	-1.2	1.486007
97	1	-4.01424	0.0	856.5263	-2.5	7607.9	0.0	1.672805
98	2	-4.01424	-2.6	642.3947	-216.6	7822.0	-2.6	1.254604
99	3	-4.01424	-0.7	796.9306	-62.1	7667.5	-0.7	1.556414
100	1	-5.01424	4.9	1274.083	415.1	7190.3	4.9	2.488298
101	2	-5.01424	4.7	1252.67	393.7	7211.7	4.7	2.446478
102	3	-5.01424	3.2	1130.451	271.4	7334.0	3.2	2.207783
103	1	-6.01424	23.5	2847.866	1988.9	5616.5	23.5	5.561914
104	2	-6.01424	27.2	3160.853	2301.8	5303.6	27.2	6.173181
105	3	-6.01424	28.7	3290.309	2431.3	5174.1	28.7	6.42601
106	1	-7.01424	78.6	7512.397	6653.4	952.0	78.6	14.6718

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule:	18.21%	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"	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2365.81	7334.01	TRUE		Unknown 3
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2430.21	7533.65	TRUE		Unknown 3
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2866.60	8886.46	TRUE		Unknown 3
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2825.16	8757.98	TRUE		Unknown 3
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	3132.54	9710.87	TRUE		Unknown 3
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3031.59	9397.93	TRUE		Unknown 3
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2911.50	9025.65	TRUE		Unknown 3
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2960.70	9178.15	TRUE		Unknown 3
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3149.81	9764.40	TRUE		Unknown 3
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2906.67	9010.68	TRUE		Unknown 3
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2810.70	8713.15	TRUE		Unknown 3
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	3102.39	9617.42	TRUE		Unknown 4
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	2563.90	7948.10	TRUE		Unknown 4
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	3076.31	9536.55	TRUE		Unknown 4
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	2921.88	9057.81	TRUE		Unknown 4
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	2842.44	8811.56	TRUE		Unknown 4
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	3004.97	9315.40	TRUE		Unknown 4
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	2985.83	9256.06	TRUE		Unknown 4
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3292.53	10206.83	TRUE		Unknown 4
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	3041.51	9428.67	TRUE		Unknown 4
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3003.23	9310.00	TRUE		Unknown 4
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3112.52	9648.82	TRUE		Unknown 4
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3170.27	9827.82	TRUE		Unknown 4
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	3125.65	9689.51	TRUE		Unknown 4
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2714.65	8415.42	TRUE		Unknown 4
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2987.44	9261.05	TRUE		Unknown 4
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2949.51	9143.47	TRUE		Unknown 4
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3004.77	9314.77	TRUE		Unknown 4
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2800.99	8683.08	TRUE		Unknown 4
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2814.81	8725.91	TRUE		Unknown 4
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	3092.37	9586.36	TRUE		Unknown 4
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2909.27	9018.73	TRUE		Unknown 4
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3027.59	9385.52	TRUE		Unknown 4
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2898.29	8984.69	TRUE		Unknown 4
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3225.08	9997.75	TRUE		Unknown 4
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2302.98	7139.24	TRUE		Unknown 5
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2628.31	8147.75	TRUE		Unknown 5
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2818.79	8738.24	TRUE		Unknown 5
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	3308.70	10256.96	TRUE		Unknown 5
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2848.40	8830.04	TRUE		Unknown 5
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2898.83	8986.36	TRUE		Unknown 5
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2894.25	8972.16	TRUE		Unknown 5
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	3034.80	9407.88	TRUE		Unknown 5
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	3246.55	10064.29	TRUE		Unknown 5
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3257.39	10097.92	TRUE		Unknown 5
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3028.96	9389.77	TRUE		Unknown 5
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	3163.32	9806.30	TRUE		Unknown 5
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3038.04	9417.93	TRUE		Unknown 5
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2735.30	8479.43	TRUE		Unknown 5
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3239.73	10043.16	TRUE		Unknown 5
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3082.00	9554.19	TRUE		Unknown 5
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3079.80	9547.39	TRUE		Unknown 5
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	3078.89	9544.55	TRUE		Unknown 5

Values for analysis by nonlinear regression
Standard Curve
unknown

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
107	2	-7.01424	76.5	7334.008	6475.0	1130.4	76.5	14.3234
108	3	-7.01424	78.9	7533.651	6674.6	930.8	78.9	14.7133
109	1	-8.01424	94.8	8886.46	8027.4	-422.0	94.8	17.35535
110	2	-8.01424	93.3	8757.981	7899.0	-293.6	93.3	17.10443
111	3	-8.01424	104.6	9710.868	8851.9	-1246.5	104.6	18.96543
112	1	-9.01424	100.9	9397.929	8538.9	-933.5	100.9	18.35426
113	2	-9.01424	96.5	9025.647	8166.6	-561.2	96.5	17.62719
114	3	-9.01424	98.3	9178.155	8319.1	-713.7	98.3	17.92504
115	1	-10.01424	105.2	9764.399	8905.4	-1300.0	105.2	19.06998
116	2	-10.01424	96.3	9010.677	8151.7	-546.3	96.3	17.59795
117	3	-10.01424	92.8	8713.155	7854.1	-248.7	92.8	17.01689
118	1	-3.01424	103.5	9617.415	8758.4	-1153.0	103.5	18.78292
119	2	-3.01424	83.8	7948.096	7089.1	516.3	83.8	15.52272
120	3	-3.01424	102.5	9536.552	8677.5	-1072.1	102.5	18.62499
121	1	-4.01424	96.9	9057.813	8198.8	-593.4	96.9	17.69001
122	2	-4.01424	94.0	8811.561	7952.5	-347.2	94.0	17.20908
123	3	-4.01424	99.9	9315.398	8456.4	-851.0	99.9	18.19307
124	1	-5.01424	99.2	9256.064	8397.1	-791.7	99.2	18.07719
125	2	-5.01424	110.4	10206.83	9347.8	-1742.4	110.4	19.93406
126	3	-5.01424	101.2	9428.672	8569.7	-964.3	101.2	18.4143
127	1	-6.01424	99.8	9310.004	8451.0	-845.6	99.8	18.18254
128	2	-6.01424	103.8	9648.824	8789.8	-1184.4	103.8	18.84426
129	3	-6.01424	106.0	9827.825	8968.8	-1363.4	106.0	19.19385
130	1	-7.01424	104.3	9689.506	8830.5	-1225.1	104.3	18.92371
131	2	-7.01424	89.3	8415.415	7556.4	49.0	89.3	16.4354
132	3	-7.01424	99.3	9261.049	8402.0	-796.6	99.3	18.08693
133	1	-8.01424	97.9	9143.466	8284.5	-679.1	97.9	17.85729
134	2	-8.01424	99.9	9314.772	8455.8	-850.4	99.9	18.19185
135	3	-8.01424	92.4	8683.081	7824.1	-218.7	92.4	16.95815
136	1	-9.01424	92.9	8725.908	7866.9	-261.5	92.9	17.04179
137	2	-9.01424	103.1	9586.356	8727.3	-1121.9	103.1	18.72226
138	3	-9.01424	96.4	9018.728	8159.7	-554.3	96.4	17.61368
139	1	-10.01424	100.7	9385.52	8526.5	-921.1	100.7	18.33002
140	2	-10.01424	96.0	8984.69	8125.7	-520.3	96.0	17.5472
141	3	-10.01424	108.0	9997.751	9138.7	-1533.3	108.0	19.52572
142	1	-3.01424	74.2	7139.244	6280.2	1325.2	74.2	13.94302
143	2	-3.01424	86.1	8147.749	7288.7	316.7	86.1	15.91264
144	3	-3.01424	93.1	8738.243	7879.2	-273.8	93.1	17.06588
145	1	-4.01424	111.0	10256.96	9397.9	-1792.5	111.0	20.03195
146	2	-4.01424	94.2	8830.037	7971.0	-365.6	94.2	17.24516
147	3	-4.01424	96.0	8986.364	8127.4	-522.0	96.0	17.55047
148	1	-5.01424	95.9	8972.16	8113.1	-507.7	95.9	17.52273
149	2	-5.01424	101.0	9407.88	8548.9	-943.5	101.0	18.37369
150	3	-5.01424	108.8	10064.29	9205.3	-1599.9	108.8	19.65567
151	1	-6.01424	109.2	10097.92	9238.9	-1633.5	109.2	19.72135
152	2	-6.01424	100.8	9389.77	8530.8	-925.4	100.8	18.33832
153	3	-6.01424	105.7	9806.301	8947.3	-1341.9	105.7	19.15182
154	1	-7.01424	101.1	9417.933	8558.9	-953.5	101.1	18.39333
155	2	-7.01424	90.0	8479.43	7620.4	-15.0	90.0	16.56042
156	3	-7.01424	108.5	10043.16	9184.1	-1578.7	108.5	19.6144
157	1	-8.01424	102.7	9554.194	8695.2	-1089.8	102.7	18.65945
158	2	-8.01424	102.6	9547.389	8688.4	-1083.0	102.6	18.64616
159	3	-8.01424	102.6	9544.547	8685.5	-1080.1	102.6	18.64061

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 18.21% 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"	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3091.78	9584.52	TRUE		Unknown 5
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3046.23	9443.30	TRUE		Unknown 5
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	3239.88	10043.64	TRUE		Unknown 5
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3243.36	10054.43	TRUE		Unknown 5
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2827.85	8766.33	TRUE		Unknown 5
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2848.41	8830.08	TRUE		Unknown 5
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	2991.83	9274.66	TRUE		
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	2968.44	9202.17	TRUE		
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3001.33	9304.11	TRUE		
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	246.79	740.38	TRUE		
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	308.49	925.48	TRUE		
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	276.30	828.91	TRUE		
172	1	none	Hot		**—**	—	—	30	—	—	—	—		52527.51	52527.51	TRUE		
173	2	none	Hot		**—**	—	—	30	—	—	—	—		51359.62	51359.62	TRUE		
174	3	none	Hot		**—**	—	—	30	—	—	—	—		50356.64	50356.64	TRUE		
175	1	none	Hot		**—**	—	—	30	—	—	—	—		50910.92	50910.92	TRUE		
176	2	none	Hot		**—**	—	—	30	—	—	—	—		50841.12	50841.12	TRUE		
177	3	none	Hot		**—**	—	—	30	—	—	—	—		51222.11	51222.11	TRUE		

Values for analysis by nonlinear regression

Standard Curve

unknown

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
160	1	-9.01424	103.1	9584.521	8725.5	-1120.1	103.1	18.71868
161	2	-9.01424	101.4	9443.304	8584.3	-978.9	101.4	18.44288
162	3	-9.01424	108.5	10043.64	9184.6	-1579.2	108.5	19.61533
163	1	-10.01424	108.6	10054.43	9195.4	-1590.0	108.6	19.6364
164	2	-10.01424	93.4	8766.329	7907.3	-301.9	93.4	17.12074
165	3	-10.01424	94.2	8830.083	7971.1	-365.7	94.2	17.24525
166	1	—	99.4	9274.664	8415.7	-810.3	99.4	18.11352
167	2	—	98.6	9202.173	8343.2	-737.8	98.6	17.97195
168	3	—	99.8	9304.114	8445.1	-839.7	99.8	18.17104
169	1	-6.00	-1.4	740.382	-118.6	7724.0	-1.4	1.445974
170	2	-6.00	0.8	925.4775	66.5	7538.9	0.8	1.807468
171	3	-6.00	-0.4	828.9054	-30.1	7635.5	-0.4	1.618861
172	1			52527.51	51668.5			
173	2			51359.62	50500.6			
174	3			50356.64	49497.6			
175	1			50910.92	50051.9			
176	2			50841.12	49982.1			
177	3			51222.11	50363.1			

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:	**C**		
Run identification:	3(Seq#6865)		
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:	0725-06-01		
Protein (cytosol):	600	**micro gram per tube**	
Standard Curve IC50:	2.86E-09	**M**	
Weak Positive, Max Concentration:	**3.00E-02**	**M**	
Weak Positive IC50:	7.12E-05	**M**	
RBA:	4.0236E-05		
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)
IC50, Unknown 1:	1.84E-05	**CR42405**	
RBA, Unknown 1:	0.01559%		
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 2:	1.67E-07	**CR42406**	
RBA, Unknown 2:	1.72012%		
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 3:	2.94E-07	**CR42407**	
RBA, Unknown 3:	0.97349%		
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 4:	0.00E+00	**CR42408**	
RBA, Unknown 4:	#DIV/0!		
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)
IC50, Unknown 5:	4.84E-03	**CR42409**	
RBA, Unknown 5:	0.00006%		
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

Summary values				
	n	Mean	SD	
EtOH	6	9265.8	414.55	Total Binding, solvent control, tubes
Hot	6	46539.3	397.87	Total hot R1881 added to each tube
NSB	6	884.5	158.17	Nonspecific Binding
Specific EtOH	6	8381.3	414.55	

Assay Characterization Values		
EtOH / Hot	0.20	less than 0.1?
NSB / EtOH	0.10	around 0.25 ?

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 19.91% 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	3177.39	9849.91	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2918.64	9047.77	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2951.17	9148.62	TRUE	
4	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	297.08	891.23	TRUE	
5	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	362.64	1087.91	TRUE	
6	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	321.62	964.86	TRUE	
7	1	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	347.72	1077.92	TRUE	
8	2	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	411.60	1275.97	TRUE	
9	3	Inert R1881	S	1	**E-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	449.02	1391.96	TRUE	
10	1	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	832.91	2582.01	TRUE	
11	2	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	853.06	2644.48	TRUE	
12	3	Inert R1881	S	2	**E-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	781.49	2422.63	TRUE	
13	1	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2387.22	7400.38	TRUE	
14	2	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2358.88	7312.52	TRUE	
15	3	Inert R1881	S	3	**E-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2203.96	6832.28	TRUE	
16	1	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3232.66	10021.25	TRUE	
17	2	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2814.29	8724.31	TRUE	
18	3	Inert R1881	S	4	**E-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3085.33	9564.54	TRUE	
19	1	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	2940.41	9115.27	TRUE	
20	2	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	2993.49	9279.81	TRUE	
21	3	Inert R1881	S	5	**E-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3060.87	9488.69	TRUE	
22	1	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	503.45	1510.35	TRUE	
23	2	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	387.79	1163.38	TRUE	
24	3	Weak Positive	P	1	**E-1-P1**	3.00E-02	300	30	10	50	300	1.0E-03	100	475.73	1427.20	TRUE	
25	1	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1536.90	4610.69	TRUE	
26	2	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1494.44	4483.33	TRUE	
27	3	Weak Positive	P	2	**E-1-P2**	3.00E-03	300	30	10	50	300	1.0E-04	100	1418.51	4255.52	TRUE	
28	1	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2773.32	8319.96	TRUE	
29	2	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2592.99	7778.97	TRUE	
30	3	Weak Positive	P	3	**E-1-P3**	3.00E-04	300	30	10	50	300	1.0E-05	100	2752.60	8257.79	TRUE	
31	1	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	2899.90	8699.69	TRUE	
32	2	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	3145.17	9435.52	TRUE	
33	3	Weak Positive	P	4	**E-1-P4**	3.00E-05	300	30	10	50	300	1.0E-06	100	2949.46	8848.37	TRUE	
34	1	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3024.10	9072.31	TRUE	
35	2	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	2810.65	8431.95	TRUE	
36	3	Weak Positive	P	5	**E-1-P5**	3.00E-06	300	30	10	50	300	1.0E-07	100	3122.14	9366.43	TRUE	
37	1	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3404.64	10213.92	TRUE	
38	2	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	3072.78	9218.33	TRUE	
39	3	Weak Positive	P	6	**E-1-P6**	3.00E-07	300	30	10	50	300	1.0E-08	100	2966.73	8900.18	TRUE	
40	1	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	2731.82	8195.45	TRUE	
41	2	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	2988.89	8966.66	TRUE	
42	3	Weak Positive	P	7	**E-1-P7**	3.00E-08	300	30	10	50	300	1.0E-09	100	2768.24	8304.71	TRUE	
43	1	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	2803.80	8411.39	TRUE	
44	2	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	2814.08	8442.23	TRUE	
45	3	Weak Positive	P	8	**E-1-P8**	3.00E-09	300	30	10	50	300	1.0E-10	100	2968.31	8904.94	TRUE	
46	1	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	601.03	1863.18	TRUE	
47	2	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	716.87	2222.28	TRUE	
48	3	Unknown 1	U1	1	**E-1-U1**	3.00E-02	300	30	10	50	310	9.7E-04	100	880.43	2729.32	TRUE	
49	1	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	760.94	2358.90	TRUE	
50	2	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	816.97	2532.61	TRUE	
51	3	Unknown 1	U1	2	**E-1-U1**	3.00E-03	300	30	10	50	310	9.7E-05	100	2811.33	8715.11	TRUE	
52	1	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	1541.62	4779.01	TRUE	
53	2	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	1554.58	4819.20	TRUE	

Values for analysis by nonlinear regression

Standard Curve

unknown

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		—	107.0	9849.906	8965.5	-1468.6	107.0	21.1647
2	2		—	97.4	9047.772	8163.3	-666.5	97.4	19.44113
3	3		—	98.6	9148.618	8264.2	-767.3	98.6	19.65782
4	1		-6.00	0.1	891.2328	6.8	7490.1	0.1	1.91501
5	2		-6.00	2.4	1087.909	203.5	7293.4	2.4	2.337613
6	3		-6.00	0.0	964.8639	80.4	7416.5	1.0	2.073223
7	1	cold R1881	-7.01	2.3	1077.919	193.5	7303.4	2.3	2.316147
8	2	cold R1881	-7.01	4.7	1275.968	391.5	7105.3	4.7	2.741698
9	3	cold R1881	-7.01	6.1	1391.965	507.5	6989.3	6.1	2.990944
10	1	cold R1881	-8.01	20.3	2582.014	1697.6	5799.3	20.3	5.548027
11	2	cold R1881	-8.01	21.0	2644.483	1760.0	5736.8	21.0	5.682255
12	3	cold R1881	-8.01	18.4	2422.63	1538.2	5958.7	18.4	5.205556
13	1	cold R1881	-9.01	77.7	7400.379	6515.9	980.9	77.7	15.90135
14	2	cold R1881	-9.01	76.7	7312.516	6428.1	1068.8	76.7	15.71255
15	3	cold R1881	-9.01	71.0	6832.279	5947.8	1549.0	71.0	14.68066
16	1	cold R1881	-10.01	109.0	10021.25	9136.8	-1639.9	109.0	21.53287
17	2	cold R1881	-10.01	93.5	8724.305	7839.9	-343.0	93.5	18.7461
18	3	cold R1881	-10.01	103.6	9564.535	8680.1	-1183.2	103.6	20.55152
19	1	cold R1881	-11.01	98.2	9115.268	8230.8	-734.0	98.2	19.58616
20	2	cold R1881	-11.01	100.2	9279.813	8395.4	-898.5	100.2	19.93973
21	3	cold R1881	-11.01	102.7	9488.688	8604.2	-1107.4	102.7	20.38854
22	1	Weak Positive	-3	7.5	1510.351	625.9	6871.0	7.5	3.245322
23	2	Weak Positive	-3	3.3	1163.378	278.9	7217.9	3.3	2.499775
24	3	Weak Positive	-3	6.5	1427.202	542.7	6954.1	6.5	3.066658
25	1	Weak Positive	-4	44.5	4610.688	3726.2	3770.6	44.5	9.907081
26	2	Weak Positive	-4	42.9	4483.329	3598.9	3898.0	42.9	9.633422
27	3	Weak Positive	-4	40.2	4255.515	3371.1	4125.8	40.2	9.143913
28	1	Weak Positive	-5	88.7	8319.96	7435.5	61.4	88.7	17.87727
29	2	Weak Positive	-5	82.3	7778.97	6894.5	602.3	82.3	16.71483
30	3	Weak Positive	-5	88.0	8257.794	7373.3	123.5	88.0	17.74369
31	1	Weak Positive	-6	93.2	8699.694	7815.2	-318.4	93.2	18.69321
32	2	Weak Positive	-6	102.0	9435.516	8551.1	-1054.2	102.0	20.27429
33	3	Weak Positive	-6	95.0	8848.374	7963.9	-467.1	95.0	19.01268
34	1	Weak Positive	-7	97.7	9072.312	8187.9	-691.0	97.7	19.49386
35	2	Weak Positive	-7	90.1	8431.95	7547.5	-50.6	90.1	18.11791
36	3	Weak Positive	-7	101.2	9366.432	8482.0	-985.1	101.2	20.12585
37	1	Weak Positive	-8	111.3	10213.92	9329.5	-1832.6	111.3	21.94686
38	2	Weak Positive	-8	99.4	9218.334	8333.9	-837.0	99.4	19.80763
39	3	Weak Positive	-8	95.6	8900.181	8015.7	-518.9	95.6	19.124
40	1	Weak Positive	-9	87.2	8195.445	7311.0	185.9	87.2	17.60972
41	2	Weak Positive	-9	96.4	8966.658	8082.2	-585.3	96.4	19.26684
42	3	Weak Positive	-9	88.5	8304.714	7420.3	76.6	88.5	17.84451
43	1	Weak Positive	-10	89.8	8411.385	7526.9	-30.1	89.8	18.07372
44	2	Weak Positive	-10	90.2	8442.234	7557.8	-60.9	90.2	18.14
45	3	Weak Positive	-10	95.7	8904.936	8020.5	-523.6	95.7	19.13422
46	1	Unknown 1	-3.01424	11.7	1863.179	978.7	6518.1	11.7	4.003452
47	2	Unknown 1	-3.01424	16.0	2222.283	1337.8	6159.0	16.0	4.775066
48	3	Unknown 1	-3.01424	22.0	2729.323	1844.9	5652.0	22.0	5.864553
49	1	Unknown 1	-4.01424	17.6	2358.902	1474.4	6022.4	17.6	5.068621
50	2	Unknown 1	-4.01424	19.7	2532.614	1648.2	5848.7	19.7	5.441879
51	3	Unknown 1	-4.01424	93.4	8715.108	7830.7	-333.8	93.4	18.72633
52	1	Unknown 1	-5.01424	46.5	4779.013	3894.6	3602.3	46.5	10.26876
53	2	Unknown 1	-5.01424	46.9	4819.201	3934.7	3562.1	46.9	10.35512

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	19.91%	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"	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	1607.56	4983.45	TRUE		Unknown 1
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2721.53	8436.74	TRUE		Unknown 1
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2986.70	9258.77	TRUE		Unknown 1
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2849.32	8832.88	TRUE		Unknown 1
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	3007.11	9322.04	TRUE		Unknown 1
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	2939.08	9111.14	TRUE		Unknown 1
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	3119.35	9669.98	TRUE		Unknown 1
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2940.92	9116.84	TRUE		Unknown 1
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2851.80	8840.57	TRUE		Unknown 1
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2970.20	9207.61	TRUE		Unknown 1
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2759.52	8554.51	TRUE		Unknown 1
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2871.69	8902.25	TRUE		Unknown 1
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2790.10	8649.31	TRUE		Unknown 1
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2870.04	8897.12	TRUE		Unknown 1
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2956.09	9163.86	TRUE		Unknown 1
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2866.59	8886.41	TRUE		Unknown 1
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	191.95	595.04	TRUE		Unknown 2
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	224.41	695.66	TRUE		Unknown 2
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	185.09	573.79	TRUE		Unknown 2
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	92.95	288.14	TRUE		Unknown 2
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	261.00	809.09	TRUE		Unknown 2
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	241.76	749.46	TRUE		Unknown 2
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	314.29	974.29	TRUE		Unknown 2
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	285.36	884.60	TRUE		Unknown 2
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	257.90	799.50	TRUE		Unknown 2
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	671.63	2082.05	TRUE		Unknown 2
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	756.36	2344.73	TRUE		Unknown 2
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	767.79	2380.15	TRUE		Unknown 2
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2075.05	6432.67	TRUE		Unknown 2
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	1890.52	5860.62	TRUE		Unknown 2
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	1902.35	5897.29	TRUE		Unknown 2
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2835.52	8790.10	TRUE		Unknown 2
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2748.60	8520.64	TRUE		Unknown 2
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2865.52	8883.12	TRUE		Unknown 2
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2636.34	8172.65	TRUE		Unknown 2
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2959.50	9174.46	TRUE		Unknown 2
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2714.58	8415.19	TRUE		Unknown 2
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2945.90	9132.28	TRUE		Unknown 2
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	3008.46	9326.22	TRUE		Unknown 2
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2828.61	8768.69	TRUE		Unknown 2
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	195.80	606.99	TRUE		Unknown 3
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	191.95	595.04	TRUE		Unknown 3
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	187.10	579.99	TRUE		Unknown 3
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	263.93	818.18	TRUE		Unknown 3
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	234.72	727.63	TRUE		Unknown 3
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	260.50	807.56	TRUE		Unknown 3
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	333.37	1033.44	TRUE		Unknown 3
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	329.06	1020.07	TRUE		Unknown 3
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	253.65	786.30	TRUE		Unknown 3
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	908.33	2815.83	TRUE		Unknown 3
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	984.31	3051.37	TRUE		Unknown 3
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	935.96	2901.48	TRUE		Unknown 3
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2394.22	7422.07	TRUE		Unknown 3

Values for analysis by nonlinear regression
Standard Curve
unknown

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
54	3	-5.01424	48.9	4983.445	4099.0	3397.9	48.9	10.70803
55	1	-6.01424	90.1	8436.74	7552.3	-55.4	90.1	18.1282
56	2	-6.01424	99.9	9258.773	8374.3	-877.5	99.9	19.89452
57	3	-6.01424	94.8	8832.88	7948.4	-451.6	94.8	18.97939
58	1	-7.01424	100.7	9322.044	8437.6	-940.7	100.7	20.03047
59	2	-7.01424	98.2	9111.139	8226.7	-729.8	98.2	19.57729
60	3	-7.01424	104.8	9669.979	8785.5	-1288.7	104.8	20.77808
61	1	-8.01424	98.2	9116.837	8232.4	-735.5	98.2	19.58954
62	2	-8.01424	94.9	8840.571	7956.1	-459.3	94.9	18.99592
63	3	-8.01424	99.3	9207.611	8323.2	-826.3	99.3	19.78458
64	1	-9.01424	91.5	8554.512	7670.1	-173.2	91.5	18.38126
65	2	-9.01424	95.7	8902.251	8017.8	-520.9	95.7	19.12845
66	3	-9.01424	92.6	8649.307	7764.9	-268.0	92.6	18.58494
67	1	-10.01424	95.6	8897.121	8012.7	-515.8	95.6	19.11743
68	2	-10.01424	98.8	9163.864	8279.4	-782.5	98.8	19.69058
69	3	-10.01424	95.5	8886.414	8002.0	-505.1	95.5	19.09442
70	1	-3.01424	-3.5	595.0382	-289.4	7786.3	-3.5	1.278571
71	2	-3.01424	-2.3	695.6583	-188.8	7685.7	-2.3	1.494775
72	3	-3.01424	-3.7	573.7871	-310.7	7807.5	-3.7	1.232908
73	1	-4.01424	-7.1	288.1388	-596.3	8093.2	-7.1	0.61913
74	2	-4.01424	-0.9	809.0879	-75.4	7572.2	-0.9	1.738504
75	3	-4.01424	-1.6	749.4566	-135.0	7631.9	-1.6	1.610373
76	1	-5.01424	1.1	974.2934	89.8	7407.0	1.1	2.093484
77	2	-5.01424	0.0	884.6027	0.1	7496.7	0.0	1.900764
78	3	-5.01424	-1.0	799.4971	-85.0	7581.8	-1.0	1.717896
79	1	-6.01424	14.3	2082.053	1197.6	6299.3	14.3	4.47375
80	2	-6.01424	17.4	2344.728	1460.3	6036.6	17.4	5.038166
81	3	-6.01424	17.8	2380.153	1495.7	6001.2	17.8	5.114285
82	1	-7.01424	66.2	6432.667	5548.2	1948.6	66.2	13.82201
83	2	-7.01424	59.4	5860.624	4976.2	2520.7	59.4	12.59284
84	3	-7.01424	59.8	5897.285	5012.8	2484.0	59.8	12.67162
85	1	-8.01424	94.3	8790.1	7905.6	-408.8	94.3	18.88747
86	2	-8.01424	91.1	8520.645	7636.2	-139.3	91.1	18.30849
87	3	-8.01424	95.4	8883.118	7998.7	-501.8	95.4	19.08734
88	1	-9.01424	87.0	8172.651	7288.2	208.7	87.0	17.56074
89	2	-9.01424	98.9	9174.459	8290.0	-793.1	98.9	19.71335
90	3	-9.01424	89.9	8415.192	7530.7	-33.9	89.9	18.0819
91	1	-10.01424	98.4	9132.278	8247.8	-751.0	98.4	19.62271
92	2	-10.01424	100.7	9326.217	8441.8	-944.9	100.7	20.03943
93	3	-10.01424	94.1	8768.688	7884.2	-387.4	94.1	18.84146
94	1	-3.01424	-3.3	606.993	-277.5	7774.3	-3.3	1.304258
95	2	-3.01424	-3.5	595.0416	-289.4	7786.3	-3.5	1.278578
96	3	-3.01424	-3.6	579.9945	-304.5	7801.3	-3.6	1.246246
97	1	-4.01424	-0.8	818.1821	-66.3	7563.1	-0.8	1.758045
98	2	-4.01424	-1.9	727.6295	-156.8	7653.7	-1.9	1.563473
99	3	-4.01424	-0.9	807.5562	-76.9	7573.8	-0.9	1.735213
100	1	-5.01424	1.8	1033.445	149.0	7347.9	1.8	2.220584
101	2	-5.01424	1.6	1020.071	135.6	7361.2	1.6	2.191848
102	3	-5.01424	-1.2	786.3048	-98.2	7595.0	-1.2	1.689549
103	1	-6.01424	23.0	2815.83	1931.4	5565.5	23.0	6.050432
104	2	-6.01424	25.9	3051.375	2166.9	5329.9	25.9	6.556553
105	3	-6.01424	24.1	2901.483	2017.0	5479.8	24.1	6.234476
106	1	-7.01424	78.0	7422.073	6537.6	959.2	78.0	15.94796

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	19.91%	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"	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2354.80	7299.88	TRUE		Unknown 3
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2152.91	6674.01	TRUE		Unknown 3
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2780.49	8619.53	TRUE		Unknown 3
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2692.89	8347.94	TRUE		Unknown 3
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2714.37	8414.56	TRUE		Unknown 3
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3002.63	9308.15	TRUE		Unknown 3
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2973.67	9218.36	TRUE		Unknown 3
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	3064.33	9499.41	TRUE		Unknown 3
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	3068.07	9511.00	TRUE		Unknown 3
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2978.27	9232.64	TRUE		Unknown 3
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2882.24	8934.95	TRUE		Unknown 3
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	2920.38	9053.18	TRUE		Unknown 4
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	3034.55	9407.10	TRUE		Unknown 4
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	3115.77	9658.89	TRUE		Unknown 4
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	2844.97	8819.41	TRUE		Unknown 4
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	2803.04	8689.42	TRUE		Unknown 4
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	2519.34	7809.96	TRUE		Unknown 4
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	2758.82	8552.33	TRUE		Unknown 4
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	2578.52	7993.42	TRUE		Unknown 4
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	2908.49	9016.33	TRUE		Unknown 4
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	2825.17	8758.03	TRUE		Unknown 4
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	2909.27	9018.73	TRUE		Unknown 4
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3003.74	9311.60	TRUE		Unknown 4
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2683.88	8320.04	TRUE		Unknown 4
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2824.41	8755.66	TRUE		Unknown 4
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2670.18	8277.57	TRUE		Unknown 4
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2886.10	8946.92	TRUE		Unknown 4
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	3011.67	9336.19	TRUE		Unknown 4
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2863.16	8875.80	TRUE		Unknown 4
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2742.28	8501.07	TRUE		Unknown 4
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2815.55	8728.19	TRUE		Unknown 4
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	2994.47	9282.86	TRUE		Unknown 4
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2838.99	8800.85	TRUE		Unknown 4
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2857.47	8858.14	TRUE		Unknown 4
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2923.19	9061.88	TRUE		Unknown 4
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2535.06	7858.67	TRUE		Unknown 5
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2636.35	8172.69	TRUE		Unknown 5
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2515.92	7799.34	TRUE		Unknown 5
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2759.55	8554.60	TRUE		Unknown 5
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2663.57	8257.06	TRUE		Unknown 5
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2659.39	8244.11	TRUE		Unknown 5
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2752.42	8532.51	TRUE		Unknown 5
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2927.24	9074.43	TRUE		Unknown 5
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2752.66	8533.23	TRUE		Unknown 5
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2868.98	8893.84	TRUE		Unknown 5
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2913.54	9031.98	TRUE		Unknown 5
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2880.45	8929.38	TRUE		Unknown 5
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2903.26	9000.10	TRUE		Unknown 5
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2879.26	8925.72	TRUE		Unknown 5
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3121.56	9676.85	TRUE		Unknown 5
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2913.82	9032.83	TRUE		Unknown 5
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2826.87	8763.28	TRUE		Unknown 5
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2738.76	8490.16	TRUE		Unknown 5

Values for analysis by nonlinear regression
Standard Curve
unknown

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
107	2	-7.01424	76.5	7299.883	6415.4	1081.4	76.5	15.68541
108	3	-7.01424	69.1	6674.006	5789.6	1707.3	69.1	14.34057
109	1	-8.01424	92.3	8619.528	7735.1	-238.2	92.3	18.52096
110	2	-8.01424	89.0	8347.944	7463.5	33.4	89.0	17.9374
111	3	-8.01424	89.8	8414.559	7530.1	-33.2	89.8	18.08054
112	1	-9.01424	100.5	9308.15	8423.7	-926.8	100.5	20.00061
113	2	-9.01424	99.4	9218.365	8333.9	-837.1	99.4	19.80769
114	3	-9.01424	102.8	9499.411	8615.0	-1118.1	102.8	20.41158
115	1	-10.01424	102.9	9511.002	8626.5	-1129.7	102.9	20.43649
116	2	-10.01424	99.6	9232.643	8348.2	-851.3	99.6	19.83837
117	3	-10.01424	96.1	8934.953	8050.5	-553.6	96.1	19.19872
118	1	-3.01424	97.5	9053.178	8168.7	-671.9	97.5	19.45275
119	2	-3.01424	101.7	9407.096	8522.6	-1025.8	101.7	20.21322
120	3	-3.01424	104.7	9658.893	8774.4	-1277.6	104.7	20.75426
121	1	-4.01424	94.7	8819.41	7935.0	-438.1	94.7	18.95045
122	2	-4.01424	93.1	8689.418	7805.0	-308.1	93.1	18.67113
123	3	-4.01424	82.6	7809.96	6925.5	571.4	82.6	16.78142
124	1	-5.01424	91.5	8552.327	7667.9	-171.0	91.5	18.37656
125	2	-5.01424	84.8	7993.421	7109.0	387.9	84.8	17.17563
126	3	-5.01424	97.0	9016.325	8131.9	-635.0	97.0	19.37356
127	1	-6.01424	93.9	8758.027	7873.6	-376.7	93.9	18.81855
128	2	-6.01424	97.1	9018.728	8134.3	-637.4	97.1	19.37873
129	3	-6.01424	100.5	9311.597	8427.1	-930.3	100.5	20.00802
130	1	-7.01424	88.7	8320.037	7435.6	61.3	88.7	17.87744
131	2	-7.01424	93.9	8755.656	7871.2	-374.3	93.9	18.81346
132	3	-7.01424	88.2	8277.567	7393.1	103.7	88.2	17.78618
133	1	-8.01424	96.2	8946.922	8062.5	-565.6	96.2	19.22444
134	2	-8.01424	100.8	9336.186	8451.7	-954.9	100.8	20.06086
135	3	-8.01424	95.3	8875.799	7991.3	-494.5	95.3	19.07161
136	1	-9.01424	90.9	8501.068	7616.6	-119.8	90.9	18.26642
137	2	-9.01424	93.6	8728.19	7843.7	-346.9	93.6	18.75444
138	3	-9.01424	100.2	9282.86	8398.4	-901.5	100.2	19.94627
139	1	-10.01424	94.5	8800.854	7916.4	-419.5	94.5	18.91058
140	2	-10.01424	95.1	8858.145	7973.7	-476.8	95.1	19.03368
141	3	-10.01424	97.6	9061.88	8177.4	-680.6	97.6	19.47145
142	1	-3.01424	83.2	7858.671	6974.2	522.6	83.2	16.88609
143	2	-3.01424	87.0	8172.694	7288.2	208.6	87.0	17.56084
144	3	-3.01424	82.5	7799.337	6914.9	582.0	82.5	16.7586
145	1	-4.01424	91.5	8554.602	7670.1	-173.3	91.5	18.38145
146	2	-4.01424	88.0	8257.058	7372.6	124.3	88.0	17.74211
147	3	-4.01424	87.8	8244.109	7359.7	137.2	87.8	17.71429
148	1	-5.01424	91.3	8532.514	7648.1	-151.2	91.3	18.33399
149	2	-5.01424	97.7	9074.432	8190.0	-693.1	97.7	19.49842
150	3	-5.01424	91.3	8533.234	7648.8	-151.9	91.3	18.33554
151	1	-6.01424	95.6	8893.838	8009.4	-512.5	95.6	19.11037
152	2	-6.01424	97.2	9031.977	8147.5	-650.7	97.2	19.4072
153	3	-6.01424	96.0	8929.383	8044.9	-548.1	96.0	19.18675
154	1	-7.01424	96.8	9000.097	8115.6	-618.8	96.8	19.33869
155	2	-7.01424	95.9	8925.715	8041.3	-544.4	95.9	19.17887
156	3	-7.01424	104.9	9676.845	8792.4	-1295.5	104.9	20.79284
157	1	-8.01424	97.2	9032.83	8148.4	-651.5	97.2	19.40903
158	2	-8.01424	94.0	8763.282	7878.8	-382.0	94.0	18.82984
159	3	-8.01424	90.7	8490.162	7605.7	-108.8	90.7	18.24299

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 19.91% 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"	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2839.68	8803.02	TRUE		Unknown 5
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2790.65	8651.01	TRUE		Unknown 5
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2876.99	8918.68	TRUE		Unknown 5
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2752.66	8533.23	TRUE		Unknown 5
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	3075.11	9532.84	TRUE		Unknown 5
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2902.32	8997.20	TRUE		Unknown 5
166	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	2949.52	9143.52	TRUE		
167	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3118.76	9668.14	TRUE		
168	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	2818.28	8736.66	TRUE		
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	250.22	750.67	TRUE		
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	319.77	959.30	TRUE		
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	217.59	652.76	TRUE		
172	1	none	Hot		**—**	—	—	30	—	—	—	—		45991.22	45991.22	TRUE		
173	2	none	Hot		**—**	—	—	30	—	—	—	—		46964.59	46964.59	TRUE		
174	3	none	Hot		**—**	—	—	30	—	—	—	—		46245.07	46245.07	TRUE		
175	1	none	Hot		**—**	—	—	30	—	—	—	—		46446.86	46446.86	TRUE		
176	2	none	Hot		**—**	—	—	30	—	—	—	—		47000.57	47000.57	TRUE		
177	3	none	Hot		**—**	—	—	30	—	—	—	—		46587.61	46587.61	TRUE		

Values for analysis by nonlinear regression
Standard Curve
unknown

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
160	1	-9.01424	94.5	8803.017	7918.6	-421.7	94.5	18.91523
161	2	-9.01424	92.7	8651.009	7766.6	-269.7	92.7	18.5886
162	3	-9.01424	95.9	8918.675	8034.2	-537.4	95.9	19.16374
163	1	-10.01424	91.3	8533.234	7648.8	-151.9	91.3	18.33554
164	2	-10.01424	103.2	9532.844	8648.4	-1151.5	103.2	20.48342
165	3	-10.01424	96.8	8997.201	8112.7	-615.9	96.8	19.33247
166	1	—	98.5	9143.515	8259.1	-762.2	98.5	19.64686
167	2	—	104.8	9668.144	8783.7	-1286.8	104.8	20.77414
168	3	—	93.7	8736.662	7852.2	-355.3	93.7	18.77265
169	1	-6.00	-1.6	750.6651	-133.8	7630.6	-1.6	1.61297
170	2	-6.00	0.9	959.2971	74.8	7422.0	0.9	2.061262
171	3	-6.00	-2.8	652.7628	-231.7	7728.6	-2.8	1.402605
172	1			45991.22	45106.8			
173	2			46964.59	46080.1			
174	3			46245.07	45360.6			
175	1			46446.86	45562.4			
176	2			47000.57	46116.1			
177	3			46587.61	45703.2			

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:	**C**			
Run identification:	4(Seq#6954)			
Assay start date:	12/19/2005			
Tracer lot number:	3559-507			
Specific activity on day of assay:	78.97	**Ci/mmole**		
Cytosol vial or lot identification:	0725-06-01			
Protein (cytosol):	600	**micro gram per tube**		
Standard Curve IC50:	1.83E-09	**M**		
Weak Positive, Max Concentration:	**3.00E-02**	**M**		
Weak Positive IC50:	6.94E-05	**M**		
RBA:	2.64303E-05			
Max Concentration, Unknown 1:	3.00E-02	**M**	5e-3)	
IC50, Unknown 1:	1.43E-05	**CR42405**		
RBA, Unknown 1:	0.01280%			
Max Concentration, Unknown 2:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 2:	3.11E-07	**CR42406**		
RBA, Unknown 2:	0.59066%			
Max Concentration, Unknown 3:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 3:	4.69E-07	**CR42407**		
RBA, Unknown 3:	0.39121%			
Max Concentration, Unknown 4:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 4:	0.00E+00	**CR42408**		
RBA, Unknown 4:	#DIV/0!			
Max Concentration, Unknown 5:	3.00E-02	**M**	(example 5e-3)	
IC50, Unknown 5:	1.28E-02	**CR42409**		
RBA, Unknown 5:	0.00001%			
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	

Summary values				
	n	Mean	SD	
EtOH	6	7568.6	782.47	Total Binding, solvent control, tubes
Hot	6	49454.5	697.79	Total hot R1881 added to each tube
NSB	6	549.6	60.62	Nonspecific Binding
Specific EtOH	6	7019.0	782.47	

Assay Characterization Values		
EtOH / Hot	0.15	less than 0.1?
NSB / EtOH	0.07	around 0.25 ?

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 15.30% 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	2444.76	7578.75	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2515.03	7796.60	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2540.60	7875.85	TRUE	
4	1	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	174.86	524.58	TRUE	
5	2	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	216.10	648.31	TRUE	
6	3	Inert R1881	NSB		E-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	197.96	593.87	TRUE	
7	1	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	190.96	591.98	TRUE	
8	2	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	315.85	979.12	TRUE	
9	3	Inert R1881	S	1	E-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	295.90	917.28	TRUE	
10	1	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	678.24	2102.53	TRUE	
11	2	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	671.16	2080.61	TRUE	
12	3	Inert R1881	S	2	E-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	696.85	2160.22	TRUE	
13	1	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1068.72	3313.04	TRUE	
14	2	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1815.83	5629.07	TRUE	
15	3	Inert R1881	S	3	E-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1735.43	5379.82	TRUE	
16	1	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2378.67	7373.88	TRUE	
17	2	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2370.51	7348.59	TRUE	
18	3	Inert R1881	S	4	E-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	2358.99	7312.87	TRUE	
19	1	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2378.79	7374.24	TRUE	
20	2	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2484.36	7701.53	TRUE	
21	3	Inert R1881	S	5	E-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2511.15	7784.55	TRUE	
22	1	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	399.60	1198.80	TRUE	
23	2	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	369.96	1109.88	TRUE	
24	3	Weak Positive	P	1	E-1-P1	3.00E-02	300	30	10	50	300	1.0E-03	100	311.57	934.72	TRUE	
25	1	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1257.03	3771.08	TRUE	
26	2	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1153.67	3461.02	TRUE	
27	3	Weak Positive	P	2	E-1-P2	3.00E-03	300	30	10	50	300	1.0E-04	100	1138.25	3414.76	TRUE	
28	1	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2111.72	6335.16	TRUE	
29	2	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2072.43	6217.29	TRUE	
30	3	Weak Positive	P	3	E-1-P3	3.00E-04	300	30	10	50	300	1.0E-05	100	2210.52	6631.57	TRUE	
31	1	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2428.28	7284.83	TRUE	
32	2	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2444.77	7334.32	TRUE	
33	3	Weak Positive	P	4	E-1-P4	3.00E-05	300	30	10	50	300	1.0E-06	100	2418.38	7255.13	TRUE	
34	1	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2521.06	7563.18	TRUE	
35	2	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2547.05	7641.15	TRUE	
36	3	Weak Positive	P	5	E-1-P5	3.00E-06	300	30	10	50	300	1.0E-07	100	2329.30	6987.89	TRUE	
37	1	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2566.67	7700.02	TRUE	
38	2	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2525.17	7575.52	TRUE	
39	3	Weak Positive	P	6	E-1-P6	3.00E-07	300	30	10	50	300	1.0E-08	100	2613.22	7839.65	TRUE	
40	1	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	1877.29	5631.88	TRUE	
41	2	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	2615.91	7847.74	TRUE	
42	3	Weak Positive	P	7	E-1-P7	3.00E-08	300	30	10	50	300	1.0E-09	100	2304.94	6914.82	TRUE	
43	1	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2329.30	6987.89	TRUE	
44	2	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2428.28	7284.83	TRUE	
45	3	Weak Positive	P	8	E-1-P8	3.00E-09	300	30	10	50	300	1.0E-10	100	2549.53	7648.60	TRUE	
46	1	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	607.07	1881.91	TRUE	
47	2	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	541.93	1679.98	TRUE	
48	3	Unknown 1	U1	1	E-1-U1	3.00E-02	300	30	10	50	310	9.7E-04	100	625.33	1938.54	TRUE	
49	1	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	706.05	2188.76	TRUE	
50	2	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	615.07	1906.73	TRUE	
51	3	Unknown 1	U1	2	E-1-U1	3.00E-03	300	30	10	50	310	9.7E-05	100	576.24	1786.36	TRUE	
52	1	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	1413.81	4382.80	TRUE	
53	2	Unknown 1	U1	3	E-1-U1	3.00E-04	300	30	10	50	310	9.7E-06	100	1389.01	4305.92	TRUE	

Values for analysis by nonlinear regression

Standard Curve

unknown

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	7578.753	7029.2	-559.7	100.1	15.3247
2	2		—	103.2	7796.599	7247.0	-777.6	103.2	15.7652
3	3		—	104.4	7875.854	7326.3	-856.8	104.4	15.92546
4	1		-6.00	-0.4	524.5839	-25.0	6494.4	-0.4	1.060741
5	2		-6.00	1.4	648.3138	98.8	6370.7	1.4	1.31093
6	3		-6.00	0.0	593.8686	44.3	6425.2	0.6	1.200839
7	1	cold R1881	-7.01	0.6	591.9841	42.4	6427.0	0.6	1.197028
8	2	cold R1881	-7.01	6.1	979.1201	429.6	6039.9	6.1	1.979841
9	3	cold R1881	-7.01	5.2	917.281	367.7	6101.7	5.2	1.854799
10	1	cold R1881	-8.01	22.1	2102.532	1553.0	4916.5	22.1	4.251448
11	2	cold R1881	-8.01	21.8	2080.605	1531.1	4938.4	21.8	4.207112
12	3	cold R1881	-8.01	22.9	2160.223	1610.7	4858.8	22.9	4.368104
13	1	cold R1881	-9.01	39.4	3313.035	2763.5	3706.0	39.4	6.699161
14	2	cold R1881	-9.01	72.4	5629.073	5079.5	1390.0	72.4	11.38233
15	3	cold R1881	-9.01	68.8	5379.818	4830.3	1639.2	68.8	10.87832
16	1	cold R1881	-10.01	97.2	7373.877	6824.3	-354.8	97.2	14.91043
17	2	cold R1881	-10.01	96.9	7348.593	6799.0	-329.6	96.9	14.85931
18	3	cold R1881	-10.01	96.4	7312.869	6763.3	-293.8	96.4	14.78707
19	1	cold R1881	-11.01	97.2	7374.237	6824.7	-355.2	97.2	14.91116
20	2	cold R1881	-11.01	101.9	7701.525	7152.0	-682.5	101.9	15.57296
21	3	cold R1881	-11.01	103.1	7784.55	7235.0	-765.5	103.1	15.74084
22	1	Weak Positive	-3	9.2	1198.802	649.2	5820.2	9.2	2.424052
23	2	Weak Positive	-3	8.0	1109.88	560.3	5909.1	8.0	2.244246
24	3	Weak Positive	-3	5.5	934.7169	385.2	6084.3	5.5	1.890055
25	1	Weak Positive	-4	45.9	3771.084	3221.5	3247.9	45.9	7.625364
26	2	Weak Positive	-4	41.5	3461.016	2911.5	3558.0	41.5	6.998387
27	3	Weak Positive	-4	40.8	3414.762	2865.2	3604.3	40.8	6.904859
28	1	Weak Positive	-5	82.4	6335.157	5785.6	683.9	82.4	12.81008
29	2	Weak Positive	-5	80.7	6217.293	5667.7	801.7	80.7	12.57175
30	3	Weak Positive	-5	86.7	6631.566	6082.0	387.5	86.7	13.40943
31	1	Weak Positive	-6	96.0	7284.825	6735.3	-265.8	96.0	14.73036
32	2	Weak Positive	-6	96.7	7334.316	6784.8	-315.3	96.7	14.83044
33	3	Weak Positive	-6	95.5	7255.134	6705.6	-236.1	95.5	14.67033
34	1	Weak Positive	-7	99.9	7563.177	7013.6	-544.1	99.9	15.29321
35	2	Weak Positive	-7	101.0	7641.147	7091.6	-622.1	101.0	15.45087
36	3	Weak Positive	-7	91.7	6987.891	6438.3	31.1	91.7	14.12995
37	1	Weak Positive	-8	101.9	7700.016	7150.5	-681.0	101.9	15.56991
38	2	Weak Positive	-8	100.1	7575.519	7026.0	-556.5	100.1	15.31817
39	3	Weak Positive	-8	103.9	7839.654	7290.1	-820.6	103.9	15.85226
40	1	Weak Positive	-9	72.4	5631.882	5082.3	1387.1	72.4	11.38801
41	2	Weak Positive	-9	104.0	7847.739	7298.2	-828.7	104.0	15.86861
42	3	Weak Positive	-9	90.7	6914.82	6365.3	104.2	90.7	13.98219
43	1	Weak Positive	-10	91.7	6987.891	6438.3	31.1	91.7	14.12995
44	2	Weak Positive	-10	96.0	7284.825	6735.3	-265.8	96.0	14.73036
45	3	Weak Positive	-10	101.1	7648.596	7099.0	-629.6	101.1	15.46593
46	1	Unknown 1	-3.01424	19.0	1881.913	1332.4	5137.1	19.0	3.805344
47	2	Unknown 1	-3.01424	16.1	1679.982	1130.4	5339.0	16.1	3.397026
48	3	Unknown 1	-3.01424	19.8	1938.536	1389.0	5080.5	19.8	3.919839
49	1	Unknown 1	-4.01424	23.4	2188.759	1639.2	4830.3	23.4	4.425805
50	2	Unknown 1	-4.01424	19.3	1906.728	1357.2	5112.3	19.3	3.855521
51	3	Unknown 1	-4.01424	17.6	1786.357	1236.8	5232.7	17.6	3.612124
52	1	Unknown 1	-5.01424	54.6	4382.805	3833.3	2636.2	54.6	8.862301
53	2	Unknown 1	-5.01424	53.5	4305.922	3756.4	2713.1	53.5	8.706838

Column O, Rows 10 through 28 will contain output parameters

working volume **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule:	15.30%	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"	
54	3	Unknown 1	U1	3	**E-1-U1**	3.00E-04	300	30	10	50	310	9.7E-06	100	1400.41	4341.26	TRUE		Unknown 1
55	1	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2352.40	7292.45	TRUE		Unknown 1
56	2	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2167.64	6719.69	TRUE		Unknown 1
57	3	Unknown 1	U1	4	**E-1-U1**	3.00E-05	300	30	10	50	310	9.7E-07	100	2516.81	7802.12	TRUE		Unknown 1
58	1	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	1882.84	5836.82	TRUE		Unknown 1
59	2	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	2570.01	7967.03	TRUE		Unknown 1
60	3	Unknown 1	U1	5	**E-1-U1**	3.00E-06	300	30	10	50	310	9.7E-08	100	2482.26	7695.00	TRUE		Unknown 1
61	1	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2563.54	7946.98	TRUE		Unknown 1
62	2	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2455.50	7612.05	TRUE		Unknown 1
63	3	Unknown 1	U1	6	**E-1-U1**	3.00E-07	300	30	10	50	310	9.7E-09	100	2659.85	8245.53	TRUE		Unknown 1
64	1	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2484.97	7703.40	TRUE		Unknown 1
65	2	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2330.63	7224.95	TRUE		Unknown 1
66	3	Unknown 1	U1	7	**E-1-U1**	3.00E-08	300	30	10	50	310	9.7E-10	100	2458.78	7622.20	TRUE		Unknown 1
67	1	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2266.10	7024.91	TRUE		Unknown 1
68	2	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2480.24	7688.75	TRUE		Unknown 1
69	3	Unknown 1	U1	8	**E-1-U1**	3.00E-09	300	30	10	50	310	9.7E-11	100	2659.78	8245.31	TRUE		Unknown 1
70	1	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	130.96	405.98	TRUE		Unknown 2
71	2	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	110.56	342.73	TRUE		Unknown 2
72	3	Unknown 2	U2	1	**E-1-U2**	3.00E-02	300	30	10	50	310	9.7E-04	100	194.31	602.38	TRUE		Unknown 2
73	1	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	152.94	474.11	TRUE		Unknown 2
74	2	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	204.56	634.13	TRUE		Unknown 2
75	3	Unknown 2	U2	2	**E-1-U2**	3.00E-03	300	30	10	50	310	9.7E-05	100	183.34	568.37	TRUE		Unknown 2
76	1	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	181.46	562.53	TRUE		Unknown 2
77	2	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	243.66	755.35	TRUE		Unknown 2
78	3	Unknown 2	U2	3	**E-1-U2**	3.00E-04	300	30	10	50	310	9.7E-06	100	279.40	866.13	TRUE		Unknown 2
79	1	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	622.06	1928.39	TRUE		Unknown 2
80	2	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	717.01	2222.72	TRUE		Unknown 2
81	3	Unknown 2	U2	4	**E-1-U2**	3.00E-05	300	30	10	50	310	9.7E-07	100	640.07	1984.21	TRUE		Unknown 2
82	1	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	1986.19	6157.20	TRUE		Unknown 2
83	2	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	1805.33	5596.53	TRUE		Unknown 2
84	3	Unknown 2	U2	5	**E-1-U2**	3.00E-06	300	30	10	50	310	9.7E-08	100	2054.69	6369.54	TRUE		Unknown 2
85	1	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2365.61	7333.40	TRUE		Unknown 2
86	2	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2079.30	6445.84	TRUE		Unknown 2
87	3	Unknown 2	U2	6	**E-1-U2**	3.00E-07	300	30	10	50	310	9.7E-09	100	2536.78	7864.01	TRUE		Unknown 2
88	1	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2417.12	7493.08	TRUE		Unknown 2
89	2	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2791.23	8652.80	TRUE		Unknown 2
90	3	Unknown 2	U2	7	**E-1-U2**	3.00E-08	300	30	10	50	310	9.7E-10	100	2573.47	7977.76	TRUE		Unknown 2
91	1	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2622.96	8131.18	TRUE		Unknown 2
92	2	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2596.57	8049.35	TRUE		Unknown 2
93	3	Unknown 2	U2	8	**E-1-U2**	3.00E-09	300	30	10	50	310	9.7E-11	100	2642.76	8192.55	TRUE		Unknown 2
94	1	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	116.96	362.57	TRUE		Unknown 3
95	2	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	117.86	365.38	TRUE		Unknown 3
96	3	Unknown 3	U3	1	**E-1-U3**	3.00E-02	300	30	10	50	310	9.7E-04	100	155.07	480.71	TRUE		Unknown 3
97	1	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	144.06	446.58	TRUE		Unknown 3
98	2	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	166.97	517.62	TRUE		Unknown 3
99	3	Unknown 3	U3	2	**E-1-U3**	3.00E-03	300	30	10	50	310	9.7E-05	100	240.85	746.64	TRUE		Unknown 3
100	1	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	269.67	835.97	TRUE		Unknown 3
101	2	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	275.95	855.46	TRUE		Unknown 3
102	3	Unknown 3	U3	3	**E-1-U3**	3.00E-04	300	30	10	50	310	9.7E-06	100	275.02	852.55	TRUE		Unknown 3
103	1	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	779.22	2415.57	TRUE		Unknown 3
104	2	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	815.67	2528.59	TRUE		Unknown 3
105	3	Unknown 3	U3	4	**E-1-U3**	3.00E-05	300	30	10	50	310	9.7E-07	100	877.44	2720.05	TRUE		Unknown 3
106	1	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2053.26	6365.09	TRUE		Unknown 3

Values for analysis by nonlinear regression
Standard Curve
unknown

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
54	3	-5.01424	54.0	4341.262	3791.7	2677.8	54.0	8.778298
55	1	-6.01424	96.1	7292.452	6742.9	-273.4	96.1	14.74579
56	2	-6.01424	87.9	6719.693	6170.1	299.3	87.9	13.58763
57	3	-6.01424	103.3	7802.123	7252.6	-783.1	103.3	15.77637
58	1	-7.01424	75.3	5836.816	5287.3	1182.2	75.3	11.8024
59	2	-7.01424	105.7	7967.031	7417.5	-948.0	105.7	16.10983
60	3	-7.01424	101.8	7694.997	7145.4	-676.0	101.8	15.55976
61	1	-8.01424	105.4	7946.98	7397.4	-928.0	105.4	16.06928
62	2	-8.01424	100.6	7612.05	7062.5	-593.0	100.6	15.39203
63	3	-8.01424	109.6	8245.532	7696.0	-1226.5	109.6	16.67297
64	1	-9.01424	101.9	7703.398	7153.8	-684.4	101.9	15.57674
65	2	-9.01424	95.1	7224.953	6675.4	-205.9	95.1	14.6093
66	3	-9.01424	100.8	7622.203	7072.6	-603.2	100.8	15.41256
67	1	-10.01424	92.3	7024.913	6475.4	-5.9	92.3	14.20481
68	2	-10.01424	101.7	7688.75	7139.2	-669.7	101.7	15.54713
69	3	-10.01424	109.6	8245.312	7695.8	-1226.3	109.6	16.67253
70	1	-3.01424	-2.0	405.976	-143.6	6613.1	-2.0	0.820908
71	2	-3.01424	-2.9	342.7314	-206.8	6676.3	-2.9	0.693024
72	3	-3.01424	0.8	602.3762	52.8	6416.7	0.8	1.218042
73	1	-4.01424	-1.1	474.1053	-75.4	6544.9	-1.1	0.95867
74	2	-4.01424	1.2	634.1264	84.6	6384.9	1.2	1.282243
75	3	-4.01424	0.3	568.3664	18.8	6450.7	0.3	1.149272
76	1	-5.01424	0.2	562.5316	13.0	6456.5	0.2	1.137473
77	2	-5.01424	2.9	755.3469	205.8	6263.7	2.9	1.527358
78	3	-5.01424	4.5	866.1313	316.6	6152.9	4.5	1.751371
79	1	-6.01424	19.6	1928.386	1378.8	5090.6	19.6	3.899316
80	2	-6.01424	23.8	2222.719	1673.2	4796.3	23.8	4.494475
81	3	-6.01424	20.4	1984.212	1434.7	5034.8	20.4	4.012199
82	1	-7.01424	79.9	6157.195	5607.6	861.8	79.9	12.45023
83	2	-7.01424	71.9	5596.532	5047.0	1422.5	71.9	11.31653
84	3	-7.01424	82.9	6369.536	5820.0	649.5	82.9	12.87959
85	1	-8.01424	96.6	7333.403	6783.9	-314.4	96.6	14.82859
86	2	-8.01424	84.0	6445.842	5896.3	573.2	84.0	13.03389
87	3	-8.01424	104.2	7864.006	7314.5	-845.0	104.2	15.9015
88	1	-9.01424	98.9	7493.081	6943.5	-474.1	98.9	15.15147
89	2	-9.01424	115.4	8652.801	8103.2	-1633.8	115.4	17.49649
90	3	-9.01424	105.8	7977.76	7428.2	-958.7	105.8	16.13152
91	1	-10.01424	108.0	8131.179	7581.6	-1112.2	108.0	16.44174
92	2	-10.01424	106.8	8049.355	7499.8	-1030.3	106.8	16.27629
93	3	-10.01424	108.9	8192.547	7643.0	-1173.5	108.9	16.56583
94	1	-3.01424	-2.7	362.5686	-187.0	6656.5	-2.7	0.733136
95	2	-3.01424	-2.6	365.3803	-184.2	6653.6	-2.6	0.738821
96	3	-3.01424	-1.0	480.7111	-68.8	6538.3	-1.0	0.972027
97	1	-4.01424	-1.5	446.5761	-103.0	6572.5	-1.5	0.903004
98	2	-4.01424	-0.5	517.6222	-31.9	6501.4	-0.5	1.046664
99	3	-4.01424	2.8	746.6366	197.1	6272.4	2.8	1.509745
100	1	-5.01424	4.1	835.9748	286.4	6183.1	4.1	1.690393
101	2	-5.01424	4.4	855.4552	305.9	6163.6	4.4	1.729783
102	3	-5.01424	4.3	852.5543	303.0	6166.5	4.3	1.723917
103	1	-6.01424	26.6	2415.57	1866.0	4603.5	26.6	4.884432
104	2	-6.01424	28.2	2528.588	1979.0	4490.4	28.2	5.112961
105	3	-6.01424	30.9	2720.054	2170.5	4299.0	30.9	5.500117
106	1	-7.01424	82.9	6365.091	5815.5	653.9	82.9	12.8706

Column O, Rows 10 through 28 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule:	15.30%	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"	
107	2	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2283.13	7077.71	TRUE		Unknown 3
108	3	Unknown 3	U3	5	**E-1-U3**	3.00E-06	300	30	10	50	310	9.7E-08	100	2052.81	6363.71	TRUE		Unknown 3
109	1	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2596.46	8049.04	TRUE		Unknown 3
110	2	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2619.90	8121.67	TRUE		Unknown 3
111	3	Unknown 3	U3	6	**E-1-U3**	3.00E-07	300	30	10	50	310	9.7E-09	100	2582.81	8006.72	TRUE		Unknown 3
112	1	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2433.71	7544.50	TRUE		Unknown 3
113	2	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2397.63	7432.65	TRUE		Unknown 3
114	3	Unknown 3	U3	7	**E-1-U3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2517.72	7804.93	TRUE		Unknown 3
115	1	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2443.70	7575.46	TRUE		Unknown 3
116	2	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2407.39	7462.91	TRUE		Unknown 3
117	3	Unknown 3	U3	8	**E-1-U3**	3.00E-09	300	30	10	50	310	9.7E-11	100	2576.69	7987.73	TRUE		Unknown 3
118	1	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	2498.09	7744.08	TRUE		Unknown 4
119	2	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	2599.88	8059.62	TRUE		Unknown 4
120	3	Unknown 4	U4	1	**E-1-U4**	3.00E-02	300	30	10	50	310	9.7E-04	100	2543.84	7885.90	TRUE		Unknown 4
121	1	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	2410.64	7472.98	TRUE		Unknown 4
122	2	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	1458.73	4522.06	TRUE		Unknown 4
123	3	Unknown 4	U4	2	**E-1-U4**	3.00E-03	300	30	10	50	310	9.7E-05	100	2391.15	7412.55	TRUE		Unknown 4
124	1	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	2435.88	7551.23	TRUE		Unknown 4
125	2	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	1722.22	5338.89	TRUE		Unknown 4
126	3	Unknown 4	U4	3	**E-1-U4**	3.00E-04	300	30	10	50	310	9.7E-06	100	2619.91	8121.71	TRUE		Unknown 4
127	1	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	2618.56	8117.55	TRUE		Unknown 4
128	2	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	2735.52	8480.12	TRUE		Unknown 4
129	3	Unknown 4	U4	4	**E-1-U4**	3.00E-05	300	30	10	50	310	9.7E-07	100	1953.59	6056.13	TRUE		Unknown 4
130	1	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2533.47	7853.74	TRUE		Unknown 4
131	2	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2085.75	6465.84	TRUE		Unknown 4
132	3	Unknown 4	U4	5	**E-1-U4**	3.00E-06	300	30	10	50	310	9.7E-08	100	2619.68	8121.00	TRUE		Unknown 4
133	1	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2398.62	7435.72	TRUE		Unknown 4
134	2	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	2322.74	7200.48	TRUE		Unknown 4
135	3	Unknown 4	U4	6	**E-1-U4**	3.00E-07	300	30	10	50	310	9.7E-09	100	1155.85	3583.14	TRUE		Unknown 4
136	1	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	1128.37	3497.96	TRUE		Unknown 4
137	2	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	1345.63	4171.45	TRUE		Unknown 4
138	3	Unknown 4	U4	7	**E-1-U4**	3.00E-08	300	30	10	50	310	9.7E-10	100	877.62	2720.64	TRUE		Unknown 4
139	1	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2784.64	8632.39	TRUE		Unknown 4
140	2	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2237.56	6936.44	TRUE		Unknown 4
141	3	Unknown 4	U4	8	**E-1-U4**	3.00E-09	300	30	10	50	310	9.7E-11	100	2563.59	7947.12	TRUE		Unknown 4
142	1	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2384.65	7392.41	TRUE		Unknown 5
143	2	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2261.45	7010.49	TRUE		Unknown 5
144	3	Unknown 5	U5	1	**E-1-U5**	3.00E-02	300	30	10	50	310	9.7E-04	100	2160.86	6698.68	TRUE		Unknown 5
145	1	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2460.32	7626.99	TRUE		Unknown 5
146	2	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2553.75	7916.62	TRUE		Unknown 5
147	3	Unknown 5	U5	2	**E-1-U5**	3.00E-03	300	30	10	50	310	9.7E-05	100	2427.07	7523.93	TRUE		Unknown 5
148	1	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2609.42	8089.20	TRUE		Unknown 5
149	2	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2596.59	8049.44	TRUE		Unknown 5
150	3	Unknown 5	U5	3	**E-1-U5**	3.00E-04	300	30	10	50	310	9.7E-06	100	2636.19	8172.18	TRUE		Unknown 5
151	1	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2566.11	7954.95	TRUE		Unknown 5
152	2	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2659.60	8244.77	TRUE		Unknown 5
153	3	Unknown 5	U5	4	**E-1-U5**	3.00E-05	300	30	10	50	310	9.7E-07	100	2485.38	7704.66	TRUE		Unknown 5
154	1	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2511.37	7785.23	TRUE		Unknown 5
155	2	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2722.54	8439.88	TRUE		Unknown 5
156	3	Unknown 5	U5	5	**E-1-U5**	3.00E-06	300	30	10	50	310	9.7E-08	100	2832.06	8779.37	TRUE		Unknown 5
157	1	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2637.04	8174.81	TRUE		Unknown 5
158	2	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2439.89	7563.66	TRUE		Unknown 5
159	3	Unknown 5	U5	6	**E-1-U5**	3.00E-07	300	30	10	50	310	9.7E-09	100	2550.49	7906.51	TRUE		Unknown 5

Values for analysis by nonlinear regression
Standard Curve
unknown

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
107	2	-7.01424	93.0	7077.706	6528.2	-58.7	93.0	14.31156
108	3	-7.01424	82.8	6363.708	5814.2	655.3	82.8	12.86781
109	1	-8.01424	106.8	8049.035	7499.5	-1030.0	106.8	16.27564
110	2	-8.01424	107.9	8121.675	7572.1	-1102.6	107.9	16.42253
111	3	-8.01424	106.2	8006.72	7457.2	-987.7	106.2	16.19008
112	1	-9.01424	99.7	7544.498	6994.9	-525.5	99.7	15.25544
113	2	-9.01424	98.1	7432.653	6883.1	-413.6	98.1	15.02928
114	3	-9.01424	103.4	7804.932	7255.4	-785.9	103.4	15.78205
115	1	-10.01424	100.1	7575.458	7025.9	-556.4	100.1	15.31804
116	2	-10.01424	98.5	7462.906	6913.4	-443.9	98.5	15.09045
117	3	-10.01424	106.0	7987.73	7438.2	-968.7	106.0	16.15168
118	1	-3.01424	102.5	7744.076	7194.5	-725.0	102.5	15.659
119	2	-3.01424	107.0	8059.625	7510.1	-1040.6	107.0	16.29706
120	3	-3.01424	104.5	7885.904	7336.4	-866.9	104.5	15.94578
121	1	-4.01424	98.6	7472.978	6923.4	-453.9	98.6	15.11082
122	2	-4.01424	56.6	4522.057	3972.5	2497.0	56.6	9.143877
123	3	-4.01424	97.8	7412.55	6863.0	-393.5	97.8	14.98863
124	1	-5.01424	99.8	7551.234	7001.7	-532.2	99.8	15.26906
125	2	-5.01424	68.2	5338.894	4789.3	1680.1	68.2	10.79557
126	3	-5.01424	107.9	8121.715	7572.2	-1102.7	107.9	16.42261
127	1	-6.01424	107.8	8117.548	7568.0	-1098.5	107.8	16.41418
128	2	-6.01424	113.0	8480.118	7930.6	-1461.1	113.0	17.14732
129	3	-6.01424	78.5	6056.126	5506.6	962.9	78.5	12.24586
130	1	-7.01424	104.1	7853.742	7304.2	-834.7	104.1	15.88075
131	2	-7.01424	84.3	6465.837	5916.3	553.2	84.3	13.07432
132	3	-7.01424	107.9	8120.996	7571.4	-1102.0	107.9	16.42115
133	1	-8.01424	98.1	7435.722	6886.2	-416.7	98.1	15.03549
134	2	-8.01424	94.8	7200.479	6650.9	-181.5	94.8	14.55981
135	3	-8.01424	43.2	3583.138	3033.6	3435.9	43.2	7.245326
136	1	-9.01424	42.0	3497.959	2948.4	3521.1	42.0	7.073089
137	2	-9.01424	51.6	4171.447	3621.9	2847.6	51.6	8.434922
138	3	-9.01424	30.9	2720.635	2171.1	4298.4	30.9	5.501292
139	1	-10.01424	115.2	8632.39	8082.8	-1613.4	115.2	17.45522
140	2	-10.01424	91.0	6936.439	6386.9	82.6	91.0	14.02591
141	3	-10.01424	105.4	7947.12	7397.6	-928.1	105.4	16.06956
142	1	-3.01424	97.5	7392.406	6842.9	-373.4	97.5	14.9479
143	2	-3.01424	92.0	7010.489	6460.9	8.5	92.0	14.17564
144	3	-3.01424	87.6	6698.675	6149.1	320.4	87.6	13.54513
145	1	-4.01424	100.8	7626.992	7077.4	-608.0	100.8	15.42225
146	2	-4.01424	105.0	7916.616	7367.1	-897.6	105.0	16.00788
147	3	-4.01424	99.4	7523.926	6974.4	-504.9	99.4	15.21384
148	1	-5.01424	107.4	8089.202	7539.6	-1070.2	107.4	16.35686
149	2	-5.01424	106.9	8049.441	7499.9	-1030.4	106.9	16.27647
150	3	-5.01424	108.6	8172.177	7622.6	-1153.1	108.6	16.52464
151	1	-6.01424	105.5	7954.953	7405.4	-935.9	105.5	16.08541
152	2	-6.01424	109.6	8244.769	7695.2	-1225.7	109.6	16.67143
153	3	-6.01424	101.9	7704.663	7155.1	-685.6	101.9	15.5793
154	1	-7.01424	103.1	7785.232	7235.7	-766.2	103.1	15.74222
155	2	-7.01424	112.4	8439.877	7890.3	-1420.8	112.4	17.06595
156	3	-7.01424	117.3	8779.371	8229.8	-1760.3	117.3	17.75243
157	1	-8.01424	108.6	8174.812	7625.3	-1155.8	108.6	16.52997
158	2	-8.01424	99.9	7563.659	7014.1	-544.6	99.9	15.29418
159	3	-8.01424	104.8	7906.51	7357.0	-887.5	104.8	15.98745

Column O, Rows 10 through 28 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

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

Check the 10% rule:	15.30%	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"	
160	1	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2723.80	8443.79	TRUE		Unknown 5
161	2	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2566.90	7957.39	TRUE		Unknown 5
162	3	Unknown 5	U5	7	**E-1-U5**	3.00E-08	300	30	10	50	310	9.7E-10	100	2834.15	8785.86	TRUE		Unknown 5
163	1	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2443.14	7573.73	TRUE		Unknown 5
164	2	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2679.08	8305.14	TRUE		Unknown 5
165	3	Unknown 5	U5	8	**E-1-U5**	3.00E-09	300	30	10	50	310	9.7E-11	100	2363.02	7325.35	TRUE		Unknown 5
166	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2747.25	8516.49	TRUE		
167	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	2417.15	7493.16	TRUE		
168	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1984.08	6150.64	TRUE		
169	1	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	160.43	481.29	TRUE		
170	2	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	177.57	532.70	TRUE		
171	3	Inert R1881	NSB		**E-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	172.19	516.57	TRUE		
172	1	none	Hot		—	—	—	30	—	—	—	—		49657.64	49657.64	TRUE		
173	2	none	Hot		—	—	—	30	—	—	—	—		49521.55	49521.55	TRUE		
174	3	none	Hot		—	—	—	30	—	—	—	—		49534.62	49534.62	TRUE		
175	1	none	Hot		—	—	—	30	—	—	—	—		48088.30	48088.30	TRUE		
176	2	none	Hot		—	—	—	30	—	—	—	—		49960.48	49960.48	TRUE		
177	3	none	Hot		—	—	—	30	—	—	—	—		49964.29	49964.29	TRUE		

Values for analysis by nonlinear regression
Standard Curve
unknown

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
160	1	-9.01424	112.5	8443.786	7894.2	-1424.8	112.5	17.07385
161	2	-9.01424	105.5	7957.39	7407.8	-938.4	105.5	16.09033
162	3	-9.01424	117.3	8785.859	8236.3	-1766.8	117.3	17.76555
163	1	-10.01424	100.1	7573.731	7024.2	-554.7	100.1	15.31455
164	2	-10.01424	110.5	8305.142	7755.6	-1286.1	110.5	16.79351
165	3	-10.01424	96.5	7325.353	6775.8	-306.3	96.5	14.81231
166	1	—	113.5	8516.487	7966.9	-1497.5	113.5	17.22086
167	2	—	98.9	7493.159	6943.6	-474.1	98.9	15.15163
168	3	—	79.8	6150.636	5601.1	868.4	79.8	12.43696
169	1	-6.00	-1.0	481.2858	-68.3	6537.7	-1.0	0.973189
170	2	-6.00	-0.2	532.6992	-16.9	6486.3	-0.2	1.077151
171	3	-6.00	-0.5	516.5679	-33.0	6502.5	-0.5	1.044532
172	1			49657.64	49108.1			
173	2			49521.55	48972.0			
174	3			49534.62	48985.1			
175	1			48088.3	47538.7			
176	2			49960.48	49410.9			
177	3			49964.29	49414.7			

Appendix 4: Copy of Biological Method

OFFICIAL
QA
ISSUE

ANDROGEN RECEPTOR COMPETITIVE BINDING ASSAY USING [^{3}H]-R1881

Written By: Rebecca C. M°Gee Date: 06 JUN 05
Rebecca C. M°Gee, B.Sc.
Principal Research Scientist, Technology Development

Approved By: James E Russo for Aruna Koganti Date: 06 June 05
Aruna Koganti, Ph. D.
Study Director

Approved By: Date: 06 JUN 2005
Neil S. Jensen, Ph.D.
Director, Technology Development

Approved By: Date: 07 JUNE 2005
Paul Silber, Ph.D.
President & CEO

I. Introduction

A. This document describes a radioligand binding assay for the evaluation of the ability of test compounds to inhibit binding of [^{3}H]-R1881 to the Androgen receptor.

II. Safety Considerations

A. General laboratory safety and radiation safety procedures apply.

III. Materials and Equipment

A. pH meter
B. Timer
C. 20 ml scintillation vials
D. assorted **amber** vials
E. 12 × 75 mm siliconized borosilicate glass test tubes
F. 4°C refrigerator
G. Tabletop centrifuge
H. LS6500 T/A Liquid Scintillation Counter
I. Assorted pipettes and tips
J. Shaker
K. Stir/hot plate
L. Assorted graduated cylinders
M. Parafilm®
N. Vacuum concentrator

IV. Reagents and Chemicals (ACS reagent grade)

A. dithiothreitol (DTT), (FW 154.3)
B. hydrochloric acid (HCl) (FW 36.46), 1 N
C. sodium hydroxide (NaOH) (FW 40.0), 1 N
D. absolute ethanol (FW 46.07)
E. [^{3}H]-R1881 (PerkinElmer, purity >97%)
F. deionized water (dH_2O)
G. ethylenediaminetetraacetic acid disodium salt (EDTA) (FW 372.2)
H. glycerol (FW 92.09, 99%+)
I. hydroxyapatite, hydrated (HAP) (BioRad catalog # 130-0151)
J. phenylmethylsulfonyl fluoride (PMSF) (FW 174.2)
K. R1881 (non-radioactive methyltrienolone) (PerkinElmer, FW 284.4, 5 mg/bottle)
L. Formula 989 scintillation cocktail
M. 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris Base) (FW 121.1)
N. Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (FW 157.6)
O. Sodium molybdate dehydrate (FW 241.95, Sigma S-6646)
P. Triamcinolone acetonide (FW 434.5, >99%)
Q. Dexamethasone (FW 392.5)

V. Preparation of Reagents and Solutions

A. 200 mM EDTA Stock Solution
1. Add 7.444 g EDTA to 50 ml dH_2O.
2. Bring pH to 7.4 with 1N NaOH.
3. When EDTA has dissolved, Q.S. to 95 ml with dH_2O.
4. Adjust pH to 7.4 with 1N NaOH.
5. Q.S. to 100 ml.
6. Sterile filter the solution.
7. Store up to 180 days at 4°C.

B. 100 mM PMSF Stock Solution
1. Dissolve 1.742 g of PMSF in 100 ml of ethanol.
2. Store up to 180 days at 4°C.

C. 1 M Sodium Molybdate Stock Solution
1. Dissolve 2.419 g sodium molybdate to 8 mL of dH_2O
2. Q.S. to 10 mL with dH_2O.
3. Sterile filter the solution.
4. Store up to 180 days at 4°C.

D. 1 M Tris Stock Buffer
1. Dissolve 147.24 g of Tris-HCl and 8 g of Tris base to 800 ml of cold (~4°C) dH_2O.
2. Adjust pH to 7.4 ± 0.1 at 4 (±3) °C using 1N HCl or 1N NaOH
3. Q.S. to 1 L.
4. Sterile filter the buffer.
5. Store up to 180 days at 4°C.

E. Low Salt TEDG + PMSF Buffer
1. 871.5 ml dH_2O.
2. 10 ml 1M Tris Stock Buffer
3. 1 mL sodium molybdate stock solution
4. 100 ml glycerol
5. 7.5 ml 200 mM EDTA Stock Solution
6. Store at 4°C until addition of DTT and PMSF.
7. Dissolve 154 mg DTT in the buffer immediately before use.
8. 10 ml 100 mM PMSF Stock Solution
9. Check pH and adjust to 7.4 ± 0.1 (at 4 ± 3°C).
10. Prepare fresh daily.

F. 50 mM Tris Buffer
1. 950 mL of dH_2O
2. 50 mL of 1 M Tris Stock Buffer
3. Check pH and adjust to 7.4 ± 0.1 at 4 ±3 °C.
4. Sterile filter the buffer.
5. Store up to 30 days at 4°C.

G. HAP Slurry
1. Begin preparation on the day the assay incubation starts.
2. 100 mL HAP
   (a) HAP comes hydrated at 2.5 ml/g (0.4 g/ml)
   (b) Mix by gently rolling the bottle on the countertop, shaking will destroy the HAP crystals.
   (c) Pour 100 ml into a graduated cylinder.
3. Allow it to settle for at least 2 hours at 4°C.
4. Aspirate the supernatant and discard.
5. Q.S. to 100 ml of 50 mM Tris buffer, cover with Parafilm® and invert several times to resuspend the HAP.
6. Allow the HAP to settle for at least 2 hours at 4°C.
7. Aspirate the supernatant and discard.
8. Q.S. to 100 ml of 50 mM Tris buffer, cover with Parafilm® and invert several times to resuspend the HAP.
9. Allow the HAP to settle overnight at 4°C.
10. Aspirate the supernatant and discard.
11. Q.S. to 100 ml of 50 mM Tris buffer, cover with Parafilm® and invert several times to resuspend the HAP.
12. Allow the HAP to settle for at least 2 hours at 4°C.
13. Measure the volume of HAP remaining in the graduated cylinder.
14. Divide that volume by 0.6; this will give you the total volume of HAP slurry to be made.
15. Q.S. to the total volume calculated in step V.G.14
16. Store at 4°C until ready for use in the extraction.

H. Labelling of assay tubes
1. Each tube will be labelled with a unique identifying code consisting of a position code, a "tube type code", a replicate code and a concentration code.
2. The position code represents the number in the counting sequence of the final sample in the scintillation counter.
3. The replicate code identifies the tube as being the first, second or third tube of each triplicate.
4. The concentration code refers to the number of different concentrations of the competitor that are used.
5. The tube type code identifies the type of sample:
   (a) EtOH = tubes containing cytosol, buffer, triamcinolone, EtOH (vehicle) and [^{3}H]-R1881
   (b) NSB = tubes containing cytosol, buffer, triamcinolone, R1881 at a final concentration of 1 µM and [^{3}H]-R1881.
   (c) S = tubes containing cytosol, buffer, triamcinolone, R1881 at 5 different concentrations, and [^{3}H]-R1881. Each concentration is assigned a number (S1, S2, S3, S4 and S5).
   (d) WP = tubes containing cytosol, buffer, triamcinolone, dexamethasone at 8 different concentrations, and [^{3}H]-

R1881. Each concentration is assigned a number (WP1, WP2, WP3, WP4, WP5, WP6, WP7, AND WP8).

(e) Hot = sample of [^{3}H]-R1881 in scintillation vial containing only the amount of [^{3}H]-R1881 used in the incubation tubes.

6. Label two sets of tubes, one for the incubation (incubation tubes) and one for the harvest (HAP tubes) except for the "Hot" samples in scintillation vials.

VI. Androgen Receptor Competition Binding Assay Start Solutions

A. [^{3}H]-R1881 Stock solutions will be provided by the sponsor

1. 0.1 µM [^{3}H]-R1881 Stock Solution (**amber** vial)
   (a) Dispense 1 µL per specific activity unit into a 20 mL **amber** vial and bringing the volume to 10 mL with absolute ethanol.
   (b) **Example:** if the specific activity is 86 Ci/mmol, dispense 86 µL of the original bottle into a 20 mL **amber** vial and add 9.914 mL of absolute ethanol.
   (c) Store at -20°C.

2. 10 nM [^{3}H]-R1881 Stock Solution (**amber** vial)
   (a) 1 mL of 0.1 µM [^{3}H]-R1881 Stock Solution
   (b) 9 mL absolute ethanol
   (c) Store at -20°C in an **amber** vial.

B. 60 µM triamcinolone acetonide

1. First prepare 6 mM triamcinolone acetonide
   (a) 2.61 mg
   (b) dissolved in 1 mL absolute ethanol
2. 60 µM triamcinolone acetonide
   (a) 0.1 mL of 6 mM triamcinolone acetonide
   (b) 9.9 mL absolute ethanol

C. 1X Cytosol

1. Thaw on ice while the assay tube contents are in the vacuum concentrator.
2. Dilute to 3.3 mg of protein per mL in low salt TEDG + PMSF buffer.
   (a) Keep on ice until addition to incubation tubes.

D. 30X stock drug solutions provided by the sponsor

1. R1881 concentrations
   (a) 10 µM (NSB)
   (b) 3 µM
   (c) 300 nM
   (d) 30 nM

(e) 3 nM
(f) 0.3 nM

2. weak positive (dexamethasone)
(a) 30 mM
(b) 3 mM
(c) 300 µM
(d) 30 µM
(e) 3 µM
(f) 300 nM
(g) 30 nM
(h) 3 nM

VII. Androgen receptor binding assay incubation

A. Place labelled incubation tubes on ice.
B. Add 50 µL of 60 µM triamcinolone to each assay tube.
C. Add 30 µL of 10 µM R1881 to each tube labeled "NSB".
D. Add 30 µL of 10 nM [^{3}H]R1881 to each tube, including the scintillation vials labeled "Hot".
E. Add 14 mL of Formula 989 to "hot" tubes (#52-57) and cap the tubes.
F. Place the incubation tubes (#1-51) in the vacuum concentrator and dry according to the instrument instructions.
G. When the tube contents are dry, transfer the tubes to a rack on ice.
H. Add the following components to each incubation tube:

Tube #	Tube type	Replicate	Competitor	Competitor Initial concentration	Volume of initial concentration
1	EtOH	1	Ethanol	100%	10 µL
2	EtOH	2	Ethanol	100%	10 µL
3	EtOH	3	Ethanol	100%	10 µL
4	NSB	1	R1881	10 µM	***N/A***
5	NSB	2	R1881	10 µM	***N/A***
6	NSB	3	R1881	10 µM	***N/A***
7	S1	1	R1881	3 µM	10 µL
8	S1	2	R1881	3 µM	10 µL
9	S1	3	R1881	3 µM	10 µL
10	S2	1	R1881	300 nM	10 µL
11	S2	2	R1881	300 nM	10 µL
12	S2	3	R1881	300 nM	10 µL
13	S3	1	R1881	30 nM	10 µL
14	S3	2	R1881	30 nM	10 µL
15	S3	3	R1881	30 nM	10 µL
16	S4	1	R1881	3 nM	10 µL

Tube #	Tube type	Replicate	Competitor	Competitor Initial concentration	Volume of initial concentration
17	S4	2	R1881	3 nM	10 µL
18	S4	3	R1881	3 nM	10 µL
19	S5	1	R1881	0.3 nM	10 µL
20	S5	2	R1881	0.3 nM	10 µL
21	S5	3	R1881	0.3 nM	10 µL
22	WP1	1	Weak positive	30 mM	10 µL
23	WP1	2	Weak positive	30 mM	10 µL
24	WP1	3	Weak positive	30 mM	10 µL
25	WP2	1	Weak positive	3 mM	10 µL
26	WP2	2	Weak positive	3 mM	10 µL
27	WP2	3	Weak positive	3 mM	10 µL
28	WP3	1	Weak positive	300 µM	10 µL
29	WP3	2	Weak positive	300 µM	10 µL
30	WP3	3	Weak positive	300 µM	10 µL
31	WP4	1	Weak positive	30 µM	10 µL
32	WP4	2	Weak positive	30 µM	10 µL
33	WP4	3	Weak positive	30 µM	10 µL
34	WP5	1	Weak positive	3 µM	10 µL
35	WP5	2	Weak positive	3 µM	10 µL
36	WP5	3	Weak positive	3 µM	10 µL
37	WP6	1	Weak positive	300 nM	10 µL
38	WP6	2	Weak positive	300 nM	10 µL
39	WP6	3	Weak positive	300 nM	10 µL
40	WP7	1	Weak positive	30 nM	10 µL
41	WP7	2	Weak positive	30 nM	10 µL
42	WP7	3	Weak positive	30 nM	10 µL
43	WP8	1	Weak positive	3 nM	10 µL
44	WP8	2	Weak positive	3 nM	10 µL
45	WP8	3	Weak positive	3 nM	10 µL
46	EtOH	1	ethanol	100%	10 µL
47	EtOH	2	ethanol	100%	10 µL
48	EtOH	3	ethanol	100%	10 µL
49	NSB	1	R1881	10 µM	***N/A***
50	NSB	2	R1881	10 µM	***N/A***
51	NSB	3	R1881	10 µM	***N/A***

I. Add 300 µL of 1X Cytosol to all incubation tubes (#1-51).

J. Incubate for 20 ± 1 hour in the refrigerator on an orbital shaker at approximately 50 rpm.

VIII. Completion of the androgen receptor binding assay

A. Separation of [^{3}H]-R1881 bound to receptor from free (unbound) [^{3}H]-R1881
   1. Add 500 µL of HAP to empty, labeled tubes in an ice-water bath in centrifuge rotor buckets (one HAP tube for each incubation tube). NOTE: stir the HAP continuously while adding it to the tubes.
   2. Remove the incubation tubes from the refrigerator and place in an ice-water bath.
   3. Transfer 100 µL from each incubation tube to its corresponding HAP tube.
   4. Transfer the HAP tubes to centrifuge rotor buckets on ice.
   5. Vortex the tubes (entire rotor bucket) for 15 ± 5 seconds in 5 minutes intervals for a total of 20 minutes with the tubes remaining on ice between vortexing. Tubes should be vortexed a total of 5 times. (NOTE: cover the tubes with Parafilm when performing any vortexing step to prevent splashing of radioactive material.)
   6. Add 2 ml of 50 mM Tris buffer to each tube.
   7. Quickly vortex the tubes.
   8. Centrifuge the tubes for 2-3 minutes at 600 × *g* at 4°C.
   9. Decant or carefully aspirate the supernatant from the tubes.
   10. Add 2 ml of 50 mM Tris buffer to each tube.
   11. Quickly vortex the tubes.
   12. Centrifuge the tubes for 2-3 minutes at 600 × *g* at 4°C.
   13. Decant or carefully aspirate the supernatant from the tubes.
   14. Repeat steps 7 through 10 two more times.

B. Extraction and quantifying [^{3}H]-R1881 bound to receptor

   1. Add 2 ml of absolute ethanol to each HAP tube.
   2. Allow the tubes to sit at room temparature, vortexing at 5 minute intervals, for 10 minutes. Tubes should be vortexed a total of 3 times.
   3. Centrifuge for 10 minutes at 600 × *g* at 4°C.
   4. Carefully decant the entire supernatant into a scintillation vial.
   5. Add 14 ml Formula 989 to each scintillation vial.
   6. Measure the radioactivity from each sample using a liquid scintillation counter.

IX. Data calculations

A. Enter the total radioactivity of each sample into the data calculation spreadsheet prepared by the sponsor for this study.
B. Complete all other fields in the data calculation spreadsheet.
C. Transfer data from spreadsheet into GraphPad Prism tables:
   1. The X-axis is the final log concentration of the non-radioactive compound.
   2. The Y-axis is the DPM.

3. Perform non-linear regression, sigmoidal dose-response analysis on the data.
4. Determine the K_I using the Cheng-Prusoff equation
   (a)
$$K_I = \frac{IC_{50}}{1 + \frac{[\text{radioligand}]}{K_D}}$$

Appendix 5: Battelle QAU Statement