DRAFT REPORT

Validation of an Androgen Receptor Binding Assay–Task 2

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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:	270-1126-04
In Vitro Technologies Protocol Number:	1126
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Date of Issue:	01 February 2006

This report contains 122 pages, including eight appendices.

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Participation

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

Study Director:	Aruna Koganti, Ph.D.
Scientists:	Neil Jensen, Ph.D.
	Rebecca C. McGee, B.Sc.
Technical Writers:	Barbara Biery, Ph.D.
	Blaise Considine, B.A.

Study Dates and Data Retention

Study initiation date:	11 January 2005
Date protocol signed by Sponsor:	05 January 2005
Date test articles received:	13 January 2005
Experimental start date:	17 February 2005
Experimental end date:	21 March 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 (excluding proprietary information) 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 8.

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:
17 February 2005	Standard and sample preparation	18 February 2005
07 March 2005	Sample preparation	07 March 2005
11 March 2005	Incubation and analyses	14 March 2005
17 March 2005	Sample preparation	21 March 2005
10–16 August 2005	Data and report	16 August 2005

Quality Assurance	Signature	Date

Glossary of Abbreviations

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

Summary

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

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

A total of three saturation experiments were conducted and the amount of cytosolic protein used for each incubation in the saturation binding experiments was 0.6 mg. The K_d observed across the three saturation experiments was 1.01, 0.481, and 1.21 nM and the B_{max} observed was 13.81, 10.03, and 15.61 fmole/100 µg. A total of three competitive binding experiments were conducted and the amount of cytosolic protein used for each incubation in the competitive binding experiments was 1.0 mg. The IC_{50} values for R1881 were determined to be 1.42, 1.35, and 1.29 nM. The IC_{50} values for dexamethasone were determined to be 34.3, 36.7, and 29.6 µM. The RBA values for dexamethasone in comparison to R1881 were, 0.0000413, 0.0000367, and 0.0000436. The K_i values for R1881 and dexamethasone were 0.673, 0.639, 0.611, and 16,247, 17,384, and 14,021 nM, respectively.

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 Article Information and Preparation

Battelle provided the following test materials at the necessary dilutions:

- Substrate: ^{3}H-R1881 (CAS No. 965-93-5)
- Reference Article: Unlabelled R1881–Methyltrienolone (CAS No. 965-93-5)
- Test Article: Dexamethasone (CAS No. 50-02-2)

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, ethylene diamine tetraacetic acid, dithiothreitol, glycerol, and phenylmethylsulfonyl fluoride (TEDG + PMSF Buffer) (pH 7.4) (lot 0677-10-01)
- Absolute ethanol (Sigma, lot 01043MC)
- Hydroxylapatite (HAP) (lot 0677-14-18)

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.

Substrate Preparation

The substrate solution was prepared from the radiolabeled R1881 received from Battelle and stored at 4 to 5°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.

Preparation of Rat Ventral Prostate Cytosol

Rat ventral prostate cytosol was prepared by Battelle and provided to In Vitro Technologies for use in the assays.

Assays

Evaluation of the Cytosol for Conducting Androgen Receptor Competitive Binding Assays

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

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

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

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

Saturation Radioligand Binding Assay

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

The experimental setup and details of the saturation binding assay are specified in the In Vitro Technologies biological method B065.A. The saturation binding experiments were conducted as three independent replicates. The same technician conducted all three replicate experiments.

Competitive Binding Assay (Inhibition of Androgen Receptor Binding of [^{3}H]-R1881 by Test 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 article. The experimental setup and details of the competitive binding assay are specified in the In Vitro Technologies biological method B066.A.

These experiments tested the androgen receptor binding of [^{3}H]-R1881 in the presence of multiple concentrations of a test article. The inhibition experiments were conducted as three independent replicates. All three replicate experiments for a given test 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) were 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 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 reflected 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 was determined experimentally.

Reporting of Ambiguities

For run 2 of the saturation binding assay: Test articles C-1-C2 and C-1-C1 were stored in the refrigerator. Test articles C-1-H1 and C-1-H2 were stored at –20°C.

In the assay, tubes rested for >5 minutes at room temperature.

For run 3 of the saturation binding assay: Test articles C-1-C2 and C-1-C1 were stored in the refrigerator. Test articles C-1-H1 and C-1-H2 were stored at –20°C.

A step was added in the middle of the assay (between steps 30 and 31), based on sponsor comment, to add 2 mL of 50 mM Tris to each tube.

The tubes were dried in a SpeedVac for 46 minutes, then the rotor was turned off. However, the bleeder valve was mishandled so the rotor slowed under vacuum. The samples were not completely dry at that point, so they were dried for an additional 15 minutes.

Samples 22–24 and 46–48 looked crystalline.

During the assays, test article C-1-H1 was exhausted for samples 22-24 and 46–48. These samples were mistakenly made with C-2-H1 and 50 mL of 6 mM triamcinolone.

It appeared that the traimcinolone in tubes 22–24 and 46–48 did not go completely back into solution in the cytosol.

For run 1 of the competition binding assay: Cytosol stock was spilled, and another vial was thawed. Part of the diluted cytosol prepared for the assay was not used for 26 minutes while the additional vial was thawed.

Not all of the cytosol was in the bottom of tube 22.

Criteria for Data Acceptance

All data obtained were reported.

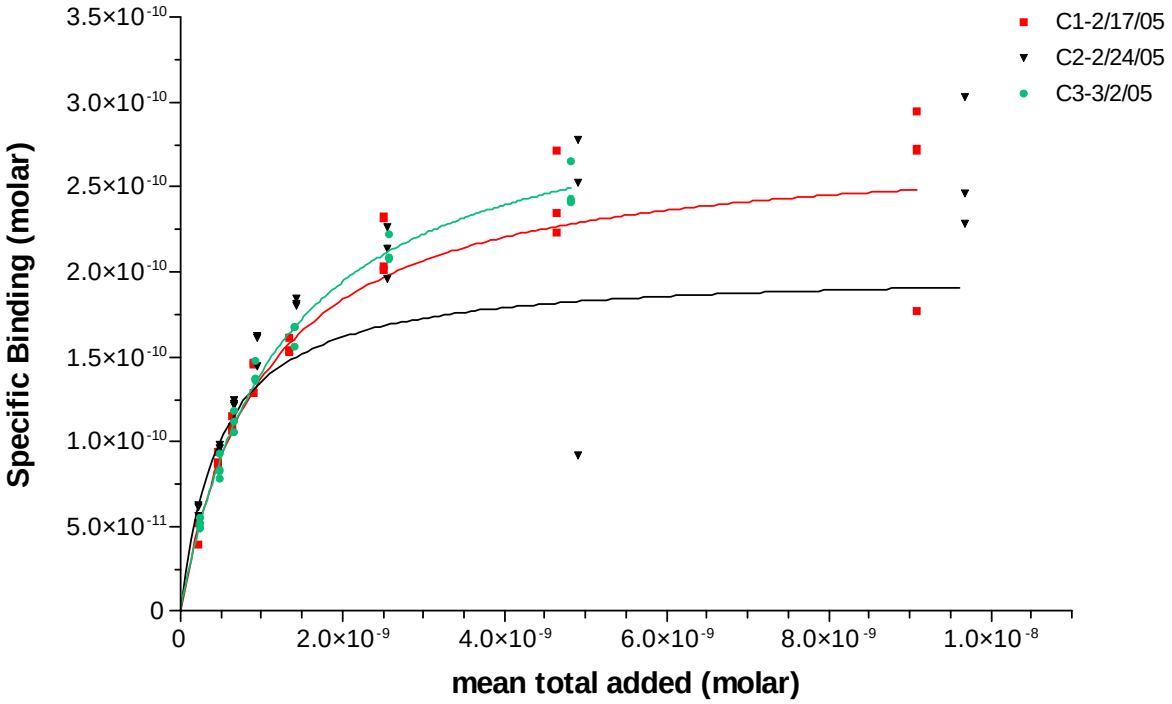
Results

Rat ventral prostate cytosol was provided to In Vitro Technologies by Battelle at a protein concentration of 5.206 mg/mL. A series of saturation and competitive binding experiments were conducted using the cytosol.

A total of three saturation assays (runs 1, 2, and 4) were conducted and the data are presented in Table 1 and Figure 1. During saturation assay run 3, several of the samples were accidentally dropped. Therefore, no data was generated from this run. The raw data and all supporting calculations for run 1, 2, and 4 are presented in Appendices 2 and 3. The amount of cytosolic protein used in the saturation binding experiments was 0.6 mg. The K_d observed across the three saturation experiments was 1.01, 0.481, and 1.21 nM and the B_{max} observed was 13.81, 10.03, and 15.61 fmole/100 µg.

A total of three competitive binding experiments were conducted and the data are presented in Table 2 and Figure 2. The raw data and all supporting calculations are presented in Appendices 4 and 5. The amount of cytosolic protein used for each incubation in the competitive binding experiments was 1.0 mg. The IC_{50} values for R1881 were determined to be 1.42, 1.35, and 1.29 nM. The IC_{50} values for dexamethasone were determined to be 34.3, 36.7, and 29.6 µM. The RBA values for dexamethasone in comparison to R1881 were, 0.0000413, 0.0000367, and 0.0000436. The K_i values for R1881 and dexamethasone were 0.673, 0.639, 0.611, and 16,247, 17,384, and 14,021 nM, respectively.

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



specific bound	C1-2/17/05	C2-2/24/05	C3-3/2/05
BMAX	2.761e-010	2.005e-010	3.122e-010
KD	1.009e-009	4.813e-010	1.215e-009
Std. Error			
BMAX	1.538e-011	2.162e-011	1.038e-011
KD	1.084e-010	1.329e-010	7.013e-011
95% Confidence Intervals			
BMAX	2.442e-010 to 3.080e-010	1.557e-010 to 2.453e-010	2.905e-010 to 3.340e-010
KD	7.845e-010 to 1.234e-009	2.056e-010 to 7.569e-010	1.068e-009 to 1.362e-009
Goodness of Fit			
Degrees of Freedom	22	22	19
R^2 (unweighted)	0.9024	0.5669	0.9880
Weighted Sum of Squares ($1/Y^2$)	0.3593	1.733	0.05706
Absolute Sum of Squares	1.201e-020	5.048e-020	1.068e-021
Sy.x	2.336e-011	4.790e-011	7.497e-012
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	24	24	21
Number of missing values	0	0	3

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

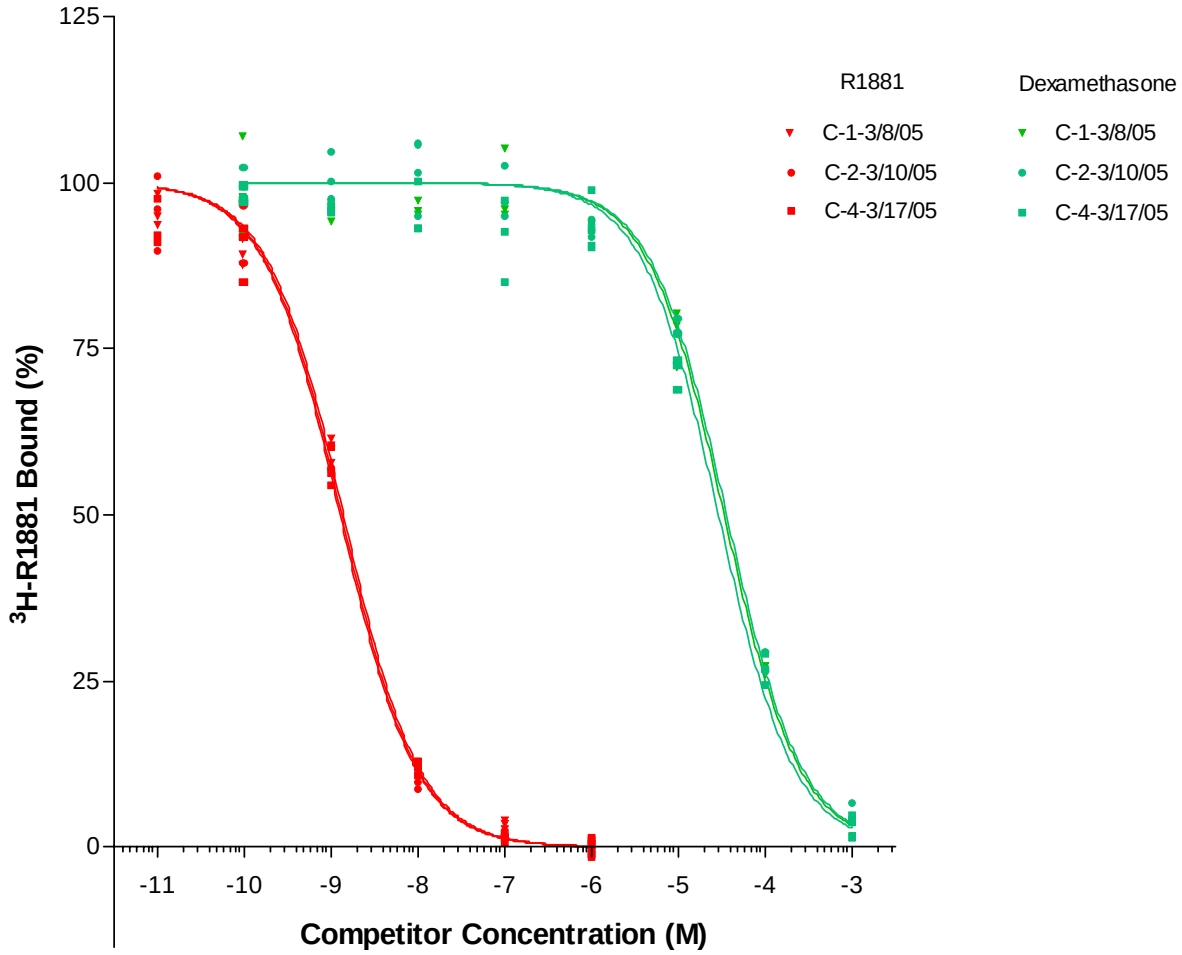


Table 1: Saturation Binding Experiments

Run number	Assay date	Technician	K_d (nM)	B_{max} (fmole/100 µg)
1	17 February 2005	RCM	1.01	13.81
2	24 February 2005	RCM	0.481	10.03
3	02 March 2005	RCM	1.21	15.61
Mean			0.900	13.15

Table 2: Competitive Binding Experiments

			R1881			Dexamethasone		
Run number	Assay date	Technician	IC_{50} (nM)	RBA	K_i (nM)	IC_{50} (nM)	RBA	K_i (nM)
1	08 March 2005	RCM	1.42	100	0.673	34300	0.0000413	16247
2	10 March 2005	RCM	1.35	100	0.639	36700	0.0000367	17384
4	17 March 2005	RCM	1.29	100	0.611	29600	0.0000436	14021

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

$K_i = IC_{50} /(1 + (L/Kd))$; L = 1 nM; mean K_d = 0.900 nM

Appendix 1: Copy of In Vitro Technologies Protocol No. 1126

In Vitro Technologies, Inc.
Protocol No. 1126
Version: Final (05 January 2005)

Validation of an Androgen Receptor Binding Assay–Task 2

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:	Neil S. Jensen, Ph.D.
EPA Contract Number:	68-W-01-023 (Battelle Prime Contractor)
Lab Study Number:	270-1126-04

Objective

The objective of this study is to establish interlaboratory variability using the same assay and common preparations of rat ventral prostate tissue homogenate, radiolabeled R1881, unradiolabeled R1881, and dexamethasone. This protocol is specific to the study to be conducted at In Vitro Technologies, Inc (IVT).

Test Article Identification

Battelle will provide the following test articles at the necessary dilutions:

- Marker: ^{3}H-R1881 (CAS No. 965-93-5)
- Reference Substance: Unlabelled R1881–Methyltrienolone (CAS No. 965-93-5)
- Test Substance: Dexamethasone (CAS No. 50-28-2)

These test articles will be used in the androgen receptor (AR)-binding assay to determine relative binding affinities (RBAs).

Test System Identification

The test system that will be used in this study is rat ventral prostate tissue homogenate supplied by Battelle. Each tube within the assay will be labeled as defined in the assay.

Test System Justification

Rat ventral prostate tissue homogenate will be used because of the data that exist for comparison and the inability to identify appropriate recombinant system(s) without false negative- and false positive-acting chemicals in the *in vitro* binding assay.

Description of Study

In Vitro Technologies, Inc. will conduct competitive binding receptor assays of a known chemical using the homogenate, radiolabeled and inert R1881, protocol, and reporting criteria provided by Battelle.

Note: Battelle will supply rat ventral prostate tissue homogenate and the series of dilutions of non-radiolabeled R1881, and of a weak binder necessary to perform the assay.

Experimental Methods

Standardization of AR Competitive Binding Assays

Prior to routinely conducting the AR competitive binding assays, the methods will be standardized within each laboratory. This can be accomplished by conducting a series of saturation radioligand binding assays to demonstrate AR specificity and saturation. Nonlinear regression analysis of these data and subsequent Scatchard plots will document AR binding affinity (K_d) and maximum specific binding number (B_{max}).

Assays

A. Run saturation binding experiments and competitive binding experiments with R1881 according to the updated Battelle assay protocol (IVT Biological Methods B058 [Androgen Receptor Saturation Binding Assay Using [^{3}H]-R1881] and B059 [Androgen Receptor Competitive Binding Assay Using [^{3}H]-R1881]). Battelle will modify the AR binding protocol developed for a previous work assignment to conduct each run in triplicate rather than in duplicate, and to require the use of a weak positive control, dexamethasone, in addition to R1881. Each experiment will be conducted once each day on 3 separate days by the same technician to control bias. Concentrations required for different chemicals in the assay will be identified as defined in the assay protocol.

B. Run competitive binding experiments with the weak AR binder dexamethasone (at this time assume eight dilutions). Each experiment will be conducted once each day on 3 separate days by the same technician to control bias. Concentrations required for different chemicals in the assay will be identified as defined in the assay protocol.

Description of Data Calculations

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

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

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

Calculation Check

The numbers used in the calculations below include constants/conversion factors.

$$\frac{\text{X DPM}}{2.22 \times 10^{12}\ \text{dpm/Ci}} = \frac{4.5045 \times 10^{-13}\ \text{Ci}}{\text{*83.2 Ci/mmole}} = \frac{5.4141 \times 10^{-15}\ \text{mmole}}{\text{1,000 mmole/mole}} = \frac{5.4141 \times 10^{-18}\ \text{moles}}{\text{0.003 liters}}$$

$$\frac{.8047 \times 10^{-14} \text{ moles/liter}}{1 \times 10^{-9} \text{ moles/nmole}} = X\ (1.8047 \times 10^{-5})\ \text{nM}$$

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

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

1. Total binding is calculated by multiplying the DPM from the tubes that contained only radiolabeled R1881 by 1.6242×10^{-2}. This value will be total binding in fmoles.

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

3. Specific binding is calculated by subtracting nonspecific binding from total binding (i.e., fmoles total binding – fmoles nonspecific binding = specific binding in fmoles).

Graphical Presentation of the Data

Standard Curve and Test Chemical Competitive Binding Curves

Data for the standard curve and each test chemical will be plotted as the percent ^{3}H_R1881 bound versus the molar concentration. Estimates of the IC_{50} will be determined using appropriate non-linear curve fitting software such as GraphPad Prism (GraphPad Software, Inc., San Diego, CA). A Scatchard analysis may also be performed for the standard curve using R1881 to demonstrate that the assay meets acceptable quality assurance standards.

Relative Binding Affinity

The relative binding affinity (RBA) for each competitor will be calculated by dividing the IC_{50} for R1881 by the IC_{50} of the competitor and expressing as a percent (e.g., RBA for R1881 =100%).

Maximal binding capacity (B_{max}) and association/dissociation constants (K_a/K_d) will be estimated using a number of commercially available iterative non-linear regression analysis programs (e.g. GraphPad Prism).

Reporting of Competitive Binding Curves

Prism files showing the competitive binding curves shall be provided to Environmental Protection Agency (EPA). The Prism files need not be supplied by each individual laboratory; they may be generated at Battelle from the data supplied by each laboratory.

Reporting of Ambiguities

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

Criteria for Data Acceptance

Establishment of Performance Criteria

On the basis of the data generated in previous activities and Task 2, EPA will set performance criteria for Tasks 3 and 4.

Study Report

At completion of Task 2, a formal report will be submitted summarizing K_d and number of receptors for the saturation binding experiment, IC_{50} for R1881and dexamethasone, and RBA for dexamethasone as compared to R1881 from the competitive binding studies.

Raw data in standard spreadsheet will be provided within 10 days of completion of the laboratory phase of this task and a final report summarizing data generated.

Prism files showing the competitive binding curves will be provided to Battelle within 15 days of completion of the laboratory phase of this task. A report of the methods and results will also be prepared within 15 days of completion of the laboratory phase of this task.

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.

Protocol Approval

This protocol has been reviewed and approved by the following:

Sponsor Representatives

David P. Houchens, Ph.D. — Signature — 1/5/05
Program Manager
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

James E. Morris, Ph.D. — Signature — 1/6/05
Study Director
Endocrine Disruptor
Screening Program
Battelle Memorial Institute

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.

Neil S. Jensen, Ph.D. — Signature — 11JAN2005
Study Director
In Vitro Technologies

Review

Terri L. Pollock, B.A. — Signature — 1-5-05
Quality Assurance Manager
Battelle Memorial Institute

Sharon Isbell — Signature — 1-11-05
Director, Quality Systems
In Vitro Technologies

Appendix 2: Copy of Protocol Amendment

PROTOCOL AMENDMENT FORM

IVT Study Number: 270-1126-04 Document Number: PAM05-006

Date of Sponsor's Verbal Approval: 02 Feb 2005

Briefly describe the amendment:
Study director responsibilities will be transferred from Neil Jensen, Ph.D. to Aruna Koganti, Ph.D.

Briefly describe the reason for the amendment:
Study director responsibilities are transferred to redistribute study workloads.

Approved by: _____ Date: 2/15/2005
Sponsor Representative

Approved by: K. Aruna Prabhu Date: 17 Feb 2005
Study Director

Effective Date: 04 June 2002

PROTOCOL AMENDMENT FORM

IVT Study Number: 270-1126-04 Document Number: PAM 05-035

Date of Sponsor's Verbal Approval: n/a

Briefly describe the amendment:
The CAS number of dexamethasone is documented in the protocol as 50-28-2. The protocol will be amended to state that the CAS number of dexamethasone as 50-02-2.

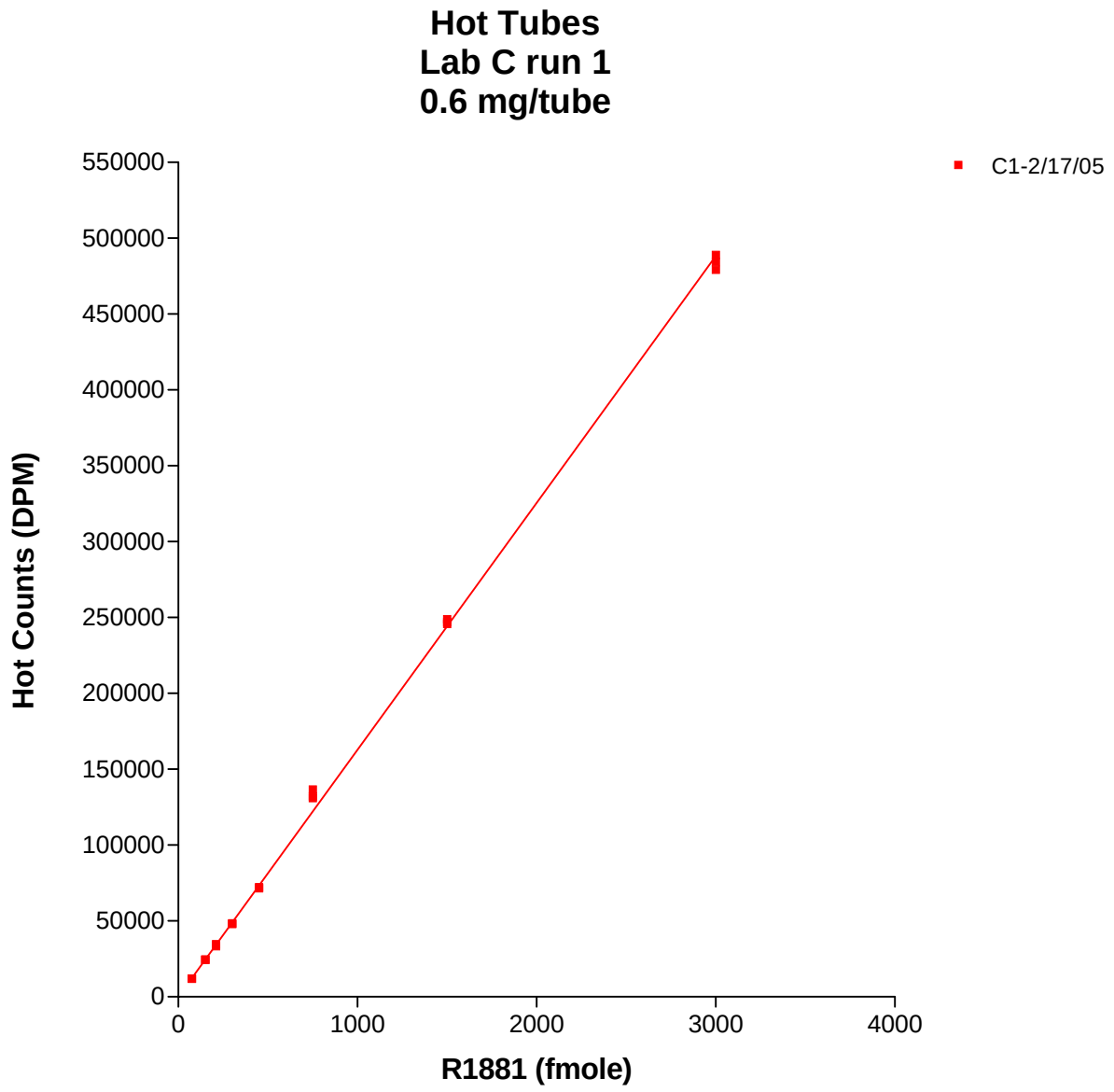
Briefly describe the reason for the amendment:
The CAS number of dexamethasone as presented in the protocol is incorrect.

Approved by: James E. Morris Date: 11-8-05
Sponsor Representative

Approved by: K. Divina Prathia Date: 11 Nov 2005
Study Director

Effective Date: 04 June 2002

Appendix 3: Prism Files for Saturation Runs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C1-2/17/05
Hot Counts (DPM)
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500000
450000
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250000
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150000
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50000
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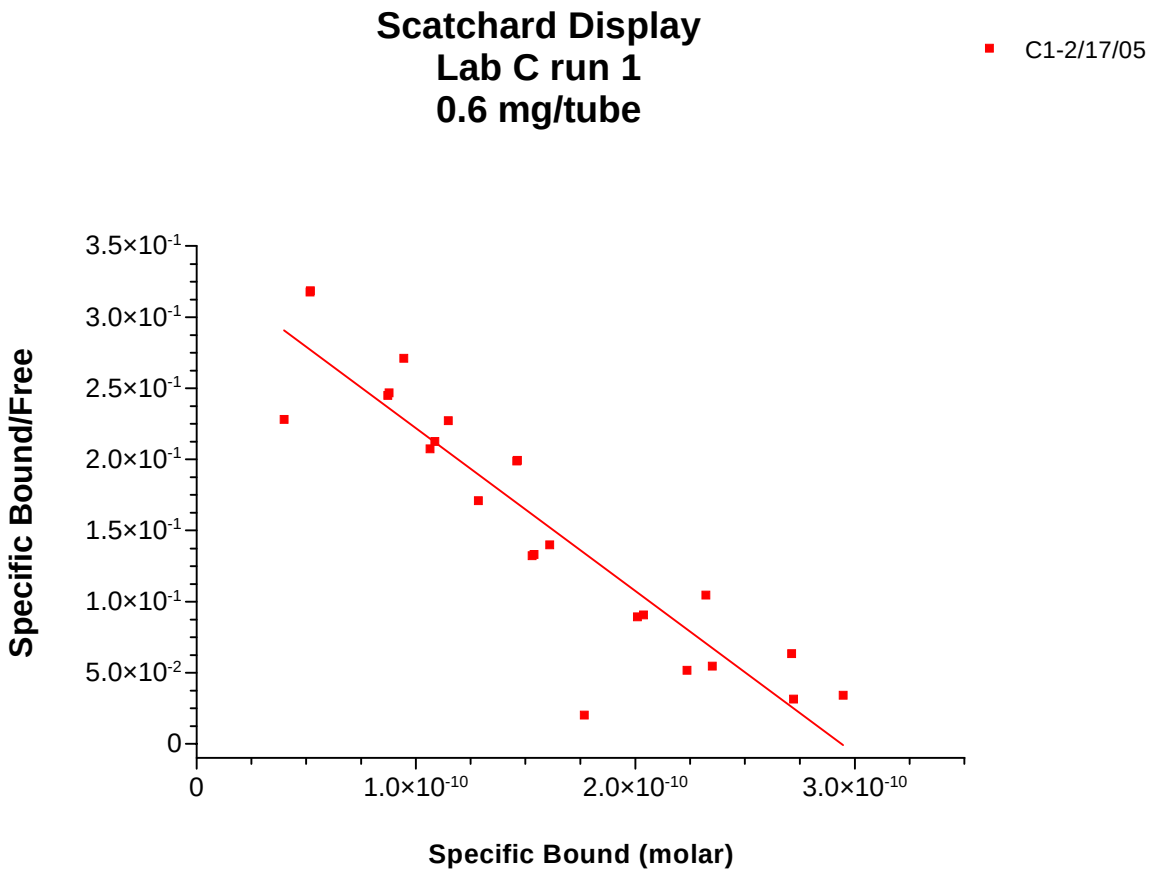
0
1000
2000
3000
4000
R1881 (fmole)


Scatchard Display
Lab C run 1
0.6 mg/tube
C1-2/17/05
Specific Bound/Free
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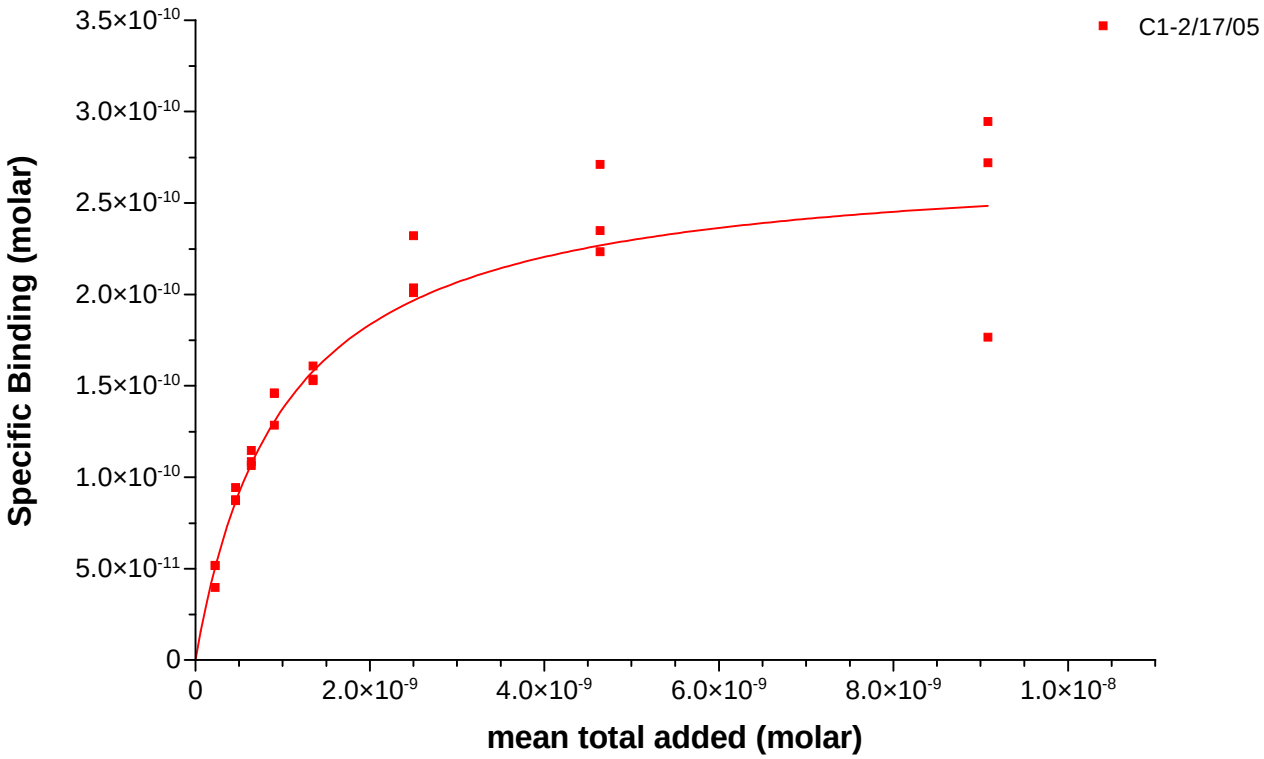
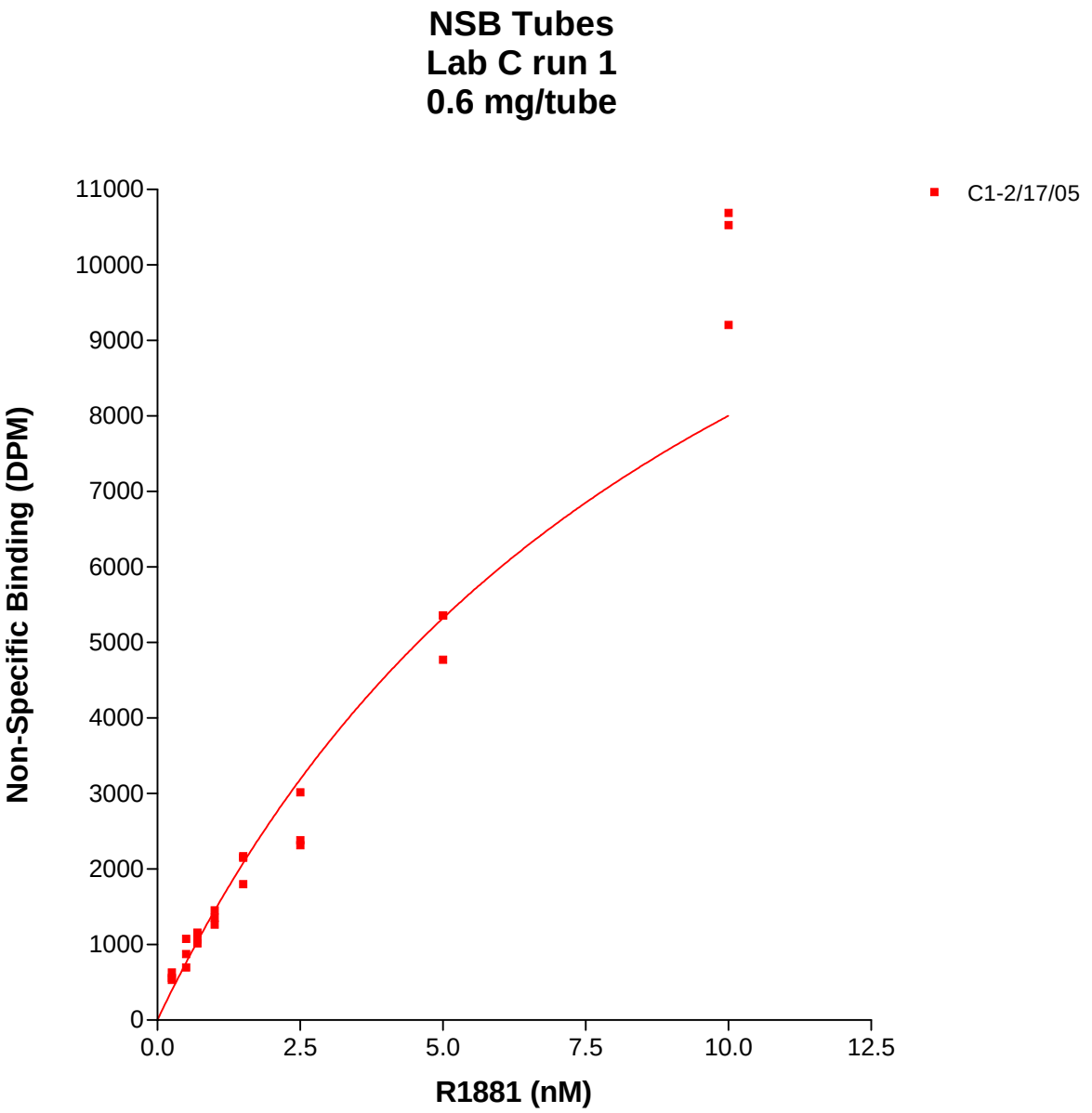
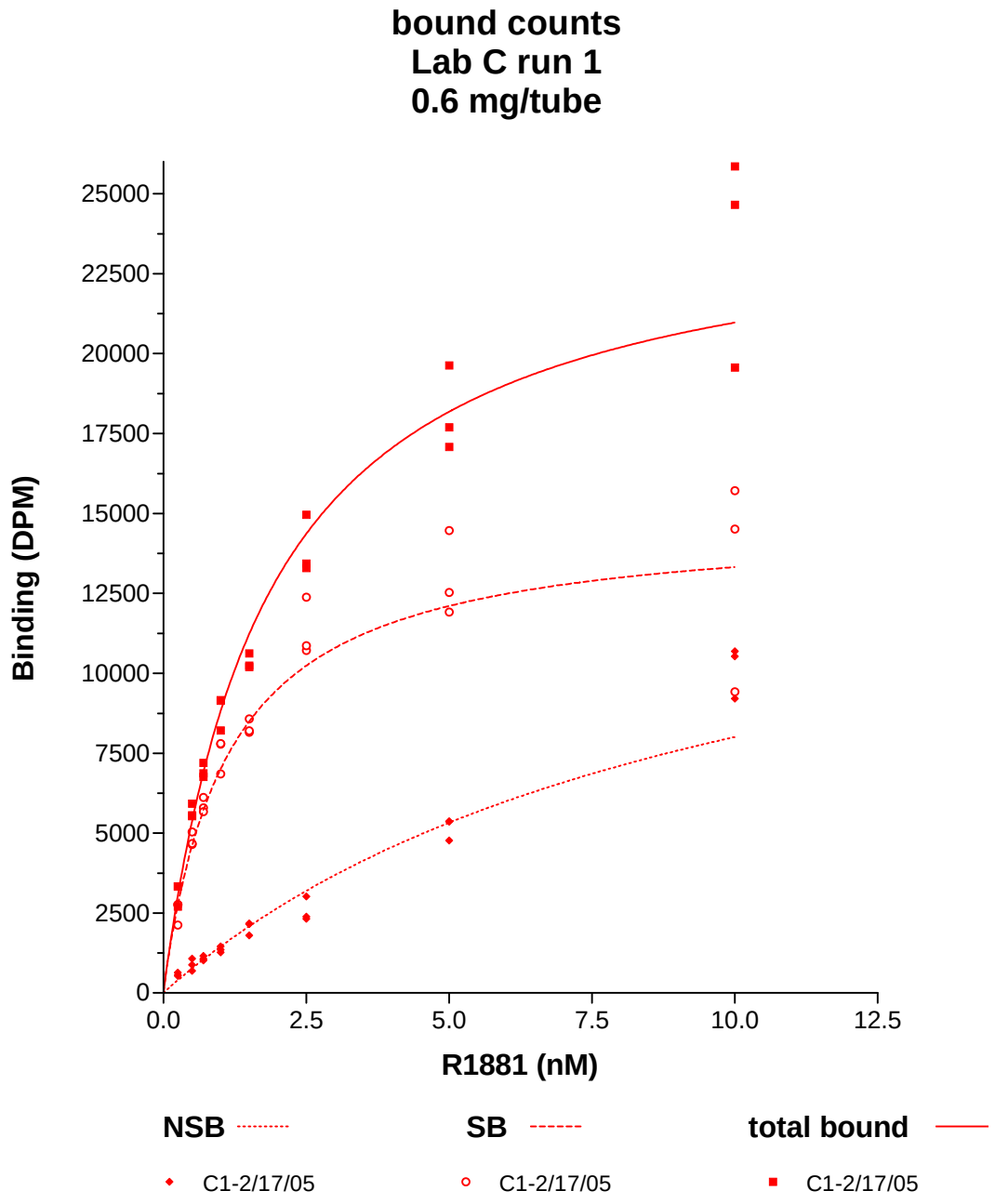
3.0×10-1
2.5×10-1
2.0×10-1
1.5×10-1
1.0×10-1
5.0×10-2
0
0
1.0×10-10
2.0×10-10
3.0×10-10
Specific Bound (molar)

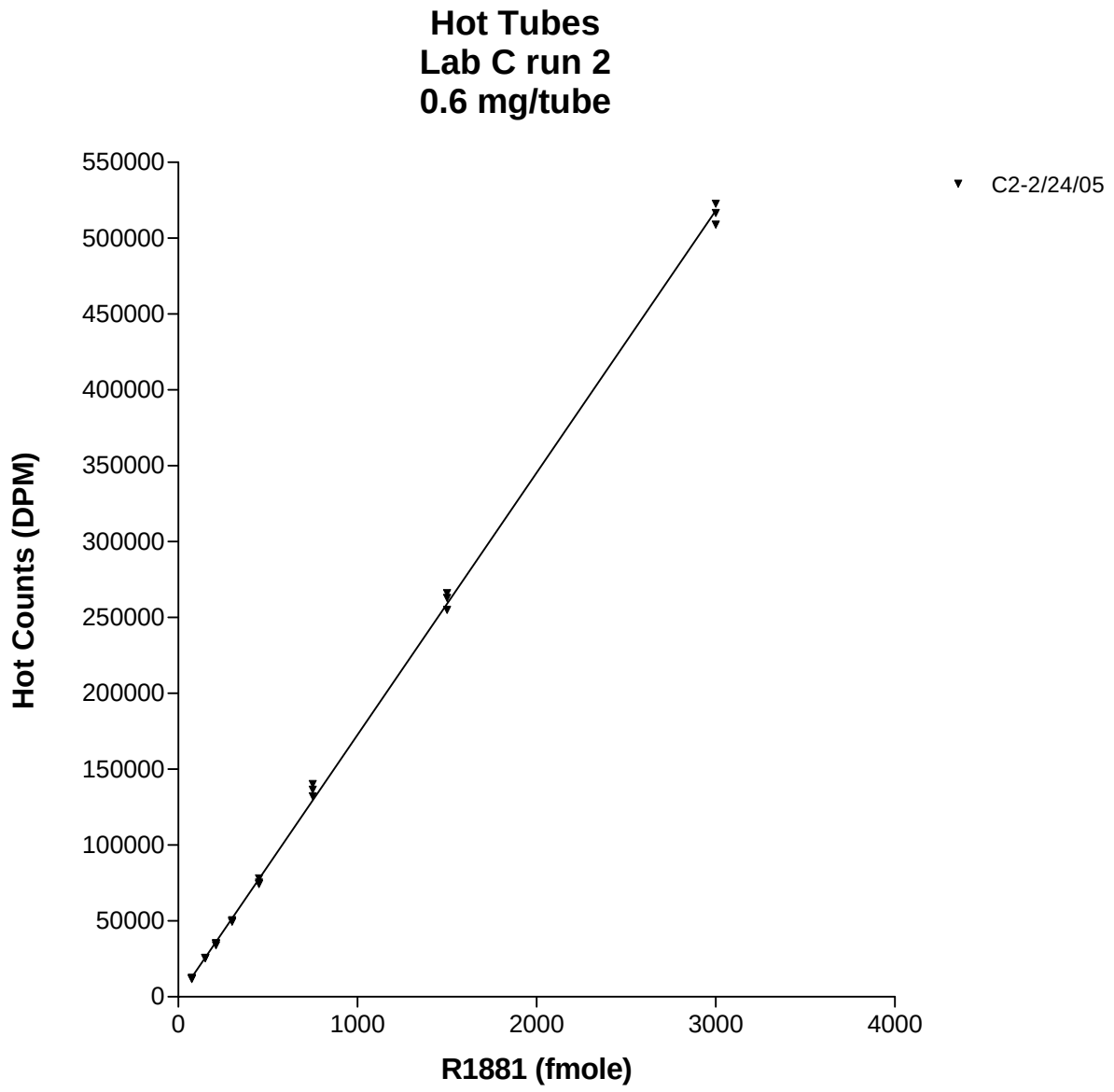
Lab C run 1
0.6 mg/tube



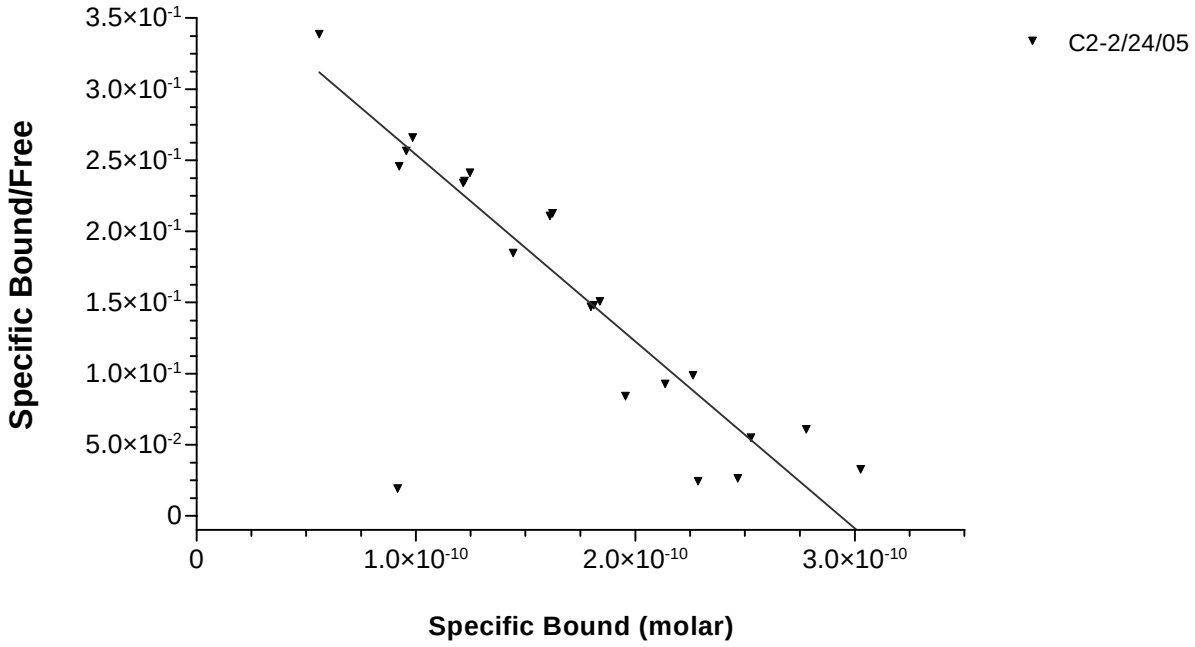
specific bound	C1-2/17/05
BMAX	2.7611e-010
KD	1.0094e-009
Std. Error	
BMAX	1.5377e-011
KD	1.0843e-010
95% Confidence Intervals	
BMAX	2.4422e-010 to 3.0800e-01
KD	7.8451e-010 to 1.2343e-00
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.9024
Weighted Sum of Squares ($1/Y^2$)	0.3593
Absolute Sum of Squares	1.2006e-020
Sy.x	2.3360e-011
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0


NSB Tubes
Lab C run 1
0.6 mg/tube
Non-Specific Binding (DPM)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
C1-2/17/05

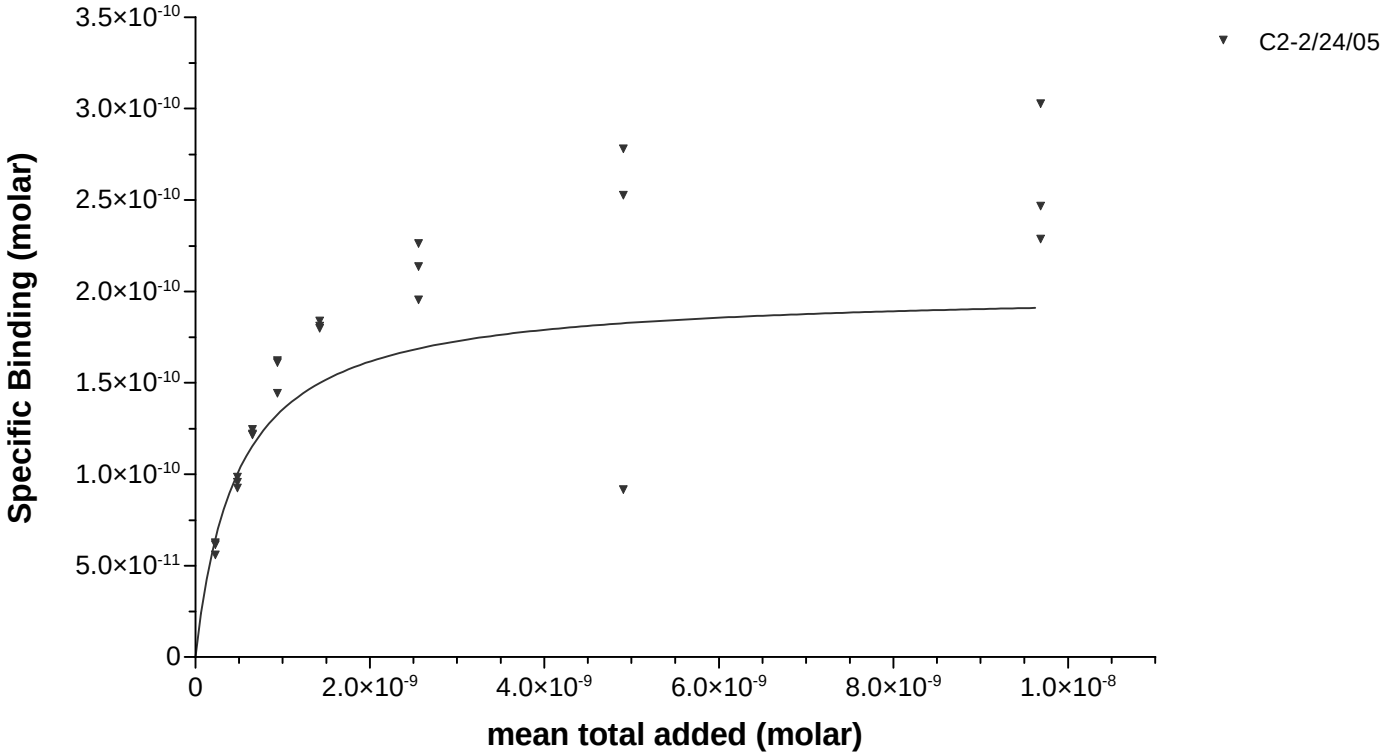

bound counts
Lab C run 1
0.6 mg/tube
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
SB
total bound
C1-2/17/05
C1-2/17/05
C1-2/17/05


Hot Tubes
Lab C run 2
0.6 mg/tube
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
1000
2000
3000
4000
R1881 (fmole)
C2-2/24/05

Scatchard Display
Lab C run 2
0.6 mg/tube

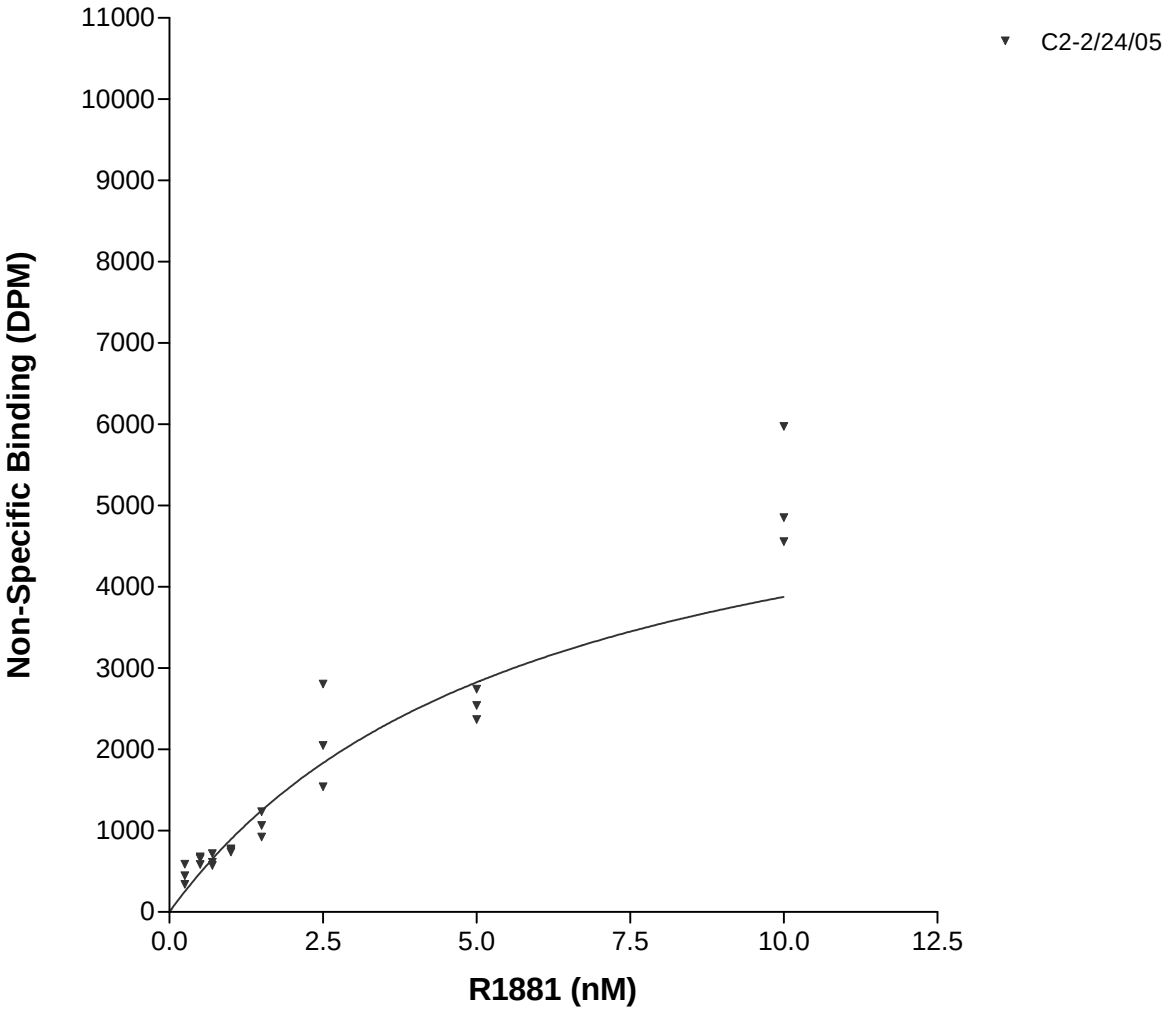


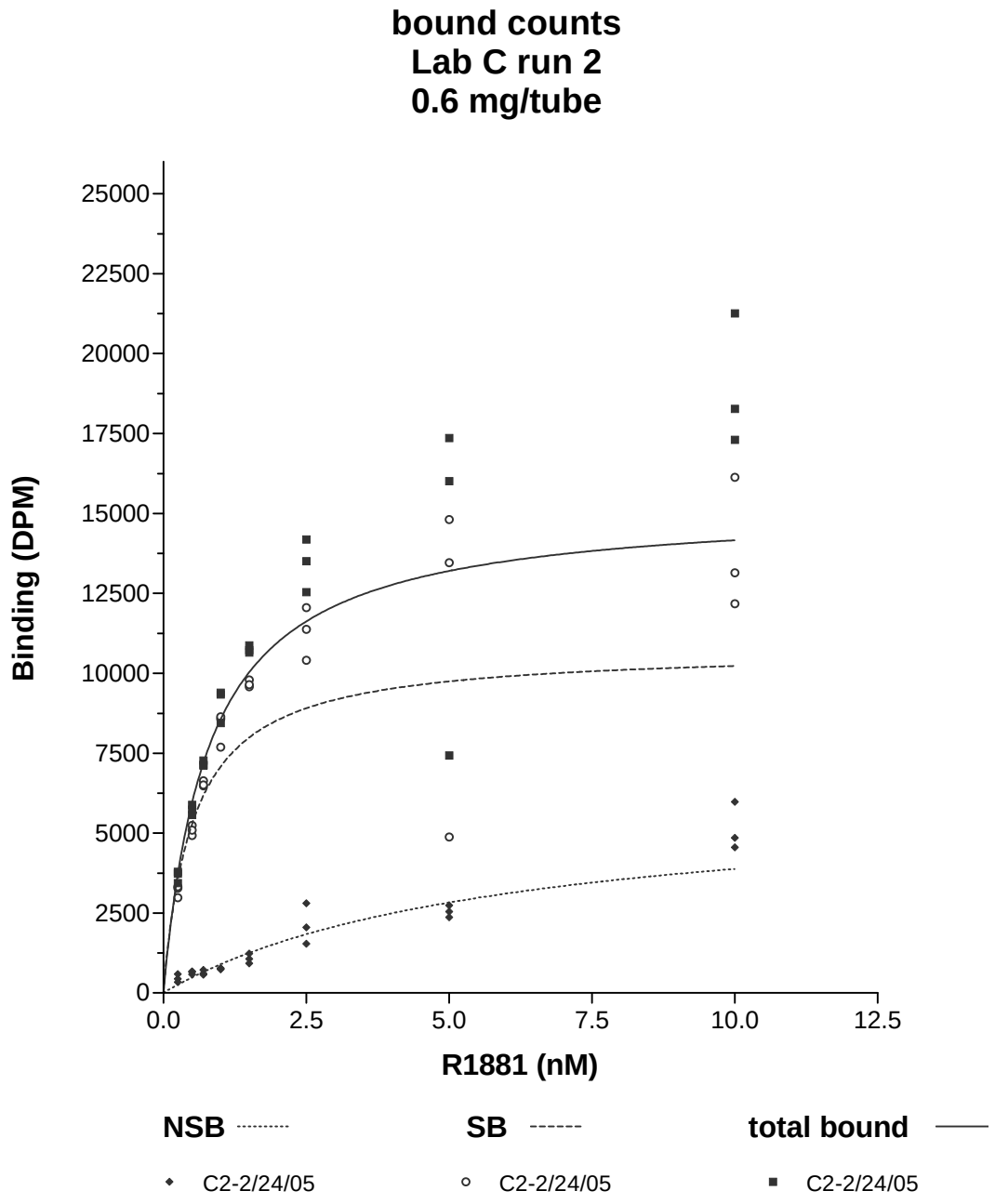
Lab C run 2
0.6 mg/tube

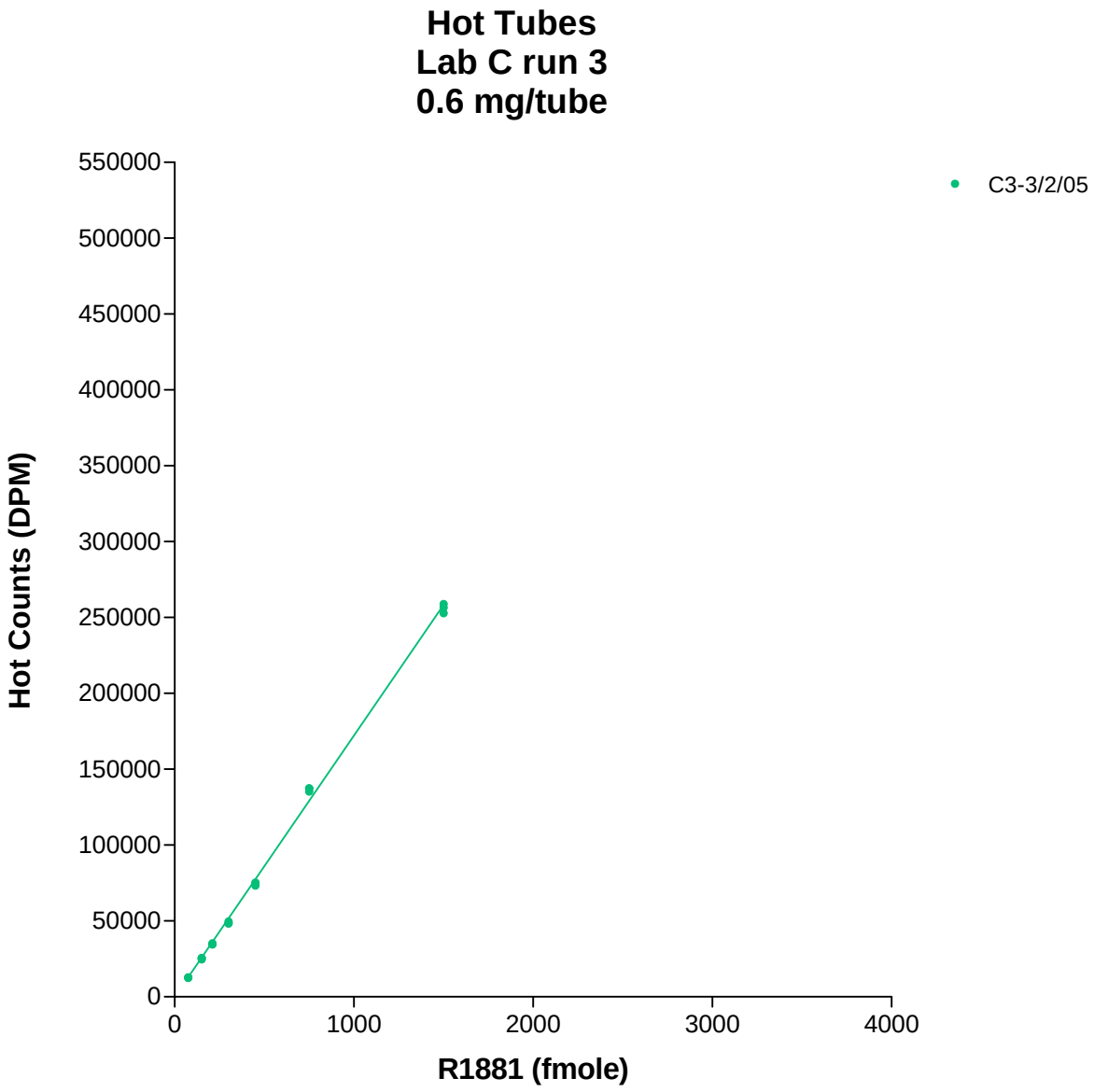


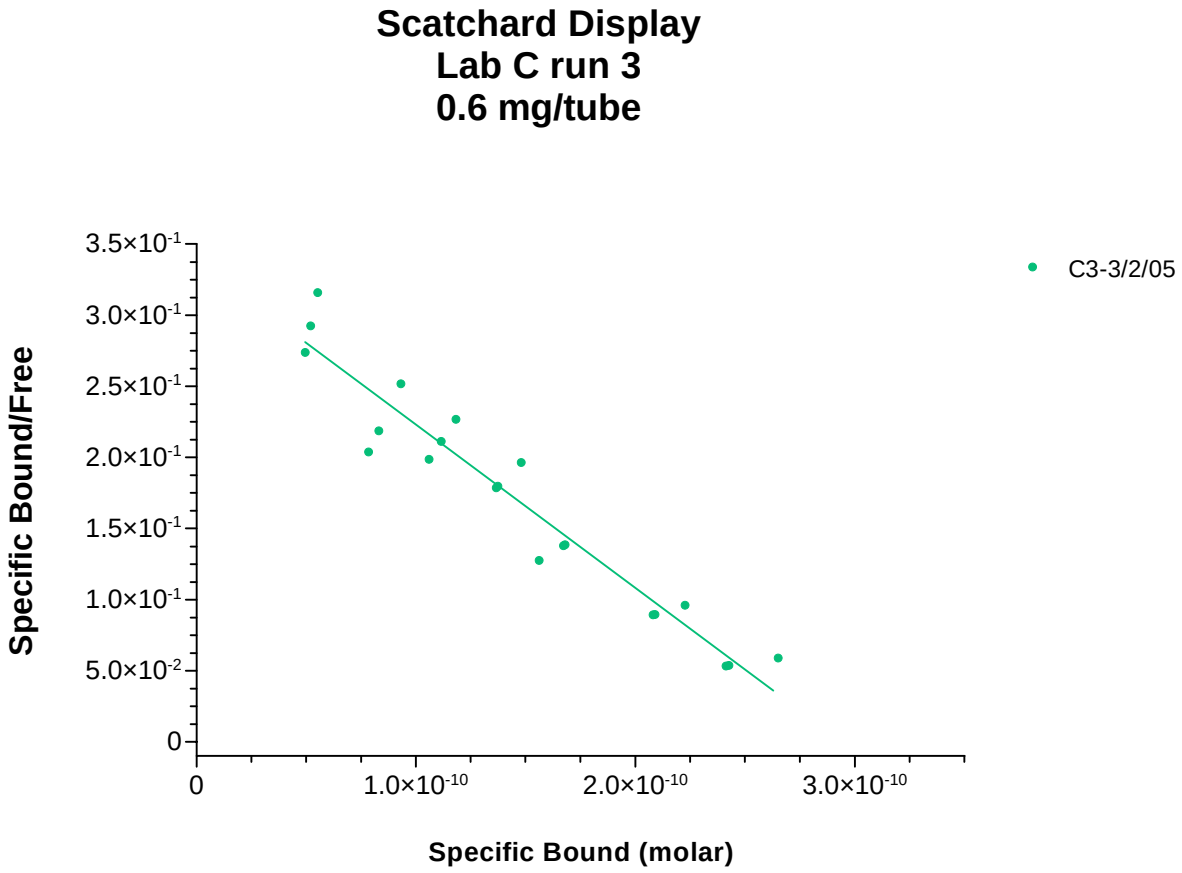
specific bound	C2-2/24/05
BMAX	2.0051e-010
KD	4.8127e-010
Std. Error	
BMAX	2.1618e-011
KD	1.3291e-010
95% Confidence Intervals	
BMAX	1.5568e-010 to 2.4535e-010
KD	2.0563e-010 to 7.5692e-010
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.5669
Weighted Sum of Squares ($1/Y^2$)	1.733
Absolute Sum of Squares	5.0479e-020
Sy.x	4.7901e-011
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

NSB Tubes
Lab C run 2
0.6 mg/tube

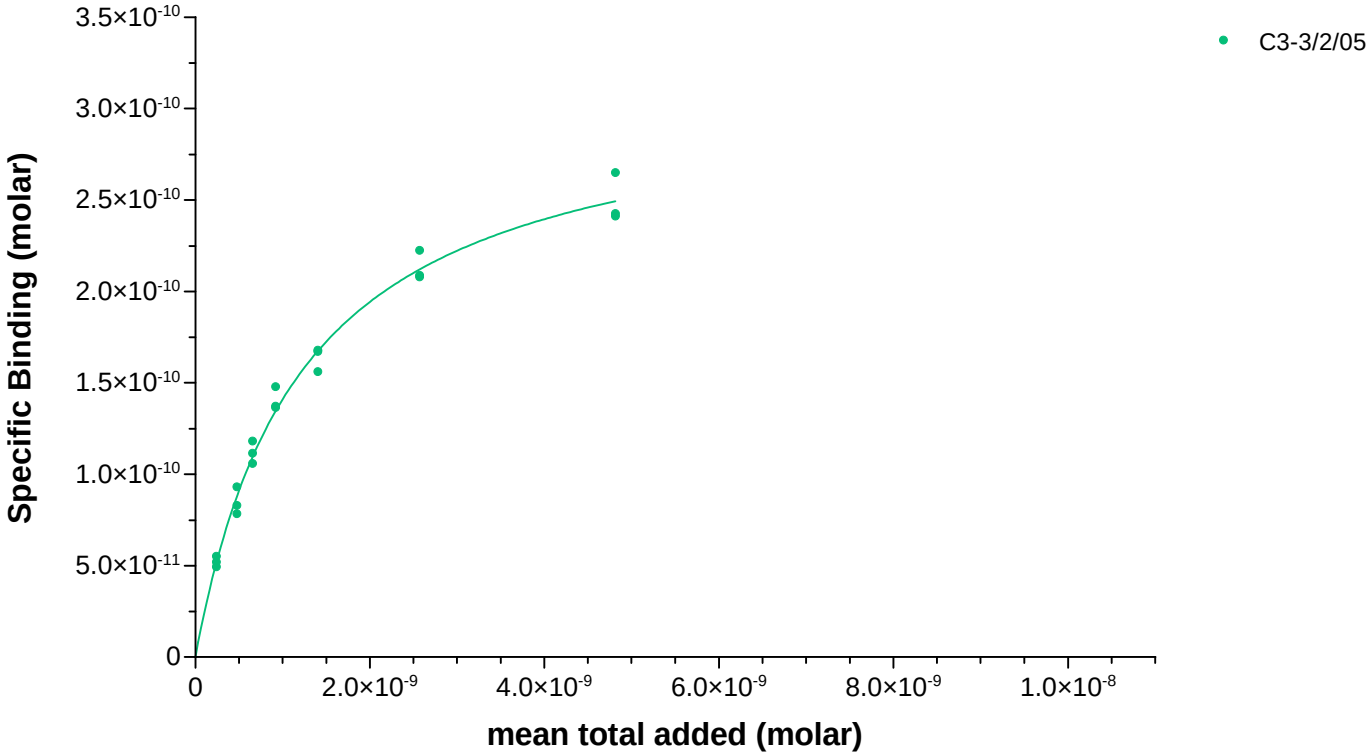



bound counts
Lab C run 2
0.6 mg/tube
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
SB
total bound
C2-2/24/05
C2-2/24/05
C2-2/24/05


Hot Tubes
Lab C run 3
0.6 mg/tube
C3-3/2/05
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
1000
2000
3000
4000
R1881 (fmole)

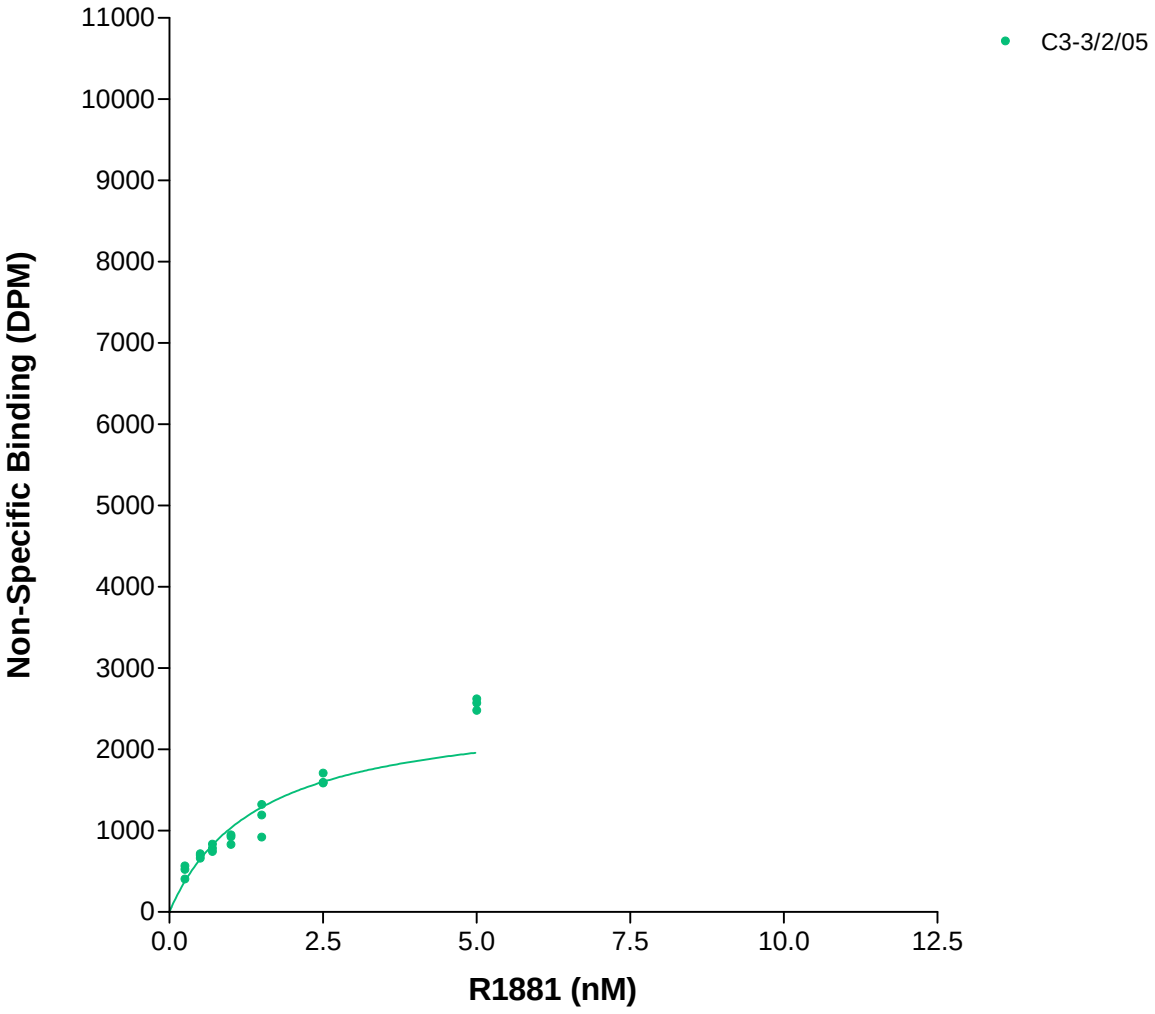

Scatchard Display
Lab C run 3
0.6 mg/tube
Specific Bound/Free
3.5×10-1
3.0×10-1
2.5×10-1
2.0×10-1
1.5×10-1
1.0×10-1
5.0×10-2
0
0
1.0×10-10
2.0×10-10
3.0×10-10
Specific Bound (molar)
C3-3/2/05

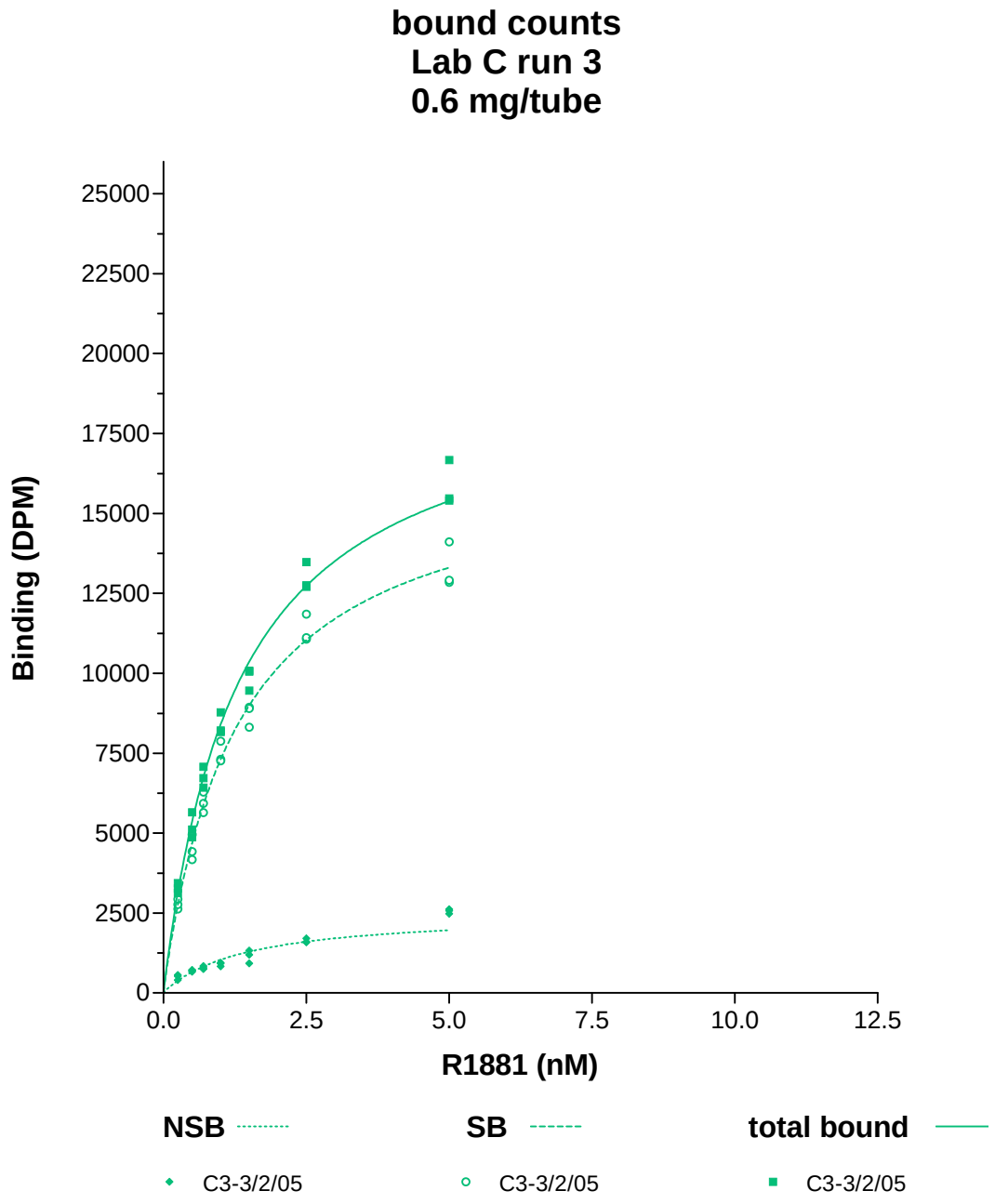
Lab C run 3
0.6 mg/tube

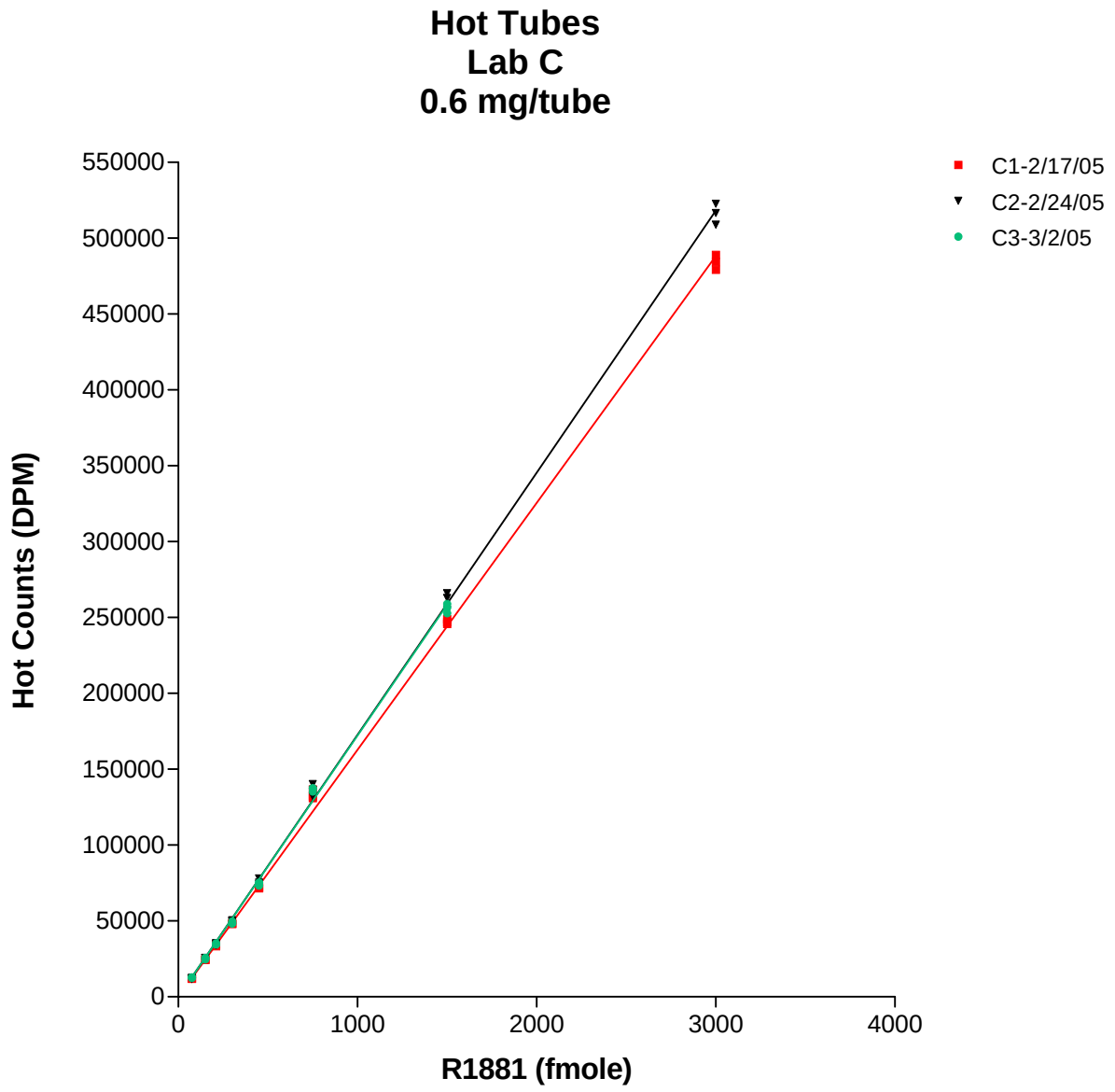


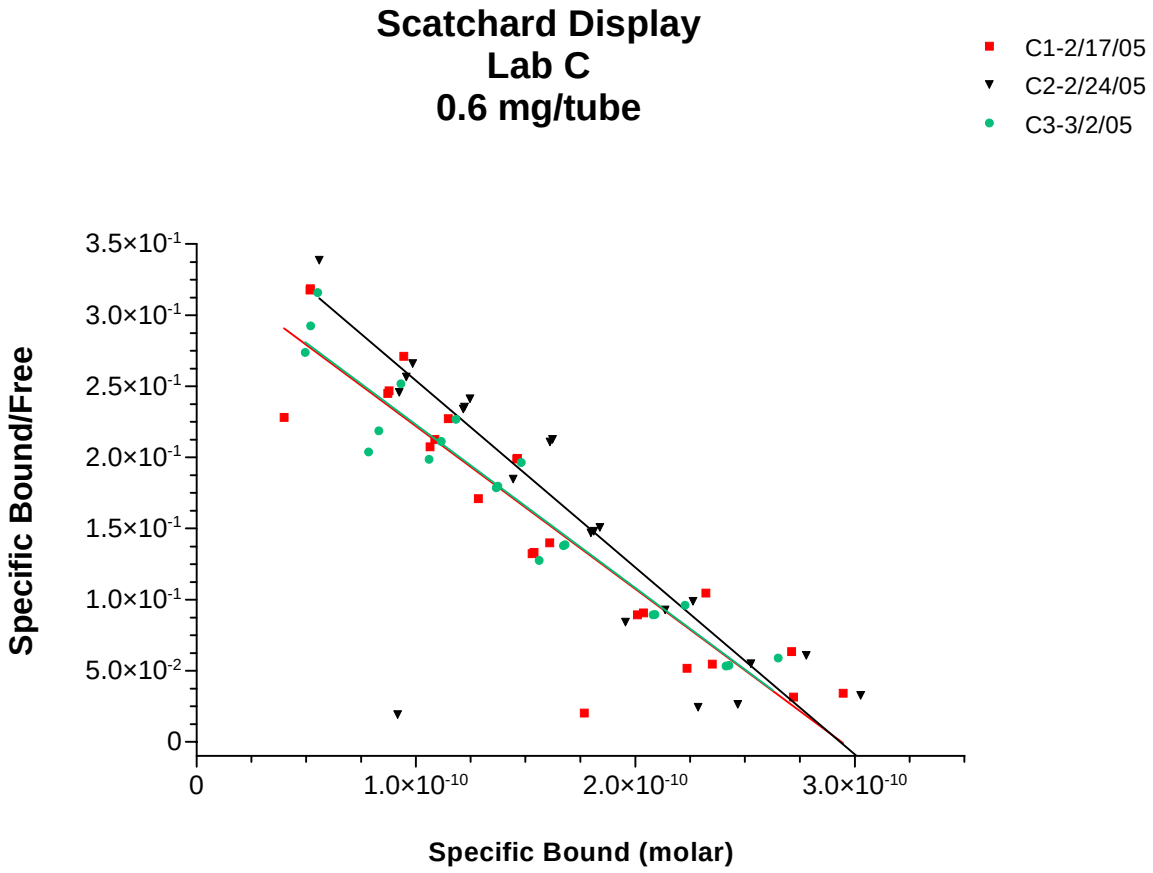
specific bound	C3-3/2/05
BMAX	3.1224e-010
KD	1.2148e-009
Std. Error	
BMAX	1.0378e-011
KD	7.0134e-011
95% Confidence Intervals	
BMAX	2.9052e-010 to 3.3396e-010
KD	1.0680e-009 to 1.3616e-009
Goodness of Fit	
Degrees of Freedom	19
R^2 (unweighted)	0.9880
Weighted Sum of Squares ($1/Y^2$)	0.05706
Absolute Sum of Squares	1.0680e-021
Sy.x	7.4973e-012
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	21
Number of missing values	3

NSB Tubes
Lab C run 3
0.6 mg/tube

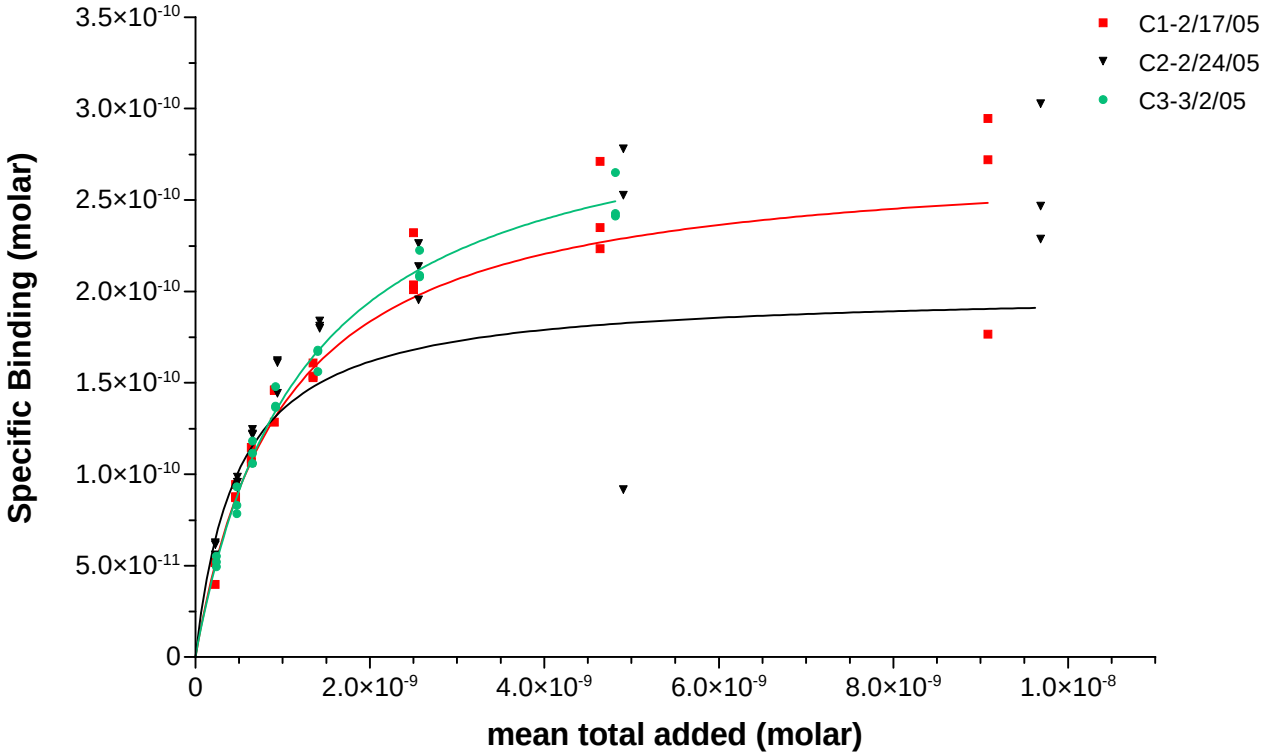



bound counts
Lab C run 3
0.6 mg/tube
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
SB
total bound
C3-3/2/05
C3-3/2/05
C3-3/2/05

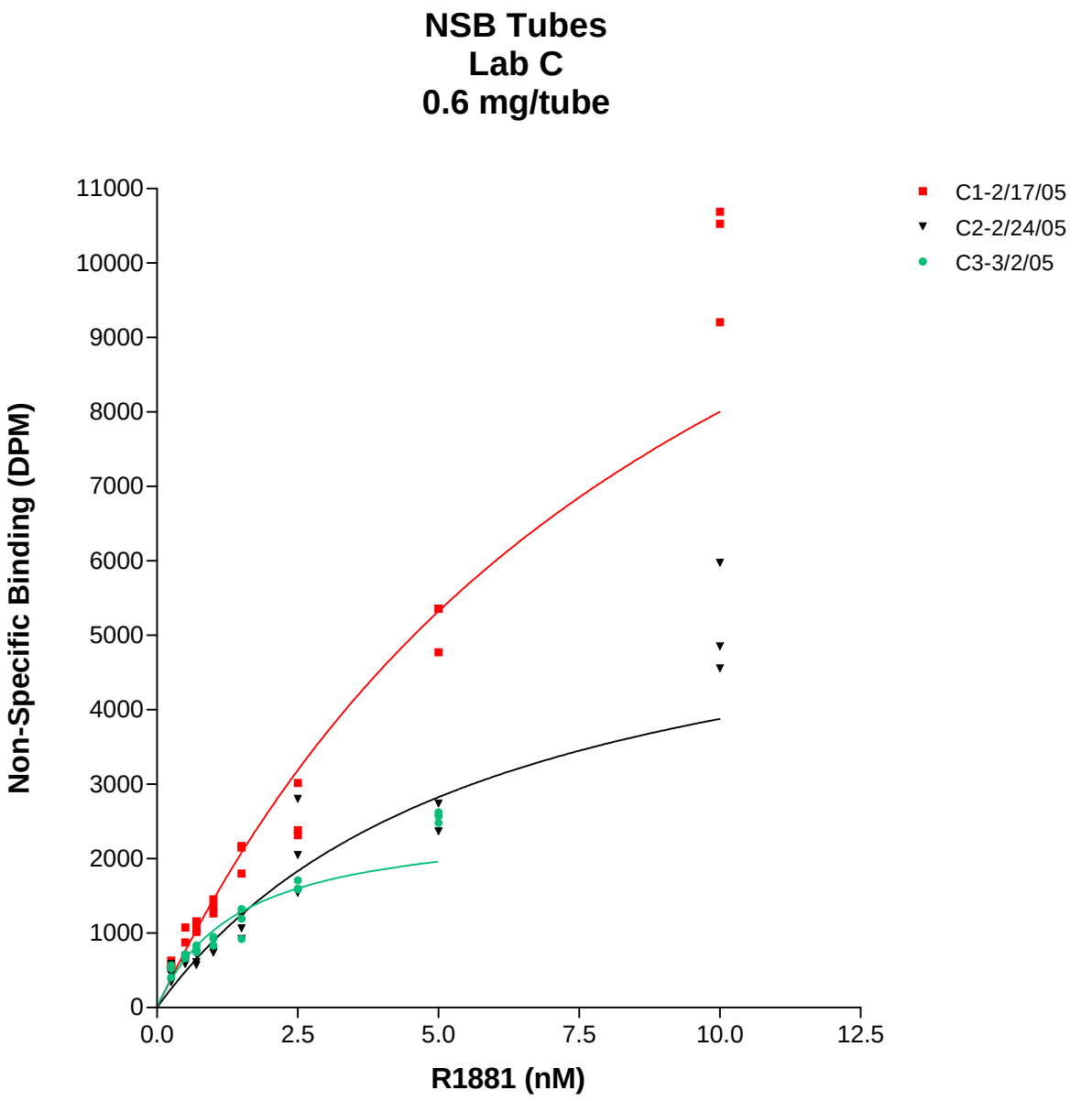

Hot Tubes
Lab C
0.6 mg/tube
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
1000
2000
3000
4000
R1881 (fmole)
C1-2/17/05
C2-2/24/05
C3-3/2/05

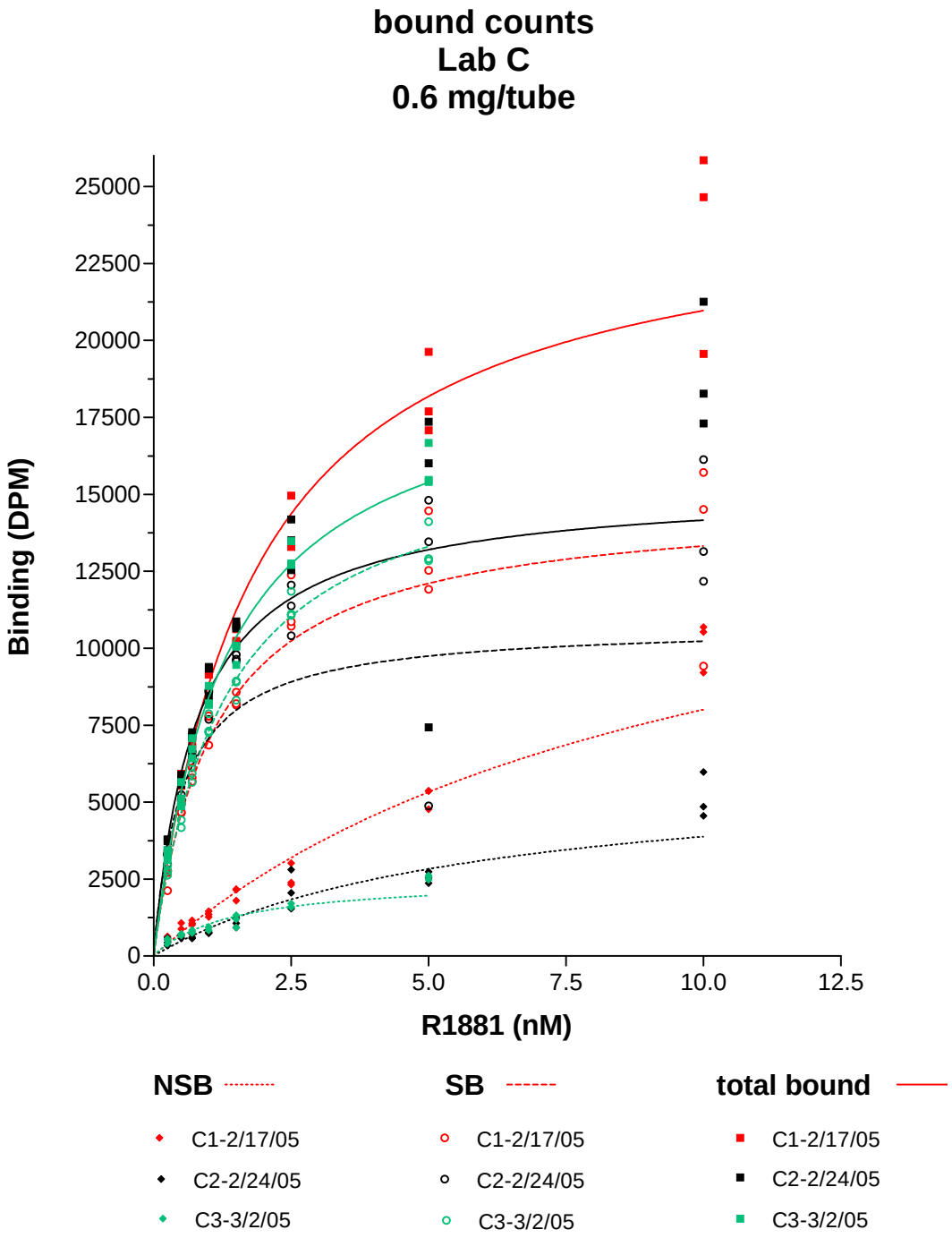

Scatchard Display
Lab C
0.6 mg/tube
C1-2/17/05
C2-2/24/05
C3-3/2/05
Specific Bound/Free
3.5×10-1
3.0×10-1
2.5×10-1
2.0×10-1
1.5×10-1
1.0×10-1
5.0×10-2
0
0
1.0×10-10
2.0×10-10
3.0×10-10
Specific Bound (molar)

Lab C
0.6 mg/tube



specific bound	C1-2/17/05	C2-2/24/05	C3-3/2/05
BMAX	2.7611e-010	2.0051e-010	3.1224e-010
KD	1.0094e-009	4.8127e-010	1.2148e-009
Std. Error			
BMAX	1.5377e-011	2.1618e-011	1.0378e-011
KD	1.0843e-010	1.3291e-010	7.0134e-011
95% Confidence Intervals			
BMAX	2.4422e-010 to 3.0800e-01	1.5568e-010 to 2.4535e-010	2.9052e-010 to 3.3396e-010
KD	7.8451e-010 to 1.2343e-00	2.0563e-010 to 7.5692e-010	1.0680e-009 to 1.3616e-009
Goodness of Fit			
Degrees of Freedom	22	22	19
R^2 (unweighted)	0.9024	0.5669	0.9880
Weighted Sum of Squares ($1/Y^2$)	0.3593	1.733	0.05706
Absolute Sum of Squares	1.2006e-020	5.0479e-020	1.0680e-021
Sy.x	2.3360e-011	4.7901e-011	7.4973e-012
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	24	24	21
Number of missing values	0	0	3


NSB Tubes
Lab C
0.6 mg/tube
Non-Specific Binding (DPM)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
C1-2/17/05
C2-2/24/05
C3-3/2/05


bound counts
Lab C
0.6 mg/tube
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
SB
total bound
C1-2/17/05
C2-2/24/05
C3-3/2/05
C1-2/17/05
C2-2/24/05
C3-3/2/05
C1-2/17/05
C2-2/24/05
C3-3/2/05

Appendix 4: Excel Files for Saturation Runs

Laboratory C

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)
72 assay tubes
Please return by eMail to n.a.Holter@pnl.gov
Provide information in all blue cells in column O
If the DPM value for a tube was judged unreliable,
Include the DPM value in column O
Provide a reason in column R
The value in column Q will change to FALSE
For your convenience, data reduction is performed in columns
U through BZ, and the values needed for analysis are presented
in columns CF through CN

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

Laboratory Code:	C	
Run identification:	1	sequence# 5971
Assay start date:	2/17/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.08	**Ci/mmole**
Cytosol lot or vial number:	31, 161, 165	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	1.01E+00	**nM**
Bmax	13.81	**fmole/100 ug**
total volume in tubes	300	**uL**
volume of ethanol counted:	2	**mL**
mulitply DPM in sample by :	3	

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

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

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	NSB y values
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	1111.079	3333.237	TRUE		27.7%	0.25	3333.2	575.0
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	899.9092	2699.7276	TRUE		22.5%	0.25	2699.7	575.0
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	1113.148	3339.444	TRUE		27.8%	0.25	3339.4	575.0
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1974.202	5922.606	TRUE		24.2%	0.5	5922.6	884.1
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1844.292	5532.876	TRUE		22.6%	0.5	5532.9	884.1
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1854.19	5562.57	TRUE		22.7%	0.5	5562.6	884.1
7	1	H	10.0	21	0.70	—	—	—	50	300	—	2400.424	7201.272	TRUE		21.1%	0.7	7201.3	1081.6
8	2	H	10.0	21	0.70	—	—	—	50	300	—	2291.552	6874.656	TRUE		20.1%	0.7	6874.7	1081.6
9	3	H	10.0	21	0.70	—	—	—	50	300	—	2252.489	6757.467	TRUE		19.8%	0.7	6757.5	1081.6
10	1	H	10.0	30	1.00	—	—	—	50	300	—	3047.346	9142.038	TRUE		18.9%	1	9142.0	1358.7
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2737.036	8211.108	TRUE		17.0%	1	8211.1	1358.7
12	3	H	10.0	30	1.00	—	—	—	50	300	—	3052.821	9158.463	TRUE		19.0%	1	9158.5	1358.7
13	1	H	10.0	45	1.50	—	—	—	50	300	—	3398.213	10194.639	TRUE		14.2%	1.5	10194.6	2041.1
14	2	H	10.0	45	1.50	—	—	—	50	300	—	3414.747	10244.241	TRUE		14.3%	1.5	10244.2	2041.1
15	3	H	10.0	45	1.50	—	—	—	50	300	—	3540.116	10620.348	TRUE		14.8%	1.5	10620.3	2041.1
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	4429.676	13289.028	TRUE		10.0%	2.5	13289.0	2574.1
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	4478.81	13436.43	TRUE		10.1%	2.5	13436.4	2574.1
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	4985.223	14955.669	TRUE		11.2%	2.5	14955.7	2574.1
19	1	H	100.0	15	5.00	—	—	—	50	300	—	6542.486	19627.458	TRUE		7.9%	5	19627.5	5164.8
20	2	H	100.0	15	5.00	—	—	—	50	300	—	5899.125	17697.375	TRUE		7.2%	5	17697.4	5164.8
21	3	H	100.0	15	5.00	—	—	—	50	300	—	5694.57	17083.71	TRUE		6.9%	5	17083.7	5164.8
22	1	H	100.0	30	10.00	—	—	—	50	300	—	6521.775	19565.325	TRUE		4.0%	10	19565.3	10141.9
23	2	H	100.0	30	10.00	—	—	—	50	300	—	8217.698	24653.094	TRUE		5.1%	10	24653.1	10141.9
24	3	H	100.0	30	10.00	—	—	—	50	300	—	8619.031	25857.093	TRUE		5.3%	10	25857.1	10141.9

25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	186.6171	559.8513	TRUE
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	177.3031	531.9093	TRUE
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	211.0651	633.1953	TRUE
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	359.068	1077.204	TRUE
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	292.574	877.722	TRUE
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	232.4528	697.3584	TRUE
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	386.0166	1158.0498	TRUE
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	339.8266	1019.4798	TRUE
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	355.7433	1067.2299	TRUE
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	422.3438	1267.0314	TRUE
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	451.8097	1355.4291	TRUE
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	484.5495	1453.6485	TRUE
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	717.0023	2151.0069	TRUE
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	600.4703	1801.4109	TRUE
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	723.6517	2170.9551	TRUE
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	795.1282	2385.3846	TRUE
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	772.66	2317.98	TRUE
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	1006.283	3018.849	TRUE
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	1591.156	4773.468	TRUE
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	1786.833	5360.499	TRUE
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	1786.833	5360.499	TRUE
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	3068.956	9206.868	TRUE
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	3509.71	10529.13	TRUE
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	3563.23	10689.69	TRUE
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11888.5	11888.5	TRUE
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	12118.63	12118.63	TRUE
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	12040.27	12040.27	TRUE
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	24620.38	24620.38	TRUE
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	24402.36	24402.36	TRUE
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	24509.41	24509.41	TRUE
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	34682.48	34682.48	TRUE
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	34257.76	34257.76	TRUE
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	33418.16	33418.16	TRUE
58	1	Hot	10.0	30	1	—	—	—	—	—	—	48199.34	48199.34	TRUE
59	2	Hot	10.0	30	1	—	—	—	—	—	—	48187.67	48187.67	TRUE
60	3	Hot	10.0	30	1	—	—	—	—	—	—	48458.53	48458.53	TRUE
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	71691.09	71691.09	TRUE
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	71553.09	71553.09	TRUE
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	72364.7	72364.7	TRUE
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	130941.4	130941.4	TRUE
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	132156.6	132156.6	TRUE
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	136632	136632	TRUE
67	1	Hot	100.0	15	5	—	—	—	—	—	—	246961.6	246961.6	TRUE
68	2	Hot	100.0	15	5	—	—	—	—	—	—	245670	245670	TRUE
69	3	Hot	100.0	15	5	—	—	—	—	—	—	248946.3	248946.3	TRUE
70	1	Hot	100.0	30	10	—	—	—	—	—	—	479266	479266	TRUE
71	2	Hot	100.0	30	10	—	—	—	—	—	—	484304.8	484304.8	TRUE
72	3	Hot	100.0	30	10	—	—	—	—	—	—	489110.9	489110.9	TRUE

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

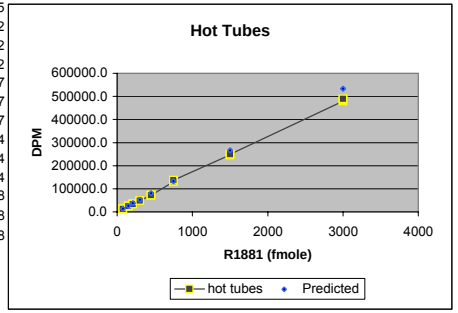
									Number of molecules					Ratio
Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
		(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
1	1	3333.2	575.0	2758.3	17.3%	27.7%	12015.8	8682.6	20	4	17	74	53	0.32
1	2	2699.7	575.0	2124.7	21.3%	22.5%	12015.8	9316.1	17	4	13	74	57	0.23
1	3	3339.4	575.0	2764.5	17.2%	27.8%	12015.8	8676.4	21	4	17	74	53	0.32
1	4	5922.6	884.1	5038.5	14.9%	24.2%	24510.7	18588.1	36	5	31	151	114	0.27
1	5	5532.9	884.1	4648.8	16.0%	22.6%	24510.7	18977.8	34	5	29	151	117	0.24
1	6	5562.6	884.1	4678.5	15.9%	22.7%	24510.7	18948.1	34	5	29	151	116	0.25
1	7	7201.3	1081.6	6119.7	15.0%	21.1%	34119.5	26918.2	44	7	38	210	165	0.23
1	8	6874.7	1081.6	5793.1	15.7%	20.1%	34119.5	27244.8	42	7	36	210	167	0.21
1	9	6757.5	1081.6	5675.9	16.0%	19.8%	34119.5	27362.0	42	7	35	210	168	0.21
1	10	9142.0	1358.7	7783.3	14.9%	18.9%	48281.8	39139.8	56	8	48	297	240	0.20
1	11	8211.1	1358.7	6852.4	16.5%	17.0%	48281.8	40070.7	50	8	42	297	246	0.17
1	12	9158.5	1358.7	7799.8	14.8%	19.0%	48281.8	39123.4	56	8	48	297	240	0.20
1	13	10194.6	2041.1	8153.5	20.0%	14.2%	71869.6	61675.0	63	13	50	442	379	0.13
1	14	10244.2	2041.1	8203.1	19.9%	14.3%	71869.6	61625.4	63	13	50	442	379	0.13
1	15	10620.3	2041.1	8579.2	19.2%	14.8%	71869.6	61249.3	65	13	53	442	376	0.14
1	16	13289.0	2574.1	10715.0	19.4%	10.0%	133243.3	119954.3	82	16	66	819	737	0.09
1	17	13436.4	2574.1	10862.4	19.2%	10.1%	133243.3	119806.9	83	16	67	819	736	0.09
1	18	14955.7	2574.1	12381.6	17.2%	11.2%	133243.3	118287.7	92	16	76	819	727	0.10
1	19	19627.5	5164.8	14462.6	26.3%	7.9%	247192.6	227565.2	121	32	89	1519	1398	0.06
1	20	17697.4	5164.8	12532.6	29.2%	7.2%	247192.6	229495.3	109	32	77	1519	1410	0.05
1	21	17083.7	5164.8	11918.9	30.2%	6.9%	247192.6	230108.9	105	32	73	1519	1414	0.05
1	22	19565.3	10141.9	9423.4	51.8%	4.0%	484227.2	464661.9	120	62	58	2975	2855	0.02
1	23	24653.1	10141.9	14511.2	41.1%	5.1%	484227.2	459574.1	151	62	89	2975	2824	0.03
1	24	25857.1	10141.9	15715.2	39.2%	5.3%	484227.2	458370.1	159	62	97	2975	2816	0.03

		Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol													
		Tube Identification			Assay tube contents									Scintillation Results	
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final		Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
					(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)		(dpm)	(dpm)
1	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		559.9	575.0
1	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		531.9	575.0
1	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25		633.2	575.0
1	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50		1077.2	884.1
1	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50		877.7	884.1
1	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50		697.4	884.1
1	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70		1158.0	1081.6
1	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70		1019.5	1081.6
1	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70		1067.2	1081.6
1	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100		1267.0	1358.7
1	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100		1355.4	1358.7
1	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100		1453.6	1358.7
1	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150		2151.0	2041.1
1	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150		1801.4	2041.1
1	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150		2171.0	2041.1
1	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		2385.4	2574.1
1	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		2318.0	2574.1
1	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250		3018.8	2574.1
1	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500		4773.5	5164.8
1	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500		5360.5	5164.8
1	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500		5360.5	5164.8
1	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000		9206.9	10141.9
1	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000		10529.1	10141.9
1	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000		10689.7	10141.9

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

Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial (nM)	Hot R1881 Volume (ul)	Molecules of R1881 (fmole)	Counts per Scintillation Vial (dpm)	Experimental number of molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (dpm)	predicted dpm
1	49	1	Hot	c1	10	7.5	75	11888.5	73	12015.8	13334
1	50	2	Hot	c1	10	7.5	75	12118.6	74	12015.8	13334
1	51	3	Hot	c1	10	7.5	75	12040.3	74	12015.8	13334
1	52	1	Hot	c2	10	15	150	24620.4	151	24510.7	26667
1	53	2	Hot	c2	10	15	150	24402.4	150	24510.7	26667
1	54	3	Hot	c2	10	15	150	24509.4	151	24510.7	26667
1	55	1	Hot	c3	10	21	210	34682.5	213	34119.5	37334
1	56	2	Hot	c3	10	21	210	34257.8	210	34119.5	37334
1	57	3	Hot	c3	10	21	210	33418.2	205	34119.5	37334
1	58	1	Hot	c4	10	30	300	48199.3	296	48281.8	53335
1	59	2	Hot	c4	10	30	300	48187.7	296	48281.8	53335
1	60	3	Hot	c4	10	30	300	48458.5	298	48281.8	53335
1	61	1	Hot	c5	10	45	450	71691.1	440	71869.6	80002
1	62	2	Hot	c5	10	45	450	71553.1	440	71869.6	80002
1	63	3	Hot	c5	10	45	450	72364.7	445	71869.6	80002
1	64	1	Hot	c6	100	7.5	750	130941.4	805	133243.3	133337
1	65	2	Hot	c6	100	7.5	750	132156.6	812	133243.3	133337
1	66	3	Hot	c6	100	7.5	750	136632.0	839	133243.3	133337
1	67	1	Hot	c7	100	15	1500	246961.6	1517	247192.6	266674
1	68	2	Hot	c7	100	15	1500	245670.0	1509	247192.6	266674
1	69	3	Hot	c7	100	15	1500	248946.3	1530	247192.6	266674
1	70	1	Hot	c8	100	30	3000	479266.0	2945	484227.2	533348
1	71	2	Hot	c8	100	30	3000	484304.8	2976	484227.2	533348
1	72	3	Hot	c8	100	30	3000	489110.9	3005	484227.2	533348

Computation Check	
2/17/05	specific activity date
80.08	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7778E+14	DPM/mmole
1.7778E+11	DPM/nmole
177.8	DPM/fmole
0.005625	fmole/DPM



Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	162.7605564 dpm/fmole	
1/slope	0.006143995 fmole/dpm	
	x	y
origin	0	0
end point	3005.1	489110.9
SLOPE function, used if missing HOT tubes		
Slope	161.8 dpm/fmole	
1/slope	0.006179 fmole/dpm	
	x	y
origin	0	0
end point	3022.4	489110.9

Prism input for bound/free		Prism input for specific bound						
specific bound/molar	bound/free	*average total added molar*	*specific bound/molar*	*spcific bound/dpm*	*total added dpm*	*total bound dpm*	NSB dpm	Hot Final Concentration (nM)
5.17158E-11	0.31768	2.2529E-10	5.17158E-11	2758.3	11888.5	3333.2	559.9	0.25
3.98378E-11	0.22807	2.2529E-10	3.98378E-11	2124.7	12118.6	2699.7	531.9	0.25
5.18322E-11	0.31862	2.2529E-10	5.18322E-11	2764.5	12040.3	3339.4	633.2	0.25
9.44694E-11	0.27106	4.59563E-10	9.44694E-11	5038.5	24620.4	5922.6	1077.2	0.50
8.71622E-11	0.24496	4.59563E-10	8.71622E-11	4648.8	24402.4	5532.9	877.7	0.50
8.7719E-11	0.24691	4.59563E-10	8.7719E-11	4678.5	24509.4	5562.6	697.4	0.50
1.14741E-10	0.22734	6.39722E-10	1.14741E-10	6119.7	34682.5	7201.3	1158.0	0.70
1.08617E-10	0.21263	6.39722E-10	1.08617E-10	5793.1	34257.8	6874.7	1019.5	0.70
1.0642E-10	0.20744	6.39722E-10	1.0642E-10	5675.9	33418.2	6757.5	1067.2	0.70
1.45933E-10	0.19886	9.05259E-10	1.45933E-10	7783.3	48199.3	9142.0	1267.0	1.00
1.28479E-10	0.17101	9.05259E-10	1.28479E-10	6852.4	48187.7	8211.1	1355.4	1.00
1.46241E-10	0.19936	9.05259E-10	1.46241E-10	7799.8	48458.5	9158.5	1453.6	1.00
1.52874E-10	0.13220	1.34752E-09	1.52874E-10	8153.5	71691.1	10194.6	2151.0	1.50
1.53804E-10	0.13311	1.34752E-09	1.53804E-10	8203.1	71553.1	10244.2	1801.4	1.50
1.60856E-10	0.14007	1.34752E-09	1.60856E-10	8579.2	72364.7	10620.3	2171.0	1.50
2.009E-10	0.08933	2.49824E-09	2.009E-10	10715.0	130941.4	13289.0	2385.4	2.50
2.03664E-10	0.09067	2.49824E-09	2.03664E-10	10862.4	132156.6	13436.4	2318.0	2.50
2.32148E-10	0.10467	2.49824E-09	2.32148E-10	12381.6	136632.0	14955.7	3018.8	2.50
2.71167E-10	0.06355	4.63473E-09	2.71167E-10	14462.6	246961.6	19627.5	4773.5	5.00
2.34979E-10	0.05461	4.63473E-09	2.34979E-10	12532.6	245670.0	17697.4	5360.5	5.00
2.23473E-10	0.05180	4.63473E-09	2.23473E-10	11918.9	248946.3	17083.7	5360.5	5.00
1.76684E-10	0.02028	9.07901E-09	1.76684E-10	9423.4	479266.0	19565.3	9206.9	10.00
2.72077E-10	0.03158	9.07901E-09	2.72077E-10	14511.2	484304.8	24653.1	10529.1	10.00
2.94652E-10	0.03428	9.07901E-09	2.94652E-10	15715.2	489110.9	25857.1	10689.7	10.00

Bmax molar	2.76E-10	KD molar	1.01E-09
mole to molar conversion value	0.0003	molar to nM converison	1.00E+09
DPM/mole = (DPM/mmole)*1000	1.78E+17	**kd nM =**	1.01E+00
Bmax molar to Bmax moles	8.2833E-14		
= DPM/((DPM/mmole)*1000)	8.2833E-14		
=Bmax DPM	14726.27856		
assay date	2/17/2005		
Bmax(dpm)	14726.27856		
DPM/Ci (definition)	2.22E+12		
Ci/mmole	80.08		
DPM/mmole	1.78E+14		
DPM/pmole	1.78E+05		
1/(DPM/mmole)	5.62E-15		
1/(DPM/pmole)	5.62E-06		
SA(dpm/pmole)	1.78E+05		
protein/tube (ug)	600		
protein./tube(mg)	0.6		
bmax pmole	0.082833		
bmax pmole/mg	0.138055		
Bmax fmole/mg	138.055		
Bmax (fmole/100 ug)	13.8055		
Bmax(fmole/100 ug)/Bmax molar	5.00E+10		

Laboratory C

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,
- Include the DPM value in column O
- Provide a reason in column R
- The value in column Q will change to FALSE

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

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

Laboratory Code:	C	
Run identification:	2	sequence# 5972
Assay start date:	2/24/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.00	**Ci/mmole**
Cytosol lot or vial number:	AR 10/27/04 vials 32, 156, 157	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	4.81E-01	**nM**
Bmax	10.03	**fmole/100 ug**
total volume in tubes	300	**uL**
volume of ethanol counted:	2	**mL**
mulitply DPM in sample by :	3	

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

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

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	1245.700	3737.1	TRUE	
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	1147.017	3441.051	TRUE	
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	1262.580	3787.74	TRUE	
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1855.175	5565.525	TRUE	
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1961.565	5884.695	TRUE	
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1911.695	5735.085	TRUE	
7	1	H	10.0	21	0.70	—	—	—	50	300	—	2370.966	7112.898	TRUE	
8	2	H	10.0	21	0.70	—	—	—	50	300	—	2423.883	7271.649	TRUE	
9	3	H	10.0	21	0.70	—	—	—	50	300	—	2380.476	7141.428	TRUE	
10	1	H	10.0	30	1.00	—	—	—	50	300	—	3112.356	9337.068	TRUE	
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2815.486	8446.458	TRUE	
12	3	H	10.0	30	1.00	—	—	—	50	300	—	3132.818	9398.454	TRUE	
13	1	H	10.0	45	1.50	—	—	—	50	300	—	3550.765	10652.295	TRUE	
14	2	H	10.0	45	1.50	—	—	—	50	300	—	3623.908	10871.724	TRUE	
15	3	H	10.0	45	1.50	—	—	—	50	300	—	3574.687	10724.061	TRUE	
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	4727.854	14183.562	TRUE	
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	4502.722	13508.166	TRUE	
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	4181.099	12543.297	TRUE	
19	1	H	100.0	15	5.00	—	—	—	50	300	—	5336.931	16010.793	TRUE	
20	2	H	100.0	15	5.00	—	—	—	50	300	—	2478.171	7434.513	TRUE	
21	3	H	100.0	15	5.00	—	—	—	50	300	—	5786.292	17358.876	TRUE	
22	1	H	100.0	30	10.00	—	—	—	50	300	—	6090.858	18272.574	TRUE	
23	2	H	100.0	30	10.00	—	—	—	50	300	—	5769.413	17308.239	TRUE	
24	3	H	100.0	30	10.00	—	—	—	50	300	—	7085.756	21257.268	TRUE	

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R
45	25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		113.9081	341.7243	TRUE	
46	26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		196.1575	588.4725	TRUE	
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		148.5396	445.6188	TRUE	
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7		194.3139	582.9417	TRUE	
49	29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7		226.1853	678.5559	TRUE	
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7		219.4335	658.3005	TRUE	
51	31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6		239.6889	719.0667	TRUE	
52	32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6		190.9636	572.8908	TRUE	
53	33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6		204.3646	613.0938	TRUE	
54	34	1	HC	10.0	30	1	1.00	30	100	50	300	C5		246.4407	739.3221	TRUE	
55	35	2	HC	10.0	30	1	1.00	30	100	50	300	C5		252.6775	758.0325	TRUE	
56	36	3	HC	10.0	30	1	1.00	30	100	50	300	C5		259.0274	777.0822	TRUE	
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4		307.2069	921.6207	TRUE	
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4		355.1254	1065.3762	TRUE	
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4		412.0794	1236.2382	TRUE	
60	40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		683.4488	2050.3464	TRUE	
61	41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		934.7167	2804.1501	TRUE	
62	42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		513.1368	1539.4104	TRUE	
63	43	1	HC	100.0	15	5	10.00	15	500	50	300	C2		789.1924	2367.5772	TRUE	
64	44	2	HC	100.0	15	5	10.00	15	500	50	300	C2		914.6153	2743.8459	TRUE	
65	45	3	HC	100.0	15	5	10.00	15	500	50	300	C2		847.3509	2542.0527	TRUE	
66	46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1		1617.056	4851.168	TRUE	
67	47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1		1991.498	5974.494	TRUE	
68	48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1		1519.155	4557.465	TRUE	
69	49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—		12593.16	12593.16	TRUE	
70	50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—		12350.36	12350.36	TRUE	
71	51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—		11808.08	11808.08	TRUE	
72	52	1	Hot	10.0	15	0.5	—	—	—	—	—	—		25720.32	25720.32	TRUE	
73	53	2	Hot	10.0	15	0.5	—	—	—	—	—	—		25496.97	25496.97	TRUE	
74	54	3	Hot	10.0	15	0.5	—	—	—	—	—	—		25601.79	25601.79	TRUE	
75	55	1	Hot	10.0	21	0.7	—	—	—	—	—	—		34023.05	34023.05	TRUE	
76	56	2	Hot	10.0	21	0.7	—	—	—	—	—	—		35276.96	35276.96	TRUE	
77	57	3	Hot	10.0	21	0.7	—	—	—	—	—	—		35078.47	35078.47	TRUE	
78	58	1	Hot	10.0	30	1	—	—	—	—	—	—		49576.80	49576.8	TRUE	
79	59	2	Hot	10.0	30	1	—	—	—	—	—	—		49983.37	49983.37	TRUE	
80	60	3	Hot	10.0	30	1	—	—	—	—	—	—		50562.82	50562.82	TRUE	
81	61	1	Hot	10.0	45	1.5	—	—	—	—	—	—		74270.38	74270.38	TRUE	
82	62	2	Hot	10.0	45	1.5	—	—	—	—	—	—		75327.52	75327.52	TRUE	
83	63	3	Hot	10.0	45	1.5	—	—	—	—	—	—		78023.59	78023.59	TRUE	
84	64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—		136452.4	136452.4	TRUE	
85	65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—		140211.3	140211.3	TRUE	
86	66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—		132107.3	132107.3	TRUE	
87	67	1	Hot	100.0	15	5	—	—	—	—	—	—		254961.7	254961.7	TRUE	
88	68	2	Hot	100.0	15	5	—	—	—	—	—	—		262814.5	262814.5	TRUE	
89	69	3	Hot	100.0	15	5	—	—	—	—	—	—		266114.3	266114.3	TRUE	
90	70	1	Hot	100.0	30	10	—	—	—	—	—	—		508881.2	508881.2	TRUE	
91	71	2	Hot	100.0	30	10	—	—	—	—	—	—		522799.3	522799.3	TRUE	
92	72	3	Hot	100.0	30	10	—	—	—	—	—	—		516609.5	516609.5	TRUE	

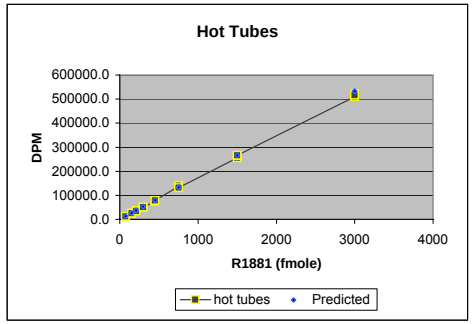
	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
17			Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)											
18			Tube Identification			Assay tube contents								
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
21	2	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
22	2	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
23	2	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
24	2	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
25	2	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
26	2	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
27	2	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
28	2	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
29	2	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
30	2	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
31	2	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
32	2	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
33	2	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
34	2	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
35	2	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
36	2	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
37	2	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
38	2	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
39	2	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
40	2	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
41	2	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
42	2	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
43	2	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
44	2	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY
17															
18										Number of molecules					Ratio
19	Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
20			(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
21	2	1	3737.1	458.6	3278.5	12.3%	30.5%	12250.5	8513.4	22	3	19	71	49	0.39
22	2	2	3441.1	458.6	2982.4	13.3%	28.1%	12250.5	8809.5	20	3	17	71	51	0.34
23	2	3	3787.7	458.6	3329.1	12.1%	30.9%	12250.5	8462.8	22	3	19	71	49	0.39
24	2	4	5565.5	639.9	4925.6	11.5%	21.7%	25606.4	20040.8	32	4	29	148	116	0.25
25	2	5	5884.7	639.9	5244.8	10.9%	23.0%	25606.4	19721.7	34	4	30	148	114	0.27
26	2	6	5735.1	639.9	5095.2	11.2%	22.4%	25606.4	19871.3	33	4	29	148	115	0.26
27	2	7	7112.9	635.0	6477.9	8.9%	20.4%	34792.8	27679.9	41	4	37	201	160	0.23
28	2	8	7271.6	635.0	6636.6	8.7%	20.9%	34792.8	27521.2	42	4	38	201	159	0.24
29	2	9	7141.4	635.0	6506.4	8.9%	20.5%	34792.8	27651.4	41	4	38	201	160	0.24
30	2	10	9337.1	758.1	8578.9	8.1%	18.7%	50041.0	40703.9	54	4	50	290	236	0.21
31	2	11	8446.5	758.1	7688.3	9.0%	16.9%	50041.0	41594.5	49	4	45	290	241	0.18
32	2	12	9398.5	758.1	8640.3	8.1%	18.8%	50041.0	40642.5	54	4	50	290	235	0.21
33	2	13	10652.3	1074.4	9577.9	10.1%	14.0%	75873.8	65221.5	62	6	55	439	378	0.15
34	2	14	10871.7	1074.4	9797.3	9.9%	14.3%	75873.8	65002.1	63	6	57	439	376	0.15
35	2	15	10724.1	1074.4	9649.6	10.0%	14.1%	75873.8	65149.8	62	6	56	439	377	0.15
36	2	16	14183.6	2131.3	12052.3	15.0%	10.4%	136257.0	122073.4	82	12	70	789	707	0.10
37	2	17	13508.2	2131.3	11376.9	15.8%	9.9%	136257.0	122748.8	78	12	66	789	711	0.09
38	2	18	12543.3	2131.3	10412.0	17.0%	9.2%	136257.0	123713.7	73	12	60	789	716	0.08
39	2	19	16010.8	2551.2	13459.6	15.9%	6.1%	261296.8	245286.0	93	15	78	1513	1420	0.05
40	2	20	7434.5	2551.2	4883.4	34.3%	2.8%	261296.8	253862.3	43	15	28	1513	1470	0.02
41	2	21	17358.9	2551.2	14807.7	14.7%	6.6%	261296.8	243938.0	100	15	86	1513	1412	0.06
42	2	22	18272.6	5127.7	13144.9	28.1%	3.5%	516096.7	497824.1	106	30	76	2987	2882	0.03
43	2	23	17308.2	5127.7	12180.5	29.6%	3.4%	516096.7	498788.4	100	30	71	2987	2887	0.02
44	2	24	21257.3	5127.7	16129.6	24.1%	4.1%	516096.7	494839.4	123	30	93	2987	2864	0.03

	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
17			Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
18			Tube Identification			Assay tube contents								Scintillation Results	
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
21	2	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	341.7	458.6
22	2	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	588.5	458.6
23	2	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	445.6	458.6
24	2	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	582.9	639.9
25	2	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	678.6	639.9
26	2	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	658.3	639.9
27	2	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	719.1	635.0
28	2	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	572.9	635.0
29	2	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	613.1	635.0
30	2	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	739.3	758.1
31	2	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	758.0	758.1
32	2	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	777.1	758.1
33	2	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	921.6	1074.4
34	2	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	1065.4	1074.4
35	2	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	1236.2	1074.4
36	2	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	2050.3	2131.3
37	2	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	2804.2	2131.3
38	2	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1539.4	2131.3
39	2	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2367.6	2551.2
40	2	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	2743.8	2551.2
41	2	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	2542.1	2551.2
42	2	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	4851.2	5127.7
43	2	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	5974.5	5127.7
44	2	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	4557.5	5127.7

	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB
17														
18			Free -- Positions 49-72, radiolabeled R1881 without cytosol											
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial	Hot R1881 Volume	Molecules of R1881	Counts per Scintillation Vial	Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)	predicted dpm		
20						(nM)	(ul)	(fmole)	(dpm)	(fmole)	(dpm)			
21	2	49	1	Hot	c1	10	7.5	75	12593.2	73	12250.5	13319	**Computation Check**	
22	2	50	2	Hot	c1	10	7.5	75	12350.4	71	12250.5	13319		
23	2	51	3	Hot	c1	10	7.5	75	11808.1	68	12250.5	13319	**2/24/05**	specific activity date
24	2	52	1	Hot	c2	10	15	150	25720.3	149	25606.4	26639	**80.00**	Ci/mMole 3H R1881
25	2	53	2	Hot	c2	10	15	150	25497.0	148	25606.4	26639	2.22E+12	DPM/Ci (definition)
26	2	54	3	Hot	c2	10	15	150	25601.8	148	25606.4	26639		
27	2	55	1	Hot	c3	10	21	210	34023.1	197	34792.8	37294	1.7759E+14	DPM/mmole
28	2	56	2	Hot	c3	10	21	210	35277.0	204	34792.8	37294	1.7759E+11	DPM/nmole
29	2	57	3	Hot	c3	10	21	210	35078.5	203	34792.8	37294	177.6	DPM/fmole
30	2	58	1	Hot	c4	10	30	300	49576.8	287	50041.0	53277	0.005631	fmole/DPM
31	2	59	2	Hot	c4	10	30	300	49983.4	289	50041.0	53277		
32	2	60	3	Hot	c4	10	30	300	50562.8	293	50041.0	53277		
33	2	61	1	Hot	c5	10	45	450	74270.4	430	75873.8	79916		
34	2	62	2	Hot	c5	10	45	450	75327.5	436	75873.8	79916		
35	2	63	3	Hot	c5	10	45	450	78023.6	452	75873.8	79916		
36	2	64	1	Hot	c6	100	7.5	750	136452.4	790	136257.0	133194		
37	2	65	2	Hot	c6	100	7.5	750	140211.3	812	136257.0	133194		
38	2	66	3	Hot	c6	100	7.5	750	132107.3	765	136257.0	133194		
39	2	67	1	Hot	c7	100	15	1500	254961.7	1476	261296.8	266387		
40	2	68	2	Hot	c7	100	15	1500	262814.5	1521	261296.8	266387		
41	2	69	3	Hot	c7	100	15	1500	266114.3	1540	261296.8	266387		
42	2	70	1	Hot	c8	100	30	3000	508881.2	2946	516096.7	532775		
43	2	71	2	Hot	c8	100	30	3000	522799.3	3026	516096.7	532775		
44	2	72	3	Hot	c8	100	30	3000	516609.5	2990	516096.7	532775		

	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
46		Linear regression results (LINEST function)									
47		*(Regression line forced through 0,0)*									
48		Slope	172.7528037	dpm/fmole							
49		1/slope	0.005788618	fmole/dpm							
50			x	y							
51		origin	0	0							
52		end point	3026.3	522799.3							
53		SLOPE function, used if missing HOT tubes									
54		Slope	172.7	dpm/fmole							
55		1/slope	0.005791	fmole/dpm							
56			x	y							
57		origin	0	0							
58		end point	3027.4	522799.3							
59											
60											
61											
62											
63											
64											



	S	T	U	V
18				
19	**Ten Percent Rule**	**Saturation X values**	**Bound y values**	**NSB y values**
20				
21	30.5%	0.25	3737.1	458.6
22	28.1%	0.25	3441.1	458.6
23	30.9%	0.25	3787.7	458.6
24	21.7%	0.5	5565.5	639.9
25	23.0%	0.5	5884.7	639.9
26	22.4%	0.5	5735.1	639.9
27	20.4%	0.7	7112.9	635.0
28	20.9%	0.7	7271.6	635.0
29	20.5%	0.7	7141.4	635.0
30	18.7%	1	9337.1	758.1
31	16.9%	1	8446.5	758.1
32	18.8%	1	9398.5	758.1
33	14.0%	1.5	10652.3	1074.4
34	14.3%	1.5	10871.7	1074.4
35	14.1%	1.5	10724.1	1074.4
36	10.4%	2.5	14183.6	2131.3
37	9.9%	2.5	13508.2	2131.3
38	9.2%	2.5	12543.3	2131.3
39	6.1%	5	16010.8	2551.2
40	2.8%	5	7434.5	2551.2
41	6.6%	5	17358.9	2551.2
42	3.5%	10	18272.6	5127.7
43	3.4%	10	17308.2	5127.7
44	4.1%	10	21257.3	5127.7

	CF	CG	CH	CI	CJ	CK	CL	CM	CN
18									
19	**Prism input for bound/free**		**Prism input for specific bound**						
20	**specific bound/molar**	**bound/free**	***average total added molar***	***specific bound/molar***	***spcific bound/dpm***	***total added dpm***	***total bound dpm***	**NSB dpm**	**Hot Final Concentration (nM)**
21	6.15362E-11	0.38510	2.29938E-10	6.15362E-11	3278.5	12593.2	3737.1	341.7	0.25
22	5.59795E-11	0.33855	2.29938E-10	5.59795E-11	2982.4	12350.4	3441.1	588.5	0.25
23	6.24867E-11	0.39338	2.29938E-10	6.24867E-11	3329.1	11808.1	3787.7	445.6	0.25
24	9.24517E-11	0.24578	4.80623E-10	9.24517E-11	4925.6	25720.3	5565.5	582.9	0.50
25	9.84424E-11	0.26594	4.80623E-10	9.84424E-11	5244.8	25497.0	5884.7	678.6	0.50
26	9.56342E-11	0.25641	4.80623E-10	9.56342E-11	5095.2	25601.8	5735.1	658.3	0.50
27	1.21588E-10	0.23403	6.53049E-10	1.21588E-10	6477.9	34023.1	7112.9	719.1	0.70
28	1.24567E-10	0.24115	6.53049E-10	1.24567E-10	6636.6	35277.0	7271.6	572.9	0.70
29	1.22123E-10	0.23530	6.53049E-10	1.22123E-10	6506.4	35078.5	7141.4	613.1	0.70
30	1.61023E-10	0.21076	9.39252E-10	1.61023E-10	8578.9	49576.8	9337.1	739.3	1.00
31	1.44307E-10	0.18484	9.39252E-10	1.44307E-10	7688.3	49983.4	8446.5	758.0	1.00
32	1.62176E-10	0.21259	9.39252E-10	1.62176E-10	8640.3	50562.8	9398.5	777.1	1.00
33	1.79774E-10	0.14685	1.42413E-09	1.79774E-10	9577.9	74270.4	10652.3	921.6	1.50
34	1.83892E-10	0.15072	1.42413E-09	1.83892E-10	9797.3	75327.5	10871.7	1065.4	1.50
35	1.81121E-10	0.14811	1.42413E-09	1.81121E-10	9649.6	78023.6	10724.1	1236.2	1.50
36	2.26217E-10	0.09873	2.5575E-09	2.26217E-10	12052.3	136452.4	14183.6	2050.3	2.50
37	2.1354E-10	0.09268	2.5575E-09	2.1354E-10	11376.9	140211.3	13508.2	2804.2	2.50
38	1.9543E-10	0.08416	2.5575E-09	1.9543E-10	10412.0	132107.3	12543.3	1539.4	2.50
39	2.52633E-10	0.05487	4.90445E-09	2.52633E-10	13459.6	254961.7	16010.8	2367.6	5.00
40	9.16589E-11	0.01924	4.90445E-09	9.16589E-11	4883.4	262814.5	7434.5	2743.8	5.00
41	2.77936E-10	0.06070	4.90445E-09	2.77936E-10	14807.7	266114.3	17358.9	2542.1	5.00
42	2.46725E-10	0.02640	9.68696E-09	2.46725E-10	13144.9	508881.2	18272.6	4851.2	10.00
43	2.28624E-10	0.02442	9.68696E-09	2.28624E-10	12180.5	522799.3	17308.2	5974.5	10.00
44	3.02746E-10	0.03260	9.68696E-09	3.02746E-10	16129.6	516609.5	21257.3	4557.5	10.00

Laboratory C

AR Saturation Assay (cold R1881 dilutions supplied by Battelle)

72 assay tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,
- Include the DPM value in column O
- Provide a reason in column R
- The value in column Q will change to FALSE

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

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

Laboratory Code:	C	
Run identification:	3	sequence# 5973
Assay start date:	3/2/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.92	**Ci/mmole**
Cytosol lot or vial number:	AR 10/27/04 vials 33, 162, 163	
protein (cytosol) per tube:	600	**ug**
protein (cytosol) per tube:	0.6	**mg**
KD	1.21E+00	**nM**
Bmax	15.61	**fmole/100 ug**
total volume in tubes	300	**uL**
volume of ethanol counted:	2	**mL**
mulitply DPM in sample by :	3	

Receptor_Notes

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

protcol calls for counting decanted EtOH supe

reflects 100ul of reaction mixture processed

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Final Cold Tube ID	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	1044.471	3133.413	TRUE	
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	1090.497	3271.491	TRUE	
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	1147.017	3441.051	TRUE	
4	1	H	10.0	15	0.50	—	—	—	50	300	—	1622.447	4867.341	TRUE	
5	2	H	10.0	15	0.50	—	—	—	50	300	—	1883.883	5651.649	TRUE	
6	3	H	10.0	15	0.50	—	—	—	50	300	—	1705.564	5116.692	TRUE	
7	1	H	10.0	21	0.70	—	—	—	50	300	—	2143.054	6429.162	TRUE	
8	2	H	10.0	21	0.70	—	—	—	50	300	—	2359.741	7079.223	TRUE	
9	3	H	10.0	21	0.70	—	—	—	50	300	—	2241.298	6723.894	TRUE	
10	1	H	10.0	30	1.00	—	—	—	50	300	—	2737.131	8211.393	TRUE	
11	2	H	10.0	30	1.00	—	—	—	50	300	—	2722.918	8168.754	TRUE	
12	3	H	10.0	30	1.00	—	—	—	50	300	—	2925.724	8777.172	TRUE	
13	1	H	10.0	45	1.50	—	—	—	50	300	—	3361.956	10085.868	TRUE	
14	2	H	10.0	45	1.50	—	—	—	50	300	—	3350.648	10051.944	TRUE	
15	3	H	10.0	45	1.50	—	—	—	50	300	—	3154.102	9462.306	TRUE	
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	4234.68	12704.04	TRUE	
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	4493.323	13479.969	TRUE	
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	4250.258	12750.774	TRUE	
19	1	H	100.0	15	5.00	—	—	—	50	300	—	5133.691	15401.073	TRUE	
20	2	H	100.0	15	5.00	—	—	—	50	300	—	5158.375	15475.125	TRUE	
21	3	H	100.0	15	5.00	—	—	—	50	300	—	5555.581	16666.743	TRUE	
22	1	H	100.0	30	10.00	—	—	—	50	300	—	4429.181	13287.543	FALSE	6 mM triamcinolone used
23	2	H	100.0	30	10.00	—	—	—	50	300	—	4990.281	14970.843	FALSE	6 mM triamcinolone used
24	3	H	100.0	30	10.00	—	—	—	50	300	—	4415.615	13246.845	FALSE	6 mM triamcinolone used

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R
45	25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		136.3129	408.9387	TRUE	
46	26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		189.0504	567.1512	TRUE	
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		175.5468	526.6404	TRUE	
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7		232.7293	698.1879	TRUE	
49	29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7		239.3787	718.1361	TRUE	
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7		221.1158	663.3474	TRUE	
51	31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6		248.8227	746.4681	TRUE	
52	32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6		261.3187	783.9561	TRUE	
53	33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6		279.2751	837.8253	TRUE	
54	34	1	HC	10.0	30	1	1.00	30	100	50	300	C5		277.1401	831.4203	TRUE	
55	35	2	HC	10.0	30	1	1.00	30	100	50	300	C5		309.5539	928.6617	TRUE	
56	36	3	HC	10.0	30	1	1.00	30	100	50	300	C5		316.3573	949.0719	TRUE	
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4		442.2429	1326.7287	TRUE	
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4		398.3562	1195.0686	TRUE	
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4		308.222	924.666	TRUE	
60	40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		530.0163	1590.0489	TRUE	
61	41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		570.5271	1711.5813	TRUE	
62	42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		532.6880	1598.064	TRUE	
63	43	1	HC	100.0	15	5	10.00	15	500	50	300	C2		827.0955	2481.2865	TRUE	
64	44	2	HC	100.0	15	5	10.00	15	500	50	300	C2		874.2347	2622.7041	TRUE	
65	45	3	HC	100.0	15	5	10.00	15	500	50	300	C2		857.6613	2572.9839	TRUE	
66	46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1		830.8593	2492.5779	FALSE	6 mM triamcinolone used
67	47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1		740.4029	2221.2087	FALSE	6 mM triamcinolone used
68	48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1		820.344	2461.0308	FALSE	6 mM triamcinolone used
69	49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—		12793.94	12793.94	TRUE	
70	50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—		12954.48	12954.48	TRUE	
71	51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—		12485.84	12485.84	TRUE	
72	52	1	Hot	10.0	15	0.5	—	—	—	—	—	—		25734.48	25734.48	TRUE	
73	53	2	Hot	10.0	15	0.5	—	—	—	—	—	—		24728.68	24728.68	TRUE	
74	54	3	Hot	10.0	15	0.5	—	—	—	—	—	—		25557.68	25557.68	TRUE	
75	55	1	Hot	10.0	21	0.7	—	—	—	—	—	—		35238.84	35238.84	TRUE	
76	56	2	Hot	10.0	21	0.7	—	—	—	—	—	—		34465.23	34465.23	TRUE	
77	57	3	Hot	10.0	21	0.7	—	—	—	—	—	—		34751.25	34751.25	TRUE	
78	58	1	Hot	10.0	30	1	—	—	—	—	—	—		48279.12	48279.12	TRUE	
79	59	2	Hot	10.0	30	1	—	—	—	—	—	—		48624.25	48624.25	TRUE	
80	60	3	Hot	10.0	30	1	—	—	—	—	—	—		49647.46	49647.46	TRUE	
81	61	1	Hot	10.0	45	1.5	—	—	—	—	—	—		73284.76	73284.76	TRUE	
82	62	2	Hot	10.0	45	1.5	—	—	—	—	—	—		75084.47	75084.47	TRUE	
83	63	3	Hot	10.0	45	1.5	—	—	—	—	—	—		75349.91	75349.91	TRUE	
84	64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—		135331.7	135331.7	TRUE	
85	65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—		137337.2	137337.2	TRUE	
86	66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—		137538.0	137538	TRUE	
87	67	1	Hot	100.0	15	5	—	—	—	—	—	—		259006.0	259006	TRUE	
88	68	2	Hot	100.0	15	5	—	—	—	—	—	—		256517.8	256517.8	TRUE	
89	69	3	Hot	100.0	15	5	—	—	—	—	—	—		252779.8	252779.8	TRUE	
90	70	1	Hot	100.0	30	10	—	—	—	—	—	—		490455.9	490455.9	FALSE	6 mM triamcinolone used
91	71	2	Hot	100.0	30	10	—	—	—	—	—	—		492381.4	492381.4	FALSE	6 mM triamcinolone used
92	72	3	Hot	100.0	30	10	—	—	—	—	—	—		503274.1	503274.1	FALSE	6 mM triamcinolone used

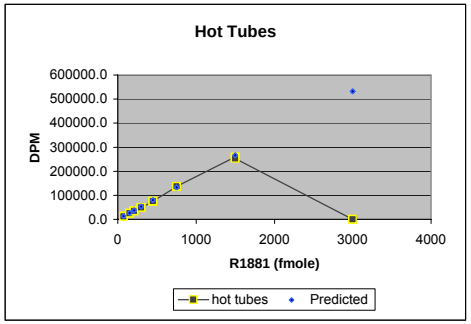
	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
17			Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)											
18			Tube Identification			Assay tube contents								
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
21	3	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
22	3	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
23	3	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
24	3	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
25	3	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
26	3	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
27	3	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
28	3	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
29	3	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
30	3	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
31	3	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
32	3	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
33	3	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
34	3	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
35	3	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
36	3	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
37	3	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
38	3	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
39	3	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
40	3	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
41	3	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
42	3	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
43	3	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
44	3	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300

	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY
17															
18										Number of molecules					Ratio
19	Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
20			(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
21	3	1	3133.4	500.9	2632.5	16.0%	24.6%	12744.8	9611.3	18	3	15	74	56	0.27
22	3	2	3271.5	500.9	2770.6	15.3%	25.7%	12744.8	9473.3	19	3	16	74	55	0.29
23	3	3	3441.1	500.9	2940.1	14.6%	27.0%	12744.8	9303.7	20	3	17	74	54	0.32
24	3	4	4867.3	693.2	4174.1	14.2%	19.2%	25340.3	20472.9	28	4	24	147	118	0.20
25	3	5	5651.6	693.2	4958.4	12.3%	22.3%	25340.3	19688.6	33	4	29	147	114	0.25
26	3	6	5116.7	693.2	4423.5	13.5%	20.2%	25340.3	20223.6	30	4	26	147	117	0.22
27	3	7	6429.2	789.4	5639.7	12.3%	18.5%	34818.4	28389.3	37	5	33	201	164	0.20
28	3	8	7079.2	789.4	6289.8	11.2%	20.3%	34818.4	27739.2	41	5	36	201	160	0.23
29	3	9	6723.9	789.4	5934.5	11.7%	19.3%	34818.4	28094.5	39	5	34	201	163	0.21
30	3	10	8211.4	903.1	7308.3	11.0%	16.8%	48850.3	40638.9	47	5	42	283	235	0.18
31	3	11	8168.8	903.1	7265.7	11.1%	16.7%	48850.3	40681.5	47	5	42	283	235	0.18
32	3	12	8777.2	903.1	7874.1	10.3%	18.0%	48850.3	40073.1	51	5	46	283	232	0.20
33	3	13	10085.9	1148.8	8937.0	11.4%	13.5%	74573.0	64487.2	58	7	52	431	373	0.14
34	3	14	10051.9	1148.8	8903.1	11.4%	13.5%	74573.0	64521.1	58	7	51	431	373	0.14
35	3	15	9462.3	1148.8	8313.5	12.1%	12.7%	74573.0	65110.7	55	7	48	431	377	0.13
36	3	16	12704.0	1633.2	11070.8	12.9%	9.3%	136735.6	124031.6	73	9	64	791	717	0.09
37	3	17	13480.0	1633.2	11846.7	12.1%	9.9%	136735.6	123255.7	78	9	69	791	713	0.10
38	3	18	12750.8	1633.2	11117.5	12.8%	9.3%	136735.6	123984.9	74	9	64	791	717	0.09
39	3	19	15401.1	2559.0	12842.1	16.6%	6.0%	256101.2	240700.1	89	15	74	1481	1392	0.05
40	3	20	15475.1	2559.0	12916.1	16.5%	6.0%	256101.2	240626.1	90	15	75	1481	1392	0.05
41	3	21	16666.7	2559.0	14107.8	15.4%	6.5%	256101.2	239434.5	96	15	82	1481	1385	0.06
42	3	22			#VALUE!							#VALUE!			#VALUE!
43	3	23			#VALUE!							#VALUE!			#VALUE!
44	3	24			#VALUE!							#VALUE!			#VALUE!

	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
17			Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
18			Tube Identification			Assay tube contents								Scintillation Results	
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
21	3	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	408.9	500.9
22	3	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	567.2	500.9
23	3	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	526.6	500.9
24	3	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	698.2	693.2
25	3	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	718.1	693.2
26	3	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	663.3	693.2
27	3	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	746.5	789.4
28	3	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	784.0	789.4
29	3	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	837.8	789.4
30	3	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	831.4	903.1
31	3	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	928.7	903.1
32	3	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	949.1	903.1
33	3	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	1326.7	1148.8
34	3	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	1195.1	1148.8
35	3	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	924.7	1148.8
36	3	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1590.0	1633.2
37	3	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1711.6	1633.2
38	3	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1598.1	1633.2
39	3	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2481.3	2559.0
40	3	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	2622.7	2559.0
41	3	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	2573.0	2559.0
42	3	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000		
43	3	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000		
44	3	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000		

	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB
17														
18			Free -- Positions 49-72, radiolabeled R1881 without cytosol											
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial	Hot R1881 Volume	Molecules of R1881	Counts per Scintillation Vial	Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)	predicted dpm		
20						(nM)	(ul)	(fmole)	(dpm)	(fmole)	(dpm)			
21	3	49	1	Hot	c1	10	7.5	75	12793.9	74	12744.8	13307	**Computation Check**	
22	3	50	2	Hot	c1	10	7.5	75	12954.5	75	12744.8	13307		
23	3	51	3	Hot	c1	10	7.5	75	12485.8	72	12744.8	13307	**3/2/05**	specific activity date
24	3	52	1	Hot	c2	10	15	150	25734.5	149	25340.3	26614	**79.92**	Ci/mMole 3H R1881
25	3	53	2	Hot	c2	10	15	150	24728.7	143	25340.3	26614	2.22E+12	DPM/Ci (definition)
26	3	54	3	Hot	c2	10	15	150	25557.7	148	25340.3	26614		
27	3	55	1	Hot	c3	10	21	210	35238.8	204	34818.4	37260	1.7743E+14	DPM/mmole
28	3	56	2	Hot	c3	10	21	210	34465.2	199	34818.4	37260	1.7743E+11	DPM/nmole
29	3	57	3	Hot	c3	10	21	210	34751.3	201	34818.4	37260	177.4	DPM/fmole
30	3	58	1	Hot	c4	10	30	300	48279.1	279	48850.3	53228	0.005636	fmole/DPM
31	3	59	2	Hot	c4	10	30	300	48624.3	281	48850.3	53228		
32	3	60	3	Hot	c4	10	30	300	49647.5	287	48850.3	53228		
33	3	61	1	Hot	c5	10	45	450	73284.8	424	74573.0	79843		
34	3	62	2	Hot	c5	10	45	450	75084.5	434	74573.0	79843		
35	3	63	3	Hot	c5	10	45	450	75349.9	436	74573.0	79843		
36	3	64	1	Hot	c6	100	7.5	750	135331.7	783	136735.6	133071		
37	3	65	2	Hot	c6	100	7.5	750	137337.2	794	136735.6	133071		
38	3	66	3	Hot	c6	100	7.5	750	137538.0	796	136735.6	133071		
39	3	67	1	Hot	c7	100	15	1500	259006.0	1498	256101.2	266142		
40	3	68	2	Hot	c7	100	15	1500	256517.8	1484	256101.2	266142		
41	3	69	3	Hot	c7	100	15	1500	252779.8	1462	256101.2	266142		
42	3	70	1	Hot	c8	100	30	3000	FALSE			532284		
43	3	71	2	Hot	c8	100	30	3000	FALSE			532284		
44	3	72	3	Hot	c8	100	30	3000	FALSE			532284		

	BV	BW	BX	BY
46	Linear regression results (LINEST function)			
47	*(Regression line forced through 0,0)*			
48	Slope	dpm/fmole		
49	1/slope	fmole/dpm		
50		x	y	
51	origin	0	0	
52	end point	1498.2	FALSE	
53	SLOPE function, used if missing HOT tubes			
54	Slope	172.9	dpm/fmole	
55	1/slope	0.005784	fmole/dpm	
56		x	y	
57	origin	0	0	
58	end point	1498.2	259006	

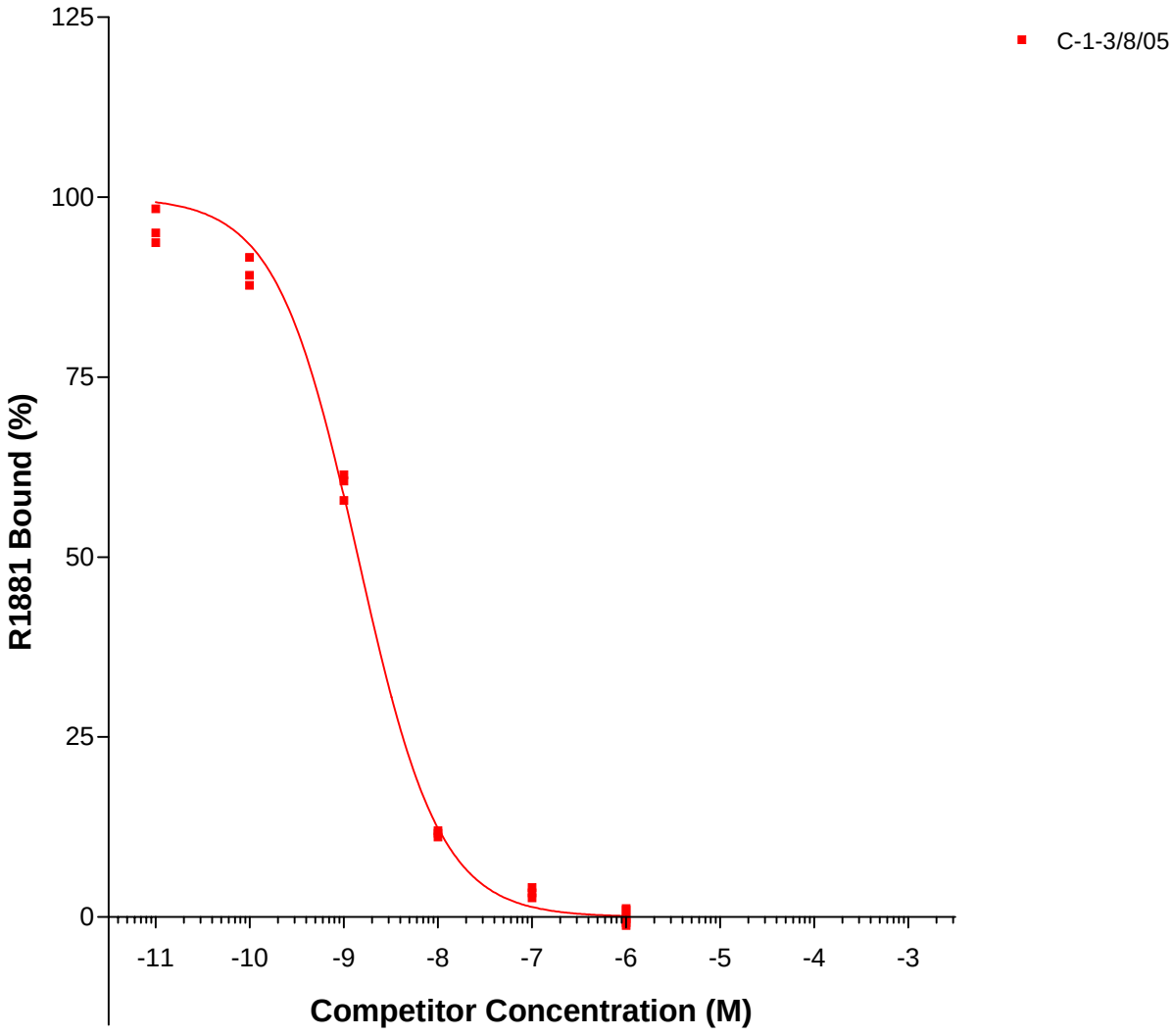


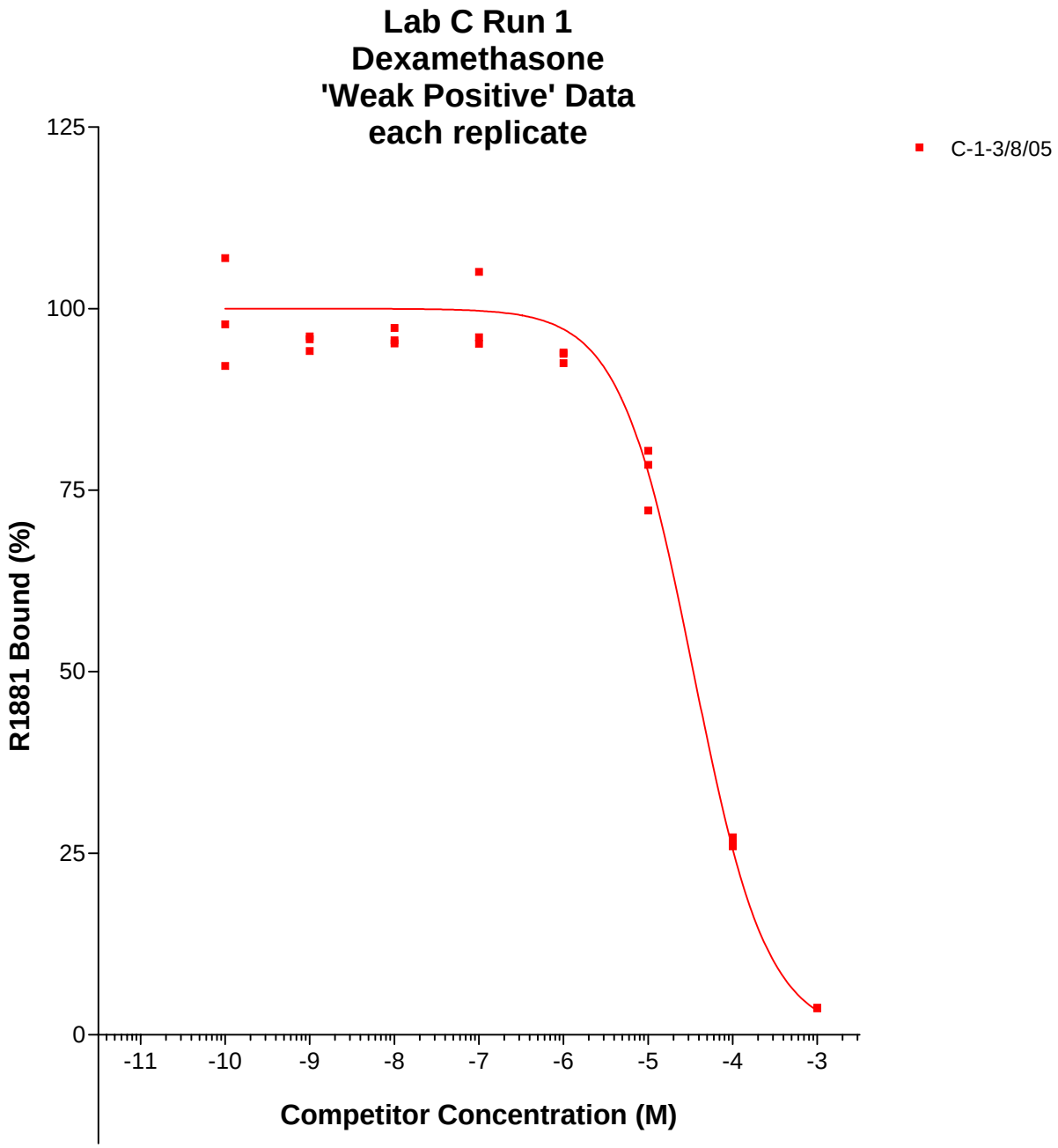
	S	T	U	V
18				
19	**Ten Percent Rule**	**Saturation X values**	**Bound y values**	**NSB y values**
20				
21	24.6%	0.25	3133.4	500.9
22	25.7%	0.25	3271.5	500.9
23	27.0%	0.25	3441.1	500.9
24	19.2%	0.5	4867.3	693.2
25	22.3%	0.5	5651.6	693.2
26	20.2%	0.5	5116.7	693.2
27	18.5%	0.7	6429.2	789.4
28	20.3%	0.7	7079.2	789.4
29	19.3%	0.7	6723.9	789.4
30	16.8%	1	8211.4	903.1
31	16.7%	1	8168.8	903.1
32	18.0%	1	8777.2	903.1
33	13.5%	1.5	10085.9	1148.8
34	13.5%	1.5	10051.9	1148.8
35	12.7%	1.5	9462.3	1148.8
36	9.3%	2.5	12704.0	1633.2
37	9.9%	2.5	13480.0	1633.2
38	9.3%	2.5	12750.8	1633.2
39	6.0%	5	15401.1	2559.0
40	6.0%	5	15475.1	2559.0
41	6.5%	5	16666.7	2559.0
42		10		
43		10		
44		10		

	CF	CG	CH	CI	CJ	CK	CL	CM	CN
18									
19	**Prism input for bound/free**		**Prism input for specific bound**						
20	**specific bound/molar**	**bound/free**	***average total added molar***	***specific bound/molar***	***spcific bound/dpm***	***total added dpm***	***total bound dpm***	**NSB dpm**	**Hot Final Concentration (nM)**
21	4.94567E-11	0.27390	2.39435E-10	4.94567E-11	2632.5	12793.9	3133.4	408.9	0.25
22	5.20508E-11	0.29246	2.39435E-10	5.20508E-11	2770.6	12954.5	3271.5	567.2	0.25
23	5.52363E-11	0.31602	2.39435E-10	5.52363E-11	2940.1	12485.8	3441.1	526.6	0.25
24	7.8419E-11	0.20388	4.76067E-10	7.8419E-11	4174.1	25734.5	4867.3	698.2	0.50
25	9.31538E-11	0.25184	4.76067E-10	9.31538E-11	4958.4	24728.7	5651.6	718.1	0.50
26	8.31036E-11	0.21873	4.76067E-10	8.31036E-11	4423.5	25557.7	5116.7	663.3	0.50
27	1.05954E-10	0.19866	6.54133E-10	1.05954E-10	5639.7	35238.8	6429.2	746.5	0.70
28	1.18166E-10	0.22675	6.54133E-10	1.18166E-10	6289.8	34465.2	7079.2	784.0	0.70
29	1.11491E-10	0.21123	6.54133E-10	1.11491E-10	5934.5	34751.3	6723.9	837.8	0.70
30	1.37302E-10	0.17984	9.17749E-10	1.37302E-10	7308.3	48279.1	8211.4	831.4	1.00
31	1.36501E-10	0.17860	9.17749E-10	1.36501E-10	7265.7	48624.3	8168.8	928.7	1.00
32	1.47931E-10	0.19649	9.17749E-10	1.47931E-10	7874.1	49647.5	8777.2	949.1	1.00
33	1.679E-10	0.13859	1.401E-09	1.679E-10	8937.0	73284.8	10085.9	1326.7	1.50
34	1.67263E-10	0.13799	1.401E-09	1.67263E-10	8903.1	75084.5	10051.9	1195.1	1.50
35	1.56185E-10	0.12768	1.401E-09	1.56185E-10	8313.5	75349.9	9462.3	924.7	1.50
36	2.07987E-10	0.08926	2.56885E-09	2.07987E-10	11070.8	135331.7	12704.0	1590.0	2.50
37	2.22564E-10	0.09612	2.56885E-09	2.22564E-10	11846.7	137337.2	13480.0	1711.6	2.50
38	2.08865E-10	0.08967	2.56885E-09	2.08865E-10	11117.5	137538.0	12750.8	1598.1	2.50
39	2.41264E-10	0.05335	4.81136E-09	2.41264E-10	12842.1	259006.0	15401.1	2481.3	5.00
40	2.42655E-10	0.05368	4.81136E-09	2.42655E-10	12916.1	256517.8	15475.1	2622.7	5.00
41	2.65042E-10	0.05892	4.81136E-09	2.65042E-10	14107.8	252779.8	16666.7	2573.0	5.00
42	#VALUE!			#VALUE!	#VALUE!				10.00
43	#VALUE!			#VALUE!	#VALUE!				10.00
44	#VALUE!			#VALUE!	#VALUE!				10.00

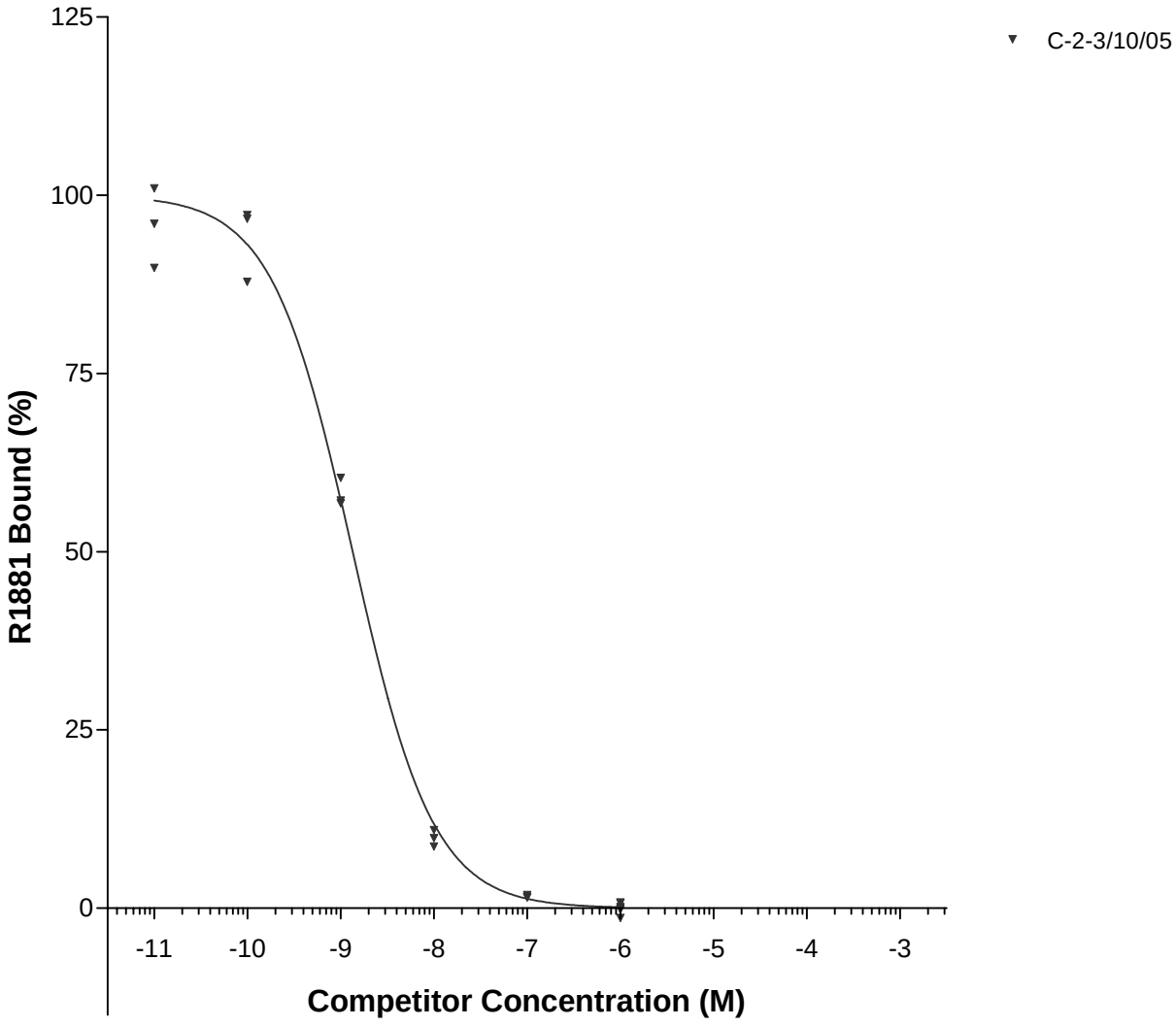
Appendix 5: Prism Files for Competitive Binding Experiments

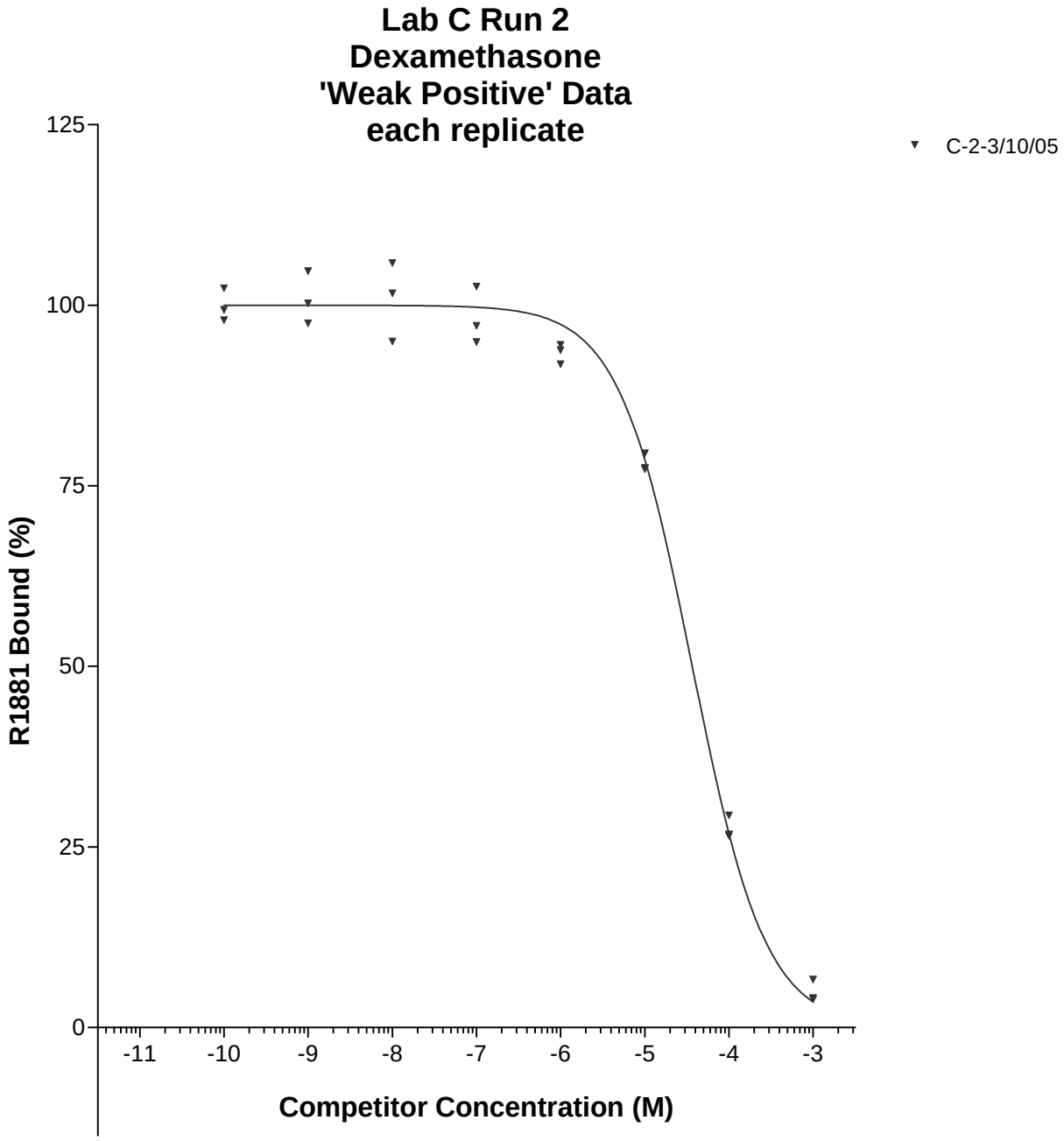
Lab C
Run 1
Standard Curve
each replicate

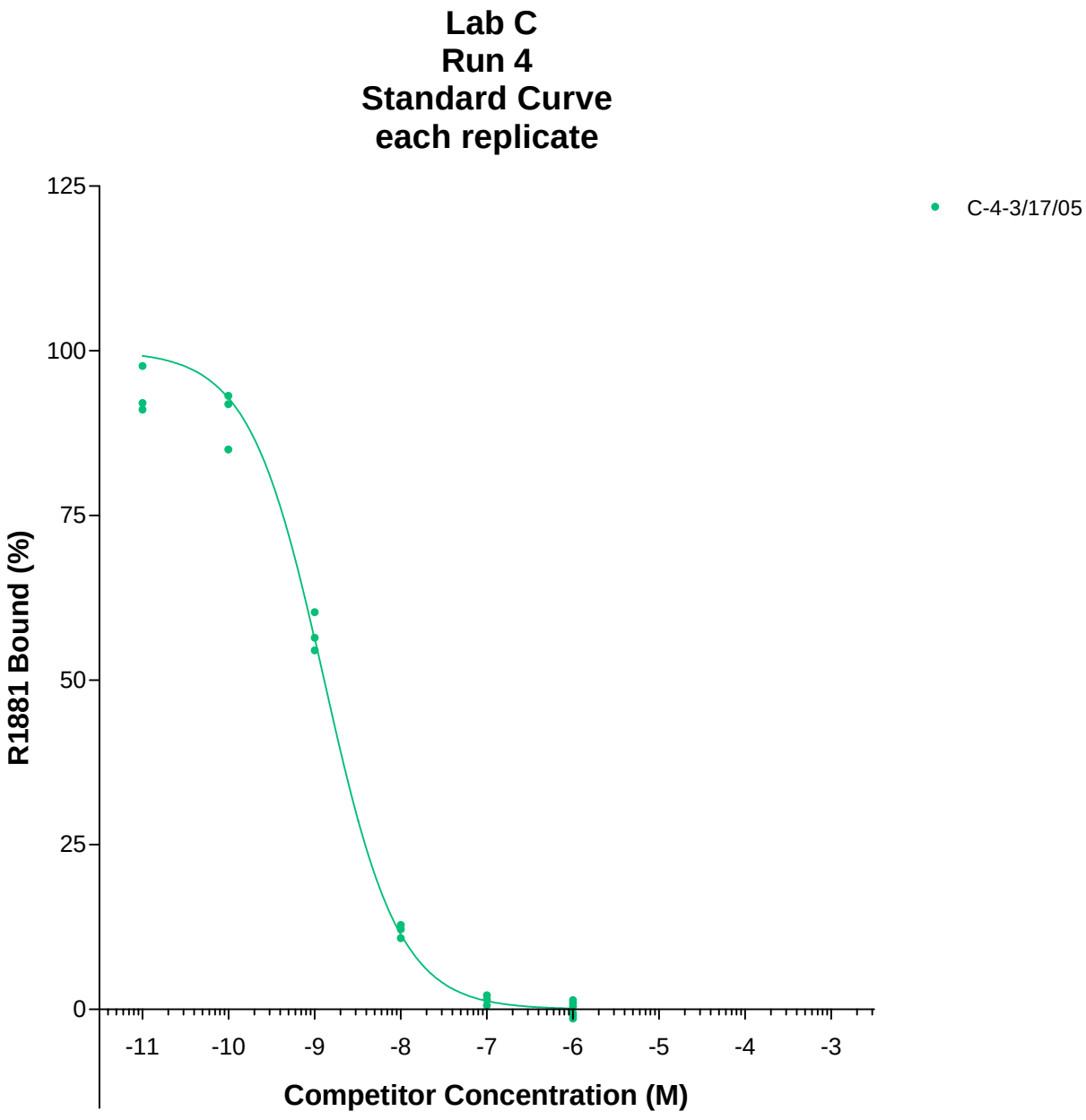


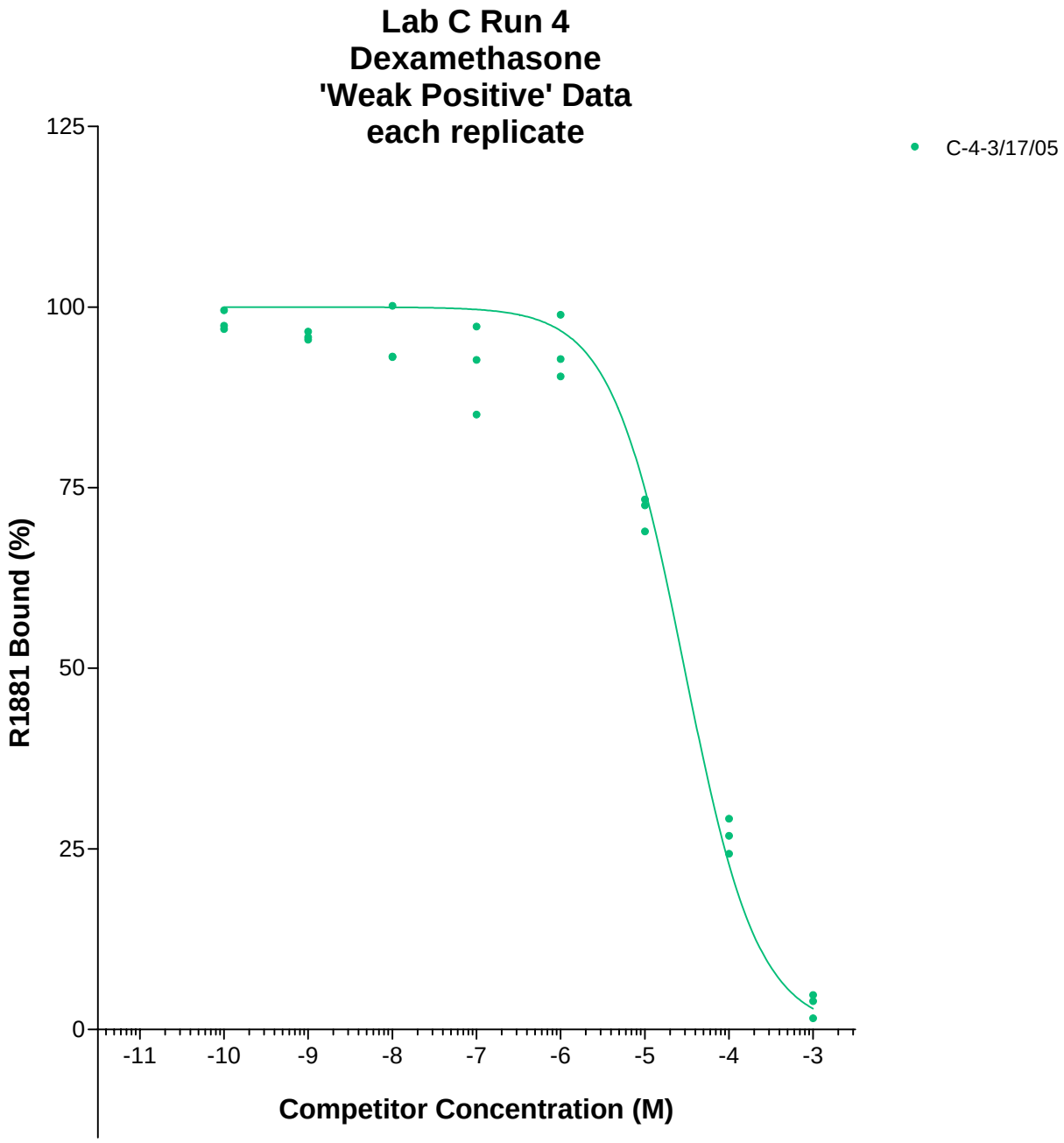

Lab C Run 1
Dexamethasone
'Weak Positive' Data
each replicate
C-1-3/8/05
R1881 Bound (%)
125
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
Competitor Concentration (M)

Lab C
Run 2
Standard Curve
each replicate

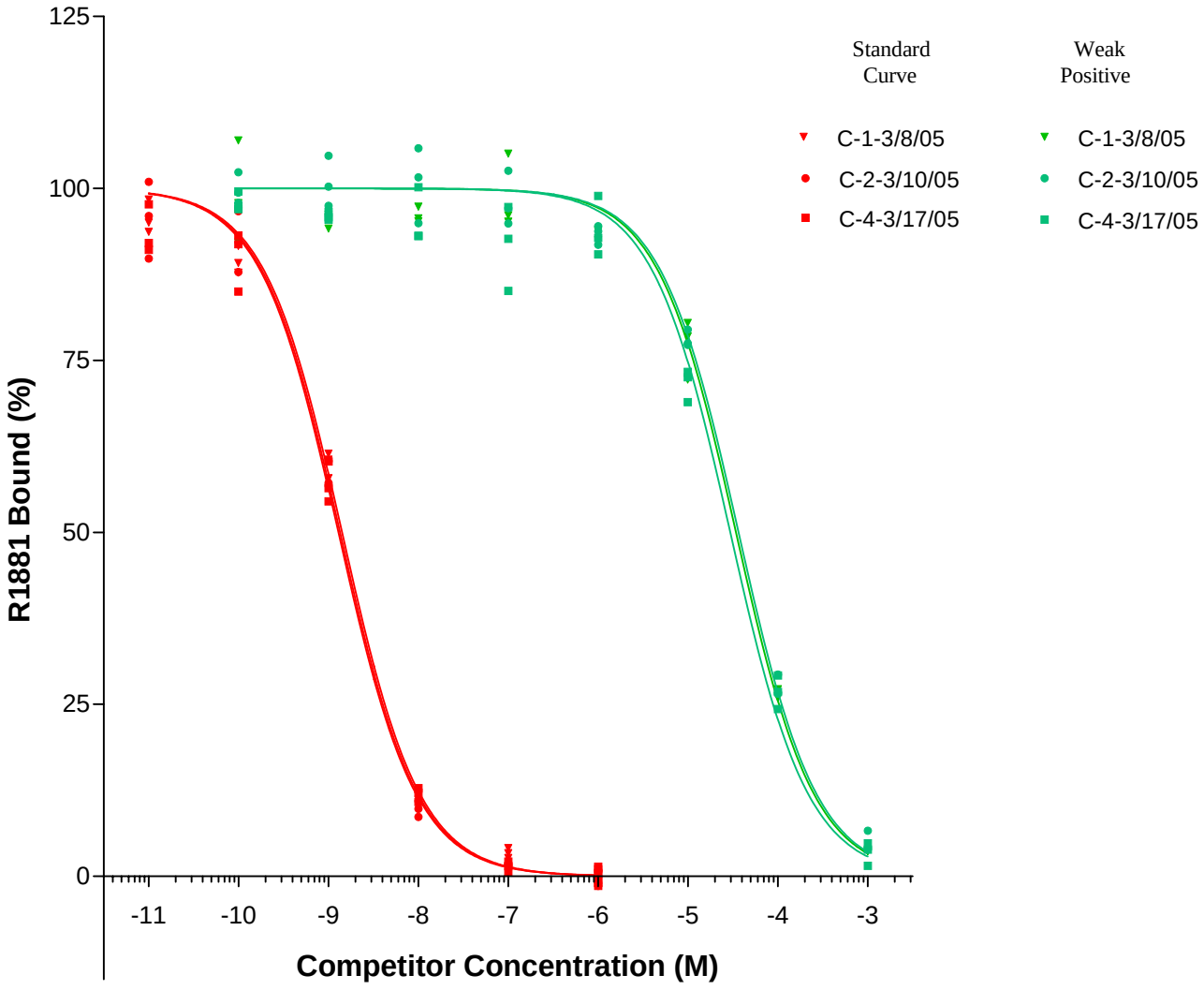



Lab C Run 2
Dexamethasone
'Weak Positive' Data
each replicate
C-2-3/10/05
R1881 Bound (%)
125
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
Competitor Concentration (M)


Lab C
Run 4
Standard Curve
each replicate
C-4-3/17/05
R1881 Bound (%)
125
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
Competitor Concentration (M)


Lab C Run 4
Dexamethasone
'Weak Positive' Data
each replicate
C-4-3/17/05
R1881 Bound (%)
125
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
Competitor Concentration (M)

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



Appendix 6: Excel Files for Competitive Binding Experiments

Competitive Assay of a known Weak Positive

57 Assay Tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

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

Provide a reason in column R

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

Provide information in all blue cells in this column

Laboratory Code:	**C**	
Run identification:	1 (sequence # 6132)	
Assay start date:	3/8/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.85	**Ci/mmole**
Cytosol vial or lot identification:	34, 35, 36, 159	
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.42E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	3.43E-05	**M**
RBA:	4.13E-05	

volume of ethanol counted:	2	**mL**
mulitply DPM in sample by :	3	

protcol calls for counting decanted EtOH s

reflects 100ul of reaction mixture processed

Summary values				
	n	Mean	SD	
EtOH	6	13694.9	513.00	Total Binding, solvent control, tubes
Hot	6	48913.6	595.20	Total hot R1881 added to each tube
NSB	6	820.9	119.37	Nonspecific Binding
Specific EtOH	6	12874.0	513.00	

Assay Characterization Values		
EtOH / Hot	0.28	less than 0.1?
NSB / EtOH	0.06	around 0.25 ?

supernate

Column O, Rows 10 through 13 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:	28.00%	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 "C"	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	4267.12	13228.0565	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4371.77	13552.4777	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4248.95	13171.7419	TRUE	
4	1	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	273.45	820.3392	TRUE	
5	2	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	241.22	723.648	TRUE	
6	3	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	315.85	947.5356	TRUE	
7	1	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	405.11	1255.82798	TRUE	
8	2	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	435.53	1350.13928	TRUE	
9	3	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	376.12	1165.96177	TRUE	
10	1	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	762.13	2362.60703	TRUE	
11	2	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	748.93	2321.69602	TRUE	
12	3	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	727.00	2253.69411	TRUE	
13	1	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2817.54	8734.3616	TRUE	
14	2	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2781.28	8621.9804	TRUE	
15	3	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2668.28	8271.6742	TRUE	
16	1	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4070.52	12618.6089	TRUE	
17	2	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3909.73	12120.1599	TRUE	
18	3	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3966.68	12296.7142	TRUE	
19	1	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4212.40	13058.44	TRUE	
20	2	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4155.88	12883.228	TRUE	
21	3	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4351.54	13489.7585	TRUE	

Values for analysis by nonlinear regression

Standard Curve

Weak Positive

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			96.4	13228.06	12407.2	-354.0	96.4	27.04373
2	2			98.9	13552.48	12731.6	-678.5	98.9	27.70698
3	3			95.9	13171.74	12350.9	-297.7	95.9	26.92859
4	1		-6.0	0.0	820.3392	-0.5	12053.7	0.0	1.677119
5	2		-6.0	-0.8	723.648	-97.2	12150.4	-0.8	1.479442
6	3		-6.0	0.0	947.5356	126.7	11926.5	1.0	1.937162
7	1	cold R1881	-7.0	3.4	1255.828	435.0	11618.2	3.4	2.567442
8	2	cold R1881	-7.0	4.1	1350.139	529.3	11523.9	4.1	2.760254
9	3	cold R1881	-7.0	2.7	1165.962	345.1	11708.1	2.7	2.383718
10	1	cold R1881	-8.0	12.0	2362.607	1541.8	10511.4	12.0	4.830165
11	2	cold R1881	-8.0	11.7	2321.696	1500.8	10552.3	11.7	4.746526
12	3	cold R1881	-8.0	11.1	2253.694	1432.8	10620.3	11.1	4.607501
13	1	cold R1881	-9.0	61.5	8734.362	7913.5	4139.7	61.5	17.85672
14	2	cold R1881	-9.0	60.6	8621.98	7801.1	4252.0	60.6	17.62696
15	3	cold R1881	-9.0	57.9	8271.674	7450.8	4602.3	57.9	16.91079
16	1	cold R1881	-10.0	91.6	12618.61	11797.8	255.4	91.6	25.79776
17	2	cold R1881	-10.0	87.8	12120.16	11299.3	753.9	87.8	24.77872
18	3	cold R1881	-10.0	89.1	12296.71	11475.9	577.3	89.1	25.13967
19	1	cold R1881	-11.0	95.1	13058.44	12237.6	-184.4	95.1	26.69696
20	2	cold R1881	-11.0	93.7	12883.23	12062.4	-9.2	93.7	26.33875
21	3	cold R1881	-11.0	98.4	13489.76	12668.9	-615.7	98.4	27.57875

Column O, Rows 10 through 13 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: 28.00% 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 "C"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
22	1	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	425.56	1319.24251	FALSE	not all the cytosol made it to the bottom of
23	2	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	418.91	1298.62937	TRUE	
24	3	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	415.43	1287.83207	TRUE	
25	1	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1393.70	4320.47	TRUE	
26	2	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1352.16	4191.6805	TRUE	
27	3	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1343.45	4164.6826	TRUE	
28	1	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3263.10	10115.5976	TRUE	
29	2	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3524.44	10925.7609	TRUE	
30	3	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3605.79	11177.9583	TRUE	
31	1	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4167.70	12919.8638	TRUE	
32	2	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4105.84	12728.1102	TRUE	
33	3	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4160.27	12896.8339	TRUE	
34	1	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4254.80	13189.8924	TRUE	
35	2	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4217.95	13075.6512	TRUE	
36	3	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4628.36	14347.9098	TRUE	
37	1	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4307.65	13353.7088	TRUE	
38	2	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4236.54	13133.2771	TRUE	
39	3	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4219.88	13081.6125	TRUE	
40	1	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4175.52	12944.1151	TRUE	
41	2	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4258.92	13202.6427	TRUE	
42	3	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4243.51	13154.8686	TRUE	
43	1	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4090.64	12680.9902	TRUE	
44	2	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4329.09	13420.1666	TRUE	
45	3	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4707.94	14594.6171	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4650.11	14415.3472	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4382.11	13584.5503	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4586.16	14217.0898	TRUE	
49	1	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	262.65	787.9548	TRUE	
50	2	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	224.47	673.398	TRUE	
51	3	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	324.09	972.2592	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		49758.21	49758.21	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		48708.30	48708.30	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		49475.77	49475.77	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		48844.06	48844.06	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		48183.00	48183.00	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		48512.19	48512.19	TRUE	

Values for analysis by nonlinear regression

Standard Curve

Weak Positive

Position	Replicate		concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
22	1	Weak Positive	-3.0						
23	2	Weak Positive	-3.0	3.7	1298.629	477.8	11575.4	3.7	2.654946
24	3	Weak Positive	-3.0	3.6	1287.832	467.0	11586.2	3.6	2.632872
25	1	Weak Positive	-4.0	27.2	4320.47	3499.6	8553.6	27.2	8.832862
26	2	Weak Positive	-4.0	26.2	4191.681	3370.8	8682.3	26.2	8.569562
27	3	Weak Positive	-4.0	26.0	4164.683	3343.8	8709.3	26.0	8.514367
28	1	Weak Positive	-5.0	72.2	10115.6	9294.7	2758.4	72.2	20.68055
29	2	Weak Positive	-5.0	78.5	10925.76	10104.9	1948.3	78.5	22.33686
30	3	Weak Positive	-5.0	80.4	11177.96	10357.1	1696.1	80.4	22.85246
31	1	Weak Positive	-6.0	94.0	12919.86	12099.0	-45.8	94.0	26.41365
32	2	Weak Positive	-6.0	92.5	12728.11	11907.3	145.9	92.5	26.02162
33	3	Weak Positive	-6.0	93.8	12896.83	12076.0	-22.8	93.8	26.36657
34	1	Weak Positive	-7.0	96.1	13189.89	12369.0	-315.9	96.1	26.9657
35	2	Weak Positive	-7.0	95.2	13075.65	12254.8	-201.6	95.2	26.73214
36	3	Weak Positive	-7.0	105.1	14347.91	13527.1	-1473.9	105.1	29.33318
37	1	Weak Positive	-8.0	97.3	13353.71	12532.9	-479.7	97.3	27.30061
38	2	Weak Positive	-8.0	95.6	13133.28	12312.4	-259.3	95.6	26.84996
39	3	Weak Positive	-8.0	95.2	13081.61	12260.8	-207.6	95.2	26.74433
40	1	Weak Positive	-9.0	94.2	12944.12	12123.3	-70.1	94.2	26.46323
41	2	Weak Positive	-9.0	96.2	13202.64	12381.8	-328.6	96.2	26.99177
42	3	Weak Positive	-9.0	95.8	13154.87	12334.0	-280.8	95.8	26.8941
43	1	Weak Positive	-10.0	92.1	12680.99	11860.1	193.0	92.1	25.92529
44	2	Weak Positive	-10.0	97.9	13420.17	12599.3	-546.1	97.9	27.43648
45	3	Weak Positive	-10.0	107.0	14594.62	13773.8	-1720.6	107.0	29.83755
46	1		—	105.6	14415.35	13594.5	-1541.3	105.6	29.47105
47	2		—	99.1	13584.55	12763.7	-710.5	99.1	27.77255
48	3		—	104.1	14217.09	13396.2	-1343.1	104.1	29.06573
49	1		-6.0	-0.3	787.9548	-32.9	12086.1	-0.3	1.610912
50	2		-6.0	-1.1	673.398	-147.5	12200.6	-1.1	1.376709
51	3		-6.0	1.2	972.2592	151.4	11901.8	1.2	1.987708
52	1				49758.21	48937.4			
53	2				48708.3	47887.4			
54	3				49475.77	48654.9			
55	1				48844.06	48023.2			
56	2				48183	47362.1			
57	3				48512.19	47691.3			

Competitive Assay of a known Weak Positive

57 Assay Tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

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

Provide a reason in column R

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

Provide information in all blue cells in this column

Laboratory Code:	**C**	
Run identification:	2 (sequence # 6133)	
Assay start date:	3/10/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.82	**Ci/mmole**
Cytosol vial or lot identification:	37, 38, 39	
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.35E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	3.67E-05	**M**
RBA:	3.67E-05	

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

Summary values	n	Mean	SD	
EtOH	6	13409.0	415.24	Total Binding, solvent control, tubes
Hot	6	48556.5	620.99	Total hot R1881 added to each tube
NSB	6	1080.1	95.95	Nonspecific Binding
Specific EtOH	6	12328.9	415.24	

Assay Characterization Values		
EtOH / Hot	0.28	less than 0.1?
NSB / EtOH	0.08	around 0.25 ?

supernate

Column O, Rows 10 through 13 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: 27.62% 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 "C"	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	4149.21	12862.5479	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4354.38	13498.5625	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4271.53	13241.7523	TRUE	
4	1	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	391.98	1175.9277	TRUE	
5	2	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	351.77	1055.3199	TRUE	
6	3	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	365.17	1095.5226	TRUE	
7	1	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	411.86	1276.75856	TRUE	
8	2	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	408.48	1266.29327	TRUE	
9	3	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	422.24	1308.92912	TRUE	
10	1	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	739.32	2291.88611	TRUE	
11	2	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	692.06	2145.37298	TRUE	
12	3	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	784.63	2432.3406	TRUE	
13	1	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2623.18	8131.8518	TRUE	
14	2	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2606.55	8080.3174	TRUE	
15	3	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2748.55	8520.5112	TRUE	
16	1	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4214.67	13065.4863	TRUE	
17	2	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3842.72	11912.4444	TRUE	
18	3	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	4194.50	13002.95	TRUE	
19	1	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3919.83	12151.4606	TRUE	
20	2	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4363.36	13526.4253	TRUE	
21	3	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4165.85	12914.1474	TRUE	

Values for analysis by nonlinear regression

Standard Curve

Weak Positive

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			95.6	12862.55	11782.4	-533.6	95.6	26.48985
2	2			100.7	13498.56	12418.5	-1169.6	100.7	27.7997
3	3			98.6	13241.75	12161.6	-912.8	98.6	27.27081
4	1		-6.0	0.8	1175.928	95.8	11153.0	0.8	2.421772
5	2		-6.0	-0.2	1055.32	-24.8	11273.6	-0.2	2.173385
6	3		-6.0	0.0	1095.523	15.4	11233.4	0.1	2.256181
7	1	cold R1881	-7.0	1.6	1276.759	196.6	11052.2	1.6	2.629428
8	2	cold R1881	-7.0	1.5	1266.293	186.2	11062.6	1.5	2.607875
9	3	cold R1881	-7.0	1.9	1308.929	228.8	11020.0	1.9	2.695682
10	1	cold R1881	-8.0	9.8	2291.886	1211.8	10037.0	9.8	4.720039
11	2	cold R1881	-8.0	8.6	2145.373	1065.3	10183.6	8.6	4.418302
12	3	cold R1881	-8.0	11.0	2432.341	1352.2	9896.6	11.0	5.009299
13	1	cold R1881	-9.0	57.2	8131.852	7051.7	4197.1	57.2	16.74719
14	2	cold R1881	-9.0	56.8	8080.317	7000.2	4248.6	56.8	16.64106
15	3	cold R1881	-9.0	60.3	8520.511	7440.4	3808.4	60.3	17.54762
16	1	cold R1881	-10.0	97.2	13065.49	11985.4	-736.6	97.2	26.9078
17	2	cold R1881	-10.0	87.9	11912.44	10832.3	416.5	87.9	24.53316
18	3	cold R1881	-10.0	96.7	13002.95	11922.8	-674.0	96.7	26.77901
19	1	cold R1881	-11.0	89.8	12151.46	11071.3	177.5	89.8	25.0254
20	2	cold R1881	-11.0	101.0	13526.43	12446.3	-1197.5	101.0	27.85708
21	3	cold R1881	-11.0	96.0	12914.15	11834.0	-585.2	96.0	26.59612

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

and the maximum concentration for the weak positive

working volume **3.1E+02** uL

Check the 10% rule: 27.62% If the ratio of EtOH / Hot is > 10% then there are problems with the assay

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

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory "C"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
22	1	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	502.54	1557.86129	TRUE	
23	2	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	509.76	1580.25879	TRUE	
24	3	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	612.30	1898.14364	TRUE	
25	1	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1515.78	4698.9149	TRUE	
26	2	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1404.37	4353.5594	TRUE	
27	3	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1412.10	4377.4945	TRUE	
28	1	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3429.91	10632.7334	TRUE	
29	2	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3422.10	10608.5131	TRUE	
30	3	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3510.55	10882.6895	TRUE	
31	1	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4078.63	12643.7468	TRUE	
32	2	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4107.39	12732.9214	TRUE	
33	3	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4000.39	12401.2059	TRUE	
34	1	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4429.02	13729.9496	TRUE	
35	2	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4212.40	13058.44	TRUE	
36	3	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4123.43	12782.6299	TRUE	
37	1	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4557.47	14128.1415	TRUE	
38	2	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4125.35	12788.585	TRUE	
39	3	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4390.78	13611.4304	TRUE	
40	1	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4335.94	13441.4202	TRUE	
41	2	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4514.04	13993.5364	TRUE	
42	3	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4226.26	13101.3998	TRUE	
43	1	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4243.40	13154.5276	TRUE	
44	2	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4418.80	13698.2707	TRUE	
45	3	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4300.90	13332.7776	TRUE	
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4425.81	13719.9955	TRUE	
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4235.11	13128.8286	TRUE	
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4516.95	14002.5574	TRUE	
49	1	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	388.99	1166.9712	TRUE	
50	2	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	303.83	911.493	TRUE	
51	3	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	358.48	1075.4325	TRUE	
52	1	none	Hot		—	—	—	30	—	—	—	—		49150.64	49150.64	TRUE	
53	2	none	Hot		—	—	—	30	—	—	—	—		48656.94	48656.94	TRUE	
54	3	none	Hot		—	—	—	30	—	—	—	—		49371.27	49371.27	TRUE	
55	1	none	Hot		—	—	—	30	—	—	—	—		48207.97	48207.97	TRUE	
56	2	none	Hot		—	—	—	30	—	—	—	—		48206.35	48206.35	TRUE	
57	3	none	Hot		—	—	—	30	—	—	—	—		47745.88	47745.88	TRUE	

Values for analysis by nonlinear regression

Standard Curve

Weak Positive

Position	Replicate		concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
22	1	Weak Positive	-3.0	3.9	1557.861	477.8	10771.1	3.9	3.208347
23	2	Weak Positive	-3.0	4.1	1580.259	500.1	10748.7	4.1	3.254474
24	3	Weak Positive	-3.0	6.6	1898.144	818.0	10430.8	6.6	3.909144
25	1	Weak Positive	-4.0	29.4	4698.915	3618.8	7630.0	29.4	9.677209
26	2	Weak Positive	-4.0	26.6	4353.559	3273.4	7975.4	26.6	8.965965
27	3	Weak Positive	-4.0	26.7	4377.495	3297.4	7951.4	26.7	9.015258
28	1	Weak Positive	-5.0	77.5	10632.73	9552.6	1696.2	77.5	21.89765
29	2	Weak Positive	-5.0	77.3	10608.51	9528.4	1720.4	77.3	21.84777
30	3	Weak Positive	-5.0	79.5	10882.69	9802.6	1446.2	79.5	22.41242
31	1	Weak Positive	-6.0	93.8	12643.75	11563.6	-314.8	93.8	26.03924
32	2	Weak Positive	-6.0	94.5	12732.92	11652.8	-404.0	94.5	26.22289
33	3	Weak Positive	-6.0	91.8	12401.21	11321.1	-72.3	91.8	25.53974
34	1	Weak Positive	-7.0	102.6	13729.95	12649.8	-1401.0	102.6	28.27623
35	2	Weak Positive	-7.0	97.2	13058.44	11978.3	-729.5	97.2	26.89328
36	3	Weak Positive	-7.0	94.9	12782.63	11702.5	-453.7	94.9	26.32527
37	1	Weak Positive	-8.0	105.8	14128.14	13048.0	-1799.2	105.8	29.09629
38	2	Weak Positive	-8.0	95.0	12788.59	11708.5	-459.7	95.0	26.33753
39	3	Weak Positive	-8.0	101.6	13611.43	12531.3	-1282.5	101.6	28.03214
40	1	Weak Positive	-9.0	100.3	13441.42	12361.3	-1112.5	100.3	27.68202
41	2	Weak Positive	-9.0	104.7	13993.54	12913.4	-1664.6	104.7	28.81907
42	3	Weak Positive	-9.0	97.5	13101.4	12021.3	-772.5	97.5	26.98176
43	1	Weak Positive	-10.0	97.9	13154.53	12074.4	-825.6	97.9	27.09117
44	2	Weak Positive	-10.0	102.3	13698.27	12618.2	-1369.3	102.3	28.21099
45	3	Weak Positive	-10.0	99.4	13332.78	12252.7	-1003.8	99.4	27.45827
46	1		—	102.5	13720	12639.9	-1391.1	102.5	28.25573
47	2		—	97.7	13128.83	12048.7	-799.9	97.7	27.03825
48	3		—	104.8	14002.56	12922.4	-1673.6	104.8	28.83765
49	1		-6.0	0.7	1166.971	86.9	11162.0	0.7	2.403326
50	2		-6.0	-1.4	911.493	-168.6	11417.4	-1.4	1.87718
51	3		-6.0	0.0	1075.433	-4.7	11253.5	0.0	2.214806
52	1				49150.64	48070.5			
53	2				48656.94	47576.8			
54	3				49371.27	48291.2			
55	1				48207.97	47127.9			
56	2				48206.35	47126.2			
57	3				47745.88	46665.8			

Competitive Assay of a known Weak Positive

57 Assay Tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

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

Provide a reason in column R

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

Provide information in all blue cells in this column

Laboratory Code:	**C**	
Run identification:	4 (sequence # 6150)	
Assay start date:	3/17/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.74	**Ci/mmole**
Cytosol vial or lot identification:	43, 44, 45	
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.29E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	2.96E-05	**M**
RBA:	4.36E-05	

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

Summary values				
	n	Mean	SD	
EtOH	6	12729.6	699.19	Total Binding, solvent control, tubes
Hot	6	49095.3	579.58	Total hot R1881 added to each tube
NSB	6	810.6	132.77	Nonspecific Binding
Specific EtOH	6	11919.0	699.19	

Assay Characterization Values		
EtOH / Hot	0.26	less than 0.1?
NSB / EtOH	0.06	around 0.25 ?

supernate

Column O, Rows 10 through 13 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:	25.93%	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 "C"	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	4354.89	13500.1528	TRUE	
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4132.59	12811.0135	TRUE	
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4179.13	12955.3061	TRUE	
4	1	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	309.20	927.5874	TRUE	
5	2	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	247.92	743.7492	TRUE	
6	3	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	289.25	867.7431	TRUE	
7	1	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	318.27	986.64041	TRUE	
8	2	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	344.34	1067.454	TRUE	
9	3	Inert R1881	S	1	**C-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	285.92	886.3613	TRUE	
10	1	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	676.74	2097.90888	TRUE	
11	2	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	729.19	2260.49055	TRUE	
12	3	Inert R1881	S	2	**C-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	755.17	2341.03134	TRUE	
13	1	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2579.96	7997.8667	TRUE	
14	2	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2432.20	7539.8076	TRUE	
15	3	Inert R1881	S	3	**C-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2357.20	7307.3293	TRUE	
16	1	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3794.49	11762.9221	TRUE	
17	2	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3531.19	10946.6921	TRUE	
18	3	Inert R1881	S	4	**C-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3843.91	11916.1241	TRUE	
19	1	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3763.57	11667.0515	TRUE	
20	2	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	4017.32	12453.6951	TRUE	
21	3	Inert R1881	S	5	**C-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3801.26	11783.9153	TRUE	

Values for analysis by nonlinear regression
Standard Curve
Weak Positive

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			106.5	13500.15	12689.6	-1581.2	106.5	27.49787
2	2			100.7	12811.01	12000.4	-892.0	100.7	26.09419
3	3			101.9	12955.31	12144.7	-1036.3	101.9	26.3881
4	1		-6.0	1.0	927.5874	117.0	10991.4	1.0	1.889362
5	2		-6.0	-0.6	743.7492	-66.8	11175.2	-0.6	1.51491
6	3		-6.0	0.0	867.7431	57.2	11051.3	0.5	1.767468
7	1	cold R1881	-7.0	1.5	986.6404	176.1	10932.4	1.5	2.009645
8	2	cold R1881	-7.0	2.2	1067.454	256.9	10851.5	2.2	2.17425
9	3	cold R1881	-7.0	0.6	886.3613	75.8	11032.6	0.6	1.805391
10	1	cold R1881	-8.0	10.8	2097.909	1287.3	9821.1	10.8	4.273139
11	2	cold R1881	-8.0	12.2	2260.491	1449.9	9658.5	12.2	4.604295
12	3	cold R1881	-8.0	12.8	2341.031	1530.4	9578.0	12.8	4.768345
13	1	cold R1881	-9.0	60.3	7997.867	7187.3	3921.1	60.3	16.29051
14	2	cold R1881	-9.0	56.5	7539.808	6729.2	4379.2	56.5	15.35751
15	3	cold R1881	-9.0	54.5	7307.329	6496.7	4611.7	54.5	14.88398
16	1	cold R1881	-10.0	91.9	11762.92	10952.3	156.1	91.9	23.95938
17	2	cold R1881	-10.0	85.0	10946.69	10136.1	972.3	85.0	22.29684
18	3	cold R1881	-10.0	93.2	11916.12	11105.5	2.9	93.2	24.27143
19	1	cold R1881	-11.0	91.1	11667.05	10856.5	251.9	91.1	23.76411
20	2	cold R1881	-11.0	97.7	12453.7	11643.1	-534.7	97.7	25.36639
21	3	cold R1881	-11.0	92.1	11783.92	10973.3	135.1	92.1	24.00214

Column O, Rows 10 through 13 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:	25.93%	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 "C"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"
22	1	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	445.62	1381.41828	TRUE	
23	2	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	320.71	994.20255	TRUE	
24	3	Weak Positive	P	1	**C-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	411.60	1275.97457	TRUE	
25	1	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1292.97	4008.207	TRUE	
26	2	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1196.89	3710.3683	TRUE	
27	3	Weak Positive	P	2	**C-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1383.65	4289.3119	TRUE	
28	1	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2911.85	9026.7288	TRUE	
29	2	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3081.93	9553.9892	TRUE	
30	3	Weak Positive	P	3	**C-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3051.81	9460.6203	TRUE	
31	1	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4065.02	12601.5651	TRUE	
32	2	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3830.30	11873.9424	TRUE	
33	3	Weak Positive	P	4	**C-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3738.09	11588.0852	TRUE	
34	1	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4003.54	12410.9616	TRUE	
35	2	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3534.50	10956.9562	TRUE	
36	3	Weak Positive	P	5	**C-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3825.23	11858.2006	TRUE	
37	1	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4113.36	12751.4005	TRUE	
38	2	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3840.03	11904.1023	TRUE	
39	3	Weak Positive	P	6	**C-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3842.72	11912.4444	TRUE	
40	1	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3933.13	12192.6875	TRUE	
41	2	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3945.96	12232.4822	TRUE	
42	3	Weak Positive	P	7	**C-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3976.04	12325.7302	TRUE	
43	1	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4006.89	12421.359	TRUE	
44	2	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3990.31	12369.9734	TRUE	
45	3	Weak Positive	P	8	**C-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4089.18	12676.4549	TRUE	
46	1	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3755.62	11642.4158	TRUE	
47	2	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	4286.13	13287.0061	TRUE	
48	3	ethanol	EtOH	0	**—**	—	300	30	10	50	310	—	100	3929.55	12181.5957	TRUE	
49	1	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	216.06	648.1728	TRUE	
50	2	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	325.82	977.463	TRUE	
51	3	Inert R1881	NSB		**C-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	232.94	698.8149	TRUE	
52	1	none	Hot		**—**	—	—	30	—	—	—	—		49449.09	49449.09	TRUE	
53	2	none	Hot		**—**	—	—	30	—	—	—	—		49852.59	49852.59	TRUE	
54	3	none	Hot		**—**	—	—	30	—	—	—	—		49338.19	49338.19	TRUE	
55	1	none	Hot		**—**	—	—	30	—	—	—	—		48190.00	48190.00	TRUE	
56	2	none	Hot		**—**	—	—	30	—	—	—	—		48899.29	48899.29	TRUE	
57	3	none	Hot		**—**	—	—	30	—	—	—	—		48842.42	48842.42	TRUE	

Values for analysis by nonlinear regression

Standard Curve

Weak Positive

Position	Replicate		concentration (log)	percent bound	Usable DPM values	Specific Binding (Total - mean NSB)	Free DPM (mean total add - total bound)	Percent Binding (specific bound / mean specific EtOH)	Ratio Total binding/ Hot
22	1	Weak Positive	-3.0	4.8	1381.418	570.8	10537.6	4.8	2.813751
23	2	Weak Positive	-3.0	1.5	994.2026	183.6	10924.8	1.5	2.025048
24	3	Weak Positive	-3.0	3.9	1275.975	465.4	10643.0	3.9	2.598977
25	1	Weak Positive	-4.0	26.8	4008.207	3197.6	7910.8	26.8	8.164142
26	2	Weak Positive	-4.0	24.3	3710.368	2899.8	8208.6	24.3	7.557487
27	3	Weak Positive	-4.0	29.2	4289.312	3478.7	7629.7	29.2	8.736712
28	1	Weak Positive	-5.0	68.9	9026.729	8216.1	2892.3	68.9	18.38615
29	2	Weak Positive	-5.0	73.4	9553.989	8743.4	2365.0	73.4	19.4601
30	3	Weak Positive	-5.0	72.6	9460.62	8650.0	2458.4	72.6	19.26992
31	1	Weak Positive	-6.0	98.9	12601.57	11791.0	-682.6	98.9	25.66758
32	2	Weak Positive	-6.0	92.8	11873.94	11063.4	45.1	92.8	24.18552
33	3	Weak Positive	-6.0	90.4	11588.09	10777.5	330.9	90.4	23.60327
34	1	Weak Positive	-7.0	97.3	12410.96	11600.4	-492.0	97.3	25.27935
35	2	Weak Positive	-7.0	85.1	10956.96	10146.4	962.0	85.1	22.31775
36	3	Weak Positive	-7.0	92.7	11858.2	11047.6	60.8	92.7	24.15345
37	1	Weak Positive	-8.0	100.2	12751.4	11940.8	-832.4	100.2	25.97277
38	2	Weak Positive	-8.0	93.1	11904.1	11093.5	14.9	93.1	24.24695
39	3	Weak Positive	-8.0	93.1	11912.44	11101.9	6.5	93.1	24.26394
40	1	Weak Positive	-9.0	95.5	12192.69	11382.1	-273.7	95.5	24.83475
41	2	Weak Positive	-9.0	95.8	12232.48	11421.9	-313.5	95.8	24.91581
42	3	Weak Positive	-9.0	96.6	12325.73	11515.1	-406.7	96.6	25.10574
43	1	Weak Positive	-10.0	97.4	12421.36	11610.8	-502.4	97.4	25.30052
44	2	Weak Positive	-10.0	97.0	12369.97	11559.4	-451.0	97.0	25.19586
45	3	Weak Positive	-10.0	99.6	12676.45	11865.9	-757.5	99.6	25.82012
46	1		—	90.9	11642.42	10831.8	276.6	90.9	23.71393
47	2		—	104.7	13287.01	12476.4	-1368.0	104.7	27.06372
48	3		—	95.4	12181.6	11371.0	-262.6	95.4	24.81216
49	1		-6.0	-1.4	648.1728	-162.4	11270.8	-1.4	1.320235
50	2		-6.0	1.4	977.463	166.9	10941.5	1.4	1.990952
51	3		-6.0	-0.9	698.8149	-111.8	11220.2	-0.9	1.423386
52	1				49449.09	48638.5			
53	2				49852.59	49042.0			
54	3				49338.19	48527.6			
55	1				48190	47379.4			
56	2				48899.29	48088.7			
57	3				48842.42	48031.8			

Appendix 7: Copies of Biological Methods

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

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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 with caps
F. 4°C refrigerator
G. Refrigerated 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 dihydrate (FW 241.95, Sigma S-6646)
P. Triamcinolone acetonide (FW 434.5, >99%)

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", and a replicate code.
2. The position code represents the number in the counting sequence of the final sample in the scintillation vial.
3. The replicate code identifies the tube as being the first, second or third tube of each triplicate.
4. The tube type code identifies the type of sample:
   (a) Incubation tubes
      (i) H = tubes containing cytosol, buffer, and [^{3}H]-R1881
      (ii) HC = tubes containing cytosol, buffer, R1881 and [^{3}H]-R1881
   (b) Dosing samples
      (i) Hot = sample of [^{3}H]-R1881 in scintillation vial containing only the amount of [^{3}H]-R1881 used in the corresponding H and HC tubes.
   (c) HAP tubes
      (i) H = tubes containing cytosol, buffer, and [^{3}H]-R1881
      (ii) HC = tubes containing cytosol, buffer, R1881 and [^{3}H]-R1881

VI. Androgen Receptor Saturation Binding Assay Start Solutions

A. [^{3}H]-R1881 Stock solutions in **amber** vials [Supplied by 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 bring 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. 100X R1881 Stock Solutions (non-radioactive) (**amber** vials) [Supplied by sponsor]
   1. Add 5 mL absolute ethanol to 5 mg bottle to yield 3.52 mM R1881.
   2. 10 µM R1881
      (a) 56.82 µL of 3.52 mM R1881
      (b) 19.943 mL absolute ethanol
      (c) Store at -20°C in an amber vial
   3. 1 µM R1881
      (a) 2 mL of 10 µM R1881
      (b) 18 mL of absolute ethanol.
      (c) Store at -20°C in an amber vial.

C. 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

D. 1X Cytosol
   1. Thaw on ice while the assay tube contents are in the vacuum concentrator.
   2. Dilute to 4 mg of protein per mL in low salt TEDG + PMSF buffer.
   3. Keep on ice until addition to assay tubes.

VII. <u>Androgen receptor saturation binding assay incubation</u>

A. Place labelled assay tubes on ice in a rack.
   1. Add components to the tubes according to the table below.

B. Contents of assay tubes

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1.

Tube #	Tube type	Replicate	[^{3}H]R-1881 initial concentration (nM)	[^{3}H]R-1881 volume (µL)	R1881 initial concentration (µM)	R1881 volume (µL)	Triamcinolone (µL)
1	H	1	10	7.5	—	—	50
2	H	2	10	7.5	—	—	50
3	H	3	10	7.5	—	—	50
4	H	1	10	15	—	—	50
5	H	2	10	15	—	—	50
6	H	3	10	15	—	—	50
7	H	1	10	21	—	—	50
8	H	2	10	21	—	—	50
9	H	3	10	21	—	—	50
10	H	1	10	30	—	—	50
11	H	2	10	30	—	—	50
12	H	3	10	30	—	—	50
13	H	1	10	45	—	—	50
14	H	2	10	45	—	—	50
15	H	3	10	45	—	—	50
16	H	1	100	7.5	—	—	50
17	H	2	100	7.5	—	—	50
18	H	3	100	7.5	—	—	50
19	H	1	100	15	—	—	50
20	H	2	100	15	—	—	50
21	H	3	100	15	—	—	50
22	H	1	100	30	—	—	50
23	H	2	100	30	—	—	50
24	H	3	100	30	—	—	50
25	HC	1	10	7.5	1	7.5	50
26	HC	2	10	7.5	1	7.5	50
27	HC	3	10	7.5	1	7.5	50
28	HC	1	10	15	1	15	50
29	HC	2	10	15	1	15	50
30	HC	3	10	15	1	15	50
31	HC	1	10	21	1	21	50
32	HC	2	10	21	1	21	50
33	HC	3	10	21	1	21	50
34	HC	1	10	30	1	30	50
35	HC	2	10	30	1	30	50
36	HC	3	10	30	1	30	50
37	HC	1	10	45	1	45	50
38	HC	2	10	45	1	45	50
39	HC	3	10	45	1	45	50
40	HC	1	100	7.5	10	7.5	50
Tube #	Tube type	Replicate	[^{3}H]R-1881 initial concentration (nM)	[^{3}H]R-1881 volume (µL)	R1881 initial concentration (µM)	R1881 volume (µL)	Triamcinolone (µL)
41	HC	2	100	7.5	10	7.5	50
42	HC	3	100	7.5	10	7.5	50
43	HC	1	100	15	10	15	50

44	HC	2	100	15	10	15	50
45	HC	3	100	15	10	15	50
46	HC	1	100	30	10	30	50
47	HC	2	100	30	10	30	50
48	HC	3	100	30	10	30	50
49	Hot	1	10	7.5	—	—	—
50	Hot	2	10	7.5	—	—	—
51	Hot	3	10	7.5	—	—	—
52	Hot	1	10	15	—	—	—
53	Hot	2	10	15	—	—	—
54	Hot	3	10	15	—	—	—
55	Hot	1	10	21	—	—	—
56	Hot	2	10	21	—	—	—
57	Hot	3	10	21	—	—	—
58	Hot	1	10	30	—	—	—
59	Hot	2	10	30	—	—	—
60	Hot	3	10	30	—	—	—
61	Hot	1	10	45	—	—	—
62	Hot	2	10	45	—	—	—
63	Hot	3	10	45	—	—	—
64	Hot	1	100	7.5	—	—	—
65	Hot	2	100	7.5	—	—	—
66	Hot	3	100	7.5	—	—	—
67	Hot	1	100	15	—	—	—
68	Hot	2	100	15	—	—	—
69	Hot	3	100	15	—	—	—
70	Hot	1	100	30	—	—	—
71	Hot	2	100	30	—	—	—
72	Hot	3	100	30	—	—	—

C. Place the incubation tubes (#1-48) in the vacuum concentrator and dry according to the instrument instructions.

D. When the tube contents are dry, transfer the tubes to centrifuge rotor buckets on ice.

E. Add 300 µL of 1X Cytosol to all tubes except those labeled "Hot" (#49-72)

F. Incubate for 20 ± 1 hour in the refrigerator.

G. Add 14 mL of Formula 989 to "Hot" tubes #49-72.

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--#1-48). NOTE: stir the HAP continuously while adding it to the tubes.

2. Remove the incubation tubes from the refrigerator and place in and ice-water bath.
3. Transfer 100 µL from each incubation tube to its corresponding HAP tube.
4. 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.)
5. Add 2 ml of 50 mM Tris buffer to each tube.
6. Quickly vortex the tubes.
7. Centrifuge the tubes for 2-3 minutes at 600 × *g* at 4°C.
8. Decant or carefully aspirate the supernatant from the tubes.
9. Add 2 ml of 50 mM Tris buffer to each tube.
10. Quickly vortex the tubes.
11. Centrifuge the tubes for 2-3 minutes at 600 × *g* at 4°C.
12. Decant or carefully aspirate the supernatant from the tubes.
13. 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 temperature, 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. Saturation isotherm
      (a) Actual concentration of [^{3}H]-R1881 on the X-axis and total binding, specific binding and non-specific binding in DPM or fmol on the Y-axis.
      (b) Perform one-site binding hyperbola regression on total and specific binding, use weighting of $1/Y^2$.
      (c) Perform linear regression on the non-specific binding.
   2. Scatchard analysis
      (a) Bound/Free is plotted on the X-axis
      (b) Bound is plotted on the Y-axis

(c) Perform linear regression. $-1/\text{slope} = K_D$, the X-intercept = B_{max}.

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

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OFFICIAL QA ISSUE

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 8: Battelle QAU Statement