

Audited Draft Final Report

to

Battelle Memorial Institute

on

Validation of an Androgen Receptor (AR) Binding Assay:

Task 3 – Establish Inter-Laboratory Variability Using

Rat Prostate Cytosol Prepared In-House

Southern Research Institute Study No.: 11232.01.03

EPA Contract No.: 68-W-01-023

Audited Draft Final Report

Validation of an Androgen Receptor (AR) Binding Assay: Task 3 – Establish Inter-Laboratory Variability Using Rat Prostate Cytosol Prepared In-House

SRI Protocol No. 11232.01.03

Submitted to:

David P. Houchens, Ph.D.
Program Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

By:

Southern Research Institute
2000 Ninth Avenue South
P. O. Box 55305
Birmingham, AL 35255-5305

Study Initiation: April 27, 2005
Study Completion:

Richard D. May, Ph.D.
Study Director

William R. Waud, Ph.D.
Director
Cancer Therapeutics and Immunology Department

Project 11232.01
Submitted: TBD

TABLE OF CONTENTS

Page

TABLE OF CONTENTS.....	ii
GLP COMPLIANCE STATEMENT	iii
QUALITY ASSURANCE STATEMENT	iv
INTRODUCTION.....	1
MATERIALS AND METHODS.....	1
RESULTS	2
CONCLUSIONS.....	3
PARTICIPATING PERSONNEL.....	3
RECORD ARCHIVES	3
PROTOCOL DEVIATIONS OR UNEXPECTED CIRCUMSTANCES THAT MIGHT HAVE AFFECTED THE RELIABILITY OF THE STUDY	3

TABLES

Table 1	Dissociation Constant (K _d) Values.....	4
Table 2	Maximum Binding Capacity (B _{max}) Values.....	5
Table 3	IC ₅₀ and Relative Binding Affinity (RBA) Values	6

FIGURES

Figure 1	Saturation Assay 1	7
Figure 2	Saturation Assay 2	8
Figure 3	Saturation Assay 3	9
Figure 4	Saturation Assays 1-3.....	10
Figure 5	Competitive Assays 1-3	11

APPENDICES

Appendix A	Study Protocol and Amendments
Appendix B	Processed Raw Data from Saturation Assay 1 and Associated Figures
Appendix C	Processed Raw Data from Saturation Assay 2 and Associated Figures
Appendix D	Processed Raw Data from Saturation Assay 3 and Associated Figures
Appendix E	Processed Raw Data from Competitive Assay 1 and Associated Figures
Appendix F	Processed Raw Data from Competitive Assay 2 and Associated Figures
Appendix G	Processed Raw Data from Competitive Assay 3 and Associated Figures

GLP COMPLIANCE STATEMENT

The study described in this final report was conducted in accordance with the EPA Good Laboratory Practice (GLP) Regulations (40 CFR Part 160). The final report accurately reflects the raw data obtained during the performance of the study. There were no adverse circumstances that affected the quality or the integrity of this study. The graphing and data analysis to determine Bmax, KD, IC₅₀, and RBA values were conducted by Battelle and reported to Southern Research without a signed report. Nevertheless, this information was audited by Battelle EDSP QAU and a QA statement was provided by Battelle.

Richard D. May, Ph.D.
Study Director

Date

Quality Assurance Statement

Draft Report

On

Protocol 11232.01.03

Validation of an Androgen Receptor (AR) Binding Assay: Task 3 – Establish Inter-Laboratory Variability Using Rat Prostate Cytosol Prepared In-House

Listed below are the phases and/or procedures performed by Southern Research Institute that were inspected and audited by the Quality Assurance Unit during the study described in the report. Findings were reported to the study director and management periodically.

<i>Phases/Procedures</i>	<i>Inspection/ Audit Date</i>	<i>Date Study Director/ Management Notified</i>
Final Protocol Review	4/28/05	4/28/05
Protocol Amendment (A1) Review	7/18/05	9/30/05
Androgen Receptor Competitive Binding Assay	8/16-8/17/05	9/30/05
Protocol Amendment (A2) Review	8/19/05	9/30/05
Protocol Amendment (A3) Review	10/13/05	10/13/05
Study File Data and Draft Report Review	12/20/05; 1/3- 1/6/06	1/6/06

Disclaimer: The Quality Assurance Unit at Southern Research Institute did not review the following sections of this final report:

1. Introduction
2. Appendices B-G: processed raw data and associated figures conducted by Battelle

The results presented in this audited final report accurately reflect the raw data.

Margery J. Wirth, Manager, Quality Assurance

Date

INTRODUCTION

The purpose of this study (under Work Assignment 4-11 from Battelle Memorial Institute; hereafter referred to as "Battelle") was to establish the sensitivity and specificity of the androgen receptor (AR) binding assay in our laboratory as part of an inter-laboratory program aimed at eventually testing various substances, primarily pesticides, for their effects on AR binding. This program is conducted for the United States Environmental Protection Agency (EPA). In this study, radiolabeled methyltrienolone (CAS 965-93-5; also referred to as "³H-R1881") was used as the standard and radioinert R1881 (CAS 965-93-5) and dexamethasone (CAS 50-02-2) were used as test substances; radioinert R1881 competes strongly with ³H-R1881, whereas dexamethasone has been shown to compete weakly with ³H-R1881 for binding in the assay. In this task, rat prostate cytosol was prepared at Southern Research Institute and used in the assays.

MATERIALS AND METHODS

Unlabeled and radiolabeled R1881 (lots 3411228 and 3559507, respectively) and dexamethasone (lot P4311) were supplied by Battelle for these assays. Rat ventral prostate tissues were prepared by Battelle and were obtained within 24 hours of castration. Tissues were trimmed of fat, weighed, snap frozen in liquid nitrogen, stored at $-80 \pm 5^{\circ}\text{C}$ prior to shipment, and shipped on dry ice from Battelle to Southern Research for processing, as described below. A brief description of the saturation and competitive assays is given below. All materials and methods used are described in detail the Study Protocol (see Appendix A). Upon completing each assay, data were entered into the Excel spreadsheet provided by Battelle and sent to Battelle for data processing to determine binding characteristics, as generated and displayed in the graphs contained in this report. All statistics were performed by personnel at Battelle.

Rat Prostate Cytosol Preparation

Prostates were processed according to SRI SOP No. 42-24-1. Briefly, thawed tissues were placed in cold TEDG buffer, minced, and homogenized on ice with a Polytron. After centrifugation at $30,000 \times g$ for 30 minutes at $2-8^{\circ}\text{C}$, supernatants were pooled, aliquoted, and frozen at -80°C for use in the assays. Protein concentration of the cytosol preparation was determined using the BioRad Protein Assay Kit (Bio-Rad Laboratories, Hercules, CA) according to SRI SOP No. 42-14-1. The protein concentration of the undiluted cytosol was 5.11 mg/mL.

Androgen Receptor Saturation Assay

The assay was performed in siliconized glass tubes according to SRI SOP No. 40-34-1. ³H-R1881 and cold R1881 were added from the appropriate stock solutions to the appropriate tubes. After adding 60 mM stock triamcinolone acetonide, tubes were placed in a speed-vac and dried. Upon drying, the tubes were placed on ice and rat prostate cytosol was added. The cytosol had been diluted to yield a protein concentration of 0.6 mg per 300 μL assay volume. The tubes were then vortexed gently using a whole rack vortex unit and immediately placed on an orbital shaker overnight at $2-8^{\circ}\text{C}$.

An aliquot of each concentration of [^3H]R1881 was also counted on the scintillation counter to determine total counts added (the aliquot was directly added to the 20 mL scintillation vials containing 14 mL of Safety Solve and numbered 49-72).

The following day, 100 μL aliquots from the above tubes were transferred to tubes containing a 60% hydroxylapatite slurry (HAP) and tubes were vortexed every five minutes for a total of 20 minutes. Tubes were kept on ice as much as possible during all steps. Tubes were then centrifuged. After centrifuging, tubes were placed in a decanting rack and placed back into the ice water bath. The supernatant from all the tubes was decanted simultaneously and then placed back in the ice water bath. After repeated washing, ethyl alcohol was added to each tube, and tubes were again vortexed and centrifuged. Supernatants were then decanted into vials containing scintillation cocktail, mixed, and counted using a single label DPM program with quench correction on the Beckman LS 6000IC scintillation counter.

Androgen Receptor Competitive Assay

The assay was performed in siliconized glass tubes according to SRI SOP No. 40-35-1. ^3H -R1881 and cold R1881 were added from the appropriate stock solutions to the appropriate tubes. In order to determine nonspecific binding, radioinert R1881 was added to various tubes. Triamcinolone acetonide was next added and tubes were placed in a speed-vac to dry. After drying, tubes were placed on ice and 10 μL of the compound stocks (*i.e.*, ethanol, inert R1881, or dexamethasone, the weak positive competitor) were added to the appropriate tubes in triplicate and rat prostate cytosol was added. The cytosol had been diluted to yield a protein concentration of 1.0 mg per 300 μL assay volume. The tubes were then vortexed gently using a whole rack vortex unit and immediately placed on an orbital shaker overnight at 2-8°C.

The following day, 100 μL aliquots from the above tubes were transferred to tubes containing a 60% hydroxylapatite slurry (HAP) and tubes were vortexed every five minutes for a total of 20 minutes. Tubes were kept on ice as much as possible during all steps. Tubes were then centrifuged. After centrifuging, tubes were placed in a decanting rack and placed back into the ice water bath. The supernatant from all the tubes was decanted simultaneously and then placed back in the ice water bath. After repeated washing, ethyl alcohol was added to each tube, and tubes were again vortexed and centrifuged. Supernatants were then decanted into vials containing scintillation cocktail, mixed, and counted using a single label DPM program with quench correction on the Beckman LS 6000IC scintillation counter.

RESULTS

Three saturation assays and three competitive assays were conducted. The processed raw data from these assays are found in Appendices B-G. One sample in the first assay was not included in the data analyses, as noted on the title page of Appendix B. The summary graphs for the saturation assays showing the nonspecific binding (NSB), specific binding (SB), and the total methyltrienolone bound (in disintegrations per minute or DPM) are shown in Figures 1-3. R1881 was evaluated from 0.25 to 10 nM in these three saturation assays. A composite of the three saturation assays is shown in Figure 4; this graph demonstrates some minor variation in the three assays conducted. From these data, the K_d and B_{max} values were calculated by Battelle. These values are shown in Tables 1 and 2, respectively.

After the saturation assays were considered acceptable, a concentration of 1.0 mg rat prostate cytosol/tube was recommended by Battelle for use in the competitive assays. Data from the three competitive assays are shown in Figure 5 as a function of the % of R1881 bound for various concentrations of the R1881 standard and the dexamethasone weak positive. The binding of the R1881 standard and the weak positive was very similar in all three assays. From these data, the IC_{50} values of the R1881 standard and the dexamethasone weak positive, as well as the relative binding affinity (RBA) values, were calculated by Battelle. These values are shown in Table 3.

CONCLUSIONS

In terms of accomplishing our purpose of preparing rat prostate cytosol in-house and using it to establish the sensitivity and specificity of the AR binding assay at Southern Research Institute and validating the assay, it appears that we have been successful. We will use this same batch of rat prostate cytosol and these assay conditions to execute Task 4 of this program. There were no ambiguities or insufficiencies in the results from this study.

PARTICIPATING PERSONNEL

Richard D. May, Ph.D.
Parrish L. Payne, B.S.
Mary Blackwelder
Margery J. Wirth, B.S.

Study Director, Senior Project Leader
Biologist
Auditor, Quality Assurance
Manager, Quality Assurance

RECORD ARCHIVES

All raw data pertaining to the conduct of this study, as well as a copy of the final report, will be stored in Southern Research's archives until the Study Monitor has specified the disposition of these materials. No specimens that can be stored were generated from this study.

PROTOCOL DEVIATIONS OR UNEXPECTED CIRCUMSTANCES THAT MIGHT HAVE AFFECTED THE RELIABILITY OF THE STUDY

There were no significant deviations to the Protocol or circumstances that may have comprised the study.

Table 1

Dissociation Constant (Kd) Values

Saturation Assay	Kd Value (M)
1	7.0×10^{-10}
2	10.0×10^{-10}
3	6.6×10^{-10}
Mean	7.9×10^{-10}
SD	1.9×10^{-10}

11232.01.03

Table 2

Maximum Binding Capacity (Bmax) Values

Saturation Assay	Bmax Value (fmole/100 µg)
1	5.30
2	6.67
3	5.32
Mean	5.76
SD	0.79

Table 3

IC₅₀ and Relative Binding Affinity (RBA) Values

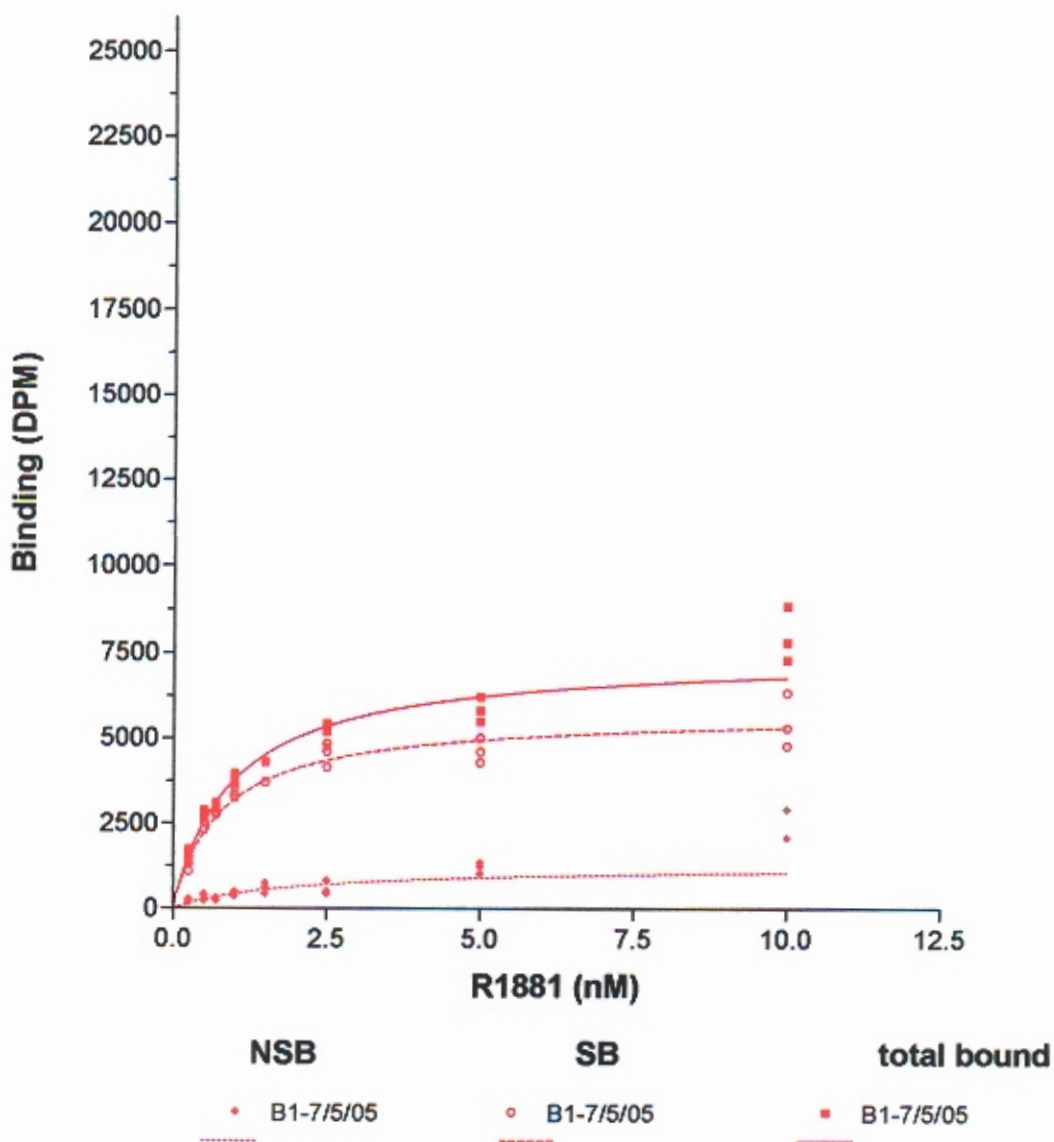
Competitive Assay	IC ₅₀ Values (M)		RBA Values (%) [*]
	R1881 Standard	Dexamethasone ^{**}	
1	1.32×10^{-9}	5.73×10^{-5}	0.0023
2	1.36×10^{-9}	5.83×10^{-5}	0.0023
3	1.76×10^{-9}	7.47×10^{-5}	0.0024
Mean	1.48×10^{-9}	6.34×10^{-5}	0.0023
SD	0.24×10^{-9}	0.98×10^{-5}	0.0000

^{*} The IC₅₀ value of R1881 ÷ the IC₅₀ of the weak positive (or competitor), expressed as a percentage.

^{**} The weak positive.

11232.01.03

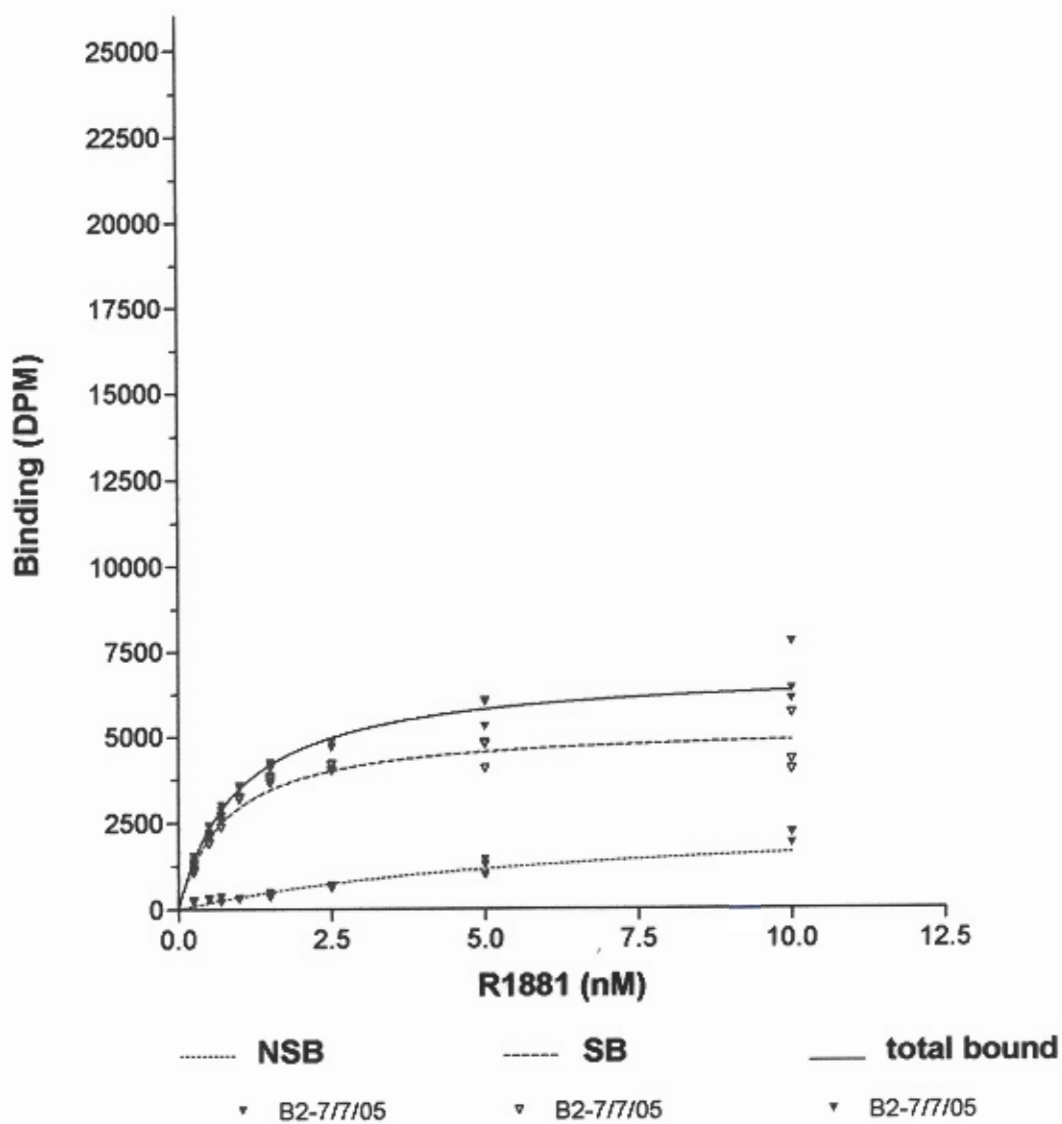
Figure 1
Saturation Assay 1



bound counts
Lab B run 1
0.6 mg/tube

Southern Research Study No. 11232.01.03

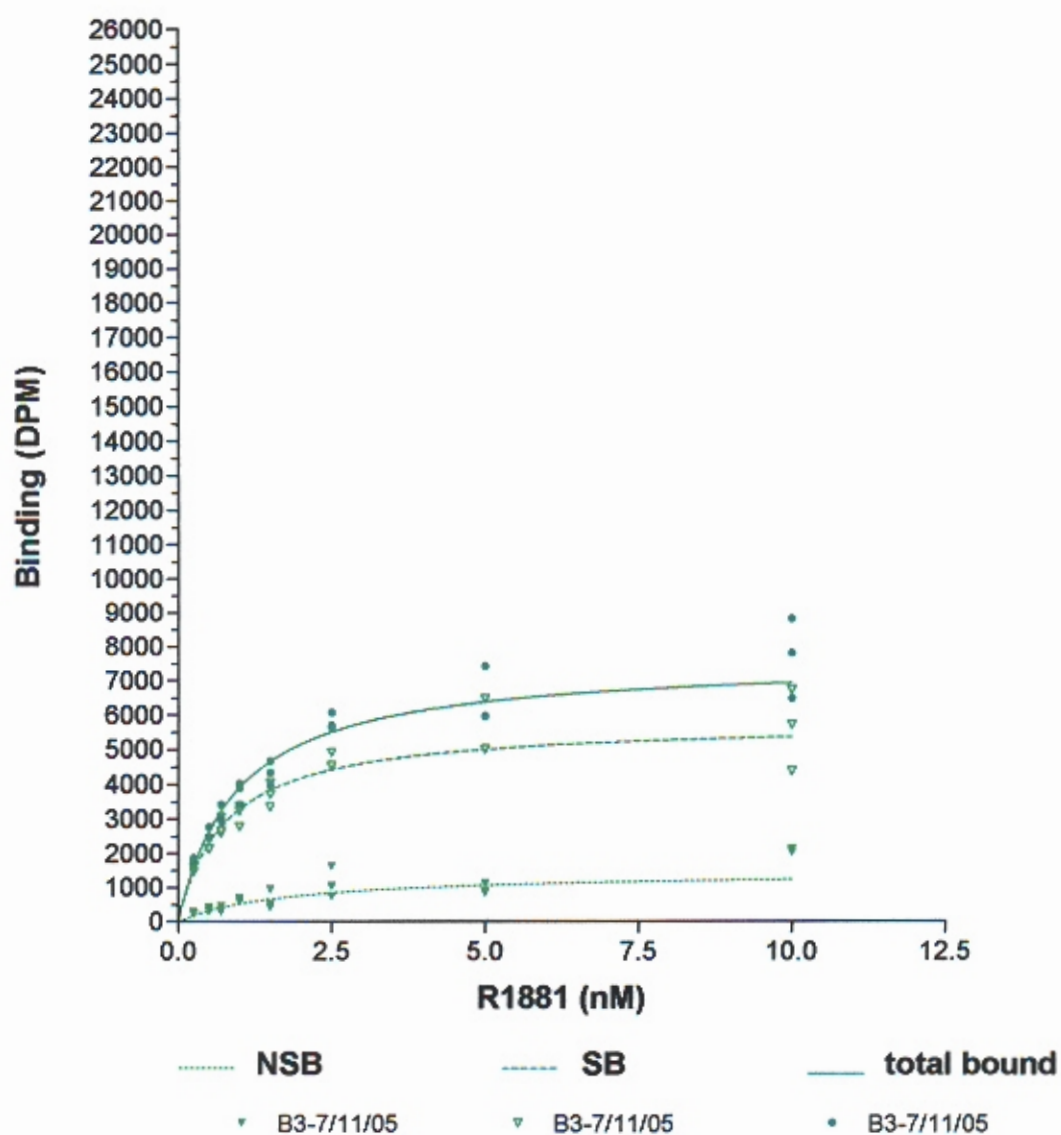
Figure 2
Saturation Assay 2



bound counts
Lab B run 2
0.6 mg/tube

Southern Research Study No. 11232.01.03

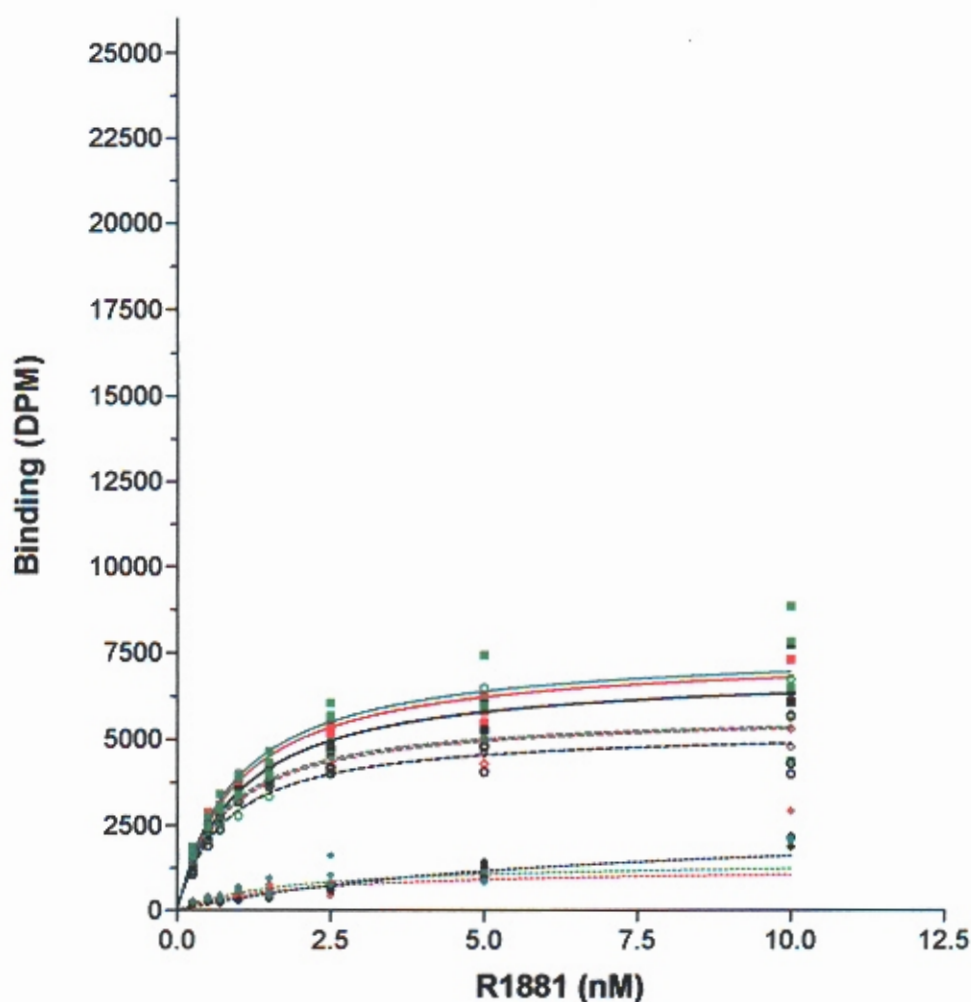
Figure 3
Saturation Assay 3



bound counts
Lab B run 3
0.6 mg/tube

Southern Research Study No. 11232.01.03

Figure 4
Saturation Assays 1-3



NSB

SB

total bound

♦ B1-7/5/05

♦ B1-7/5/05

■ B1-7/5/05

♦ B2-7/7/05

♦ B2-7/7/05

■ B2-7/7/05

♦ B3-7/11/05

♦ B3-7/11/05

■ B3-7/11/05

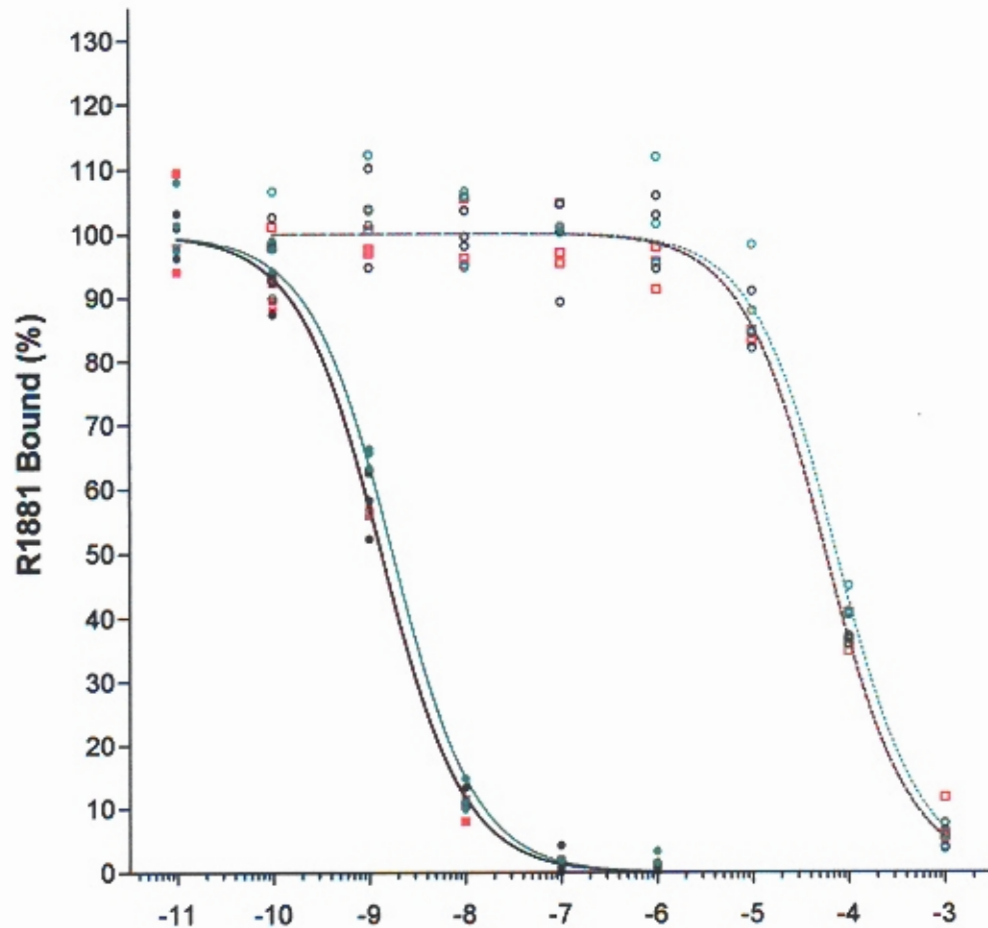
bound counts

Lab B

0.6 mg/tube

Southern Research Study No. 11232.01.03

Figure 5
Competitive Assays 1-3



Competitor Concentration (M)

Standard Curve	Weak Positive
■ B-1- 8/10/05	□ B-1- 8/10/05
• B-2-8/16/05	◊ B-2-8/16/05
• B-3-8/18/05	◊ B-3-8/18/05

Lab B

Standard Curve and 'Weak Positive'
 1.0 mg protein/tube

Southern Research Study No. 11232.01.03

APPENDIX A

Study Protocol and Amendments

Study Protocol:

**Validation of an Androgen Receptor (AR) Binding
Assay: Task 3 – Establish Inter-Laboratory
Variability Using Rat Prostate Cytosol
Prepared In-House**

Southern Research Institute Study No. : 11232.01.03

EPA Contract No. : 68-W-01-023



SOUTHERN RESEARCH
I N S T I T U T E

1.0 SPONSOR REPRESENTATIVE AND CONTACTS**Sponsor:**

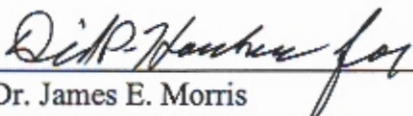
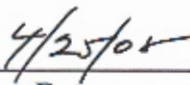
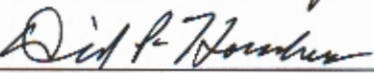
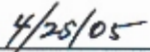
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201-2693

**Sponsor's Representatives
& Study Monitor:**

James E. Morris, Ph.D.
Principal Investigator
Battelle Pacific Northwest National Lab
P7-51
902 Battelle Boulevard
Richland, Washington 99354
Phone: (509) 376-4815
E-MAIL: james.morris@pnl.gov

David P. Houchens, Ph.D.
Program Manager
Endocrine Disruptor Screening Program
Battelle Memorial Institute
505 King Avenue
Columbus, OH 43201
Phone: (614) 424-3564
FAX: (614) 458-3564
E-MAIL: houchensd@battelle.org

Protocol Approval:
(Initial last page also)

	
Dr. James E. Morris	Date
	
Dr. David P. Houchens	Date

**Test, Control and Reference
Substances:**

Marker substance: ^3H -R1881 (methyltrienolone) (CAS 965-93-5)
Reference Substance: non-radiolabeled R1881 (CAS 965-93-5)
Test Substance: dexamethasone (CAS 50-02-2)

Ship Unused Chemicals to:

EDSP Chemical Repository
Battelle Marine Science Laboratory
1529 W. Sequim Bay Rd.
Sequim, WA 98382

2.0 TITLE:

Validation of an Androgen Receptor (AR) Binding Assay: Task 3 – Establish Inter-Laboratory Variability Using Rat Prostate Cytosol Prepared In-House

3.0 BACKGROUND AND OBJECTIVE:

3.1 BACKGROUND

The Food Quality Protection Act of 1996 requires the EPA to develop and implement a screening program using valid tests for determining the potential in humans for estrogenic effects from pesticides. EPA proposed a two-tiered screening program in a Federal Register notice in 1998 (63 FR 71542-71568, Dec. 28, 1998). One of the assays being considered for inclusion in the screening program is an AR binding assay.

Initially, EPA intended to validate and establish performance criteria for receptor binding assays retrospectively from data in the literature and not specify a particular protocol by which those performance criteria could be met. However, it proved infeasible to set up such criteria based on published literature alone. EPA is therefore standardizing and validating a protocol from which performance criteria can be derived. Battelle has previously evaluated 19 substances of various potencies including non-binders and established the intra-laboratory performance characteristics of the assay.

3.2 OBJECTIVE

The objective of this study is to establish the sensitivity and specificity of the AR binding assays at Southern Research Institute and determine variability of results when three saturation and three competitive assays are performed by the same technician on different days.

The validation study will be conducted by Southern Research Institute using a protocol and test chemicals supplied by Battelle and Standard Operating Procedures (SOPs) from Southern Research Institute. The rat cytosol reagent will be prepared at Southern Research Institute for these assays.

4.0 TESTING LABORATORY: **Southern Research Institute** (Southern Research)
Cell Biology and Immunology Group
2000 Ninth Avenue South
Birmingham, AL 35205
(205) 581-2579; FAX (205) 581-2129

5.0 KEY STUDY DATES:

Event	Proposed Study Schedule
Preparation of rat cytosol	To be determined (TBD)
Saturation assays	TBD
Competitive assays	TBD
QAU audit and management review	TBD
Data submitted to Battelle	TBD

6.0 STUDY PERSONNEL:

Study Director:	Richard D. May, Ph.D.
Team Leader:	Angela Y. Jones, M.S.
Laboratory Technician:	Parrish L. Payne, B.S.
Quality Assurance Manager:	Margery J. Wirth, B.S.

7.0 TEST SYSTEM:

The test system for this *in vitro* study is a cytosolic preparation that will be isolated from the ventral prostate gland of male rats at Southern Research. The surgical castration and removal of prostate tissues will be performed under Good Laboratory Practices (GLP) regulations by personnel at Battelle Memorial Institute. Thus, no laboratory animals are to be used at Southern Research for this task. Records relating to the number, body weight range, sex, species, strain, substrain, and age of the test system will be maintained by Battelle.

7.1 SOURCE

Rat prostate cytosol will be prepared by Southern Research using SOP No. SRI 42-24, entitled, "Rat Prostate Cytosol Preparation."

7.2 JUSTIFICATION FOR SELECTION OF THE TEST SYSTEM

The test system was identified by the Sponsor as a component of an *in vitro* androgen receptor (AR) binding assay. The test system was selected by the Sponsor and reflects the best possible system available to determine the relative binding affinities of test substances for the androgen receptor.

7.3 IDENTIFICATION

Each tube will be uniquely labeled as rat prostate cytosol as well as the date prepared, expiration date, and protein concentration value.

7.4 ROUTE OF ADMINISTRATION AND REASON FOR ITS CHOICE

All test procedures will be performed *in vitro*. The test, control and reference substances will be added directly to assay tubes containing rat prostate cytosol in the appropriate sequence with the other reagents required for measuring binding. The direct application and sequence is required for this assay type.

8.0 TEST, CONTROL, AND REFERENCE SUBSTANCES:**8.1 IDENTITY OF THE MARKER SUBSTANCE**

Name: ³H-R1881 (Methyltrienolone)
CAS No.: 965-93-5
Supplier: Sponsor (Manufacturer, Perkin-Elmer)
Lot Number(s): TBD

Characterization: Compound identity, strength, quality, and purity, as well as documentation of methods of synthesis, fabrication, or derivation, are the responsibilities of the Sponsor. Information on compound purity will be provided by the Sponsor and placed in the study file upon receipt.

Stability & Storage: The radiolabeled R1881 has a shelf-life of 6 months per the manufacturer's specifications. The compound will be stored at -20°C or lower.

8.2 IDENTITY OF THE REFERENCE SUBSTANCE

Name: Radioinert Methyltrienolone (R1881)
CAS No.: 965-93-5
Supplier: Sponsor (Manufacturer, Perkin-Elmer)
Lot Number(s): TBD

Characterization: Compound identity, strength, quality, and purity, as well as documentation of methods of synthesis, fabrication, or derivation, are the responsibilities of the Sponsor. The manufacturer's claim of purity is 98% with ≥ 97% required.

Stability & Storage: The inert R1881 has a shelf-life of 6 months per the manufacturer's specifications. The compound will be stored at -20°C or lower. The duration of the stability study was 56 days at a concentration of 30 mM in 100% ethanol (ESDP.H4-014).

8.3 IDENTITY OF THE TEST SUBSTANCE

Name: Dexamethasone
CAS No.: 50-02-2
Supplier: Sponsor (Manufacturer, Biomol)
Lot Number(s): TBD

Characterization: Compound identity, strength, quality, and purity, as well as documentation of methods of synthesis, fabrication, or derivation, are the responsibilities of the Sponsor. The manufacturer's claim of purity is 99% with ≥ 97% required.

Stability & Storage: Dexamethasone has a shelf-life of 6 months per the manufacturer's specifications. The compound will be stored at room temperature (20-25°C). The duration of the stability study was 56 days at a concentration of 30 mM in 100% ethanol (ESDP.H4-014).

9.0 EXPERIMENTAL DESIGN:

A series of androgen receptor binding assays will be performed to determine the ability of the test substance (dexamethasone) to compete with [^3H] ligand (R1881) for binding in rat ventral prostate tissue homogenate which will be prepared at the testing laboratory for this Task. Prior to routinely conducting the AR competitive binding assays as described below, the methods will be standardized by performing a series of three saturation radioligand binding assays that will be conducted by the same Technician on three separate days 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}).

9.1 SATURATION RADIOLIGAND BINDING ASSAY

AR saturation binding experiments will measure total, non-specific, and specific binding of increasing concentrations of ^3H -R1881 under conditions of equilibrium. A graph of specific ^3H -R1881 binding versus radioligand concentration should reach a plateau for maximum specific binding indicative of saturation of the AR with the radioligand. In addition, analysis of the data should document the binding of the ^3H -R1881 to a single, high-affinity binding site (e.g., $K_d = 0.05$ to 0.1 nM and a linear Scatchard plot).

At least three saturation radioligand assays will be conducted to generate a saturation curve along with K_d and B_{max} values. The sponsor will recommend an optimal concentration of rat prostate cytosol which is generally from 0.5 to 1 mg protein per assay tube, but may vary from 0.1 to 5 mg protein per assay tube, depending on the batch of cytosol used. The concentration for ^3H -R1881 will range from 0.025 to 10.0 nM in a total assay volume of 0.5 mL. Non-specific binding will be determined by adding unlabeled R1881 at $100\times$ the concentration of radiolabeled R1881. Battelle will analyze the data using a non-linear regression analysis (Motulsky & Christopoulos, 2003) with a final display of the data as a Scatchard plot. Rat prostate cytosol that will be used for this study is anticipated to yield a K_d of 0.05 to 0.1 nM and B_{max} of 36 to 44 fmol AR/ 100 μg protein (equivalent to 0.072 to 0.088 nM ER (estrogen receptor), respectively, when 100 μg protein is used in a total assay volume of 0.5 mL).

The method for conducting the saturation assay is detailed in SOP No. SRI 40-34, entitled, "Androgen Receptor Saturation Assay." The tube assignments are contained in Appendix A.

9.2 AR COMPETITIVE BINDING ASSAY

AR competitive binding assays will measure the binding of a single concentration of ^3H -R1881 in the presence of increasing concentrations of a weak binder (test substance: dexamethasone). Based on the results of the saturation assays, the sponsor will recommend an optimal concentration of rat prostate cytosol which is generally from 0.5 to 1.5 mg protein per assay tube. The competitive binding curve will be plotted by Battelle as specific ^3H -R1881 binding versus the concentration (log units) of the competitor. The concentration of the test substance that inhibits 50% of the maximum specific ^3H -R1881 binding is the IC_{50} value. At least three competitive assays will be performed to generate IC_{50} values.

The method for conducting the competitive assay is detailed in SOP No. SRI 40-35 entitled, "Androgen Receptor Competitive Binding Assay." The tube assignments are contained in Appendix B.

9.3 Detailed Procedures

Detailed protocols will be contained in SOPs for the preparation of rat cytosol and the determination of its protein concentration (SOP No. SRI 42-24 and 42-14, respectively), the saturation assays (SOP number: SRI 40-34), and the competitive assays (SOP Number: SRI 40-35).

10.0 DATA PROCESSING:

Battelle will provide Southern Research with an Excel spreadsheet with macros, so that once data are entered, all calculations described in Section 10.0 are performed automatically. Thus, Southern Research will enter data and these calculations will automatically be performed.

10.1 Free Concentration of ^3H -R1881

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

Calculation Check:

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

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

*Note this value will be the specific activity of the radioligand (^3H -R1881) used in the assay.

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

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

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

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

10.3 Graphical Presentation of the Data

10.3.1 Data will be entered into Excel template spreadsheets provided by Battelle and will be provided to Battelle following the completion of each assay for analysis and graphical presentation as described below.

10.3.2 Standard Curve and Test Chemical Competitive Binding Curves: Data for the standard curve and each test chemical will be plotted as the percent ^3H -R1881 bound versus the molar concentration. Estimates of the IC_{50} s will be determined using appropriate non linear curve fitting software such as 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 (QA) standards.

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

10.3.4 Maximal binding capacity (B_{max}) and the association/dissociation constants (K_a / K_d) can be estimated using a number of commercially available iterative nonlinear regression analysis programs. One of the better programs was developed by Munson and Rodbard and is called LIGAND (Munson, P.J., and D. Rodbard, 1980).

11.0 RECORDS:

All raw data pertaining to the conduct of this study will be stored at Southern Research for up to 1 year after the study completion date (the date the final report is signed by the Study Director). After 1 year, or at any time prior to the completion of that year if the Sponsor's Monitor so directs, the data and any samples/specimens will be shipped to the Sponsor or to the Sponsor's designated archival facility. Retention of materials at Southern Research beyond 1 year after the study completion date will accrue additional costs to the Sponsor. A copy of the final report will be retained in the central Archives at Southern Research.

All unused chemicals provided by the Sponsor, unless specified otherwise by the Sponsor, will be returned to the Sponsor or to the Sponsor-designated repository, after the study completion date.

The charges for storage of study data for this study do not include costs for handling and shipping of data, specimens, or residual test/control articles at final disposition; the Sponsor will be responsible for these costs.

12.0 FINAL REPORT:

An Excel spreadsheet template will be provided by Battelle for the assays. Data from each assay will be entered into this spreadsheet; data will undergo a 100% quality control review for accuracy in the Southern Research Cell Biology and Immunology group. The spreadsheet will then be reviewed, graphed, and approved by personnel at Battelle. The raw data and the draft final report will then be audited by the Quality Assurance Unit (QAU) at Southern Research. The audited draft final report will then be submitted to Battelle. Comments will be received and incorporated into the final report. The final report will be audited by the Southern Research QAU and approved by the Study Director at Southern Research.

13.0 REGULATORY REFERENCES:

This study will be conducted in accordance with the protocol, the Standard Operating Procedures (SOPs) of Southern Research, and the applicable regulatory requirements, as addressed below.

13.1 PROTOCOL AMENDMENTS AND DEVIATIONS

Amendments: All changes in or revisions of the approved protocol and the reasons therefore will be documented in amendments, which will be signed and dated by the Study Director and the Sponsor's Representative. Amendments will be maintained with the protocol. Written approval (a fax signature or electronic communication, such as email) for changes in the protocol may be granted by the Sponsor's Representative, but a written amendment will follow.

Deviations: All operations pertaining to this study, unless specifically defined in this protocol, will be performed according to the SOPs of Southern Research and any deviations from protocol or SOPs will be documented.

13.2 REGULATORY COMPLIANCE

Good Laboratory Practices: This nonclinical laboratory study will be conducted in strict compliance with the U.S. Environmental Protection Agency Good Laboratory Practice Standards (40 CFR Part 160).

Quality Assurance Review: Personnel from the QAU at Southern Research will inspect the study in accordance with Southern Research operating procedures and the EPA FIFRA GLPs. The QAU will audit the final report prior to submission to Battelle to assure that it

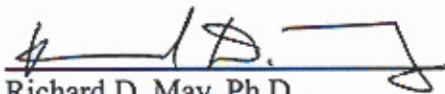
accurately represents the raw data collected during the study. QAU inspection records will be made available to Battelle, its representatives, and/or the EPA, as they request.

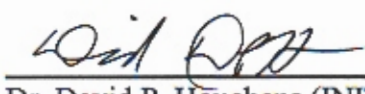
14.0 REFERENCES

- 1) Motulsky, H., and A. Christopoulos. 2003. Fitting Models to Biological Data Using Linear and Nonlinear Regression. GraphPad Software Inc., San Diego, CA.
- 2) Munson, P.J., and D. Rodbard. 1980. Ligand: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. 107:220-239.

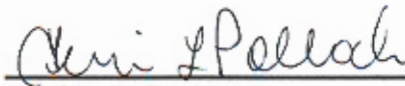
15.0 PROTOCOL APPROVALS

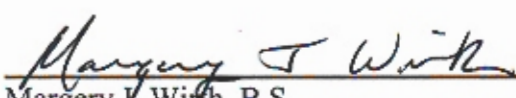
This protocol has been reviewed and approved.

Study Director:  4/27/05
Richard D. May, Ph.D. Date

Sponsor's Representative 1:  4/25/05
Dr. David P. Houchens (INITIALS ONLY; see pg. 2) Date

Sponsor's Representative 2:  4/25/05
Dr. James E. Morris (INITIALS ONLY; see pg. 2) Date

Sponsor's Quality Assurance:  4-25-05
Terri L. Pollock, B.A. Date

Southern Research Quality Assurance:  4/27/05
Margery J. Wirth, B.S. Date

Appendix A

Saturation Assay Tube Layout

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

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

Appendix B

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

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-B supplied by Battelle to laboratory "B"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	HAP (500 uL)
1	1	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
2	2	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
3	3	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
4	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	1.0E-06	100	500
5	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	1.0E-06	100	500
6	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	1.0E-06	100	500
7	1	Inert R1881	S	1	B-1-S1	3E-06	300	30	10	50	1.0E-07	100	500
8	2	Inert R1881	S	1	B-1-S1	3E-06	300	30	10	50	1.0E-07	100	500
9	3	Inert R1881	S	1	B-1-S1	3E-06	300	30	10	50	1.0E-07	100	500
10	1	Inert R1881	S	2	B-1-S2	3E-07	300	30	10	50	1.0E-08	100	500
11	2	Inert R1881	S	2	B-1-S2	3E-07	300	30	10	50	1.0E-08	100	500
12	3	Inert R1881	S	2	B-1-S2	3E-07	300	30	10	50	1.0E-08	100	500
13	1	Inert R1881	S	3	B-1-S3	3E-08	300	30	10	50	1.0E-09	100	500
14	2	Inert R1881	S	3	B-1-S3	3E-08	300	30	10	50	1.0E-09	100	500
15	3	Inert R1881	S	3	B-1-S3	3E-08	300	30	10	50	1.0E-09	100	500
16	1	Inert R1881	S	4	B-1-S4	3E-09	300	30	10	50	1.0E-10	100	500
17	2	Inert R1881	S	4	B-1-S4	3E-09	300	30	10	50	1.0E-10	100	500
18	3	Inert R1881	S	4	B-1-S4	3E-09	300	30	10	50	1.0E-10	100	500
19	1	Inert R1881	S	5	B-1-S5	3E-10	300	30	10	50	1.0E-11	100	500
20	2	Inert R1881	S	5	B-1-S5	3E-10	300	30	10	50	1.0E-11	100	500
21	3	Inert R1881	S	5	B-1-S5	3E-10	300	30	10	50	1.0E-11	100	500
22	1	Weak Positive	P	1	B-1-P1	5.00E-02	300	30	10	50	1.7E-03	100	500
23	2	Weak Positive	P	1	B-1-P1	5.00E-02	300	30	10	50	1.7E-03	100	500
24	3	Weak Positive	P	1	B-1-P1	5.00E-02	300	30	10	50	1.7E-03	100	500
25	1	Weak Positive	P	2	B-1-P2	5.00E-03	300	30	10	50	1.7E-04	100	500
26	2	Weak Positive	P	2	B-1-P2	5.00E-03	300	30	10	50	1.7E-04	100	500
27	3	Weak Positive	P	2	B-1-P2	5.00E-03	300	30	10	50	1.7E-04	100	500
28	1	Weak Positive	P	3	B-1-P3	5.00E-04	300	30	10	50	1.7E-05	100	500
29	2	Weak Positive	P	3	B-1-P3	5.00E-04	300	30	10	50	1.7E-05	100	500
30	3	Weak Positive	P	3	B-1-P3	5.00E-04	300	30	10	50	1.7E-05	100	500
31	1	Weak Positive	P	4	B-1-P4	5.00E-05	300	30	10	50	1.7E-06	100	500
32	2	Weak Positive	P	4	B-1-P4	5.00E-05	300	30	10	50	1.7E-06	100	500
33	3	Weak Positive	P	4	B-1-P4	5.00E-05	300	30	10	50	1.7E-06	100	500
34	1	Weak Positive	P	5	B-1-P5	5.00E-06	300	30	10	50	1.7E-07	100	500
35	2	Weak Positive	P	5	B-1-P5	5.00E-06	300	30	10	50	1.7E-07	100	500
36	3	Weak Positive	P	5	B-1-P5	5.00E-06	300	30	10	50	1.7E-07	100	500
37	1	Weak Positive	P	6	B-1-P6	5.00E-07	300	30	10	50	1.7E-08	100	500
38	2	Weak Positive	P	6	B-1-P6	5.00E-07	300	30	10	50	1.7E-08	100	500
39	3	Weak Positive	P	6	B-1-P6	5.00E-07	300	30	10	50	1.7E-08	100	500
40	1	Weak Positive	P	7	B-1-P7	5.00E-08	300	30	10	50	1.7E-09	100	500
41	2	Weak Positive	P	7	B-1-P7	5.00E-08	300	30	10	50	1.7E-09	100	500
42	3	Weak Positive	P	7	B-1-P7	5.00E-08	300	30	10	50	1.7E-09	100	500

43	1	Weak Positive	P	8	B-1-P8	5.00E-09	300	30	10	50	1.7E-10	100	500
44	2	Weak Positive	P	8	B-1-P8	5.00E-09	300	30	10	50	1.7E-10	100	500
45	3	Weak Positive	P	8	B-1-P8	5.00E-09	300	30	10	50	1.7E-10	100	500
46	1	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
47	2	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
48	3	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
49	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	1.0E-06	100	500
50	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	1.0E-06	100	500
51	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	1.0E-06	100	500
52	1	none	Hot		—	—	—	30	—	—	—		
53	2	none	Hot		—	—	—	30	—	—	—		
54	3	none	Hot		—	—	—	30	—	—	—		
55	1	none	Hot		—	—	—	30	—	—	—		
56	2	none	Hot		—	—	—	30	—	—	—		
57	3	none	Hot		—	—	—	30	—	—	—		

When the protocol was written, the Key Study Dates were uncertain and listed as "to be determined (TBD)," due to the availability of the rat prostates from an outside source so that we could prepare the prostate cytosol. Protocol 11232.01.03 is amended as follows:

1. Page 3. The **Key Study Dates** are revised as follows:

5.0

Event	Schedule	Dates
Preparation of rat cytosol	Weeks 1-2	June, 13-24, 2005
Receipt of other key materials	Weeks 2-3	June 20-30, 2005
Saturation assays	Weeks 4-5	July 5-15, 2005
Competitive assays	Weeks 8-9	August 1-12, 2005
QAU audit and management review	Weeks 11- 12	August 22- September 2, 2005
Data submitted to Battelle	Week 13	September 6-9, 2005

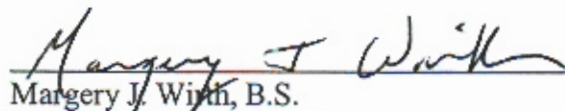
Effective Date: July 1, 2005

Approval Signatures:



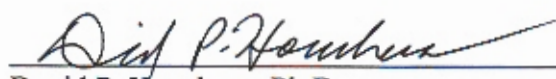
Richard D. May, Ph.D.
Study Director
Senior Project Leader
Southern Research Institute

7/14/05
Date



Margery J. Wirth, B.S.
Manager, Quality Assurance
Southern Research Institute

7/14/05
Date



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

7/13/05
Date

Due to a delay in data evaluation, the Key Study Dates were have been modified. In addition, an error in the concentration of dexamethasone (Weak Positive) shown in Appendix B was detected that requires correction (the stock concentration sent to us from Battelle was 30 mM (3.00E-2 M, which was the concentration used in the previous Task). Thus, Protocol 11232.01.03 is amended as follows:

1. Page 3. The **Key Study Dates** are revised as follows:

5.0

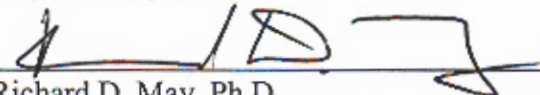
Event	Schedule	Dates
Preparation of rat cytosol	Weeks 1-2	June, 13-24, 2005
Receipt of other key materials	Weeks 2-3	June 20-30, 2005
Saturation assays	Weeks 4-5	July 5-15, 2005
Competitive assays	Weeks 9-10	August 10-19, 2005
QAU audit and management review	Weeks 12- 13	August 29- September 9, 2005
Report submitted to Battelle	Week 14	September 13-16, 2005

2. Pages 13-14 (Appendix B). The concentrations of Weak Positive listed in column 7 ["Competitor Initial Concentration (M)"], positions 22-45 are revised as follows:

Instead of 5.00E-02, 5.00E-03, 5.00E-04, etc., these concentrations are now 3.00E-02, 3.00E-03, 3.00E-04, etc. Thus, the exponents remain the same, but the first number of each concentration is now 3.00.

Effective Date: August 10, 2005

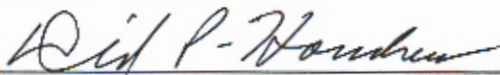
Approval Signatures:



Richard D. May, Ph.D.
Study Director
Senior Project Leader
Southern Research Institute

8/11/05

Date



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

8/10/05

Date

Due to some unclear language in Section 7.3 regarding tube labeling, Protocol 11232.01 is amended as follows:

2. Page 4. Section 7.3 now reads:

7.3 IDENTIFICATION

Each tube in the assay will be labeled according to its position, as indicated in the left-most column of Appendix B. Position will correspond to Sample Number.

Due to some unclear language in the second paragraph of Section 13.2 that reflects an error in the protocol that would allow EPA access to Southern Research QAU inspection records, Protocol 11232.01 is amended, as shown below. Note: in a regulated environment, the EPA is not allowed to review the QAU study inspection or the self-inspection records of the testing facility.

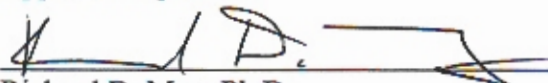
3. Pages 9-10. Section 13.2, second paragraph, now reads:

13.2 REGULATORY COMPLIANCE

Quality Assurance Review: Personnel from the Quality Assurance Unit (QAU) at Southern Research will inspect the study in accordance with Southern Research standard operating procedures and the EPA FIFRA GLPs. The QAU will audit the final report prior to submission to Battelle to assure that it accurately represents the raw data collected during the study. QAU inspection records will be made available to Battelle during visits to Southern Research and/or for special requests.

Effective Date: April 27, 2005

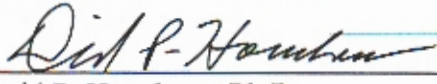
Approval Signatures:

 10/7/05
Richard D. May, Ph.D. Date

Study Director

Senior Project Leader

Southern Research Institute

 10/6/05
David P. Houchens, Ph.D. Date

Program Manager

Battelle Memorial Institute

APPENDIX B

Processed Raw Data from Saturation Assay 1 and Associated Figures

Note: The sample in position 46 was considered an outlier* in the Battelle spreadsheet and was not included in the data analysis.

*value was clearly inconsistent with other two values in the triplicate.

Counts from Saturation Assay Number 1

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Transferrin Acetate (uL)	Cytosol (uL)	Significant portion of label on Vial	dpm as counted	corrected DPM for 2mL
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	448.40	1339.2
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	521.33	1563.99
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	585.64	1756.92
4	1	H	10.0	15	0.50	—	—	—	50	300	—	893.38	2650.14
5	2	H	10.0	15	0.50	—	—	—	50	300	—	952.32	2886.96
6	3	H	10.0	15	0.50	—	—	—	50	300	—	889.22	2667.66
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1021.82	3065.46
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1035.02	3105.06
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1007.09	3021.27
10	1	H	10.0	30	1.00	—	—	—	50	300	—	1326.06	3978.18
11	2	H	10.0	30	1.00	—	—	—	50	300	—	1251.62	3754.86
12	3	H	10.0	30	1.00	—	—	—	50	300	—	1220.77	3662.31
13	1	H	10.0	45	1.50	—	—	—	50	300	—	1441.63	4324.89
14	2	H	10.0	45	1.50	—	—	—	50	300	—	1440.27	4320.81
15	3	H	10.0	45	1.50	—	—	—	50	300	—	1435.09	4305.27
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	1814.59	5443.77
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	1733.80	5200.8
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	1579.32	4737.96
19	1	H	100.0	15	5.00	—	—	—	50	300	—	1832.05	5496.15
20	2	H	100.0	15	5.00	—	—	—	50	300	—	1932.58	5797.74
21	3	H	100.0	15	5.00	—	—	—	50	300	—	2070.15	6210.45
22	1	H	100.0	30	10.00	—	—	—	50	300	—	2422.61	7267.83
23	2	H	100.0	30	10.00	—	—	—	50	300	—	2940.62	8821.85
24	3	H	100.0	30	10.00	—	—	—	50	300	—	2594.27	7782.81
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	87.90	203.7
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	84.43	253.29
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	91.60	275.4
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	140.00	420
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	128.90	386.7
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	80.09	240.27
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	101.43	304.29
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	78.87	236.61
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	103.91	311.73
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	130.54	409.62
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	118.25	354.75
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	164.63	493.89
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	207.88	623.64
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	251.82	755.46
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	146.98	440.64
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	148.52	445.56
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	173.69	521.07
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	273.87	821.61
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	345.33	1035.99
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	413.26	1239.78
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	448.93	1346.49
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	1499.12	4497.36
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	969.77	2909.31
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	693.76	2081.28
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	12159.6	12159.6
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11890.7	11890.6
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11611.4	11611.38
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23860.0	23859.96
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	23139.9	23139.93
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	24089.8	24089.8
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	34403.2	34403.17
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	33603.0	33603.01
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	34973.4	34973.39
58	1	Hot	10.0	30	1	—	—	—	—	—	—	46025.6	46025.64
59	2	Hot	10.0	30	1	—	—	—	—	—	—	47077.8	47077.81
60	3	Hot	10.0	30	1	—	—	—	—	—	—	46836.2	46836.17
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	71768.0	71768.03
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	71132.1	71132.06
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	72023.5	72023.5
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	133227.7	133227.7
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	130677.1	130677.1
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	119791.7	119790.7
67	1	Hot	100.0	15	5	—	—	—	—	—	—	222571.7	222570.7
68	2	Hot	100.0	15	5	—	—	—	—	—	—	210122.2	210121.5
69	3	Hot	100.0	15	5	—	—	—	—	—	—	215219.2	215219.2
70	1	Hot	100.0	30	10	—	—	—	—	—	—	482607.1	482607.1
71	2	Hot	100.0	30	10	—	—	—	—	—	—	476180.0	476180.0
72	3	Hot	100.0	30	10	—	—	—	—	—	—	492257.7	492257.7

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R	S	T	U
1	Laboratory B																			
2	AR Saturation Assay (cold R1681 and WP supplied by Sequim)																			
3	72 assay tubes																			
4	Please return by eMail to n.a.Holter@nrl.gov																			
5	Provide information in all blue cells in columns Q and DK																			
6	If the DPM value for a tube was judged unreliable,																			
7	Include the DPM value in column O																			
8	Provide a reason in column R																			
9	The value in column Q will automatically change to FALSE																			
10	For your convenience, data reduction is performed in columns																			
11	U through RZ, and the values needed for analysis are presented																			
12	in columns CF through CN																			
13	Cells in column S are presented with a grey background																			
14	If the total binding exceeds 10% of the hot added at that concentration,																			
15	the cytosol concentration is probably too high for good competitive assays																			
16	multiply DPM in sample by :																			
17																				
18	Saturation Assay Tube Layout																			
19	Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1681 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1681 Volume (uL)	Cold Final Concentration (nM)	Trisacetone Acetate (uL)	Cytosol (uL)	Significant portion of label on Vial	dpm as counted	Corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound Y values	
20	1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	448.40	1339.2	TRUE		11.3%	0.25	1339.2	
21	2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	521.33	1563.99	TRUE		13.2%	0.25	1564.0	
22	3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	585.64	1758.92	TRUE		14.8%	0.25	1759.0	
23	4	1	H	10.0	15	0.50	—	—	—	50	300	—	883.38	2650.14	TRUE		11.2%	0.5	2650.1	
24	5	2	H	10.0	15	0.50	—	—	—	50	300	—	952.32	2856.96	TRUE		12.2%	0.5	2857.0	
25	6	3	H	10.0	15	0.50	—	—	—	50	300	—	898.22	2694.66	TRUE		11.3%	0.5	2697.7	
26	7	1	H	10.0	21	0.70	—	—	—	50	300	—	1021.82	3065.46	TRUE		8.9%	0.7	3065.5	
27	8	2	H	10.0	21	0.70	—	—	—	50	300	—	1035.02	3105.06	TRUE		8.0%	0.7	3105.1	
28	9	3	H	10.0	21	0.70	—	—	—	50	300	—	1007.09	3021.27	TRUE		8.8%	0.7	3021.3	
29	10	1	H	10.0	30	1.00	—	—	—	50	300	—	1326.08	3978.18	TRUE		8.5%	1	3978.2	
30	11	2	H	10.0	30	1.00	—	—	—	50	300	—	1251.82	3754.86	TRUE		8.0%	1	3754.9	
31	12	3	H	10.0	30	1.00	—	—	—	50	300	—	1220.77	3662.31	TRUE		7.9%	1	3662.3	
32	13	1	H	10.0	45	1.50	—	—	—	50	300	—	1441.83	4324.89	TRUE		6.0%	1.5	4324.9	
33	14	2	H	10.0	45	1.50	—	—	—	50	300	—	1440.27	4320.81	TRUE		6.0%	1.5	4320.8	
34	15	3	H	10.0	45	1.50	—	—	—	50	300	—	1435.09	4305.27	TRUE		6.0%	1.5	4305.3	
35	16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	1814.59	5443.77	TRUE		4.3%	2.5	5443.8	
36	17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	1733.60	5200.8	TRUE		4.1%	2.5	5200.8	
37	18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	1579.32	4737.96	TRUE		3.7%	2.5	4738.0	
38	19	1	H	100.0	15	5.00	—	—	—	50	300	—	1832.05	5496.15	TRUE		2.5%	5	5496.2	
39	20	2	H	100.0	15	5.00	—	—	—	50	300	—	1932.58	5797.74	TRUE		2.7%	5	5797.7	
40	21	3	H	100.0	15	5.00	—	—	—	50	300	—	2070.15	6210.45	TRUE		2.8%	5	6210.5	
41	22	1	H	100.0	30	10.00	—	—	—	50	300	—	2422.81	7267.83	TRUE		1.5%	10	7267.8	
42	23	2	H	100.0	30	10.00	—	—	—	50	300	—	2640.82	7921.86	TRUE		1.9%	10	7921.9	
43	24	3	H	100.0	30	10.00	—	—	—	50	300	—	2594.27	7782.81	TRUE		1.8%	10	7782.8	

Receptor Notes
diluted to 2 mg/ml for use (0.6 mg/300 uL)

protocol calls for counting decanted EtOH supernate
reflects 100uL of reaction mixture processed

	V
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	NBS y values
20	
21	244.1
22	244.1
23	244.1
24	348.0
25	348.0
26	348.0
27	284.2
28	284.2
29	284.2
30	419.4
31	419.4
32	419.4
33	600.6
34	600.6
35	600.6
36	596.1
37	596.1
38	596.1
39	1207.4
40	1207.4
41	1207.4
42	2485.3
43	2485.3
44	2485.3

	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
19														
20														
21														
22														
23														
24														
25														
26														
27														
28														
29														
30														
31														
32														
33														
34														
35														
36														
37														
38														
39														
40														
41														
42														
43														
44														

	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																						
Run	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																									
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)	(dpm)	(dpm)	(dpm)	(dpm)	(dpm)	(dpm)	(dpm)	(dpm)	(dpm)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles)	(fmoles

	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN		
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
13																	
14																	
15																	
16																	
17	Non Specific Binding -- Positions 25-48 radioabeled R1881 plus 100 X Inert R1881 plus cytosol																
18	Assay tube contents																
Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial								Cytosol			Scintillation Results	
					Hot	Cold	Transcalanone Acetate	Cold Conc. Final	Hot Conc. Final	Cold Conc. Final	Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25)					
19																	
20	1	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	203.7	244.1		
21	2	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	253.3	244.1		
22	3	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	276.4	244.1		
23	1	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	420.0	349.0		
24	2	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	386.7	349.0		
25	3	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	240.3	349.0		
26	1	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	304.3	284.2		
27	2	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	236.6	284.2		
28	3	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	311.7	284.2		
29	1	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	409.6	419.4		
30	2	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	354.8	419.4		
31	3	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	483.9	419.4		
32	1	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	623.6	606.6		
33	2	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	755.5	606.6		
34	3	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	806.6	606.6		
35	1	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	445.6	596.1		
36	2	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	521.1	596.1		
37	3	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	821.6	596.1		
38	1	43	1	HC	c7	100.0	15	10.00	15	50	300	6	600	1036.0	1207.4		
39	2	44	2	HC	c7	100.0	15	10.00	15	50	300	6	600	1207.4	1207.4		
40	3	45	3	HC	c7	100.0	15	10.00	15	50	300	6	600	1239.8	1207.4		
41	1	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	1346.6	2495.3		
42	2	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	2495.3	2495.3		
43	3	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	2909.3	2495.3		
44	1													2081.3	2495.3		

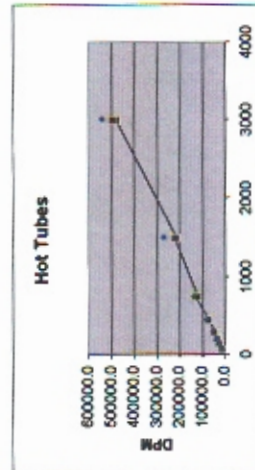
	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
13																	
14																	
15																	
16																	
17																	
18																	
19																	
20																	
21	1	49	1	Hot	c1												
22	1	50	2	Hot	c1												
23	1	51	3	Hot	c1												
24	1	52	1	Hot	c2												
25	1	53	2	Hot	c2												
26	1	54	3	Hot	c2												
27	1	55	1	Hot	c3												
28	1	56	2	Hot	c3												
29	1	57	3	Hot	c3												
30	1	58	1	Hot	c4												
31	1	59	2	Hot	c4												
32	1	60	3	Hot	c4												
33	1	61	1	Hot	c5												
34	1	62	2	Hot	c5												
35	1	63	3	Hot	c5												
36	1	64	1	Hot	c6												
37	1	65	2	Hot	c6												
38	1	66	3	Hot	c6												
39	1	67	1	Hot	c7												
40	1	68	2	Hot	c7												
41	1	69	3	Hot	c7												
42	1	70	1	Hot	c8												
43	1	71	2	Hot	c8												
44	1	72	3	Hot	c8												

Free - Positions 49-72, radioabeled R1881 without cytozol

Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial (nM)	Hot R1881 Volume (uL)	Molecules of R1881 (fmole)	Counts per Scintillation (cpm)	Vial	Experimental number of molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (cpm)
21	1	49	Hot	c1	10	7.5	75	12159.6		77	11887.2
22	1	50	Hot	c1	10	7.5	75	11890.7		75	11887.2
23	1	51	Hot	c1	10	7.5	75	11611.4		73	11887.2
24	1	52	Hot	c2	10	15	150	23980.0		151	23696.6
25	1	53	Hot	c2	10	15	150	23139.9		146	23696.6
26	1	54	Hot	c2	10	15	150	24089.8		152	23696.6
27	1	55	Hot	c3	10	21	210	34403.2		217	34326.5
28	1	56	Hot	c3	10	21	210	33903.0		212	34326.5
29	1	57	Hot	c3	10	21	210	34973.4		221	34326.5
30	1	58	Hot	c4	10	30	300	46025.6		291	46646.5
31	1	59	Hot	c4	10	30	300	47077.8		297	46646.5
32	1	60	Hot	c4	10	30	300	48336.2		296	46646.5
33	1	61	Hot	c5	10	45	450	71786.0		453	71640.5
34	1	62	Hot	c5	10	45	450	71132.1		449	71640.5
35	1	63	Hot	c5	10	45	450	72023.5		455	71640.5
36	1	64	Hot	c6	100	7.5	750	133227.7		841	127868.5
37	1	65	Hot	c6	100	7.5	750	130677.1		825	127868.5
38	1	66	Hot	c6	100	7.5	750	119790.7		756	127868.5
39	1	67	Hot	c7	100	15	1500	222570.7		1405	215970.5
40	1	68	Hot	c7	100	15	1500	210121.5		1327	215970.5
41	1	69	Hot	c7	100	15	1500	215219.2		1359	215970.5
42	1	70	Hot	c8	100	30	3000	482607.1		3047	483681.3
43	1	71	Hot	c8	100	30	3000	476180.0		3006	483681.3
44	1	72	Hot	c8	100	30	3000	492256.9		3108	483681.3

predicted dpm

Computation Check
715005 specific activity data
81.02 CfmMole 3H R1881
2.22E+12 DPM/C (definition)
1.7987E+14 DPM/mole
1.7987E+11 DPM/fmole
179.9 DPM/fmole
0.005580 fmole/DPM



	CF	CG	CH	CI	CJ	CK	CL	CM	CN	DJ	DK	DL	DM
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
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	specific bound/dpm	total added dpm	total bound dpm	NET dpm	Net Final Concentration (nM)				
21	2.02036E-11	0.10382	2.20291E-10	2.02036E-11	1095.1	12159.6	1339.2	203.7	0.25				
22	2.44694E-11	0.12705	2.20291E-10	2.44694E-11	1319.9	11690.7	1064.0	263.3	0.25				
23	2.80347E-11	0.14933	2.20291E-10	2.80347E-11	1512.8	11611.4	1756.9	275.4	0.25				
24	4.26444E-11	0.10934	4.3914E-10	4.26444E-11	2301.2	23890.0	2850.1	420.0	0.50				
25	4.70031E-11	0.12196	4.3914E-10	4.70031E-11	2638.0	23138.9	2887.0	388.7	0.50				
26	4.29691E-11	0.11026	4.3914E-10	4.29691E-11	2318.7	24098.8	2667.7	240.3	0.50				
27	5.16419E-11	0.06897	6.36132E-10	5.16419E-11	2781.3	34403.2	3065.5	304.3	0.70				
28	5.22754E-11	0.09036	6.36132E-10	5.22754E-11	2820.9	33803.0	3105.1	236.6	0.70				
29	5.07228E-11	0.08743	6.36132E-10	5.07228E-11	2737.1	34973.4	3021.3	311.7	0.70				
30	6.59502E-11	0.08341	8.64444E-10	6.59502E-11	3558.8	46025.6	3978.2	408.6	1.00				
31	6.18117E-11	0.07776	8.64444E-10	6.18117E-11	3335.4	47077.8	3754.9	354.8	1.00				
32	6.00985E-11	0.07544	8.64444E-10	6.00985E-11	3242.9	46836.2	3682.3	493.9	1.00				
33	6.89009E-11	0.05524	1.32763E-09	6.89009E-11	3718.3	71766.0	4324.9	623.8	1.50				
34	6.89313E-11	0.05517	1.32763E-09	6.89313E-11	3714.2	71132.1	4320.8	755.5	1.50				
35	6.85433E-11	0.05493	1.32763E-09	6.85433E-11	3698.7	72023.5	4305.3	440.6	1.50				
36	8.98363E-11	0.03959	2.37019E-09	8.98363E-11	4847.7	133227.7	5443.8	445.6	2.50				
37	8.53337E-11	0.03763	2.37019E-09	8.53337E-11	4804.7	130677.1	5200.8	621.1	2.50				
38	7.87645E-11	0.03363	2.37019E-09	7.87645E-11	4141.9	119790.7	4738.0	821.6	2.50				
39	7.94778E-11	0.02038	4.00232E-09	7.94778E-11	4288.7	222570.7	5496.2	1036.0	5.00				
40	8.50688E-11	0.02184	4.00232E-09	8.50688E-11	4680.3	210121.5	5797.7	1239.8	5.00				
41	9.27151E-11	0.02385	4.00232E-09	9.27151E-11	5003.0	215219.2	6210.5	1346.5	5.00				
42	8.84436E-11	0.01002	8.96348E-09	8.84436E-11	4772.5	482807.1	7267.8	2509.3	10.00				
43	1.17243E-10	0.01332	8.96348E-09	1.17243E-10	6326.6	476180.0	8821.9	2081.3	10.00				
44	9.79871E-11	0.01111	8.96348E-09	9.79871E-11	6287.5	482256.9	7782.8	2081.3	10.00				
										One site binding (hyperbola)			
										Best-fit values			
										BMAX			
										KD			
										Std Error			
										BMAX			
										KD			
										95% Confidence Intervals			
										BMAX			
										KD			
										Goodness of Fit			
										Degrees of Freedom			
										R ² (unweighted)			
										Weighted Sum of Squares			
										Absolute Sum of Squares			
										Sy.x			
										Data			
										Number of X values			
										Number of Y replicates			
										Total number of values			
										Number of missing values			
										Bmax (fmole/100 ug)			
										Bmax (fmole/100 ug)/Bmax molar			

B1-7/8/05

	DN	DO	DP
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21	KD molar molar to nM conversion		8.99E-10
22			1.00E+09
23		Kd nM =	8.99E-01
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R	S	T	U
45	25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		87.90	203.7	TRUE				
46	26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		84.43	253.29	TRUE				
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		91.80	275.4	TRUE				
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7		140.00	420	TRUE				
49	29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7		128.90	386.7	TRUE				
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7		80.09	240.27	TRUE				
51	31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C8		101.43	304.29	TRUE				
52	32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C8		78.87	238.61	TRUE				
53	33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C8		103.91	311.73	TRUE				
54	34	1	HC	10.0	30	1	1.00	30	100	50	300	C5		138.54	409.62	TRUE				
55	35	2	HC	10.0	30	1	1.00	30	100	50	300	C5		118.25	354.75	TRUE				
56	36	3	HC	10.0	30	1	1.00	30	100	50	300	C5		184.83	493.99	TRUE				
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4		207.88	623.84	TRUE				
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4		251.82	755.46	TRUE				
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4		148.88	440.64	TRUE				
60	40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		148.52	445.56	TRUE				
61	41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		173.69	521.07	TRUE				
62	42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		273.87	821.61	TRUE				
63	43	1	HC	100.0	15	5	10.00	15	500	50	300	C2		345.33	1035.99	TRUE				
64	44	2	HC	100.0	15	5	10.00	15	500	50	300	C2		413.25	1239.78	TRUE				
65	45	3	HC	100.0	15	5	10.00	15	500	50	300	C2		448.83	1346.49	TRUE				
66	46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1		1498.12	4497.36	FALSE				
67	47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1		989.77	2808.31	TRUE				
68	48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1		693.78	2081.28	TRUE				
69	49	1	Hx	10.0	7.5	0.25	—	—	—	—	—	—		12159.8	12159.6	TRUE				
70	50	2	Hx	10.0	7.5	0.25	—	—	—	—	—	—		11890.7	11890.66	TRUE				
71	51	3	Hx	10.0	7.5	0.25	—	—	—	—	—	—		11811.4	11811.38	TRUE				
72	52	1	Hx	10.0	15	0.5	—	—	—	—	—	—		23860.0	23859.96	TRUE				
73	53	2	Hx	10.0	15	0.5	—	—	—	—	—	—		23139.8	23139.93	TRUE				
74	54	3	Hx	10.0	15	0.5	—	—	—	—	—	—		24089.8	24089.8	TRUE				
75	55	1	Hx	10.0	21	0.7	—	—	—	—	—	—		34403.2	34403.17	TRUE				
76	56	2	Hx	10.0	21	0.7	—	—	—	—	—	—		33903.0	33903.01	TRUE				
77	57	3	Hx	10.0	21	0.7	—	—	—	—	—	—		34973.4	34973.39	TRUE				
78	58	1	Hx	10.0	30	1	—	—	—	—	—	—		48025.6	48025.64	TRUE				
79	59	2	Hx	10.0	30	1	—	—	—	—	—	—		47077.8	47077.81	TRUE				
80	60	3	Hx	10.0	30	1	—	—	—	—	—	—		46836.2	46836.17	TRUE				
81	61	1	Hx	10.0	45	1.5	—	—	—	—	—	—		71766.0	71766.03	TRUE				
82	62	2	Hx	10.0	45	1.5	—	—	—	—	—	—		71132.1	71132.06	TRUE				
83	63	3	Hx	10.0	45	1.5	—	—	—	—	—	—		72023.5	72023.5	TRUE				
84	64	1	Hx	100.0	7.5	2.5	—	—	—	—	—	—		133228	133227.7	TRUE				
85	65	2	Hx	100.0	7.5	2.5	—	—	—	—	—	—		130677	130677.1	TRUE				
86	66	3	Hx	100.0	7.5	2.5	—	—	—	—	—	—		119791	119790.7	TRUE				
87	67	1	Hx	100.0	15	5	—	—	—	—	—	—		222571	222570.7	TRUE				
88	68	2	Hx	100.0	15	5	—	—	—	—	—	—		210122	210121.5	TRUE				
89	69	3	Hx	100.0	15	5	—	—	—	—	—	—		215218	215218.2	TRUE				
90	70	1	Hx	100.0	30	10	—	—	—	—	—	—		482907	482907.1	TRUE				
91	71	2	Hx	100.0	30	10	—	—	—	—	—	—		476180	476180	TRUE				
92	72	3	Hx	100.0	30	10	—	—	—	—	—	—		492257	492256.9	TRUE				

	V
45	
46	
47	
48	
49	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	
61	
62	
63	
64	
65	
66	
67	
68	
69	
70	
71	
72	
73	
74	
75	
76	
77	
78	
79	
80	
81	
82	
83	
84	
85	
86	
87	
88	
89	
90	
91	
92	

	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
45														
46														
47														
48														
49														
50														
51														
52														
53														
54														
55														
56														
57														
58														
59														
60														
61														
62														
63														
64														
65														
66														
67														
68														
69														
70														
71														
72														
73														
74														
75														
76														
77														
78														
79														
80														
81														
82														
83														
84														
85														
86														
87														
88														
89														
90														
91														
92														

	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY
45															
46															
47															
48															
49															
50															
51															
52															
53															
54															
55															
56															
57															
58															
59															
60															
61															
62															
63															
64															
65															
66															
67															
68															
69															
70															
71															
72															
73															
74															
75															
76															
77															
78															
79															
80															
81															
82															
83															
84															
85															
86															
87															
88															
89															
90															
91															
92															

	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	SJ	BK	BL	BM	BN
45															
46															
47															
48															
49															
50															
51															
52															
53															
54															
55															
56															
57															
58															
59															
60															
61															
62															
63															
64															
65															
66															
67															
68															
69															
70															
71															
72															
73															
74															
75															
76															
77															
78															
79															
80															
81															
82															
83															
84															
85															
86															
87															
88															
89															
90															
91															
92															

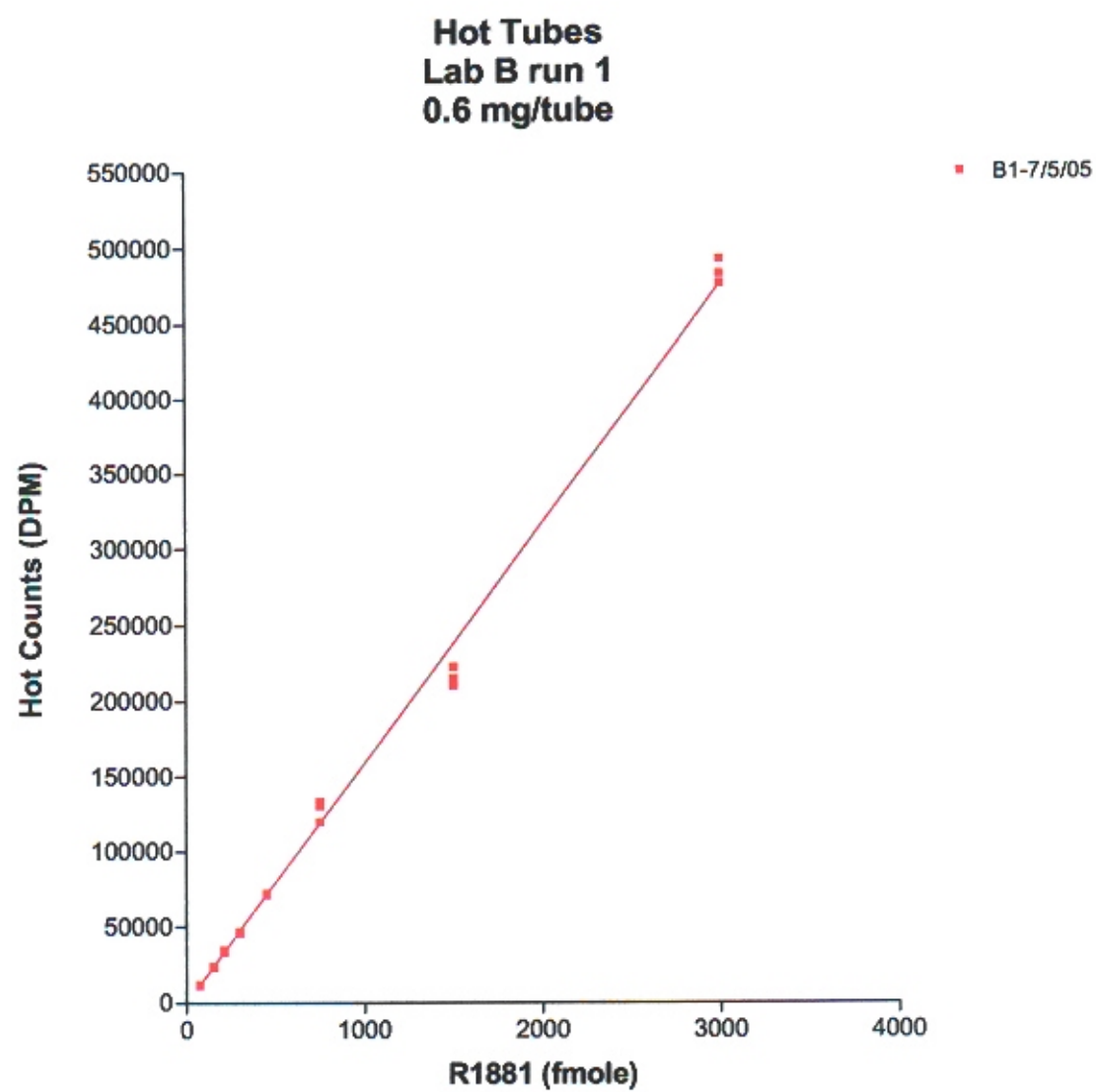
45	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
46																	
47																	
48																	
49																	
50																	
51																	
52																	
53																	
54																	
55																	
56																	
57																	
58																	
59																	
60																	
61																	
62																	
63																	
64																	
65																	
66																	
67																	
68																	
69																	
70																	
71																	
72																	
73																	
74																	
75																	
76																	
77																	
78																	
79																	
80																	
81																	
82																	
83																	
84																	
85																	
86																	
87																	
88																	
89																	
90																	
91																	
92																	

Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	153.394903	dpn/mole
Y/slope	0.00631333	fmole/dpm
origin	x	y
end point	0	0
SLOPE function used if missing HOT tubes		
Slope	156.8	dpn/mole
Y/slope	0.006397	fmole/dpm
origin	x	y
end point	0	0

—•— hot tubes • Predicted

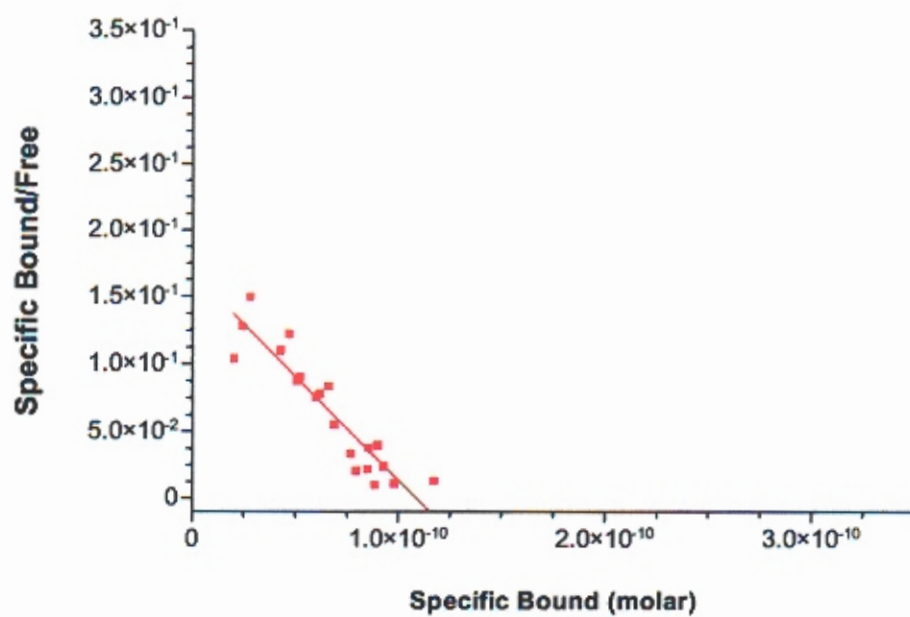
	CF	CG	CH	CI	CJ	CK	CL	CM	CN	DJ	DK	DL	DM
45													
46													
47													
48													
49													
50													
51													
52													
53													
54													
55													
56													
57													
58													
59													
60													
61													
62													
63													
64													
65													
66													
67													
68													
69													
70													
71													
72													
73													
74													
75													
76													
77													
78													
79													
80													
81													
82													
83													
84													
85													
86													
87													
88													
89													
90													
91													
92													

	DN	DO	DP
45			
46			
47			
48			
49			
50			
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			
61			
62			
63			
64			
65			
66			
67			
68			
69			
70			
71			
72			
73			
74			
75			
76			
77			
78			
79			
80			
81			
82			
83			
84			
85			
86			
87			
88			
89			
90			
91			
92			

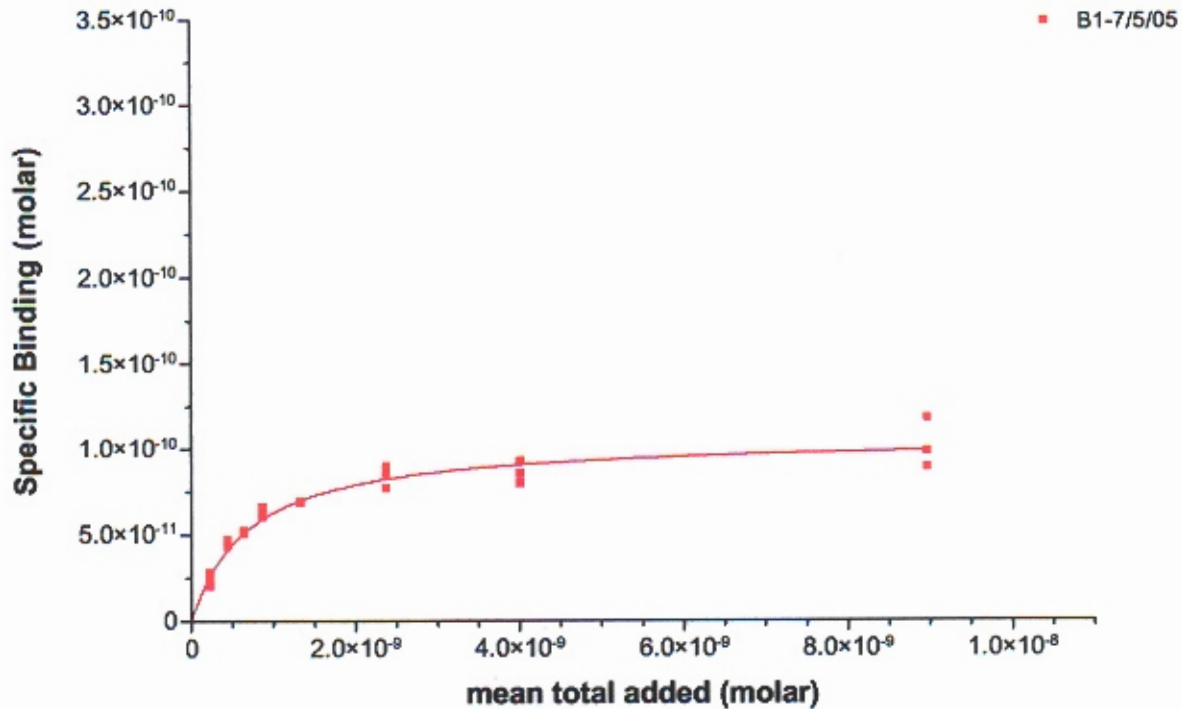


Scatchard Display
Lab B run 1
0.6 mg/tube

■ B1-7/5/05

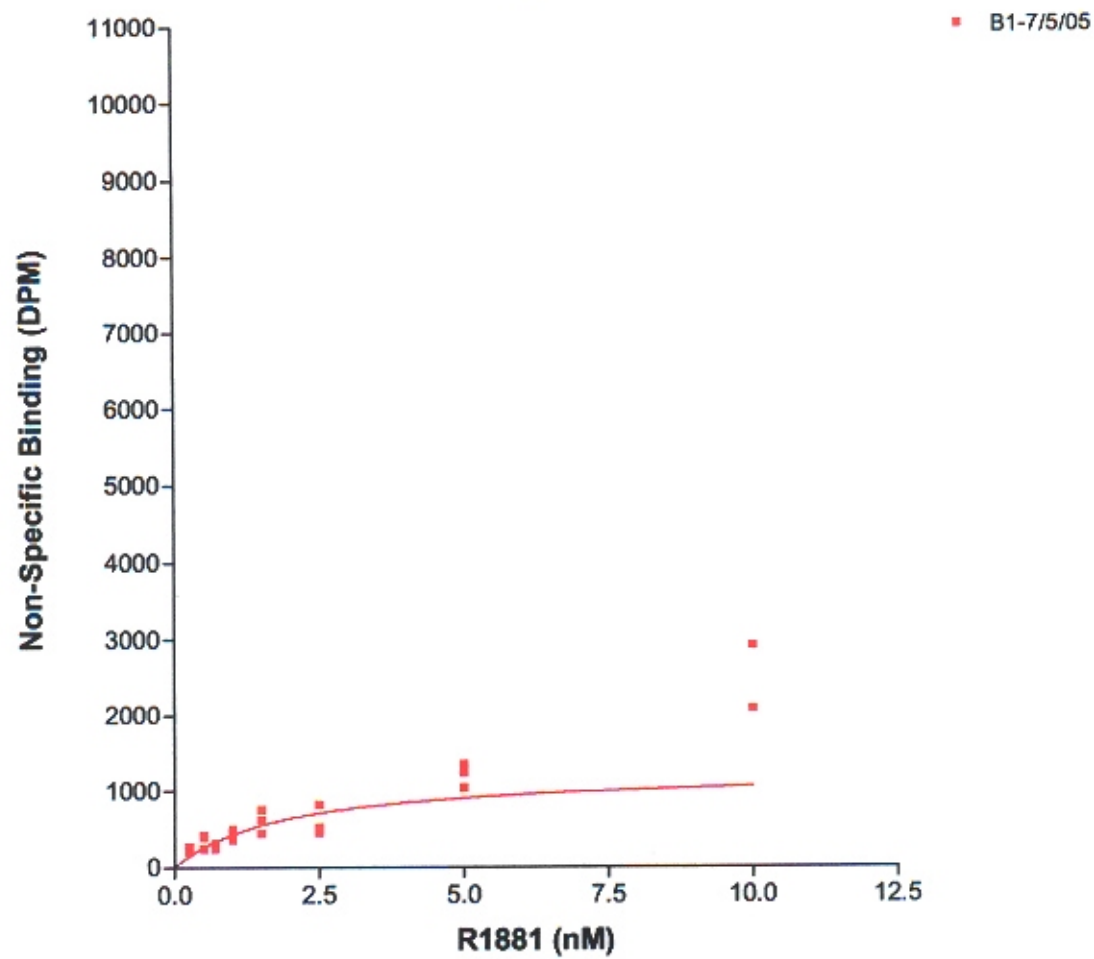


Lab B run 1 0.6 mg/tube

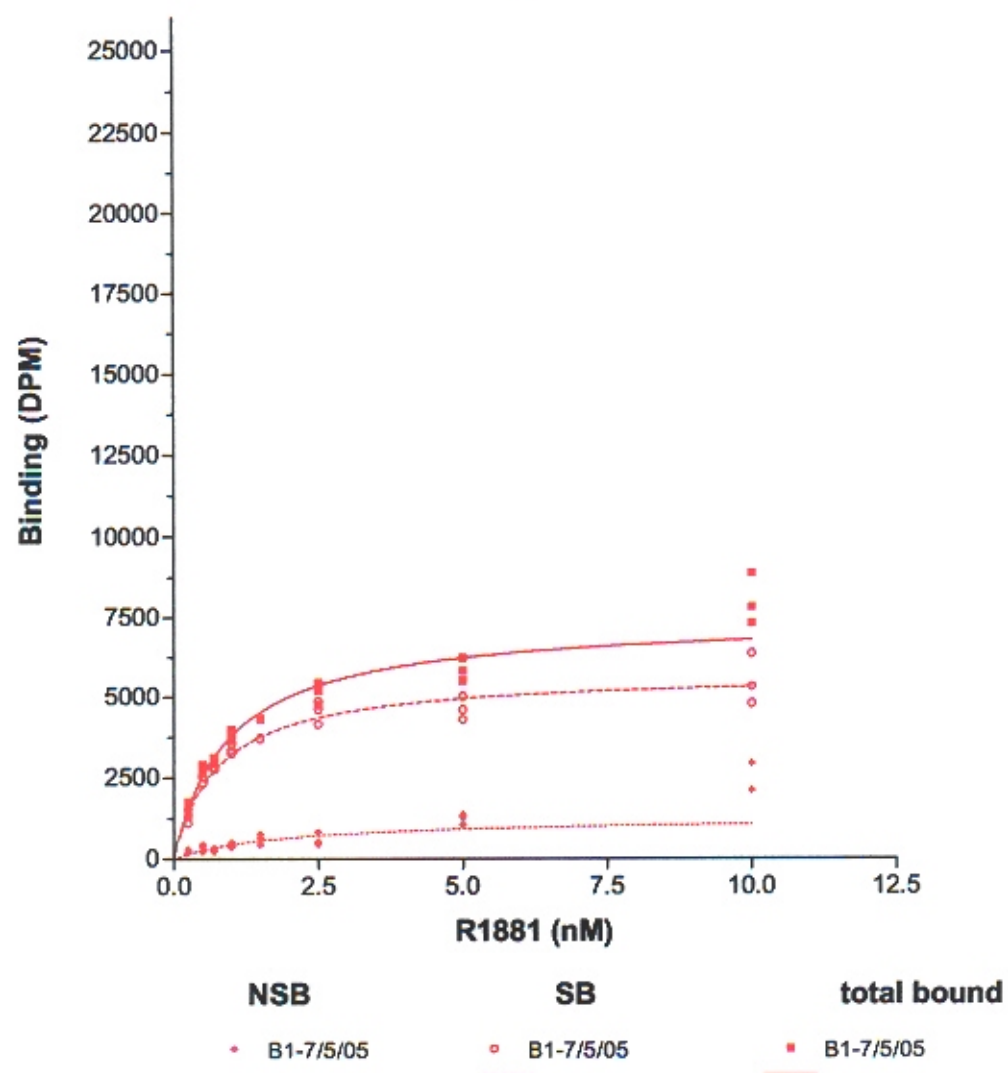


Specific bound	B1-7/5/05
BMAX	1.060e-010
KD	6.993e-010
Std. Error	
BMAX	3.984e-012
KD	5.797e-011
95% Confidence Intervals	
BMAX	9.772e-011 to 1.142e-010
KD	5.791e-010 to 8.196e-010
Goodness of Fit	
Degrees of Freedom	22
R ² (unweighted)	0.9381
Weighted Sum of Squares (1/Y ²)	0.1875
Absolute Sum of Squares	8.578e-022
Sy.x	6.244e-012
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

NSB Tubes
Lab B run 1
0.6 mg/tube



bound counts
Lab B run 1
0.6 mg/tube



APPENDIX C

Processed Raw Data from Saturation Assay 2 and Associated Figures

Counts from Saturation Assay Number 2

Saturation Assay Tube Layout

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)	Trisacetic Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial	dpm as counted	corrected DPM for 2mL
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	514.53	1543.59
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	438.71	1316.13
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	471.44	1414.32
4	1	H	10.0	15	0.50	—	—	—	50	300	—	735.39	2206.17
5	2	H	10.0	15	0.50	—	—	—	50	300	—	794.22	2382.66
6	3	H	10.0	15	0.50	—	—	—	50	300	—	805.37	2416.11
7	1	H	10.0	21	0.70	—	—	—	50	300	—	1004.10	3012.3
8	2	H	10.0	21	0.70	—	—	—	50	300	—	892.64	2677.92
9	3	H	10.0	21	0.70	—	—	—	50	300	—	954.42	2863.26
10	1	H	10.0	30	1.00	—	—	—	50	300	—	1168.50	3505.5
11	2	H	10.0	30	1.00	—	—	—	50	300	—	1185.18	3555.54
12	3	H	10.0	30	1.00	—	—	—	50	300	—	1182.65	3547.95
13	1	H	10.0	45	1.50	—	—	—	50	300	—	1354.37	4063.11
14	2	H	10.0	45	1.50	—	—	—	50	300	—	1390.23	4170.69
15	3	H	10.0	45	1.50	—	—	—	50	300	—	1413.68	4240.98
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	1616.84	4850.52
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	1607.90	4823.7
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	1557.27	4671.81
19	1	H	100.0	15	5.00	—	—	—	50	300	—	2011.53	6034.59
20	2	H	100.0	15	5.00	—	—	—	50	300	—	1997.69	5993.07
21	3	H	100.0	15	5.00	—	—	—	50	300	—	1760.25	5280.75
22	1	H	100.0	30	10.00	—	—	—	50	300	—	2019.84	6059.52
23	2	H	100.0	30	10.00	—	—	—	50	300	—	2577.29	7731.87
24	3	H	100.0	30	10.00	—	—	—	50	300	—	2121.06	6363.18
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	66.92	200.76
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	92.69	278.07
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	87.01	263.43
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	95.77	287.31
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	106.35	319.05
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	97.06	291.18
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	69.40	208.2
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	112.74	338.22
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	119.36	358.08
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	97.05	291.15
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	107.47	322.41
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	111.19	333.57
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	113.03	339.09
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	166.43	499.29
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	129.34	388.02
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	198.98	596.84
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	232.25	696.75
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	225.81	677.43
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	324.61	973.83
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	475.05	1425.15
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	424.88	1274.04
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	624.08	1872.24
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	727.83	2183.49
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	722.44	2167.32
49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11822.0	11822
50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11431.5	11431.49
51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—	11499.9	11489.89
52	1	Hot	10.0	15	0.5	—	—	—	—	—	—	23870.8	23870.76
53	2	Hot	10.0	15	0.5	—	—	—	—	—	—	24239.9	24239.86
54	3	Hot	10.0	15	0.5	—	—	—	—	—	—	23607.9	23607.9
55	1	Hot	10.0	21	0.7	—	—	—	—	—	—	33491.3	33491.34
56	2	Hot	10.0	21	0.7	—	—	—	—	—	—	34167.0	34167.01
57	3	Hot	10.0	21	0.7	—	—	—	—	—	—	33278.0	33278
58	1	Hot	10.0	30	1	—	—	—	—	—	—	47600.0	47598.96
59	2	Hot	10.0	30	1	—	—	—	—	—	—	46225.0	46225.82
60	3	Hot	10.0	30	1	—	—	—	—	—	—	47073.8	47073.84
61	1	Hot	10.0	45	1.5	—	—	—	—	—	—	72467.8	72467.84
62	2	Hot	10.0	45	1.5	—	—	—	—	—	—	73186.3	73186.27
63	3	Hot	10.0	45	1.5	—	—	—	—	—	—	71578.3	71576.26
64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—	124773.4	124773.4
65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—	129430.0	129430.2
66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—	126602.0	126601.8
67	1	Hot	100.0	15	5	—	—	—	—	—	—	257185.0	257185.4
68	2	Hot	100.0	15	5	—	—	—	—	—	—	263520.0	263519.7
69	3	Hot	100.0	15	5	—	—	—	—	—	—	243190.0	243190.8
70	1	Hot	100.0	30	10	—	—	—	—	—	—	502389.0	502389.9
71	2	Hot	100.0	30	10	—	—	—	—	—	—	506592.0	506591.8
72	3	Hot	100.0	30	10	—	—	—	—	—	—	504674.0	504673.8

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R	S	T	U
1	Laboratory B																			
2	AR Saturation Assay (cold R1881 and WP supplied by Sequim)																			
3	72 assay tubes																			
4	Please return by email to n.a.Holter@pnl.gov																			
5	Provide information in all blue cells in columns O and DK																			
6	If the DPM value for a tube was judged unreliable,																			
7	Include the DPM value in column O																			
8	Provide a reason in column R																			
9	The value in column Q will automatically change to FALSE																			
10	For your convenience, data reduction is performed in columns																			
11	U through BZ, and the values needed for analysis are presented																			
12	In columns CF through CN																			
13																				
14																				
15	Cells in column S are presented with a grey background																			
16	If the total binding exceeds 10% of the hot added at that concentration,																			
17	the cytosol concentration is probably too high for good competitive assays																			
18																				
19	Laboratory Code: B Run Identification: 2 Assay start date: 7/17/2005 Tracer lot number: 3559-507 Specific activity on day of assay: 8100 Ci/mmol Cytosol lot or vial number: 8/1505 protein (cytosol) per tube: 600 ug protein (cytosol) per tube: 0.6 mg KD 1.00E+00 nM Bmax 6.67 fmole/100 ug total volume in tubes 300 uL volume of ethanol counted: 2 mL multipy DPM in sample by: 3 Receptor Notes diluted to 2 mg/ml for use (0.6 mg/300 uL) protocol calls for counting decanted EtOH supernate reflects 100uL of reaction mixture processed																			
20	Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (nM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Trisacetone Acetate (uL)	Cytosol (uL)	Significant portion of label on Vial	dpm as counted	corrected DPM for ZmL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X values	Bound y values	
21	1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	514.53	1543.59	TRUE	—	13.3%	0.25	1543.59	
22	2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	438.71	1316.13	TRUE	—	11.4%	0.25	1316.13	
23	3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	471.44	1414.32	TRUE	—	12.2%	0.25	1414.32	
24	4	1	H	10.0	15	0.50	—	—	—	50	300	—	735.39	2206.17	TRUE	—	9.2%	0.5	2206.17	
25	5	2	H	10.0	15	0.50	—	—	—	50	300	—	794.22	2382.66	TRUE	—	9.2%	0.5	2382.7	
26	6	3	H	10.0	15	0.50	—	—	—	50	300	—	805.37	2416.11	TRUE	—	10.0%	0.5	2416.1	
27	7	1	H	10.0	21	0.70	—	—	—	50	300	—	1004.10	3012.3	TRUE	—	10.1%	0.7	3012.3	
28	8	2	H	10.0	21	0.70	—	—	—	50	300	—	892.84	2677.92	TRUE	—	8.0%	0.7	2677.9	
29	9	3	H	10.0	21	0.70	—	—	—	50	300	—	954.42	2863.26	TRUE	—	8.5%	0.7	2863.3	
30	10	1	H	10.0	30	1.00	—	—	—	50	300	—	1168.50	3505.5	TRUE	—	7.5%	1	3505.5	
31	11	2	H	10.0	30	1.00	—	—	—	50	300	—	1185.18	3555.54	TRUE	—	7.6%	1	3555.5	
32	12	3	H	10.0	30	1.00	—	—	—	50	300	—	1182.85	3547.85	TRUE	—	7.6%	1	3548.0	
33	13	1	H	10.0	45	1.50	—	—	—	50	300	—	1354.37	4083.11	TRUE	—	5.6%	1.5	4083.1	
34	14	2	H	10.0	45	1.50	—	—	—	50	300	—	1390.23	4170.69	TRUE	—	5.8%	1.5	4170.7	
35	15	3	H	10.0	45	1.50	—	—	—	50	300	—	1413.68	4240.98	TRUE	—	5.6%	1.5	4241.0	
36	16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	1616.84	4850.52	TRUE	—	3.8%	2.5	4850.5	
37	17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	1607.90	4823.7	TRUE	—	3.8%	2.5	4823.7	
38	18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	1557.27	4671.81	TRUE	—	3.7%	2.5	4671.8	
39	19	1	H	100.0	15	5.00	—	—	—	50	300	—	2011.53	6034.59	TRUE	—	2.4%	5	6034.5	
40	20	2	H	100.0	15	5.00	—	—	—	50	300	—	1997.89	5993.07	TRUE	—	2.4%	5	5993.1	
41	21	3	H	100.0	15	5.00	—	—	—	50	300	—	1760.25	5290.75	TRUE	—	2.1%	5	5290.8	
42	22	1	H	100.0	30	10.00	—	—	—	50	300	—	2019.84	6059.52	TRUE	—	1.2%	10	6059.5	
43	23	2	H	100.0	30	10.00	—	—	—	50	300	—	2019.84	6059.52	TRUE	—	1.2%	10	7731.3	
44	24	3	H	100.0	30	10.00	—	—	—	50	300	—	2121.06	6383.18	TRUE	—	1.3%	10	6393.3	

	V
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	NSB y values
20	
21	247.4
22	247.4
23	247.4
24	299.2
25	299.2
26	299.2
27	301.5
28	301.5
29	301.5
30	315.7
31	315.7
32	315.7
33	408.8
34	408.8
35	408.8
36	657.0
37	657.0
38	657.0
39	1224.3
40	1224.3
41	1224.3
42	2074.4
43	2074.4
44	2074.4

	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
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	Transketone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume	
					(nM)	(µl)	(nM)	(µl)	(µl)	(nM)	(nM)	(µM)	(µl)	(µl)
19														
20														
21	1	1	H	C1	10.0	7.5	—	—	—	300	0.25	—	300	300
22	2	2	H	C1	10.0	7.5	—	—	—	300	0.25	—	300	300
23	3	3	H	C1	10.0	7.5	—	—	—	300	0.25	—	300	300
24	4	1	H	C2	10.0	15	—	—	—	300	0.50	—	300	300
25	5	2	H	C2	10.0	15	—	—	—	300	0.50	—	300	300
26	6	3	H	C2	10.0	15	—	—	—	300	0.50	—	300	300
27	7	1	H	C3	10.0	21	—	—	—	300	0.70	—	300	300
28	8	2	H	C3	10.0	21	—	—	—	300	0.70	—	300	300
29	9	3	H	C3	10.0	21	—	—	—	300	0.70	—	300	300
30	10	1	H	C4	10.0	30	—	—	—	300	1.00	—	300	300
31	11	2	H	C4	10.0	30	—	—	—	300	1.00	—	300	300
32	12	3	H	C4	10.0	30	—	—	—	300	1.00	—	300	300
33	13	1	H	C5	10.0	45	—	—	—	300	1.50	—	300	300
34	14	2	H	C5	10.0	45	—	—	—	300	1.50	—	300	300
35	15	3	H	C5	10.0	45	—	—	—	300	1.50	—	300	300
36	16	1	H	C6	100.0	7.5	—	—	—	300	2.50	—	300	300
37	17	2	H	C6	100.0	7.5	—	—	—	300	2.50	—	300	300
38	18	3	H	C6	100.0	7.5	—	—	—	300	2.50	—	300	300
39	19	1	H	C7	100.0	15	—	—	—	300	5.00	—	300	300
40	20	2	H	C7	100.0	15	—	—	—	300	5.00	—	300	300
41	21	3	H	C7	100.0	15	—	—	—	300	5.00	—	300	300
42	22	1	H	C8	100.0	30	—	—	—	300	10.00	—	300	300
43	23	2	H	C8	100.0	30	—	—	—	300	10.00	—	300	300
44	24	3	H	C8	100.0	30	—	—	—	300	10.00	—	300	300

	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
19	Non Specific Binding - Positions 25-48 radiolabeled R1881 plus 100 X Inert R1881 plus cytosol														
	Assay tube contents														
20	Tube Identification					Hot Comp. R1881 Initial					Cold R1881 Conc. Initial				
	Rep					Hot Conc. R1881 Initial					Cold R1881 Conc. Initial				
21	Tube Type Code					Hot Conc. R1881 Initial					Cold R1881 Conc. Initial				
	Comp. Code					Hot Conc. R1881 Initial					Cold R1881 Conc. Initial				
22	25	1	HC	c1		10.0	7.5	1.00	7.5	50	300	0.25	25	200.8	247.4
23	26	2	HC	c1		10.0	7.5	1.00	7.5	50	300	0.25	25	278.1	247.4
24	27	3	HC	c1		10.0	7.5	1.00	7.5	50	300	0.25	25	283.4	247.4
25	28	1	HC	c2		10.0	15	1.00	15	50	300	0.5	50	287.3	298.2
26	29	2	HC	c2		10.0	15	1.00	15	50	300	0.5	50	319.1	298.2
27	30	3	HC	c2		10.0	15	1.00	15	50	300	0.5	50	291.2	298.2
28	31	1	HC	c3		10.0	21	1.00	21	50	300	0.7	70	208.2	301.5
29	32	2	HC	c3		10.0	21	1.00	21	50	300	0.7	70	338.2	301.5
30	33	3	HC	c3		10.0	21	1.00	21	50	300	0.7	70	368.1	301.5
31	34	1	HC	c4		10.0	30	1.00	30	50	300	1	100	291.2	315.7
32	35	2	HC	c4		10.0	30	1.00	30	50	300	1	100	322.4	315.7
33	36	3	HC	c4		10.0	30	1.00	30	50	300	1	100	333.6	315.7
34	37	1	HC	c5		10.0	45	1.00	45	50	300	1.5	150	339.1	408.8
35	38	2	HC	c5		10.0	45	1.00	45	50	300	1.5	150	489.3	408.8
36	39	3	HC	c5		10.0	45	1.00	45	50	300	1.5	150	368.0	408.8
37	40	1	HC	c6		100.0	7.5	10.00	7.5	50	300	2.5	250	696.9	657.0
38	41	2	HC	c6		100.0	7.5	10.00	7.5	50	300	2.5	250	696.8	657.0
39	42	3	HC	c6		100.0	7.5	10.00	7.5	50	300	2.5	250	677.4	657.0
40	43	1	HC	c7		100.0	15	10.00	15	50	300	5	500	973.8	1224.3
41	44	2	HC	c7		100.0	15	10.00	15	50	300	5	500	1425.2	1224.3
42	45	3	HC	c7		100.0	15	10.00	15	50	300	5	500	1274.0	1224.3
43	46	1	HC	c8		100.0	30	10.00	30	50	300	10	1000	1872.2	2074.4
44	47	2	HC	c8		100.0	30	10.00	30	50	300	10	1000	2183.5	2074.4
45	48	3	HC	c8		100.0	30	10.00	30	50	300	10	1000	2167.3	2074.4

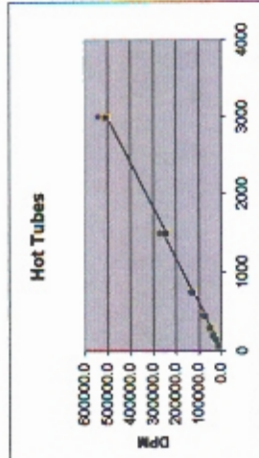
	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
13																	
14																	
15																	
16																	
17																	
18																	
19																	
20																	
21	2	49	1	Hot	c1												
22	2	50	2	Hot	c1												
23	2	51	3	Hot	c1												
24	2	52	1	Hot	c2												
25	2	53	2	Hot	c2												
26	2	54	3	Hot	c2												
27	2	55	1	Hot	c3												
28	2	56	2	Hot	c3												
29	2	57	3	Hot	c3												
30	2	58	1	Hot	c4												
31	2	59	2	Hot	c4												
32	2	60	3	Hot	c4												
33	2	61	1	Hot	c5												
34	2	62	2	Hot	c5												
35	2	63	3	Hot	c5												
36	2	64	1	Hot	c6												
37	2	65	2	Hot	c6												
38	2	66	3	Hot	c6												
39	2	67	1	Hot	c7												
40	2	68	2	Hot	c7												
41	2	69	3	Hot	c7												
42	2	70	1	Hot	c8												
43	2	71	2	Hot	c8												
44	2	72	3	Hot	c8												

Free - Positions 49-72, radiolabeled R1881 without cytosol

Run	Position	Rep	Tube Type Code	Comp. Code	Hot R1881 Conc. Initial (nM)	Hot R1881 Volume (ul)	Molecules of R1881 (fmole)	Counts per Reconstitution (dpm)	Experimental number of molecules (fmole)	Total Added (Mean of raps in pos. 49-72) (dpm)
21	49	1	Hot	c1	10	7.6	75	11822.0	70	11581.1
22	50	2	Hot	c1	10	7.6	75	11431.5	68	11581.1
23	51	3	Hot	c1	10	7.6	75	11489.9	68	11581.1
24	52	1	Hot	c2	10	16	150	23870.8	142	23906.2
25	53	2	Hot	c2	10	16	150	24239.9	144	23906.2
26	54	3	Hot	c2	10	16	150	23607.9	140	23906.2
27	55	1	Hot	c3	10	21	210	33491.3	199	33645.5
28	56	2	Hot	c3	10	21	210	34167.0	203	33645.5
29	57	3	Hot	c3	10	21	210	33278.0	198	33645.5
30	58	1	Hot	c4	10	30	300	47600.0	283	46966.6
31	59	2	Hot	c4	10	30	300	46225.8	275	46966.6
32	60	3	Hot	c4	10	30	300	47073.8	280	46966.6
33	61	1	Hot	c5	10	45	450	72467.8	431	72410.1
34	62	2	Hot	c5	10	45	450	73196.3	435	72410.1
35	63	3	Hot	c5	10	45	450	71676.3	426	72410.1
36	64	1	Hot	c6	100	7.5	750	124773.4	742	126935.2
37	65	2	Hot	c6	100	7.5	750	129430.2	769	126935.2
38	66	3	Hot	c6	100	7.5	750	126911.9	752	126935.2
39	67	1	Hot	c7	100	15	1500	257185.4	1528	254632.0
40	68	2	Hot	c7	100	15	1500	263519.7	1566	254632.0
41	69	3	Hot	c7	100	15	1500	243190.8	1445	254632.0
42	70	1	Hot	c8	100	30	3000	502389.9	2986	504551.7
43	71	2	Hot	c8	100	30	3000	506691.5	3011	504551.7
44	72	3	Hot	c8	100	30	3000	504673.8	2969	504551.7

predicted dpm

Computation Check
77705 specific activity data
81.00 Cfm/Mole 3H R1881
2.22E+12 DPM/C (definition)
1.7982E+14 DPM/mole
1.7982E+11 DPM/mole
179.8 DPM/mole
0.005561 fmole/CPM



	CF	CG	CH	CI	CJ	CK	CL	CM	CN	DJ	DK	DL	DM
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
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	specific bound/molar	total added dpm	total bound dpm	NBS dpm	Net Final Concentration (nM)				
21	2.40277E-11	0.12913	2.14695E-10	2.40277E-11	1286.2	11822.0	1543.6	200.8	0.25				
22	1.98112E-11	0.10411	2.14695E-10	1.98112E-11	1088.7	11431.5	1316.1	278.1	0.25				
23	2.16314E-11	0.11478	2.14695E-10	2.16314E-11	1168.9	11489.9	1414.3	263.4	0.25				
24	3.53508E-11	0.08788	4.4316E-10	3.53508E-11	1907.0	23870.8	2206.2	287.3	0.50				
25	3.86226E-11	0.06680	4.4316E-10	3.86226E-11	2083.5	24239.9	2382.7	319.1	0.50				
26	3.92426E-11	0.08851	4.4316E-10	3.92426E-11	2116.8	23607.9	2416.1	291.2	0.50				
27	5.02514E-11	0.08849	6.23702E-10	5.02514E-11	2710.8	33491.3	3012.3	208.2	0.70				
28	4.40028E-11	0.07674	6.23702E-10	4.40028E-11	2376.4	34167.0	2877.9	338.2	0.70				
29	4.74886E-11	0.08322	6.23702E-10	4.74886E-11	2581.8	33278.0	2863.3	358.1	0.70				
30	5.91307E-11	0.07339	8.70642E-10	5.91307E-11	3199.8	47600.0	3505.5	291.2	1.00				
31	6.00933E-11	0.07463	8.70642E-10	6.00933E-11	3239.8	46225.8	3555.5	322.4	1.00				
32	5.99176E-11	0.07444	8.70642E-10	5.99176E-11	3232.2	47073.8	3548.0	333.8	1.00				
33	6.77417E-11	0.05347	1.3423E-09	6.77417E-11	3654.3	72467.8	4063.1	339.1	1.50				
34	6.9736E-11	0.05513	1.3423E-09	6.9736E-11	3781.9	73188.3	4170.7	399.3	1.50				
35	7.1039E-11	0.05522	1.3423E-09	7.1039E-11	3832.2	71576.3	4241.0	388.0	1.50				
36	7.77368E-11	0.03435	2.35306E-09	7.77368E-11	4193.5	124773.4	4850.5	596.9	2.50				
37	7.72394E-11	0.03412	2.35306E-09	7.72394E-11	4166.7	129430.2	4823.7	686.8	2.50				
38	7.44237E-11	0.03284	2.35306E-09	7.44237E-11	4014.8	126601.9	4671.8	677.4	2.50				
39	8.91689E-11	0.01935	4.72024E-09	8.91689E-11	4810.3	257185.4	6034.8	973.8	5.00				
40	8.84002E-11	0.01918	4.72024E-09	8.84002E-11	4768.7	263519.7	5993.1	1426.2	5.00				
41	7.61966E-11	0.01627	4.72024E-09	7.61966E-11	4056.4	243190.8	5280.8	1274.0	5.00				
42	7.3875E-11	0.00799	9.35312E-09	7.3875E-11	3985.2	502389.9	6959.5	1872.2	10.00				
43	1.04876E-10	0.01139	9.35312E-09	1.04876E-10	5657.5	506591.5	7731.9	2183.5	10.00				
44	7.95041E-11	0.00981	9.35312E-09	7.95041E-11	4288.8	504673.8	6363.2	2167.3	10.00				

	DN	DO	DP
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			

KD molar 1.00E+09
molar to nM conversion 1.00E+09
Kd nM = 1.00E+09

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R	S	T	U
45	25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		66.92	200.76	TRUE				
46	26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		92.69	278.07	TRUE				
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		87.81	263.43	TRUE				
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7		95.77	287.31	TRUE				
49	29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7		106.35	319.05	TRUE				
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7		97.08	291.18	TRUE				
51	31	1	HC	10.0	21	0.7	1.00	21	70	60	300	C8		69.40	208.2	TRUE				
52	32	2	HC	10.0	21	0.7	1.00	21	70	60	300	C8		112.74	338.22	TRUE				
53	33	3	HC	10.0	21	0.7	1.00	21	70	60	300	C8		119.38	358.08	TRUE				
54	34	1	HC	10.0	30	1	1.00	30	100	100	300	C5		97.05	291.15	TRUE				
55	35	2	HC	10.0	30	1	1.00	30	100	100	300	C5		107.47	322.41	TRUE				
56	36	3	HC	10.0	30	1	1.00	30	100	100	300	C5		111.19	333.57	TRUE				
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4		113.03	339.09	TRUE				
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4		168.43	488.29	TRUE				
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4		129.34	368.02	TRUE				
60	40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		188.98	588.94	TRUE				
61	41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		232.25	698.75	TRUE				
62	42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3		225.81	677.43	TRUE				
63	43	1	HC	100.0	15	5	10.00	15	500	50	300	C2		324.81	973.83	TRUE				
64	44	2	HC	100.0	15	5	10.00	15	500	50	300	C2		475.05	1425.15	TRUE				
65	45	3	HC	100.0	15	5	10.00	15	500	50	300	C2		424.88	1274.04	TRUE				
66	46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1		624.08	1872.24	TRUE				
67	47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1		727.83	2183.49	TRUE				
68	48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1		722.44	2167.32	TRUE				
69	49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—		11822.0	11822.0	TRUE				
70	50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—		11431.5	11431.49	TRUE				
71	51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—		11489.9	11489.80	TRUE				
72	52	1	Hot	10.0	15	0.5	—	—	—	—	—	—		23870.8	23870.76	TRUE				
73	53	2	Hot	10.0	15	0.5	—	—	—	—	—	—		24238.9	24238.86	TRUE				
74	54	3	Hot	10.0	15	0.5	—	—	—	—	—	—		23607.9	23607.9	TRUE				
75	55	1	Hot	10.0	21	0.7	—	—	—	—	—	—		33491.3	33491.34	TRUE				
76	56	2	Hot	10.0	21	0.7	—	—	—	—	—	—		34167.0	34167.01	TRUE				
77	57	3	Hot	10.0	21	0.7	—	—	—	—	—	—		33278.0	33278	TRUE				
78	58	1	Hot	10.0	30	1	—	—	—	—	—	—		47600.0	47599.99	TRUE				
79	59	2	Hot	10.0	30	1	—	—	—	—	—	—		48225.8	48225.82	TRUE				
80	60	3	Hot	10.0	30	1	—	—	—	—	—	—		47073.8	47073.84	TRUE				
81	61	1	Hot	10.0	45	1.5	—	—	—	—	—	—		72467.8	72467.84	TRUE				
82	62	2	Hot	10.0	45	1.5	—	—	—	—	—	—		73186.3	73186.27	TRUE				
83	63	3	Hot	10.0	45	1.5	—	—	—	—	—	—		71578.3	71578.26	TRUE				
84	64	1	Hot	100.0	7.5	2.5	—	—	—	—	—	—		124773	124773.4	TRUE				
85	65	2	Hot	100.0	7.5	2.5	—	—	—	—	—	—		129430	129430.2	TRUE				
86	66	3	Hot	100.0	7.5	2.5	—	—	—	—	—	—		126902	126901.9	TRUE				
87	67	1	Hot	100.0	15	5	—	—	—	—	—	—		257185	257185.4	TRUE				
88	68	2	Hot	100.0	15	5	—	—	—	—	—	—		263520	263519.7	TRUE				
89	69	3	Hot	100.0	15	5	—	—	—	—	—	—		243191	243190.8	TRUE				
90	70	1	Hot	100.0	30	10	—	—	—	—	—	—		502390	502389.9	TRUE				
91	71	2	Hot	100.0	30	10	—	—	—	—	—	—		506592	506591.5	TRUE				
92	72	3	Hot	100.0	30	10	—	—	—	—	—	—		504674	504673.8	TRUE				

	V
45	
46	
47	
48	
49	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	
61	
62	
63	
64	
65	
66	
67	
68	
69	
70	
71	
72	
73	
74	
75	
76	
77	
78	
79	
80	
81	
82	
83	
84	
85	
86	
87	
88	
89	
90	
91	
92	

	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
45														
46														
47														
48														
49														
50														
51														
52														
53														
54														
55														
56														
57														
58														
59														
60														
61														
62														
63														
64														
65														
66														
67														
68														
69														
70														
71														
72														
73														
74														
75														
76														
77														
78														
79														
80														
81														
82														
83														
84														
85														
86														
87														
88														
89														
90														
91														
92														

	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY
45															
46															
47															
48															
49															
50															
51															
52															
53															
54															
55															
56															
57															
58															
59															
60															
61															
62															
63															
64															
65															
66															
67															
68															
69															
70															
71															
72															
73															
74															
75															
76															
77															
78															
79															
80															
81															
82															
83															
84															
85															
86															
87															
88															
89															
90															
91															
92															

	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
45															
46															
47															
48															
49															
50															
51															
52															
53															
54															
55															
56															
57															
58															
59															
60															
61															
62															
63															
64															
65															
66															
67															
68															
69															
70															
71															
72															
73															
74															
75															
76															
77															
78															
79															
80															
81															
82															
83															
84															
85															
86															
87															
88															
89															
90															
91															
92															

	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
45																	
46																	
47																	
48																	
49																	
50																	
51																	
52																	
53																	
54																	
55																	
56																	
57																	
58																	
59																	
60																	
61																	
62																	
63																	
64																	
65																	
66																	
67																	
68																	
69																	
70																	
71																	
72																	
73																	
74																	
75																	
76																	
77																	
78																	
79																	
80																	
81																	
82																	
83																	
84																	
85																	
86																	
87																	
88																	
89																	
90																	
91																	
92																	

Linear regression results (LINEST function)
(Regression line forced through 0,0)

Slope	168.254863	dpm/fmole
1/slope	0.00594301	fmole/dpm
origin	x	y
end point	3010.7	506591.5

SLOPE function used if missing HOT tubes

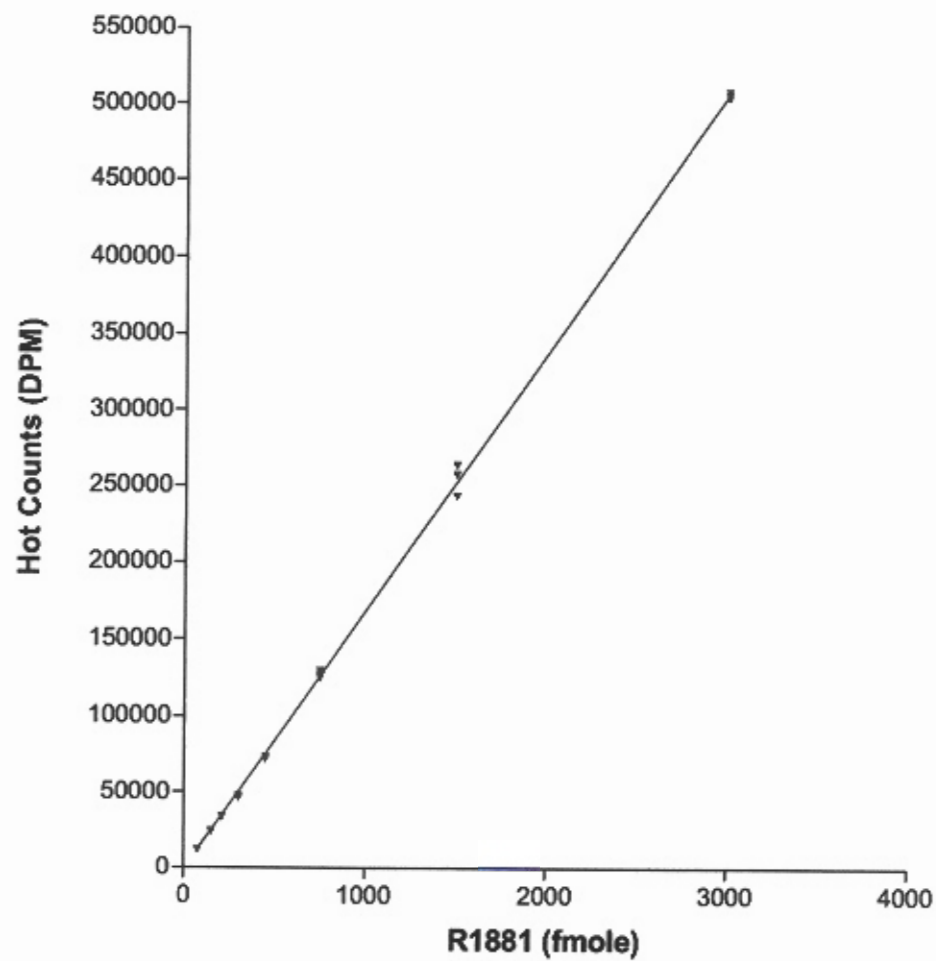
Slope	169.2	dpm/fmole
1/slope	0.005910	fmole/dpm
origin	x	y
end point	2994.0	506591.5

Hot tubes Predicted

	CF	CG	CH	CI	CJ	CK	CL	CM	CN	DJ	DK	DL	DM
45													
46													
47													
48													
49													
50													
51													
52													
53													
54													
55													
56													
57													
58													
59													
60													
61													
62													
63													
64													
65													
66													
67													
68													
69													
70													
71													
72													
73													
74													
75													
76													
77													
78													
79													
80													
81													
82													
83													
84													
85													
86													
87													
88													
89													
90													
91													
92													

	DN	DO	DP
45			
46			
47			
48			
49			
50			
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			
61			
62			
63			
64			
65			
66			
67			
68			
69			
70			
71			
72			
73			
74			
75			
76			
77			
78			
79			
80			
81			
82			
83			
84			
85			
86			
87			
88			
89			
90			
91			
92			

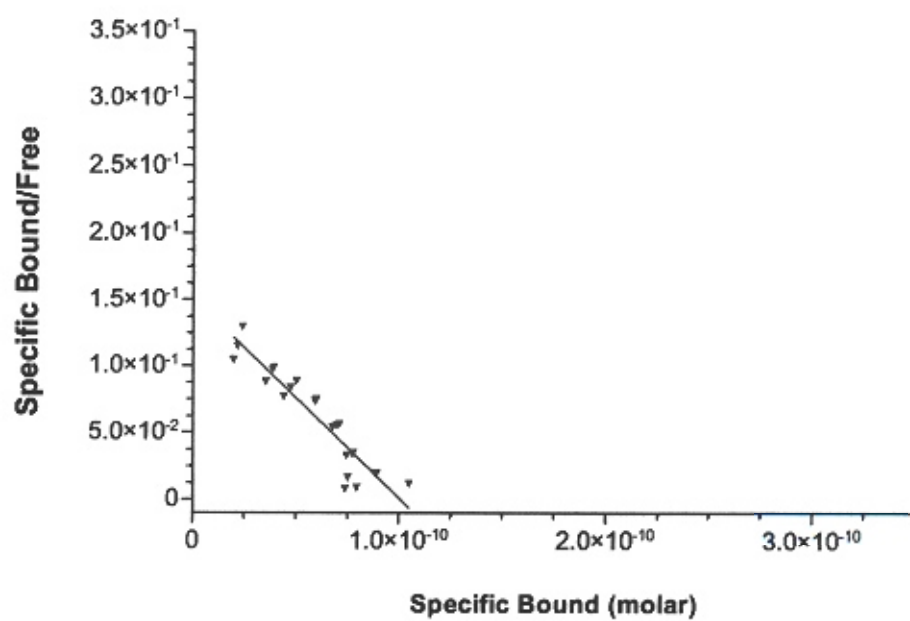
**Hot Tubes
Lab B run 2
0.6 mg/tube**



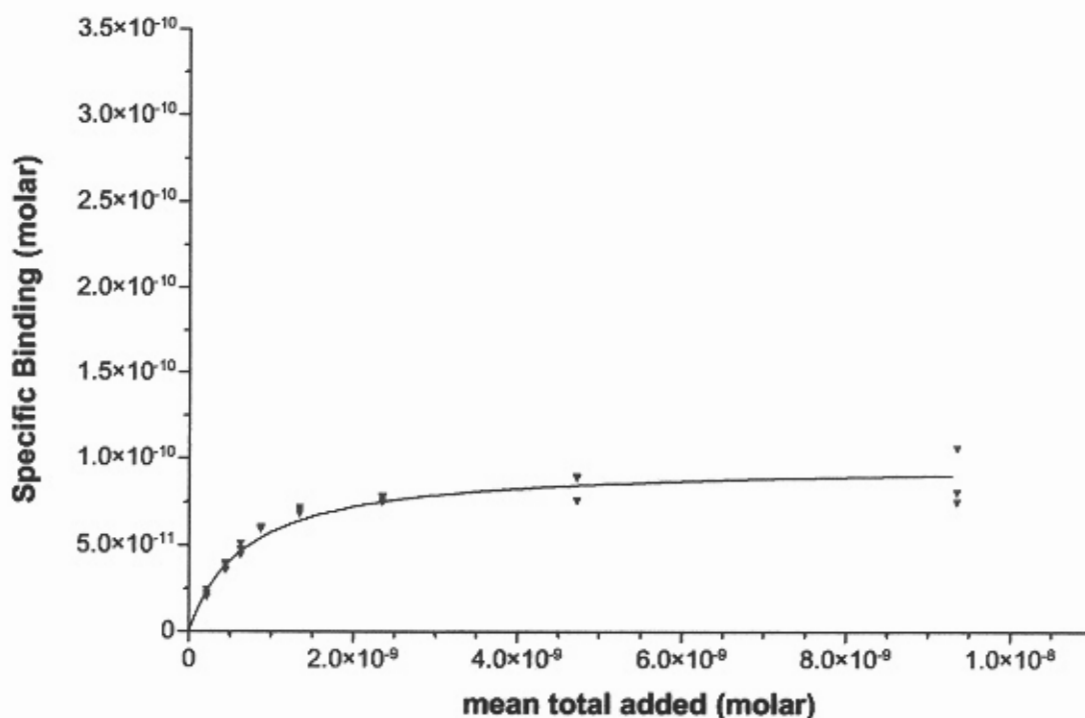
▼ B2-7/7/05

Scatchard Display
Lab B run 2
0.6 mg/tube

▼ B2-7/7/05



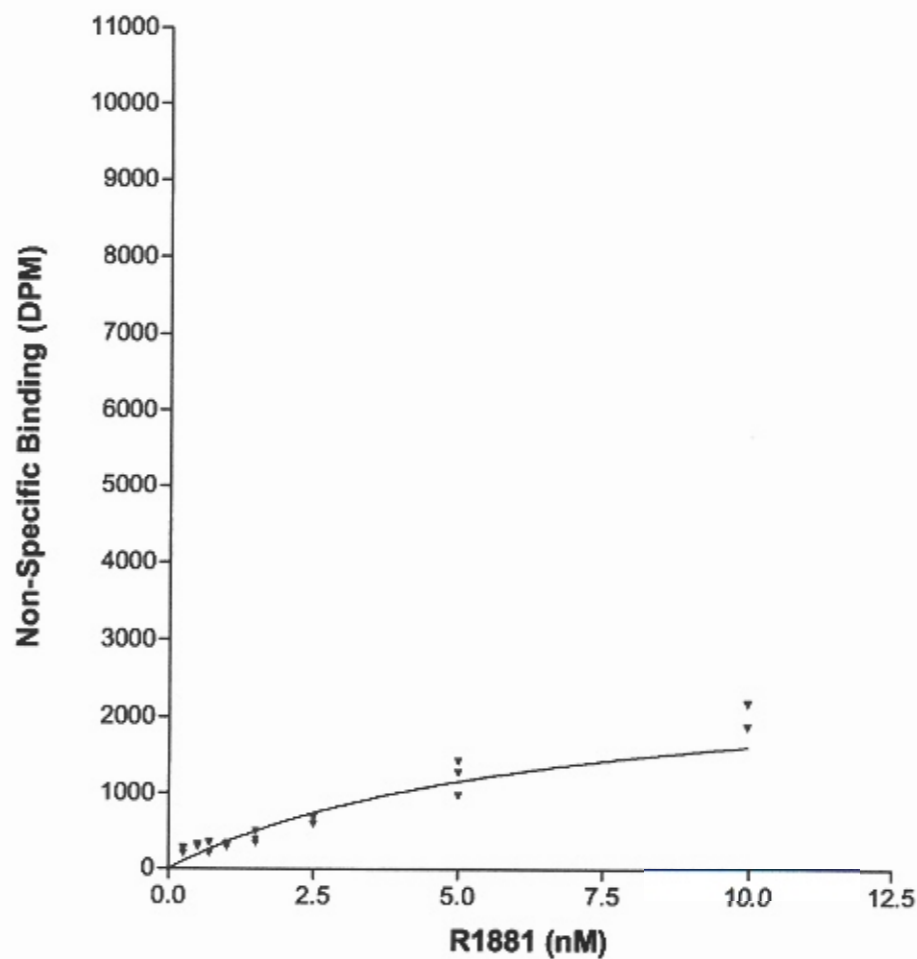
Lab B run 2 0.6 mg/tube



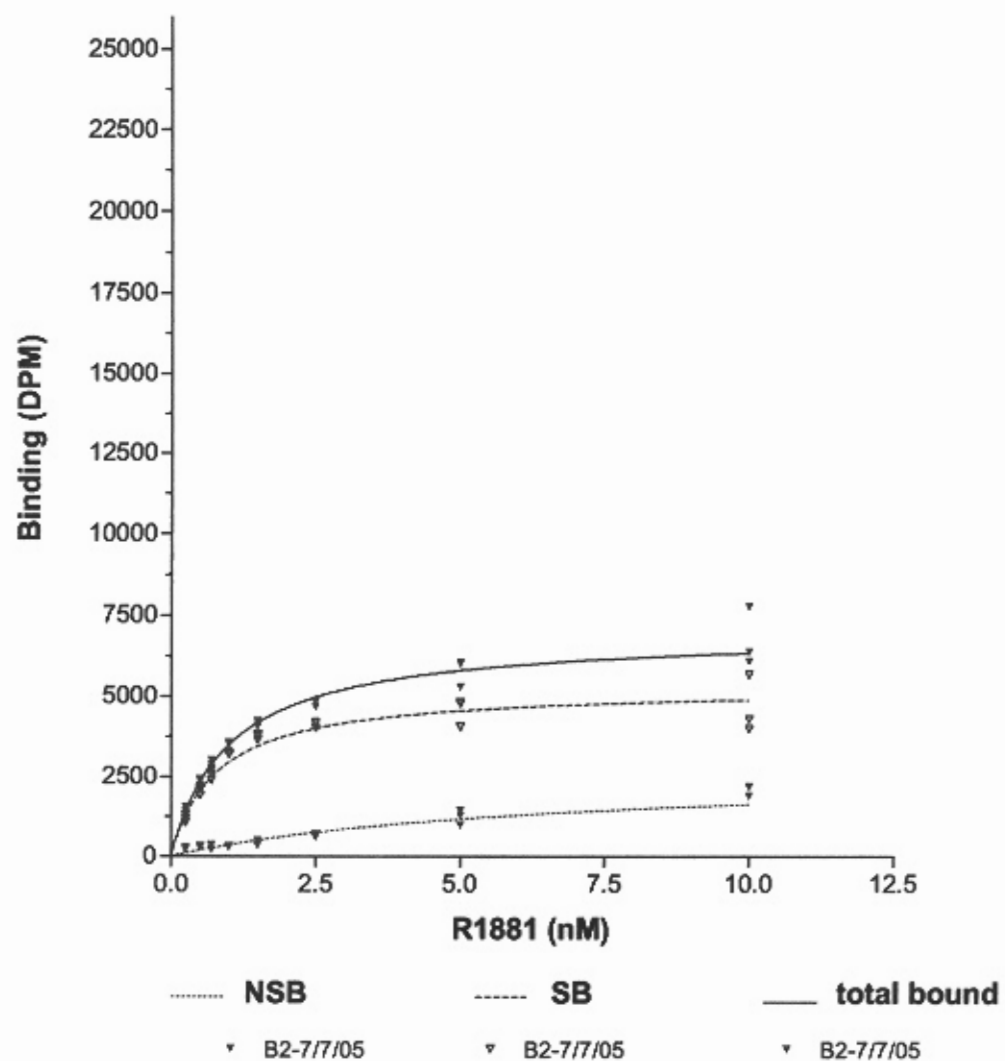
▼ B2-7/7/05

	B2-7/7/05
BMAX	9.621e-011
KD	6.773e-010
Std. Error	
BMAX	3.628e-012
KD	5.656e-011
95% Confidence Intervals	
BMAX	8.869e-011 to 1.037e-010
KD	5.600e-010 to 7.946e-010
Goodness of Fit	
Degrees of Freedom	22
R ² (unweighted)	0.9194
Weighted Sum of Squares (1/Y ²)	0.2036
Absolute Sum of Squares	9.630e-022
Sy.x	6.616e-012
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

NSB Tubes
Lab B run 2
0.6 mg/tube



bound counts
Lab B run 2
0.6 mg/tube



APPENDIX D

Processed Raw Data from Saturation Assay 3 and Associated Figures

Note: Summary figures for all three saturation assays and Bmax and Kd values (prepared by Battelle) are also included in this appendix.

Counts from Saturation Assay Number 3

Saturation Assay Tube Layout

Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Trisacetic Acid (uL)	Cytosol (uL)	Significant portion of label on Vial	dpm as counted	corrected DPM for 2mL
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	619.76	1859.28
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	571.03	1713.09
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	626.11	1878.33
4	1	H	10.0	15	0.50	—	—	—	50	300	—	631.06	2493.15
5	2	H	10.0	15	0.50	—	—	—	50	300	—	927.17	2781.51
6	3	H	10.0	15	0.50	—	—	—	50	300	—	826.00	2476
7	1	H	10.0	21	0.70	—	—	—	50	300	—	984.09	2952.27
8	2	H	10.0	21	0.70	—	—	—	50	300	—	1141.92	3425.76
9	3	H	10.0	21	0.70	—	—	—	50	300	—	1008.56	3025.66
10	1	H	10.0	30	1.00	—	—	—	50	300	—	1342.82	4028.46
11	2	H	10.0	30	1.00	—	—	—	50	300	—	1300.26	3900.76
12	3	H	10.0	30	1.00	—	—	—	50	300	—	1144.12	3432.36
13	1	H	10.0	45	1.50	—	—	—	50	300	—	1327.46	3982.36
14	2	H	10.0	45	1.50	—	—	—	50	300	—	1558.25	4674.75
15	3	H	10.0	45	1.50	—	—	—	50	300	—	1448.13	4344.39
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	2023.80	6071.4
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	1887.78	5663.34
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	1899.22	5697.66
19	1	H	100.0	15	5.00	—	—	—	50	300	—	2476.28	7428.84
20	2	H	100.0	15	5.00	—	—	—	50	300	—	1987.80	5963.4
21	3	H	100.0	15	5.00	—	—	—	50	300	—	1988.77	5966.31
22	1	H	100.0	30	10.00	—	—	—	50	300	—	2602.10	7806.3
23	2	H	100.0	30	10.00	—	—	—	50	300	—	2157.31	6471.93
24	3	H	100.0	30	10.00	—	—	—	50	300	—	2939.89	8819.87
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	77.57	232.71
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	84.74	284.22
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	78.37	235.11
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	107.20	321.6
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	104.33	312.98
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	136.51	409.53
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	112.32	336.96
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	98.66	295.98
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	160.87	452.01
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	192.66	577.98
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	220.04	660.12

	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R	S	T	U
1	Laboratory B																			
2	AR Saturation Assay (cold R1881 and WP supplied by Sequim)																			
3	72 assay tubes																			
4	Please return by email to n.a.Holler@pnl.gov																			
5	Provide information in all blue cells in columns O and DK																			
6	If the DPM value for a tube was judged unreliable,																			
7	Include the DPM value in column O																			
8	Provide a reason in column R																			
9	The value in column Q will automatically change to FALSE																			
10	For your convenience, data reduction is performed in columns																			
11	U through RZ, and the values needed for analysis are presented																			
12	in columns CF through CN																			
13	Cells in column S are presented with a grey background																			
14	if the total binding exceeds 10% of the hot added at that concentration,																			
15	the cytosol concentration is probably too high for good competitive assays																			
16																				
17																				
18																				
19	Saturation Assay Tube Layout																			
	Position	Replicate	Tube Type	Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (nM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triacetone Acetate (uL)	Cytosol (uL)	Significant portion of label on Vial	dpm as counted	corrected DPM for 2mL	Use this value?	Notes to explain why "Use this value" is set to "FALSE"	Ten Percent Rule	Saturation X value	Bound Y value
20	1	1	H		10.0	7.5	0.25				50	300		819.76	1859.28	TRUE		15.8%	0.25	1859.3
21	2	2	H		10.0	7.5	0.25				50	300		571.03	1713.09	TRUE		14.5%	0.25	1713.1
22	3	3	H		10.0	7.5	0.25				50	300		628.11	1878.33	TRUE		15.8%	0.25	1878.3
23	4	1	H		10.0	15	0.50				50	300		831.05	2483.15	TRUE		10.3%	0.5	2483.2
24	5	2	H		10.0	15	0.50				50	300		927.17	2781.51	TRUE		11.5%	0.5	2781.5
25	6	3	H		10.0	15	0.50				50	300		828.00	2478	TRUE		10.2%	0.5	2478.0
26	7	1	H		10.0	21	0.70				50	300		984.09	2952.27	TRUE		8.6%	0.7	2952.3
27	8	2	H		10.0	21	0.70				50	300		1141.82	3425.76	TRUE		10.3%	0.7	3425.8
28	9	3	H		10.0	21	0.70				50	300		1008.58	3025.68	TRUE		8.1%	0.7	3025.7
29	10	1	H		10.0	30	1.00				50	300		1342.82	4028.46	TRUE		8.6%	1	4028.5
30	11	2	H		10.0	30	1.00				50	300		1300.28	3900.78	TRUE		8.3%	1	3900.8
31	12	3	H		10.0	30	1.00				50	300		1144.12	3432.36	TRUE		7.3%	1	3432.4
32	13	1	H		10.0	45	1.50				50	300		1327.48	3982.38	TRUE		5.5%	1.5	3982.4
33	14	2	H		10.0	45	1.50				50	300		1558.25	4874.75	TRUE		6.4%	1.5	4874.8
34	15	3	H		10.0	45	1.50				50	300		1448.13	4344.39	TRUE		6.0%	1.5	4344.4
35	16	1	H		10.0	7.5	2.50				50	300		2023.80	6071.4	TRUE		5.0%	2.5	6071.4
36	17	2	H		10.0	7.5	2.50				50	300		1887.78	5663.34	TRUE		4.6%	2.5	5663.3
37	18	3	H		10.0	7.5	2.50				50	300		1899.22	5687.66	TRUE		4.7%	2.5	5687.7
38	19	1	H		10.0	15	5.00				50	300		2478.28	7428.84	TRUE		3.0%	5	7428.8
39	20	2	H		10.0	15	5.00				50	300		1887.80	5663.4	TRUE		2.4%	5	5663.4
40	21	3	H		10.0	15	5.00				50	300		1988.77	5968.31	TRUE		2.4%	5	5968.3
41	22	1	H		10.0	30	10.00				50	300		2802.10	7806.3	TRUE		1.6%	10	7806.3
42	23	2	H		10.0	30	10.00				50	300		2157.31	6471.83	TRUE		1.3%	10	6471.8
43	24	3	H		10.0	30	10.00				50	300		2939.89	8819.87	TRUE		1.8%	10	8819.7

	V
1	
2	
3	
4	
5	
6	
7	
8	
9	
10	
11	
12	
13	
14	
15	
16	
17	
18	
19	NSB y values
20	
21	250.7
22	250.7
23	250.7
24	348.0
25	348.0
26	348.0
27	361.7
28	361.7
29	361.7
30	644.4
31	644.4
32	644.4
33	628.0
34	628.0
35	628.0
36	1144.5
37	1144.5
38	1144.5
39	953.7
40	953.7
41	953.7
42	2092.7
43	2092.7
44	2092.7

	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														
Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)														
Tube Identification					Assay tube contents									
Run	Position	Tube Type Code	Conc. Code	Hot Conc. Initial (µM)	Hot R1881 Volume (µl)	Cold R1881 Conc. Initial (mM)	Cold R1881 volume (µl)	Trisacetone Acetate (µl)	Cytosol (µl)	Hot Conc. Final (µM)	Cold Conc. Final (µM)	Total Volume (µl)		
20	1	H	C1	10.0	7.5	—	—	—	300	0.25	—	300		
21	2	H	C1	10.0	7.5	—	—	—	300	0.25	—	300		
22	3	H	C1	10.0	7.5	—	—	—	300	0.25	—	300		
23	4	H	C2	10.0	15	—	—	—	300	0.50	—	300		
24	5	H	C2	10.0	15	—	—	—	300	0.50	—	300		
25	6	H	C2	10.0	15	—	—	—	300	0.50	—	300		
26	7	H	C3	10.0	21	—	—	—	300	0.70	—	300		
27	8	H	C3	10.0	21	—	—	—	300	0.70	—	300		
28	9	H	C3	10.0	21	—	—	—	300	0.70	—	300		
29	10	H	C4	10.0	30	—	—	—	300	1.00	—	300		
30	11	H	C4	10.0	30	—	—	—	300	1.00	—	300		
31	12	H	C4	10.0	30	—	—	—	300	1.00	—	300		
32	13	H	C5	10.0	45	—	—	—	300	1.50	—	300		
33	14	H	C5	10.0	45	—	—	—	300	1.50	—	300		
34	15	H	C5	10.0	45	—	—	—	300	1.50	—	300		
35	16	H	C6	10.0	7.5	—	—	—	300	2.50	—	300		
36	17	H	C6	10.0	7.5	—	—	—	300	2.50	—	300		
37	18	H	C6	10.0	7.5	—	—	—	300	2.50	—	300		
38	19	H	C7	10.0	15	—	—	—	300	5.00	—	300		
39	20	H	C7	10.0	15	—	—	—	300	5.00	—	300		
40	21	H	C7	10.0	15	—	—	—	300	5.00	—	300		
41	22	H	C8	10.0	30	—	—	—	300	10.00	—	300		
42	23	H	C8	10.0	30	—	—	—	300	10.00	—	300		
43	24	H	C8	10.0	30	—	—	—	300	10.00	—	300		
44														

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)	Ratio
AK	AL	AM	AN	AO	AP	AQ	AR	AS	Number of molecules				Ratio	
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1	1859.3	250.7	1608.6	13.5%	15.8%	11798.5	9039.2	11	2	10	72	61	0.16
2	2	1713.1	250.7	1462.4	14.6%	14.5%	11798.5	10085.4	10	2	8	72	62	0.15
3	3	1878.3	250.7	1627.7	13.3%	15.9%	11798.5	9920.1	11	2	10	72	61	0.16
4	4	2493.2	348.0	2145.1	14.0%	10.3%	24218.7	21725.6	15	2	13	148	133	0.10
5	5	2781.5	348.0	2433.5	12.5%	11.5%	24218.7	21437.2	17	2	15	148	131	0.11
6	6	2478.0	348.0	2130.0	14.0%	10.2%	24218.7	21740.7	16	2	13	148	133	0.10
7	7	2952.3	361.7	2590.6	12.2%	8.8%	33375.1	30422.9	18	2	16	204	186	0.09
8	8	3425.8	361.7	3064.1	10.6%	10.3%	33375.1	29949.4	21	2	19	204	183	0.10
9	9	3025.7	361.7	2664.0	12.0%	9.1%	33375.1	30349.4	18	2	16	204	185	0.09
10	10	4028.5	644.4	3384.1	16.0%	8.6%	46847.4	42916.9	25	4	21	286	262	0.08
11	11	3900.8	644.4	3256.4	16.5%	8.3%	46847.4	42946.6	24	4	20	286	282	0.08
12	12	3432.4	644.4	2788.0	18.8%	7.3%	46847.4	43415.0	21	4	17	286	265	0.06
13	13	3982.4	626.0	3356.3	15.7%	5.5%	72574.7	68592.3	24	4	21	443	419	0.05
14	14	4674.8	626.0	4048.7	13.4%	6.4%	72574.7	67999.9	29	4	25	443	415	0.06
15	15	4344.4	626.0	3718.4	14.4%	6.0%	72574.7	68230.3	27	4	23	443	417	0.05
16	16	6071.4	1144.5	4926.9	18.9%	5.0%	122292.0	116220.6	37	7	30	747	710	0.04
17	17	5683.3	1144.5	4518.9	20.2%	4.6%	122292.0	116528.7	35	7	28	747	713	0.04
18	18	5697.7	1144.5	4553.2	20.1%	4.7%	122292.0	116594.3	35	7	28	747	712	0.04
19	19	7428.8	953.7	6475.2	12.8%	3.0%	246146.2	238716.3	45	6	40	1504	1459	0.03
20	20	5963.4	953.7	5009.7	16.0%	2.4%	246146.2	240181.8	36	6	31	1504	1468	0.02
21	21	5986.3	953.7	5012.7	16.0%	2.4%	246146.2	240178.9	36	6	31	1504	1468	0.02
22	22	7806.3	2092.7	5713.6	26.8%	1.6%	491205.1	453396.8	48	13	35	3002	2954	0.01
23	23	6471.9	2092.7	4379.2	32.3%	1.3%	491205.1	464733.2	40	13	27	3002	2962	0.01
24	24	8819.7	2092.7	6727.0	23.7%	1.8%	491205.1	482386.5	54	13	41	3002	2948	0.01

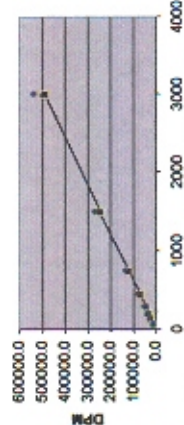
	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
1															
2															
3															
4															
5															
6															
7															
8															
9															
10															
11															
12															
13															
14															
15															
16															
17															
18															
Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X Inert R1881 plus Cytosol															
Assay tube contents															
Scintillation Results															
Tube Identification															
Counts per Scintillation															
Non Specific Binding (Mean of reps in pos. 25-48)															
Run	Position	Rep	Tube Type Code	Comp. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triacetone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final			
20															
21	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	232.7	250.7	
22	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	284.2	250.7	
23	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	235.1	250.7	
24	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	321.6	348.0	
25	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	313.0	348.0	
26	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	409.5	348.0	
27	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	337.0	361.7	
28	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	298.0	361.7	
29	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	452.0	361.7	
30	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	578.0	644.4	
31	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	660.1	644.4	
32	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	695.0	644.4	
33	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	503.8	626.0	
34	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	952.1	626.0	
35	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	422.3	626.0	
36	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1051.3	1144.5	
37	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1633.1	1144.5	
38	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	749.1	1144.5	
39	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	1121.0	953.7	
40	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	851.0	953.7	
41	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	959.0	953.7	
42	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	2126.6	2092.7	
43	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	2032.6	2092.7	
44	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	2118.9	2092.7	

	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
1																	
2																	
3																	
4																	
5																	
6																	
7																	
8																	
9																	
10																	
11																	
12																	
13																	
14																	
15																	
16																	
17																	
18																	
19																	
20																	
21	3	49	1	Hot	c1	10	7.5	75	11607.9	Counts per Scintillation	Experimental number of molecules	Total Added (Mean of raps in pos. 49-72)	13478				
22	3	50	2	Hot	c1	10	7.5	75	12119.6				13478				
23	3	51	3	Hot	c1	10	7.5	75	11767.9				13478				
24	3	52	1	Hot	c2	10	15	150	24535.5				26956				
25	3	53	2	Hot	c2	10	15	150	24040.0				26956				
26	3	54	3	Hot	c2	10	15	150	24090.7				26956				
27	3	55	1	Hot	c3	10	21	210	34443.7				37738				
28	3	56	2	Hot	c3	10	21	210	32884.6				37738				
29	3	57	3	Hot	c3	10	21	210	32797.1				37738				
30	3	58	1	Hot	c4	10	30	300	47424.3				53912				
31	3	59	2	Hot	c4	10	30	300	46712.8				53912				
32	3	60	3	Hot	c4	10	30	300	46406.0				53912				
33	3	61	1	Hot	c5	10	45	450	74221.4				80867				
34	3	62	2	Hot	c5	10	45	450	71932.2				80867				
35	3	63	3	Hot	c5	10	45	450	71670.4				80867				
36	3	64	1	Hot	c6	100	7.5	750	122261.5				134779				
37	3	65	2	Hot	c6	100	7.5	750	122314.0				134779				
38	3	66	3	Hot	c6	100	7.5	750	122300.5				134779				
39	3	67	1	Hot	c7	100	15	1500	246933.1				269568				
40	3	68	2	Hot	c7	100	15	1500	244033.6				269568				
41	3	69	3	Hot	c7	100	15	1500	244707.8				269568				
42	3	70	1	Hot	c8	100	30	3000	489302.5				539116				
43	3	71	2	Hot	c8	100	30	3000	486783.5				539116				
44	3	72	3	Hot	c8	100	30	3000	486519.4				539116				

predicted dpm

Computation Check
771103 specific activity data
80.94 Ctr/Mole 3H R1881
2.22E+12 DPM/Ci (definition)
1.7971E+14 DPM/mole
1.7971E+11 DPM/mole
179.7 DPM/mole
0.005595 fmoles/DPM

Hot Tubes



	CF	CG	CH	CI	CJ	CK	CL	CM	CN	DJ	DK	DL	DM
1													
2													
3													
4													
5													
6													
7													
8													
9													
10													
11													
12													
13													
14													
15													
16													
17													
18													
19	Prism input for bound/free				Prism input for specific bound								
20	specific bound/mole	bound/free	average total added molar	specific bound/mole	specific bound/dpm	total added dpm	total bound dpm	NBS dpm	Hot Final Concentration (nM)				
21	2.98377E-11	0.16184	2.18848E-10	2.98377E-11	1608.6	11507.9	1859.3	232.7	0.25				
22	2.71261E-11	0.14600	2.18848E-10	2.71261E-11	1462.4	12119.6	1713.1	284.2	0.25				
23	3.01911E-11	0.16408	2.18848E-10	3.01911E-11	1627.7	11767.9	1878.3	235.1	0.25				
24	3.97894E-11	0.09874	4.4923E-10	3.97894E-11	2145.1	24535.5	2493.2	321.6	0.50				
25	4.51381E-11	0.11352	4.4923E-10	4.51381E-11	2433.5	24040.0	2781.5	313.0	0.50				
26	3.96084E-11	0.09797	4.4923E-10	3.96084E-11	2130.0	24080.7	2478.0	409.5	0.50				
27	4.80531E-11	0.08515	6.19071E-10	4.80531E-11	2590.6	34443.7	2952.3	337.0	0.70				
28	5.83558E-11	0.10231	6.19071E-10	5.83558E-11	3064.1	32884.6	3425.8	296.0	0.70				
29	4.94148E-11	0.08778	6.19071E-10	4.94148E-11	2894.0	32797.1	3025.7	452.0	0.70				
30	6.27714E-11	0.07903	8.68966E-10	6.27714E-11	3394.1	47424.3	4028.5	578.0	1.00				
31	6.04031E-11	0.07583	8.68966E-10	6.04031E-11	3256.4	46712.8	3900.8	660.1	1.00				
32	5.17145E-11	0.06422	8.68966E-10	5.17145E-11	2788.0	46406.0	3432.4	695.0	1.00				
33	6.22663E-11	0.04893	1.34618E-09	6.22663E-11	3366.3	74221.4	3982.4	503.8	1.50				
34	7.50995E-11	0.05963	1.34618E-09	7.50995E-11	4048.7	71832.2	4674.8	962.1	1.50				
35	6.89712E-11	0.05450	1.34618E-09	6.89712E-11	3718.4	71670.4	4344.4	422.3	1.50				
36	9.13888E-11	0.04239	2.26839E-09	9.13888E-11	4826.9	122281.5	8071.4	1051.3	2.50				
37	8.38198E-11	0.03876	2.26839E-09	8.38198E-11	4518.9	122314.0	5663.3	1633.1	2.50				
38	8.44564E-11	0.03906	2.26839E-09	8.44564E-11	4553.2	122300.5	5697.7	749.1	2.50				
39	1.20107E-10	0.02712	4.56572E-09	1.20107E-10	6475.2	249931.1	7428.8	1121.0	5.00				
40	9.28251E-11	0.02066	4.56572E-09	9.28251E-11	6009.7	244033.6	5963.4	861.0	5.00				
41	9.29795E-11	0.02067	4.56572E-09	9.29795E-11	5012.7	244470.8	5963.4	869.0	5.00				
42	1.05981E-10	0.01182	9.1113E-09	1.05981E-10	5713.6	489302.5	7806.3	2126.6	10.00				
43	8.123E-11	0.00903	9.1113E-09	8.123E-11	4379.2	495793.5	6471.9	2032.6	10.00				
44	1.24778E-10	0.01395	9.1113E-09	1.24778E-10	6727.0	486518.4	8818.7	2118.9	10.00				

	DN	DO	DP
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			

KD molar
molar to nM conversion
6.60E-10
1.00E+09
6.60E-01

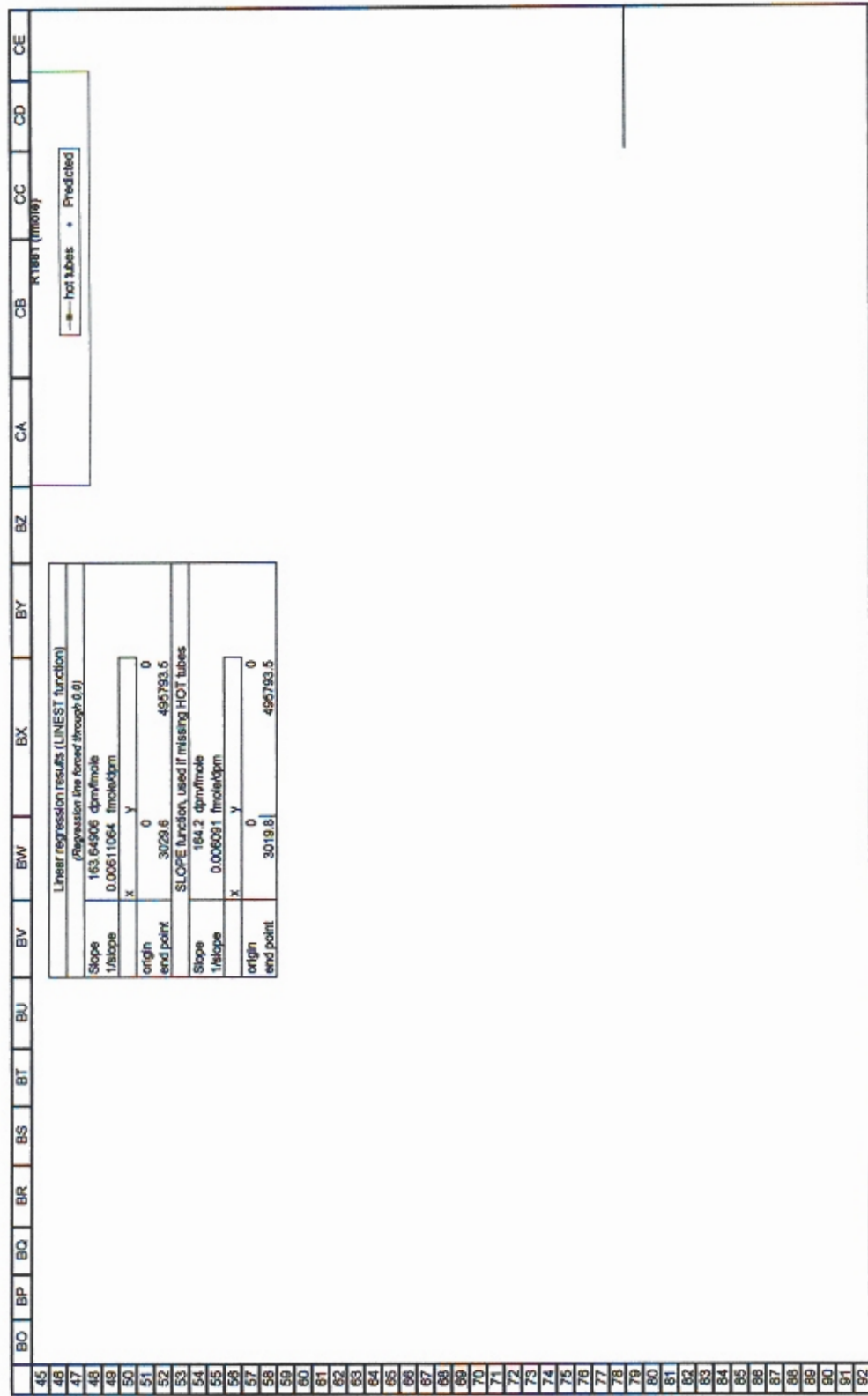
	A	B	C	D	E	F	G	H	I	J	K	L	N	O	P	Q	R	S	T	U
45	26	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		77.57	232.71	TRUE				
46	28	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		94.74	284.22	TRUE				
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8		78.37	235.11	TRUE				
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7		107.20	321.6	TRUE				
49	28	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7		104.33	312.99	TRUE				
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7		138.51	408.53	TRUE				
51	31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C8		112.32	338.96	TRUE				
52	32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C8		98.66	285.98	TRUE				
53	33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C8		150.87	452.01	TRUE				
54	34	1	HC	10.0	30	1	1.00	30	100	50	300	C5		192.66	577.98	TRUE				
55	36	2	HC	10.0	30	1	1.00	30	100	50	300	C5		220.04	660.12	TRUE				
56	38	3	HC	10.0	30	1	1.00	30	100	50	300	C5		231.65	694.95	TRUE				
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4		167.92	503.76	TRUE				
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4		317.37	952.11	TRUE				
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4		140.75	422.25	TRUE				
60	40	1	HC	10.0	7.5	2.5	10.00	7.5	250	50	300	C3		350.44	1051.32	TRUE				
61	41	2	HC	10.0	7.5	2.5	10.00	7.5	250	50	300	C3		544.35	1633.05	TRUE				
62	42	3	HC	10.0	7.5	2.5	10.00	7.5	250	50	300	C3		249.89	749.07	TRUE				
63	43	1	HC	10.0	15	5	10.00	15	500	50	300	C2		373.66	1120.98	TRUE				
64	44	2	HC	10.0	15	5	10.00	15	500	50	300	C2		283.69	850.98	TRUE				
65	45	3	HC	10.0	15	5	10.00	15	500	50	300	C2		298.34	889.02	TRUE				
66	46	1	HC	10.0	30	10	10.00	30	1000	50	300	C1		708.85	2126.55	TRUE				
67	47	2	HC	10.0	30	10	10.00	30	1000	50	300	C1		677.53	2032.59	TRUE				
68	48	3	HC	10.0	30	10	10.00	30	1000	50	300	C1		703.31	2118.93	TRUE				
69	49	1	Hot	10.0	7.5	0.25	—	—	—	—	—	—		11507.9	11507.95	TRUE				
70	50	2	Hot	10.0	7.5	0.25	—	—	—	—	—	—		12119.6	12119.62	TRUE				
71	51	3	Hot	10.0	7.5	0.25	—	—	—	—	—	—		11787.9	11787.89	TRUE				
72	52	1	Hot	10.0	15	0.5	—	—	—	—	—	—		24535.5	24535.51	TRUE				
73	53	2	Hot	10.0	15	0.5	—	—	—	—	—	—		24040.0	24039.97	TRUE				
74	54	3	Hot	10.0	15	0.5	—	—	—	—	—	—		24080.7	24080.71	TRUE				
75	55	1	Hot	10.0	21	0.7	—	—	—	—	—	—		34443.7	34443.69	TRUE				
76	56	2	Hot	10.0	21	0.7	—	—	—	—	—	—		32884.5	32884.62	TRUE				
77	57	3	Hot	10.0	21	0.7	—	—	—	—	—	—		32787.1	32787.07	TRUE				
78	58	1	Hot	10.0	30	1	—	—	—	—	—	—		47424.3	47424.27	TRUE				
79	59	2	Hot	10.0	30	1	—	—	—	—	—	—		48712.8	48712.79	TRUE				
80	60	3	Hot	10.0	30	1	—	—	—	—	—	—		48405.0	48405.04	TRUE				
81	61	1	Hot	10.0	45	1.5	—	—	—	—	—	—		74221.4	74221.39	TRUE				
82	62	2	Hot	10.0	45	1.5	—	—	—	—	—	—		71832.2	71832.21	TRUE				
83	63	3	Hot	10.0	45	1.5	—	—	—	—	—	—		71670.4	71670.36	TRUE				
84	64	1	Hot	10.0	7.5	2.5	—	—	—	—	—	—		122292	122291.5	TRUE				
85	65	2	Hot	10.0	7.5	2.5	—	—	—	—	—	—		122314	122314	TRUE				
86	66	3	Hot	10.0	7.5	2.5	—	—	—	—	—	—		122301	122300.5	TRUE				
87	67	1	Hot	10.0	15	5	—	—	—	—	—	—		249831	249831.1	TRUE				
88	68	2	Hot	10.0	15	5	—	—	—	—	—	—		244034	244033.6	TRUE				
89	69	3	Hot	10.0	15	5	—	—	—	—	—	—		244471	244470.8	TRUE				
90	70	1	Hot	10.0	30	10	—	—	—	—	—	—		489303	489302.5	TRUE				
91	71	2	Hot	10.0	30	10	—	—	—	—	—	—		495794	495793.5	TRUE				
92	72	3	Hot	10.0	30	10	—	—	—	—	—	—		489519	489519.4	TRUE				

	V
45	
46	
47	
48	
49	
50	
51	
52	
53	
54	
55	
56	
57	
58	
59	
60	
61	
62	
63	
64	
65	
66	
67	
68	
69	
70	
71	
72	
73	
74	
75	
76	
77	
78	
79	
80	
81	
82	
83	
84	
85	
86	
87	
88	
89	
90	
91	
92	

	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
45														
46														
47														
48														
49														
50														
51														
52														
53														
54														
55														
56														
57														
58														
59														
60														
61														
62														
63														
64														
65														
66														
67														
68														
69														
70														
71														
72														
73														
74														
75														
76														
77														
78														
79														
80														
81														
82														
83														
84														
85														
86														
87														
88														
89														
90														
91														
92														

	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY
45															
46															
47															
48															
49															
50															
51															
52															
53															
54															
55															
56															
57															
58															
59															
60															
61															
62															
63															
64															
65															
66															
67															
68															
69															
70															
71															
72															
73															
74															
75															
76															
77															
78															
79															
80															
81															
82															
83															
84															
85															
86															
87															
88															
89															
90															
91															
92															

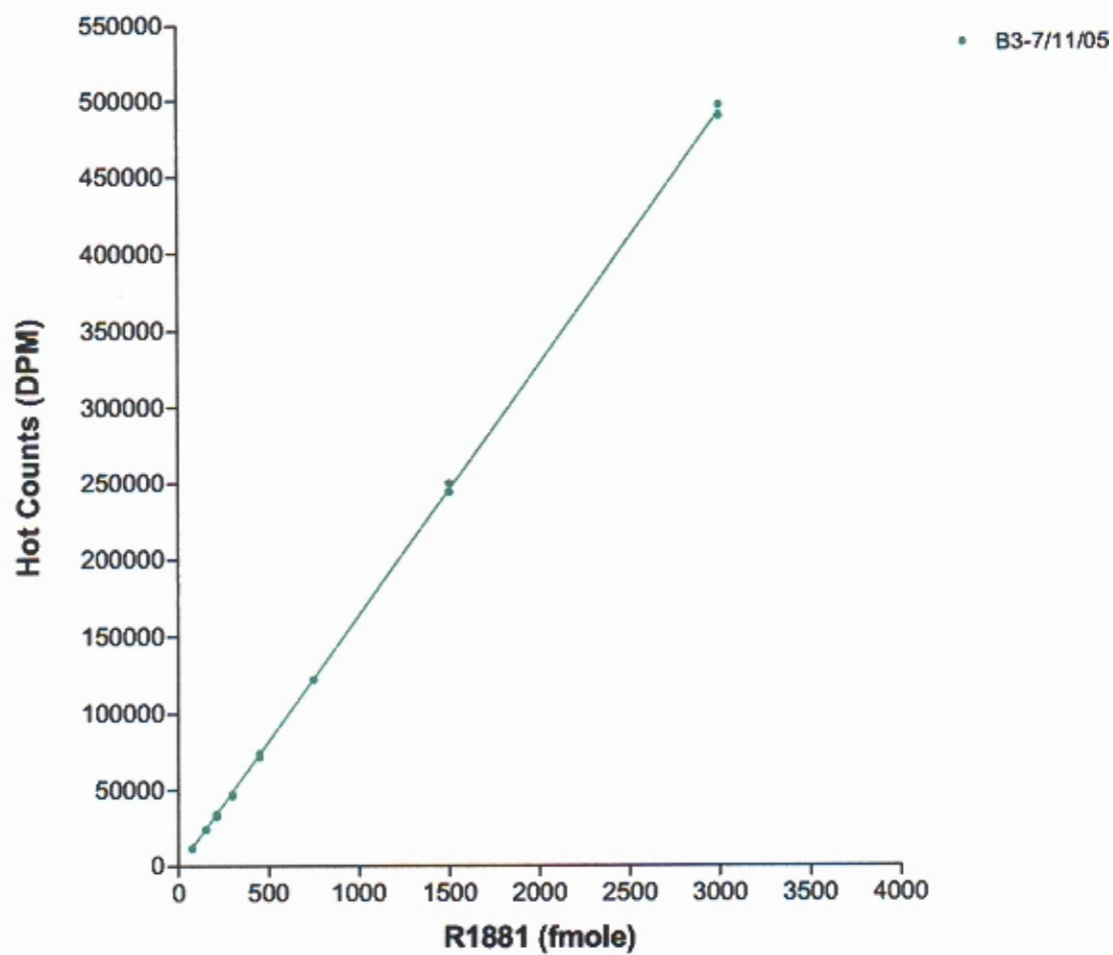
	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
45															
46															
47															
48															
49															
50															
51															
52															
53															
54															
55															
56															
57															
58															
59															
60															
61															
62															
63															
64															
65															
66															
67															
68															
69															
70															
71															
72															
73															
74															
75															
76															
77															
78															
79															
80															
81															
82															
83															
84															
85															
86															
87															
88															
89															
90															
91															
92															



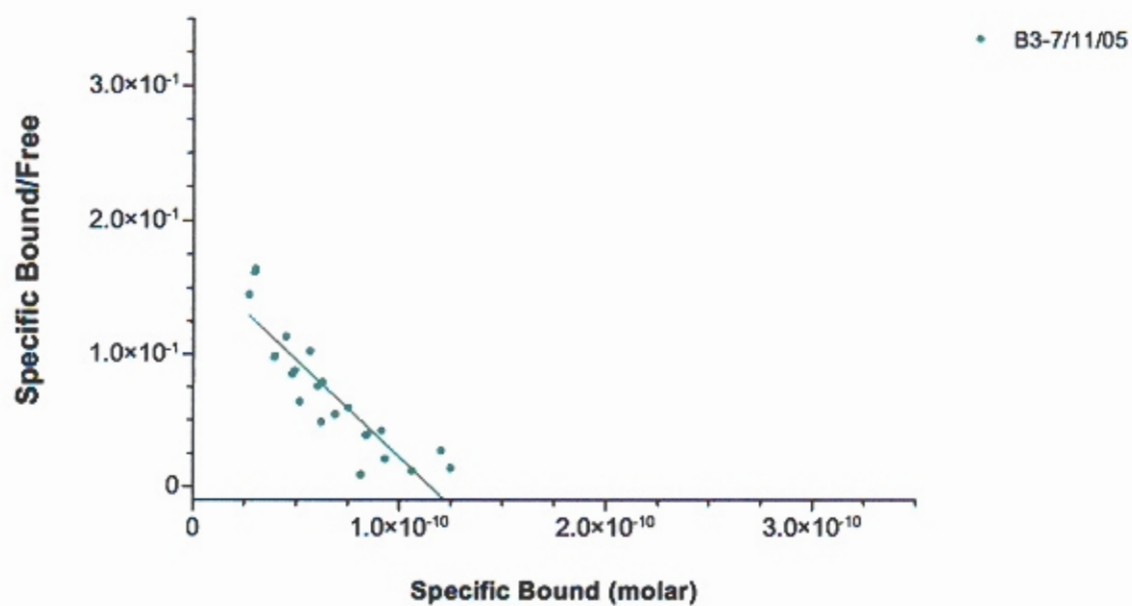
	CF	CG	CH	CI	CJ	CK	CL	CM	CN	DJ	DK	DL	DM
45													
46													
47													
48													
49													
50													
51													
52													
53													
54													
55													
56													
57													
58													
59													
60													
61													
62													
63													
64													
65													
66													
67													
68													
69													
70													
71													
72													
73													
74													
75													
76													
77													
78													
79													
80													
81													
82													
83													
84													
85													
86													
87													
88													
89													
90													
91													
92													

	DN	DO	DP
45			
46			
47			
48			
49			
50			
51			
52			
53			
54			
55			
56			
57			
58			
59			
60			
61			
62			
63			
64			
65			
66			
67			
68			
69			
70			
71			
72			
73			
74			
75			
76			
77			
78			
79			
80			
81			
82			
83			
84			
85			
86			
87			
88			
89			
90			
91			
92			

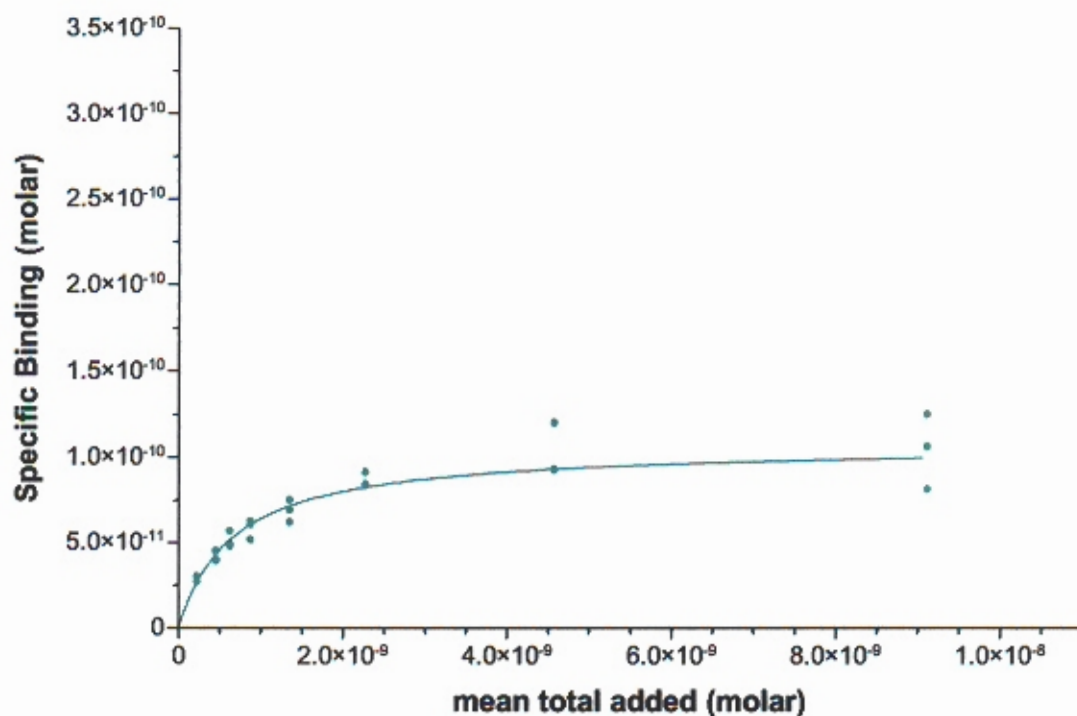
Hot Tubes
Lab B run 3
0.6 mg/tube



Scatchard Display
Lab B run 3
0.6 mg/tube



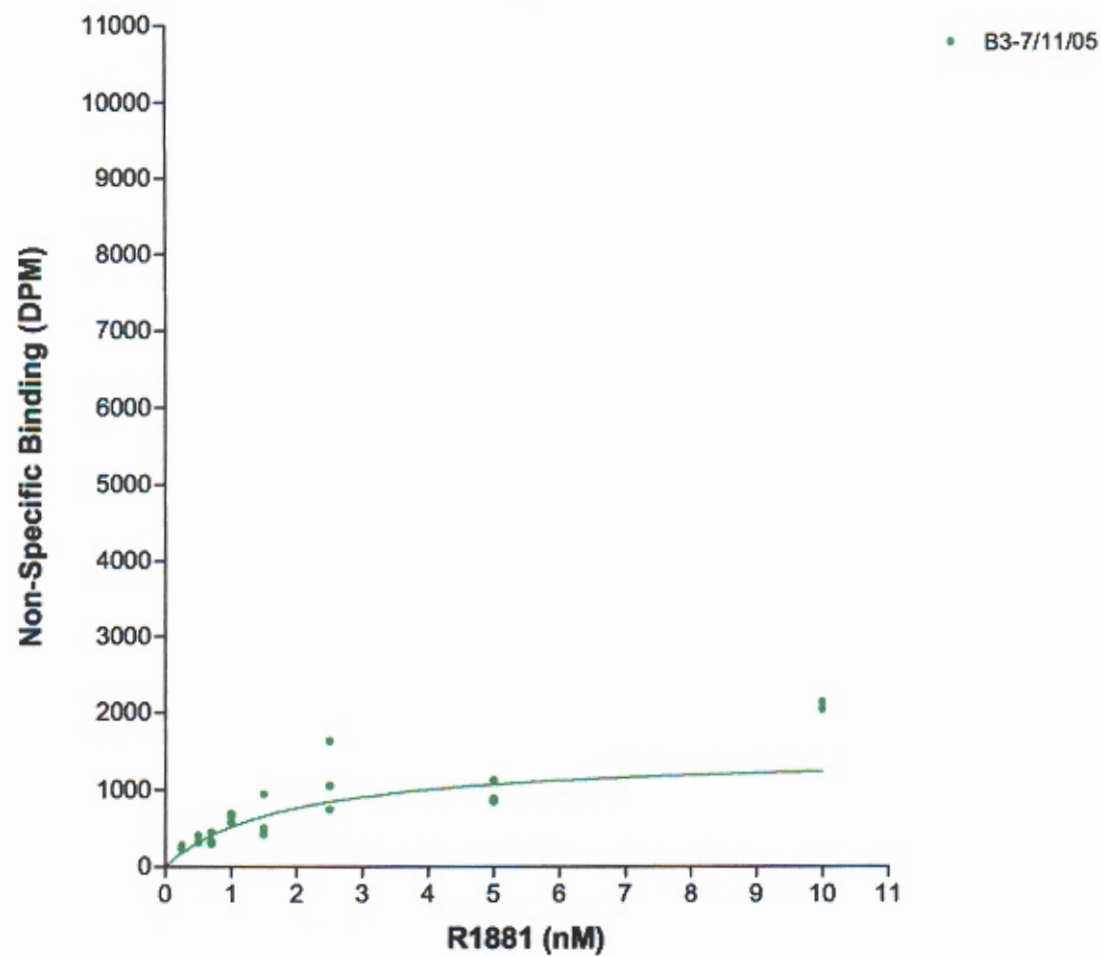
Lab B run 3 0.6 mg/tube



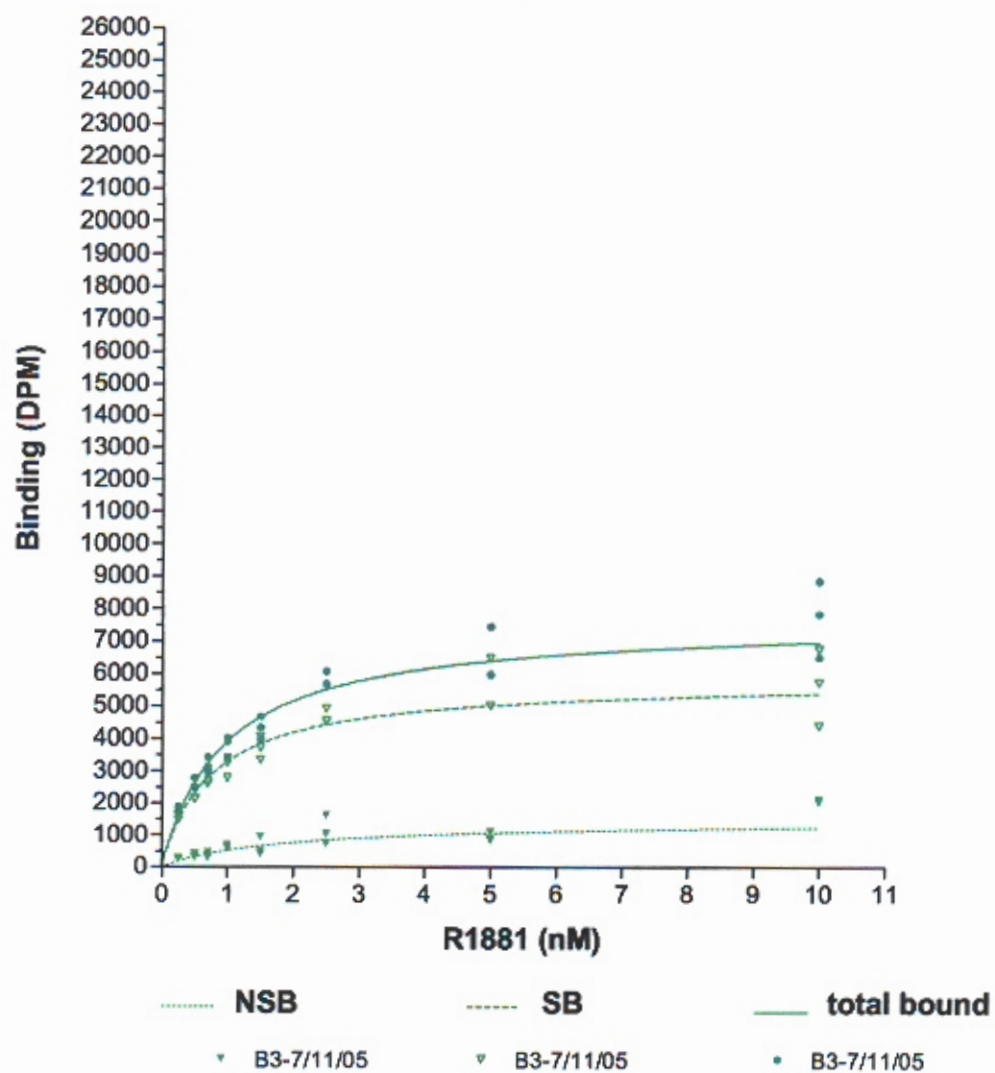
• B3-7/11/05

	B3-7/11/05
BMAX	1.063e-010
KD	6.604e-010
Std. Error	
BMAX	4.955e-012
KD	7.028e-011
95% Confidence Intervals	
BMAX	9.607e-011 to 1.166e-010
KD	5.146e-010 to 8.062e-010
Goodness of Fit	
Degrees of Freedom	22
R ² (unweighted)	0.8805
Weighted Sum of Squares (1/Y ²)	0.2728
Absolute Sum of Squares	2.134e-021
Sy.x	9.848e-012
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

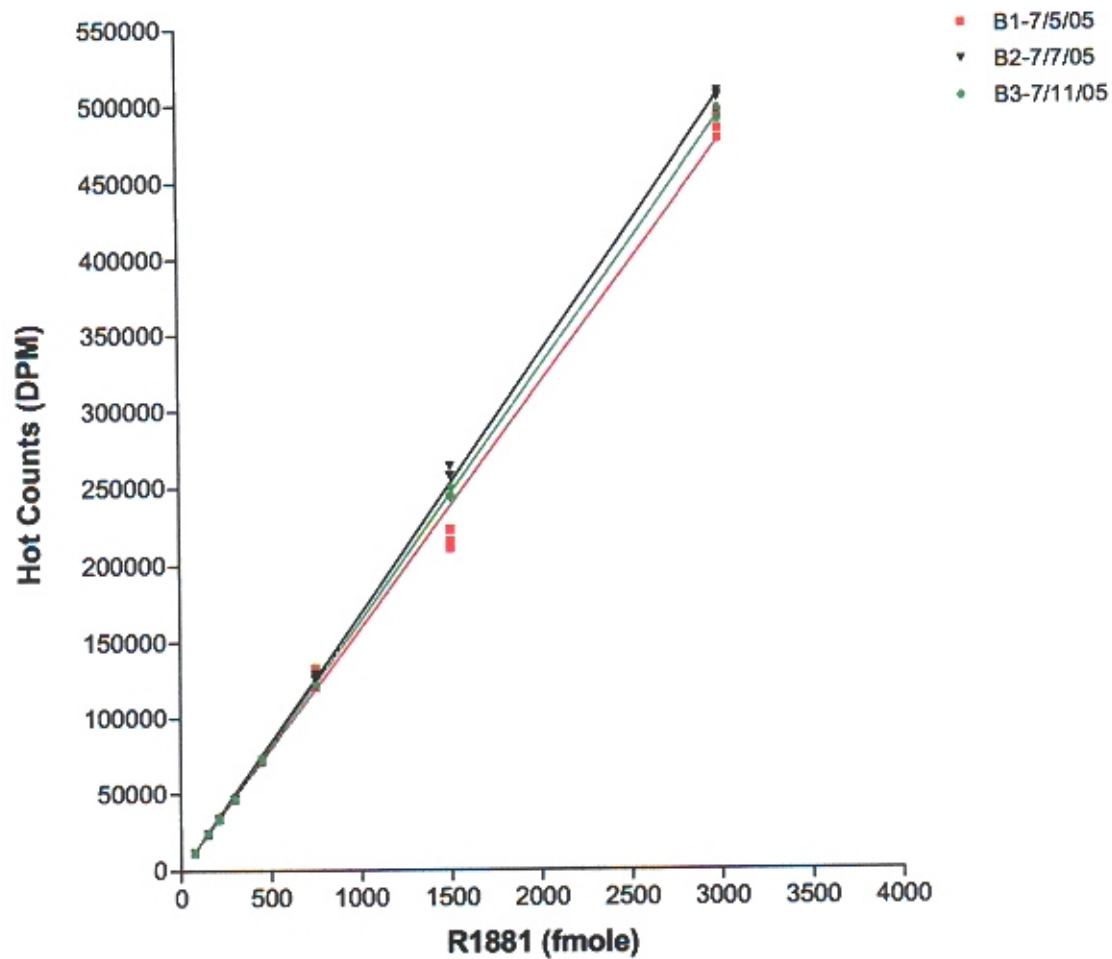
NSB Tubes
Lab B run 3
0.6 mg/tube



bound counts
Lab B run 3
0.6 mg/tube

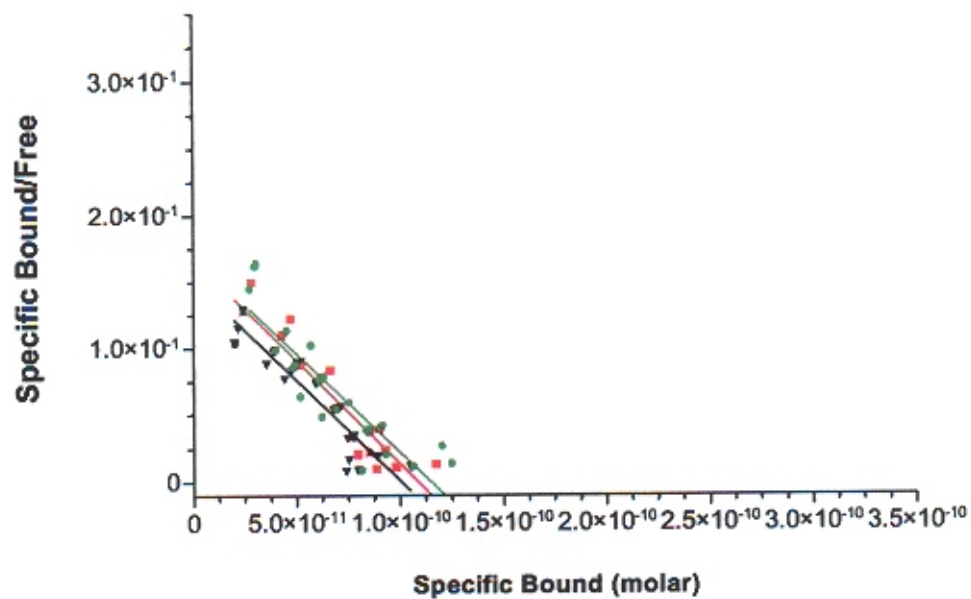


**Hot Tubes
Lab B
0.6 mg/tube**

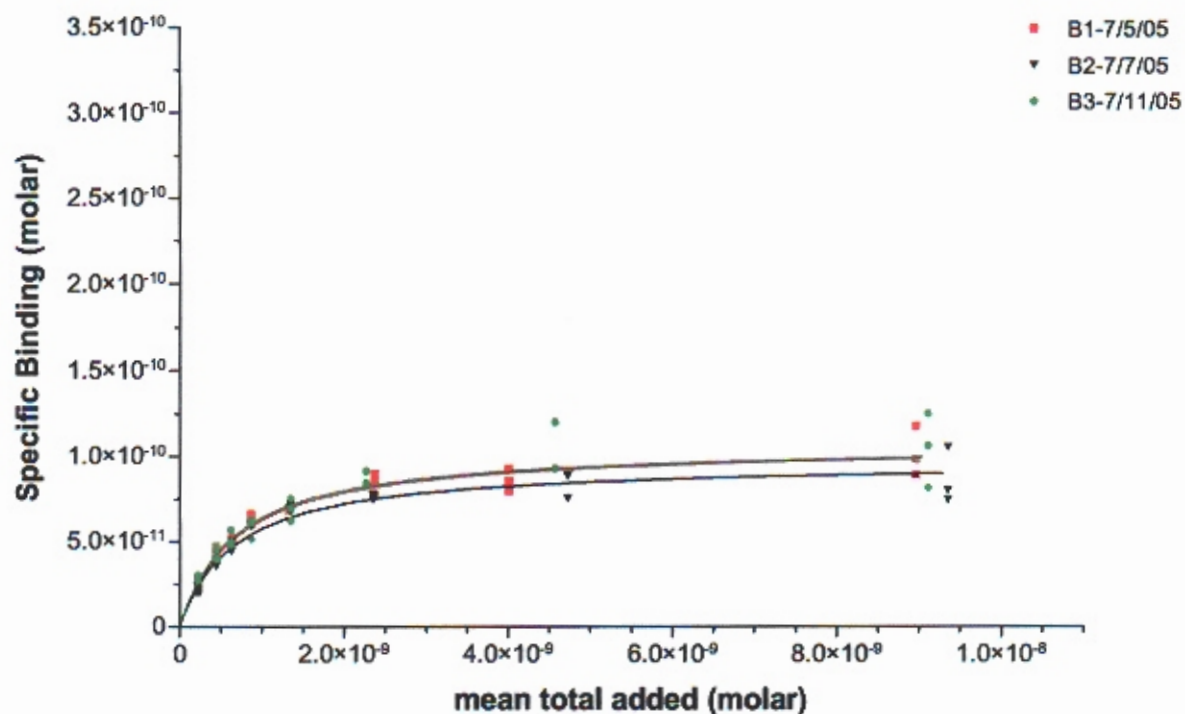


Scatchard Display
Lab B
0.6 mg/tube

- B1-7/5/05
- ▼ B2-7/7/05
- B3-7/11/05

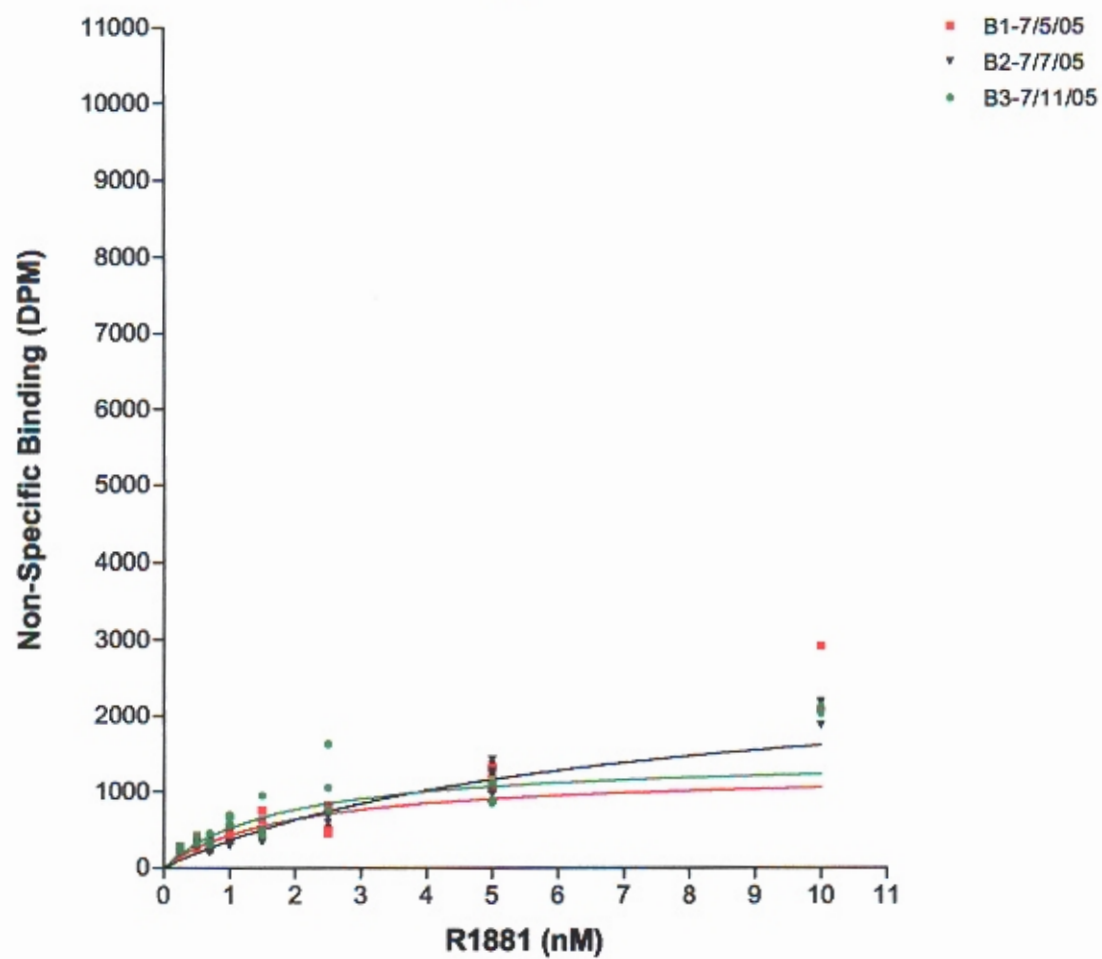


Lab B 0.6 mg/tube

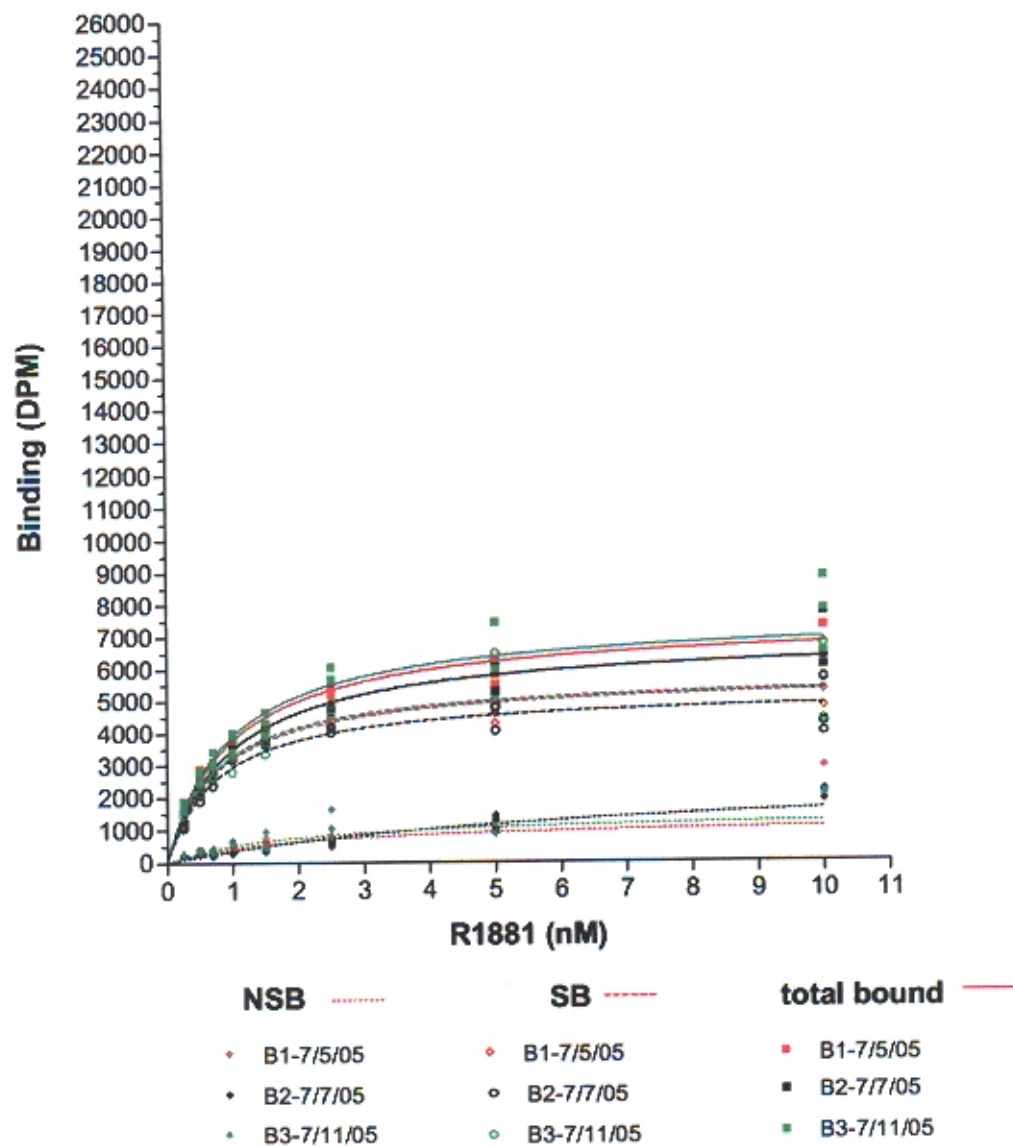


Specific bound	B1-7/5/05	B2-7/7/05	B3-7/11/05
BMAX	1.060e-010	9.621e-011	1.063e-010
KD	6.993e-010	6.773e-010	6.604e-010
Std. Error			
BMAX	3.984e-012	3.628e-012	4.955e-012
KD	5.797e-011	5.656e-011	7.028e-011
95% Confidence Intervals			
BMAX	9.772e-011 to 1.142e-010	8.869e-011 to 1.037e-010	9.607e-011 to 1.166e-010
KD	5.791e-010 to 8.196e-010	5.600e-010 to 7.946e-010	5.146e-010 to 8.062e-010
Goodness of Fit			
Degrees of Freedom	22	22	22
R ² (unweighted)	0.9381	0.9194	0.8805
Weighted Sum of Squares (1/Y ²)	0.1875	0.2036	0.2728
Absolute Sum of Squares	8.578e-022	9.630e-022	2.134e-021
Sy.x	6.244e-012	6.616e-012	9.848e-012
Data			
Number of X values	24	24	24
Number of Y replicates	1	1	1
Total number of values	24	24	24
Number of missing values	0	0	0

NSB Tubes
Lab B
0.6 mg/tube



bound counts
Lab B
0.6 mg/tube



Bmax-Kd

LabB date	1 7/5/2005	2 7/7/2005	3 7/11/2005
Bmax (fmole/100 ug)	5.30	6.67	5.32
Kd (nM)	0.70	1.00	0.66

APPENDIX E

Processed Raw Data from Competitive Assay 1 and Associated Figures

Counts from Competitive Assay Number 1

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

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Triamcetonone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	Corrected DPM for 2.0 mL
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1859.66	5764.946
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1943.59	6025.129
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1889.55	5857.605
4	1	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	170.75	512.25
5	2	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	167.17	501.51
6	3	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	153.77	461.31
7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	176.80	548.08
8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	136.55	423.305
9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	131.64	408.084
10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	339.52	1052.512
11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	280.84	870.604
12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	332.34	1030.254
13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1212.97	3760.207
14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1100.50	3411.55
15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1115.75	3458.825
16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1675.10	5192.81
17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1647.50	5107.25
18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1722.51	5339.781
19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2017.71	6254.901
20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1821.51	5646.681
21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1754.28	5438.268
22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	247.89	768.459
23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	235.23	729.213
24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	343.63	1065.253
25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	736.08	2281.848
26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	755.79	2342.949
27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	838.47	2599.257
28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1591.85	4934.735
29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1568.07	4861.017
30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1561.10	4839.41
31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1777.63	5510.653
32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1702.42	5277.502
33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1814.14	5623.834
34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1800.90	5582.79
35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1931.69	5988.239
36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1773.98	5499.338
37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1788.86	5539.266
38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1948.86	6035.266
39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1772.10	5493.51
40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1864.89	5781.159
41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1812.29	5618.099
42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1802.33	5587.223
43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1735.49	5380.019
44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1872.98	5806.238
45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1819.64	5640.884
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1872.31	5804.161
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1860.59	5767.829
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1700.42	5271.302
49	1	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	111.13	333.39
50	2	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	155.15	465.45
51	3	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	132.66	397.98
52	1	none	Hot	—	—	—	30	—	—	—	—	—	47046.66	47046.66	
53	2	none	Hot	—	—	—	30	—	—	—	—	—	46440.13	46440.13	
54	3	none	Hot	—	—	—	30	—	—	—	—	—	46156.72	46156.72	
55	1	none	Hot	—	—	—	30	—	—	—	—	—	46302.16	46302.16	
56	2	none	Hot	—	—	—	30	—	—	—	—	—	46487.76	46487.76	
57	3	none	Hot	—	—	—	30	—	—	—	—	—	45685.73	45685.73	

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1																	
2																	
3	Competitive Assay of a known Weak Positive																
4	57 Assay Tubes																
5	Please return by eMail to n.a.Holter@pnl.gov																
6	Provide information in all blue cells																
7	In columns O, AE and AI																
8	If the DPM value for a tube was judged unreliable,																
9	Include the DPM value in column O																
10	Provide a reason in column R																
11	The value in column Q will automatically change to FALSE																
12	Weak Positive, Max Concentration:																
13	Weak Positive IC50:																
14	RBA:																
15	Columns T and U contain values to be analyzed																
16	by nonlinear regression software																
17	They are also presented in table form in columns																
18	AC through AJ																
	Laboratory Code:																
	Run Identification:																
	Assay start date:																
	Tracer lot number:																
	Specific activity on day of assay:																
	Cytosol vial or lot Identification:																
	Protein (cytosol):																
	Standard Curve IC50:																
	Weak Positive, Max Concentration:																
	Weak Positive IC50:																
	RBA:																
	volume of ethanol counted:																

	A	B	R
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			

protocol calls for counting decanted EtOH su
reflects 100ul of reaction mixture processed

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														

Summary values			
	n	Mean	SD
EtOH	6	5748.5	252.94
Hot	6	46353.2	445.71
NSB	6	445.3	67.92
Specific EtOH	6	5303.2	252.94

Assay Characterization Values	
EtOH / Hot	0.12 less than 0.1?
NSB / EtOH	0.08 around 0.25?

pernate

	A	B	AF	AG	AH	AI	AJ
1							
2							
3							
4							
5							
6							
7							
8							
9							
10							
11							
12							
13							
14							
15							
16							
17							
18							

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19																	
20																	
21																	
22																	
23																	
24	Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Triamcetenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Alliquot (uL)	DPM as sampled	Corrected DPM for 2.0 mL	Use this value?
25	1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1859.66	5764.946	TRUE
26	2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1943.59	6025.129	TRUE
27	3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1889.55	5857.605	TRUE
28	4	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	170.75	512.25	TRUE
29	5	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	167.17	501.51	TRUE
30	6	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	153.77	461.31	TRUE
31	7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	176.80	548.08	TRUE
32	8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	136.55	423.305	TRUE
33	9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	131.84	408.084	TRUE
34	10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	339.52	1052.512	TRUE
35	11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	280.84	870.604	TRUE
36	12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	332.34	1030.254	TRUE
37	13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1212.97	3760.207	TRUE
38	14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1100.50	3411.55	TRUE
39	15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1115.75	3458.825	TRUE
40	16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1675.10	5192.81	TRUE
41	17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1647.50	5107.25	TRUE
42	18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1722.51	5339.781	TRUE
43	19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2017.71	6254.901	TRUE
44	20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1821.51	5646.681	TRUE
45	21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1751.28	5438.268	TRUE

	A	B	R
19			
20			
21			
22			
If the ratio of EtOH / H ₂ O is > 10% then there are problems with the assay			
23	Notes to explain why "Use this value" is set to "FALSE"		
24	Position	Replicate	
25	1	1	
26	2	2	
27	3	3	
28	4	1	
29	5	2	
30	6	3	
31	7	1	
32	8	2	
33	9	3	
34	10	1	
35	11	2	
36	12	3	
37	13	1	
38	14	2	
39	15	3	
40	16	1	
41	17	2	
42	18	3	
43	19	1	
44	20	2	
	21	3	

Values for analysis by nonlinear regression	Standard Curve	Weak Positive
---	----------------	---------------

[illegible]

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19																	
20																	
21																	
22																	
23																	
45	22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Triacelone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	Use this value?
46	23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	247.89	768.459	TRUE
47	24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	235.23	729.213	TRUE
48	25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	343.63	1065.253	TRUE
49	26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	736.08	2281.848	TRUE
50	27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	755.79	2342.949	TRUE
51	28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	838.47	2599.257	TRUE
52	29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1591.85	4934.735	TRUE
53	30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1568.07	4861.017	TRUE
54	31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1561.10	4839.41	TRUE
55	32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1777.63	5510.653	TRUE
56	33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1702.42	5277.502	TRUE
57	34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1814.14	5623.834	TRUE
58	35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1800.90	5582.79	TRUE
59	36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1931.69	5988.239	TRUE
60	37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1773.98	5499.338	TRUE
61	38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1786.86	5539.266	TRUE
62	39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1946.86	6035.266	TRUE
63	40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1772.10	5493.51	TRUE
64	41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1864.89	5781.159	TRUE
65	42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1812.29	5618.099	TRUE
66	43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1802.33	5587.223	TRUE
67	44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1735.49	5380.019	TRUE
68	45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1872.98	5806.238	TRUE
69	46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1819.64	5640.884	TRUE
70	47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1872.31	5804.161	TRUE
71	48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1860.59	5767.829	TRUE
72	49	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	1700.42	5271.302	TRUE
73	50	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	111.13	333.39	TRUE
															155.15	465.45	TRUE

3.1E+00 uL

total volume in tubes

Column O, Rows 10 through 13 will contain output parameters

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:

corrected DPM for 2.0 mL

DPM as sampled

Aliquot (uL)

Competitor Final Concentration (M)

Final Volume (uL)

Triacelone Volume (uL)

Competitor Volume (uL)

Tracer (Hot R1881) Volume (uL)

Cytosol (uL)

Competitor Initial Concentration (M)

Labels on vials in set 1-1-A

Concentration Code

Competitor Code

Competitor

Replicate

Position

	A	B	R
19			
20			
21			
22			
If the ratio of ETOH / H ₂ O is > 10% then there are problems with the assay			
23	Notes to explain why "Use this value" is set to "FALSE"		
45	Position	Replicate	
46	22	1	
47	23	2	
48	24	3	
49	25	1	
50	26	2	
51	27	3	
52	28	1	
53	29	2	
54	30	3	
55	31	1	
56	32	2	
57	33	3	
58	34	1	
59	35	2	
60	36	3	
61	37	1	
62	38	2	
63	39	3	
64	40	1	
65	41	2	
66	42	3	
67	43	1	
68	44	2	
69	45	3	
70	46	1	
71	47	2	
72	48	3	
73	49	1	
	50	2	

	A	B	AF	AG	AH	AI	AJ
19							
20							
21							
22							
23							
45							
46							
47							
48							
49							
50							
51							
52							
53							
54							
55							
56							
57							
58							
59							
60							
61							
62							
63							
64							
65							
66							
67							
68							
69							
70							
71							
72							
73							

Prism data

weak positive

(log)

Position
Replicate

Y3-SC y1-PC y2-PC y3-PC

Goodness of Fit
Degrees of Freedom 23
R² 0.9878
Absolute Sum of Sq 317.6
Sy.x 3.716

Constraints
BOTTOM BOTTOM = 0.0
TOP TOP = 100.0

Data
Number of X values 8
Number of Y replica 3
Total number of val 24
Number of missing 0

	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23			
74	Position	Replicate	Notes to explain why "Use this value" is set to "FALSE"
75	51	3	
76	52	1	
77	53	2	
78	54	3	
79	55	1	
80	56	2	
	57	3	

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
19														
20														
21														
22														
23														
74	Position	Replicate												
75	51	3												
76	52	1												
77	53	2												
78	54	3												
79	55	1												
80	56	2												
	57	3												

concentration (log)

percent bound

Standard Curve

Weak Positive

Values for analysis by nonlinear regression

Usable DPM values

Specific Binding (Total - mean NSB)

Free DPM (mean total add - total bound)

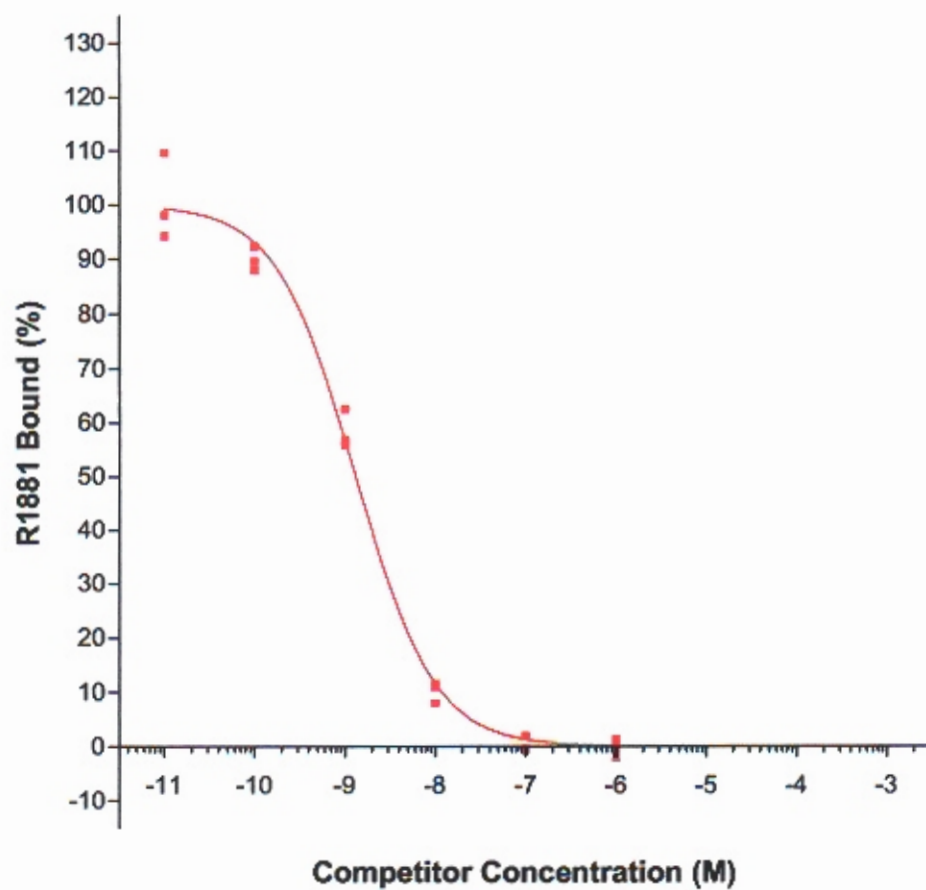
Percent Binding (specific bound / mean specific Error)

Ratio Total binding/ Hot

	Y1-SC	Y2-SC
concentration (log)		
standard curve		

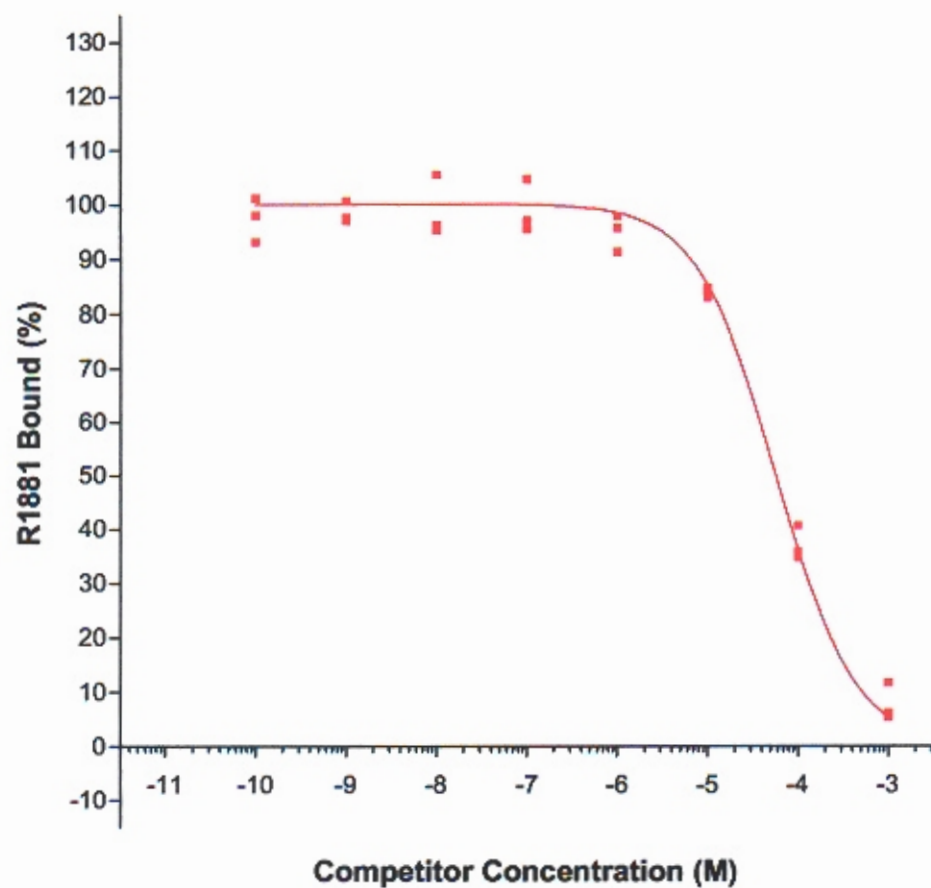
**Lab B
Run 1
Standard Curve
each Replicate**

■ B-1- 8/10/05



**Lab B Run 1
Dexamethasone
'Weak Positive' Data
each Replicate**

■ B-1- 8/10/05



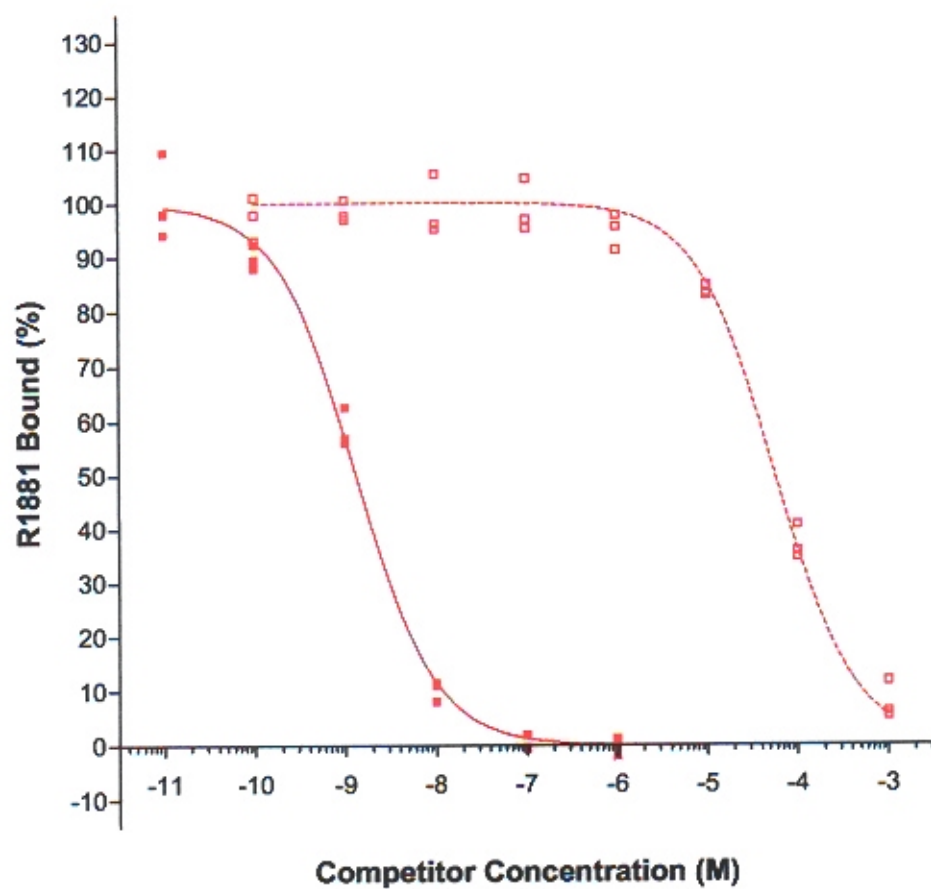
Lab B Run 1 **Standard Curve and 'Weak Positive'**

Standard
Curve

Weak
Positive

■ B-1- 8/10/05

□ B-1- 8/10/05



APPENDIX F

Processed Raw Data from Competitive Assay 2 and Associated Figures

Counts from Competitive Assay Number 2

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

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	transcenenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1751.93	5430.963
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1843.99	5716.369
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1691.36	5243.216
4	1	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	102.48	307.44
5	2	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	151.06	453.18
6	3	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	106.25	318.75
7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	122.81	380.711
8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	189.87	588.597
9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	133.58	414.098
10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	338.54	1049.474
11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	287.19	890.289
12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	287.82	892.242
13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	978.55	3033.505
14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1078.89	3338.359
15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1151.73	3570.363
16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1554.41	4818.671
17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1732.69	5371.339
18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1663.30	5156.23
19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1701.79	5275.549
20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1815.10	5626.81
21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1778.86	5514.466
22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	217.01	672.731
23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	246.20	763.22
24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	181.13	561.503
25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	721.07	2235.317
26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	728.05	2256.955
27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	707.45	2193.095
28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1611.42	4995.402
29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1504.83	4664.973
30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1463.70	4537.47
31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1806.01	5598.631
32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1855.84	5753.104
33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1670.13	5177.403
34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1836.16	5692.096
35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1764.52	5470.012
36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1585.31	4914.461
37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1819.94	5641.814
38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1730.53	5364.643
39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1753.59	5436.129
40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1876.02	5195.662
41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1927.16	5974.196
42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1825.20	5658.12
43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1804.78	5594.818
44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1644.69	5098.539
45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1804.82	5594.942
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1804.89	5595.159
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1693.98	5251.338
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1786.10	5536.91
49	1	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	132.90	398.7
50	2	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	140.24	420.72
51	3	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	114.05	342.15
52	1	none	Hot	—	—	—	30	—	—	—	—	—	100	47461.47	47461.47
53	2	none	Hot	—	—	—	30	—	—	—	—	—	100	46335.24	46335.24
54	3	none	Hot	—	—	—	30	—	—	—	—	—	100	46316.38	46316.38
55	1	none	Hot	—	—	—	30	—	—	—	—	—	100	47827.21	47827.21
56	2	none	Hot	—	—	—	30	—	—	—	—	—	100	46584.37	46584.37
57	3	none	Hot	—	—	—	30	—	—	—	—	—	100	46584.61	46584.61

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1	Competitive Assay of a known Weak Positive 57 Assay Tubes Please return by eMail to n.a.Holter@pnl.gov																
2	Provide information in all blue cells in columns O, AE and AI																
3	If the DPM value for a tube was judged unreliable, Include the DPM value in column O																
4	Provide a reason in column R																
5	The value in column Q will automatically change to FALSE																
6	Weak Positive, Max Concentration:																
7	Weak Positive IC50:																
8	RBA:																
9	Columns T and U contain values to be analyzed by nonlinear regression software They are also presented in table form in columns AC through AJ																
10	Laboratory Code:																
11	Run Identification:																
12	Assay start date:																
13	Tracer lot number:																
14	Specific activity on day of assay:																
15	Cytosol vial or lot identification:																
16	Protein (cytosol):																
17	Standard Curve IC50:																
18	Weak Positive, Max Concentration:																
	Weak Positive IC50:																
	RBA:																
	volume of ethanol counted:																

	A	B	R
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			

protocol calls for counting decanted EtOH su
reflects 100ul of reaction mixture processed

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														

Summary values			
	n	Mean	SD
EtOH	6	5462.3	190.40
Hot	6	46851.5	635.54
NSB	6	373.5	59.26
Specific EtOH	6	5088.8	190.40

Total Binding, solvent control, tubes
Total hot R1881 added to each tube
Nonspecific Binding

Assay Characterization Values	
EtOH / Hot	0.12 less than 0.1?
NSB / EtOH	0.07 around 0.25 ?

permate

	A	B	AF	AG	AH	AI	AJ	AK	AL	AM	AN
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19																	
20																	
21																	
22																	
23																	
24		1	ethanol	EtOH	0												
25		2	ethanol	EtOH	0												
26		3	ethanol	EtOH	0												
27		4	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100			
28		5	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100			
29		6	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100			
30		7	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	30	50	300	9.7E-08	100			
31		8	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	30	50	300	9.7E-08	100			
32		9	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	30	50	300	9.7E-08	100			
33		10	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	30	50	300	9.7E-09	100			
34		11	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	30	50	300	9.7E-09	100			
35		12	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	30	50	300	9.7E-09	100			
36		13	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	30	50	300	9.7E-10	100			
37		14	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	30	50	300	9.7E-10	100			
38		15	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	30	50	300	9.7E-10	100			
39		16	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	30	50	300	9.7E-11	100			
40		17	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	30	50	300	9.7E-11	100			
41		18	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	30	50	300	9.7E-11	100			
42		19	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	30	50	300	9.7E-12	100			
43		20	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	30	50	300	9.7E-12	100			
44		21	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	30	50	300	9.7E-12	100			
Column O, Rows 10 through 13 will contain output parameters from the nonlinear regression software. and the maximum concentration for the weak positive Competitive Assay Tube Layout - One Test Chemical (Weak Positive) total volume in tubes 3.1E+00 uL																	
Check the 10% rule: 11.66%																	
corrected DPM for 2.0 mL																	
Use this value?																	
23																	
24																	
25																	
26																	
27																	
28																	
29																	
30																	
31																	
32																	
33																	
34																	
35																	
36																	
37																	
38																	
39																	
40																	
41																	
42																	
43																	
44																	

	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23			
24	Position	Replicate	Notes to explain why "Use this value" is set to "FALSE"
25	1	1	
26	2	2	
27	3	3	
28	4	1	
29	5	2	
30	6	3	
31	7	1	
32	8	2	
33	9	3	
34	10	1	
35	11	2	
36	12	3	
37	13	1	
38	14	2	
39	15	3	
40	16	1	
41	17	2	
42	18	3	
43	19	1	
44	20	2	
	21	3	

[illegible]

[illegible]

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19	Column O, Rows 10 through 13 will contain output parameters from the nonlinear regression software. and the maximum concentration for the weak positive																3.1E+00 uL
20																	
21																	
22																	
23																	
45	Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Tramcetenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	Corrected DPM for 2.0 mL	Use this value?
46	22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	217.01	672.731	TRUE
47	23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	246.20	763.22	TRUE
48	24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	181.13	561.503	TRUE
49	25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	721.07	2235.317	TRUE
50	26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	728.05	2256.955	TRUE
51	27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	707.45	2193.095	TRUE
52	28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1611.42	4995.402	TRUE
53	29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1504.83	4664.973	TRUE
54	30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1463.70	4537.47	TRUE
55	31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1806.01	5598.631	TRUE
56	32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1855.84	5753.104	TRUE
57	33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1670.13	5177.403	TRUE
58	34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1836.16	5692.096	TRUE
59	35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1764.52	5470.012	TRUE
60	36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1585.31	4914.461	TRUE
61	37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1819.94	5641.814	TRUE
62	38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1730.53	5364.643	TRUE
63	39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1753.59	5436.129	TRUE
64	40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1676.02	5195.862	TRUE
65	41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1927.16	5974.196	TRUE
66	42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1825.20	5658.12	TRUE
67	43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1804.78	5594.818	TRUE
68	44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1644.69	5098.539	TRUE
69	45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1804.82	5594.942	TRUE
70	46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1804.89	5595.159	TRUE
71	47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1693.98	5251.338	TRUE
72	48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1786.10	5536.91	TRUE
73	49	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	132.90	398.7	TRUE
74	50	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	140.24	420.72	TRUE

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
19														
20														
21														
22														
23														
45														
46														
47														
48														
49														
50														
51														
52														
53														
54														
55														
56														
57														
58														
59														
60														
61														
62														
63														
64														
65														
66														
67														
68														
69														
70														
71														
72														
73														

Values for analysis by nonlinear regression
Standard Curve
Weak Positive

concentration (log)	Y1-SC	Y2-SC
Goodness of Fit		
Degrees of Freedom		20
R ²		0.9954
Absolute Sum of Squi		168.5
Sy.x		2.903
Constraints		
BOTTOM		BOTTOM =
TOP		TOP = 100
Data		
Number of X values		7
Number of Y replica		3
Total number of val		21
Number of missing		0

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 EROH)	Ratio Total binding/ Hot
-3.0	5.9	672.731	299.2	4416.1	5.9	1.435878
-3.0	7.7	763.22	389.7	4325.6	7.7	1.629018
-3.0	3.7	561.503	188.0	4527.3	3.7	1.198473
-4.0	36.6	2235.317	1861.8	2853.5	36.6	4.771063
-4.0	37.0	2256.955	1883.5	2831.9	37.0	4.817248
-4.0	35.8	2193.095	1819.6	2895.7	35.8	4.680945
-5.0	90.8	4995.402	4621.9	93.4	90.8	10.66219
-5.0	84.3	4664.973	4291.5	423.9	84.3	9.956924
-5.0	81.8	4537.47	4164.0	551.4	81.8	9.684782
-6.0	102.7	5598.631	5225.1	-509.8	102.7	11.94973
-6.0	105.7	5753.104	5379.6	-664.3	105.7	12.27943
-6.0	94.4	5177.403	4803.9	-88.6	94.4	11.05066
-7.0	104.5	5692.096	5318.6	-603.3	104.5	12.14922
-7.0	100.2	5470.012	5096.5	-381.2	100.2	11.6752
-7.0	89.2	4914.461	4541.0	174.4	89.2	10.48943
-8.0	103.5	5641.814	5268.3	-553.0	103.5	12.04189
-8.0	98.1	5364.643	4991.2	-275.8	98.1	11.4503
-8.0	99.5	5436.129	5062.6	-347.3	99.5	11.60288
-8.0	94.8	5195.662	4822.2	-106.8	94.8	11.08963
-9.0	110.1	5974.196	5600.7	-885.4	110.1	12.75133
-9.0	103.8	5658.12	5284.6	-569.3	103.8	12.0767
-10.0	102.6	5594.818	5221.3	-506.0	102.6	11.94159
-10.0	92.9	5098.539	4725.0	-9.7	92.9	10.88233
-10.0	102.6	5594.942	5221.5	-506.1	102.6	11.94185
-	102.6	5595.159	5221.7	-506.3	102.6	11.94231
-	95.9	5251.338	4877.8	-162.5	95.9	11.20846
-	101.5	5536.91	5163.4	-448.1	101.5	11.81799
-6.0	0.5	398.7	25.2	4690.1	0.5	0.850986
-6.0	0.9	420.72	47.2	4668.1	0.9	0.897985

	A	B	AF	AG	AH	AI	AJ	AK	AL	AM	AN
19											
20											
21											
22											
23											
45											
46											
47											
48											
49											
50											
51											
52											
53											
54											
55											
56											
57											
58											
59											
60											
61											
62											
63											
64											
65											
66											
67											
68											
69											
70											
71											
72											
73											

Prism data

weak positive

(log)

Position
Replicate

Y3-SC	y1-PC	y2-PC	y3-PC
22 1			
23 2			
24 3			
25 1			
26 2			
27 3			
28 1 : 0.0			
29 2 0			
30 3			
31 1			
32 2			
33 3			
34 1			
35 2			
36 3			
37 1			
38 2			
39 3			
40 1			
41 2			
42 3			
43 1			
44 2			
45 3			
46 1			
47 2			
48 3			
49 1			
50 2			

Goodness of Fit
 Degrees of Freedom 23
 R² 0.9827
 Absolute Sum of Sq 500.7
 Sy.x 4.666
 Constraints
 BOTTOM BOTTOM = 0.0
 TOP TOP = 100.0

Data

Number of X values 8
 Number of Y replica 3
 Total number of val 24
 Number of missing 0

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19																	
20																	
21																	
22																	
23																	
74																	
75																	
76																	
77																	
78																	
79																	
80																	

3.1E+00 uL

total volume in tubes

Column O, Rows 10 through 13 will contain output parameters

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the
10% rule:

11.66%

corrected DPM for 2.0 mL

Competitor Code

Competitor

Replicate

Position

Concentration Code

Labels on Vials in set 1-1-A

Competitor Initial
Concentration (M)

Cytosol (uL)

Tracer (Hot R1881) Volume
(uL)

Competitor Volume (uL)

triamcifenone Volume (uL)

Final Volume (uL)

Competitor Final
Concentration (M)

Aliquot (uL)

100

DPM as sampled

Use
this
value?

114.05

342.15

47461.47

47461.47

46335.24

46335.24

46316.38

46316.38

47827.21

47827.21

46584.37

46584.37

46584.61

46584.61

TRUE

TRUE

TRUE

TRUE

TRUE

TRUE

TRUE

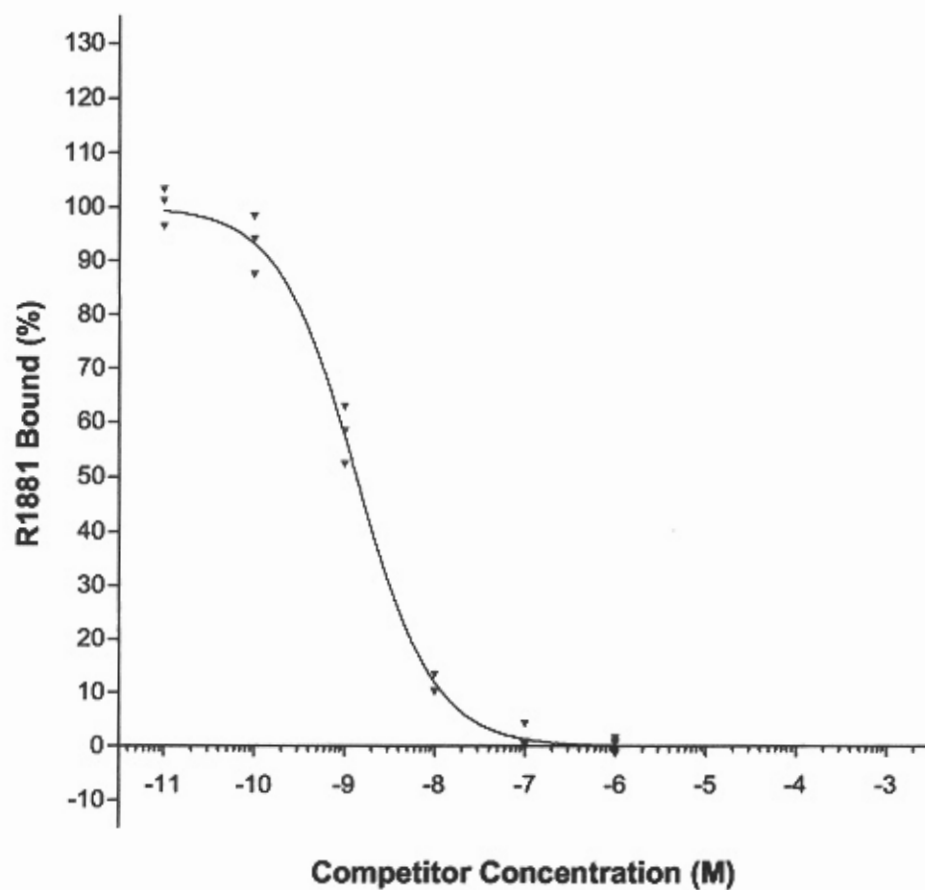
	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23			
74	Position	Replicate	Notes to explain why "Use this value" is set to "FALSE"
75	51	3	
76	52	1	
77	53	2	
78	54	3	
79	55	1	
80	56	2	
	57	3	

	A	B	AF	AG	AH	AI	AJ	AK	AL	AM	AN
19											
20											
21											
22											
23											
74											
75											
76											
77											
78											
79											
80											

Position	Replicate	Y3-SC	Y1-PC	Y2-PC	Y3-PC
51	3				
52	1				
53	2				
54	3				
55	1				
56	2				
57	3				

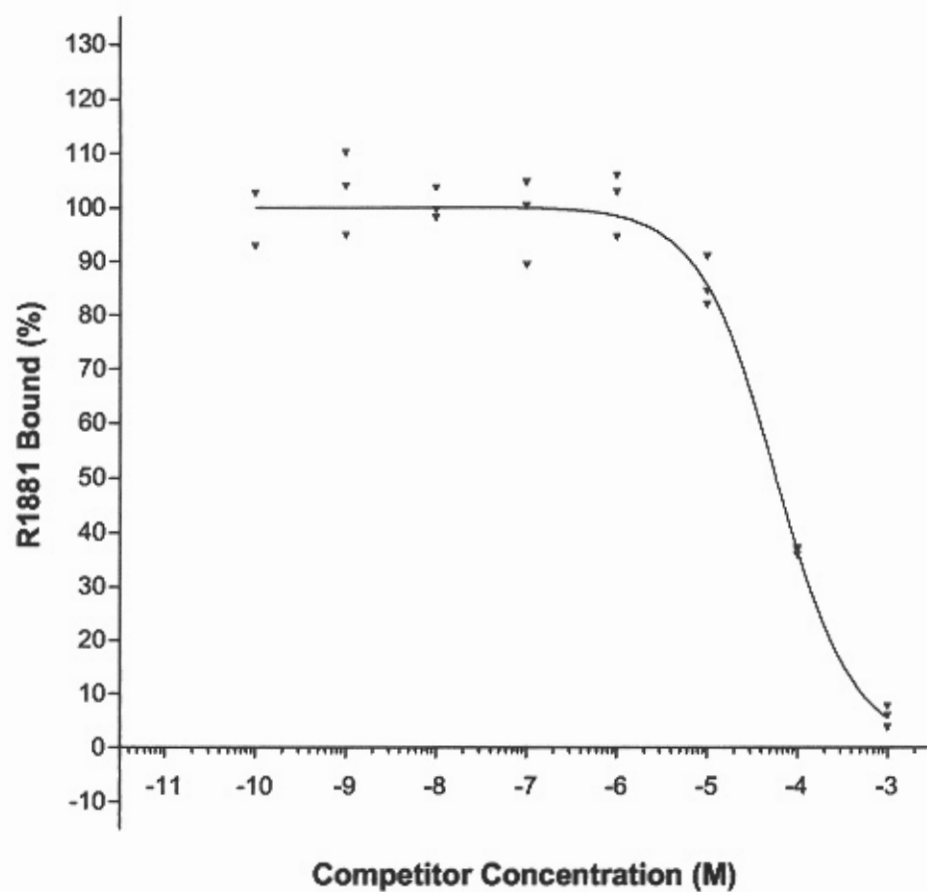
Prism data	
weak positive	
concentration (log)	

**Lab B Run 2
Standard Curve
each Replicate**



**Lab B Run 2
Dexamethasone
'Weak Positive' Data
each Replicate**

▼ B-2-8/16/05



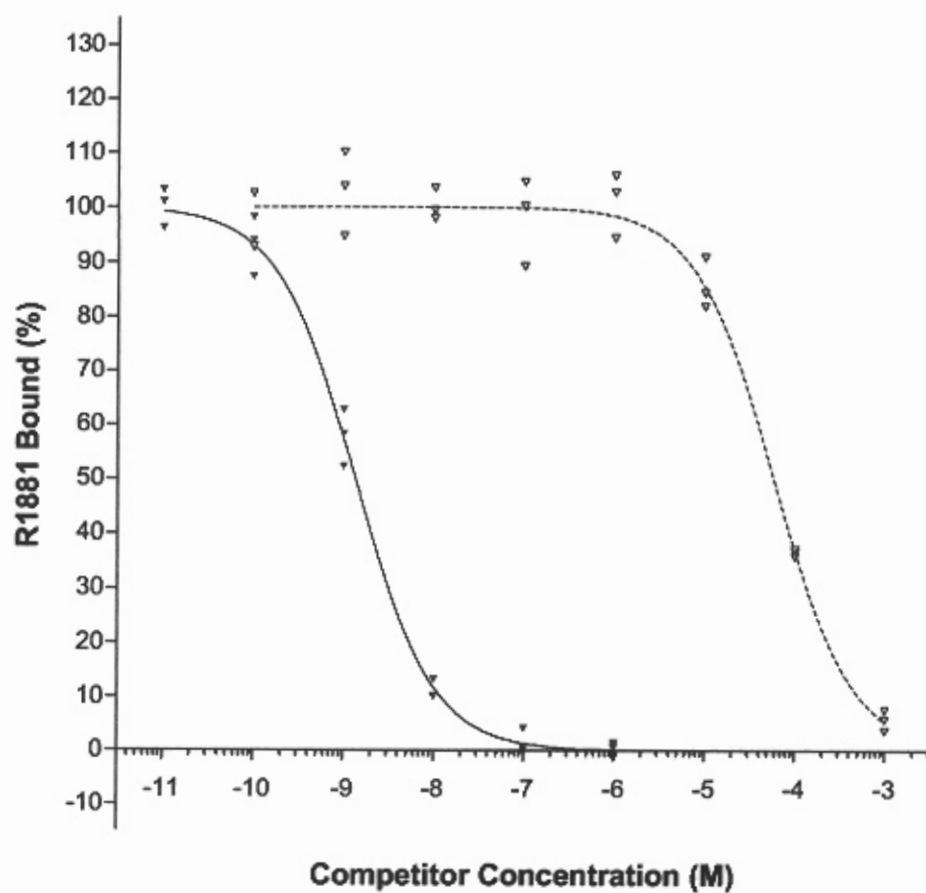
Lab B Run 2
Standard Curve and 'Weak Positive'

Standard
Curve

▼ B-2-8/16/05

Weak
Positive

▼ B-2-8/16/05



APPENDIX G

Processed Raw Data from Competitive Assay 3 and Associated Figures

Note: Summary figures for all three competitive assays and IC_{50} and RBA values (prepared by Battelle) are also included in this appendix.

Counts from Competitive Assay Number 3

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

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Triamcetenone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1583.21	4907.951
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1889.10	5856.21
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1784.18	5530.958
4	1	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	137.68	413.04
5	2	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	178.15	534.45
6	3	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	207.83	623.49
7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	120.06	372.186
8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	206.11	638.941
9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	210.21	651.651
10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	334.54	1037.074
11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	412.58	1278.998
12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	352.43	1092.533
13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1232.37	3820.347
14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1221.59	3786.929
15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	1183.33	3668.323
16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1732.22	5369.882
17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1678.17	5202.327
18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	1754.58	5439.198
19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1782.34	5556.254
20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1730.05	5363.155
21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	1898.09	5884.079
22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	241.40	748.34
23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	269.61	835.791
24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	296.19	918.189
25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	826.91	2563.421
26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	891.89	2764.859
27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	820.05	2542.155
28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1522.65	4720.835
29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1574.66	4881.446
30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1739.07	5391.117
31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1780.63	5551.573
32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1694.15	5251.865
33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1955.47	6061.957
34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1786.44	5537.964
35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1780.82	5520.542
36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1782.68	5526.308
37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1861.32	5770.092
38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1687.15	5230.165
39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1872.86	5805.866
40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1791.69	5554.859
41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1963.38	6086.478
42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1826.62	5662.522
43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1874.73	5811.663
44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1609.63	4989.853
45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1748.44	5420.164
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1718.28	5326.668
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1904.20	5903.02
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1737.24	5385.444
49	1	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	237.25	711.75
50	2	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	170.68	512.04
51	3	Inert R1881	NSB	—	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	165.74	497.22
52	1	none	Hot	—	—	—	30	—	—	—	—	—	45896.78	45896.78	
53	2	none	Hot	—	—	—	30	—	—	—	—	—	47263.98	47263.98	
54	3	none	Hot	—	—	—	30	—	—	—	—	—	45795.75	45795.75	
55	1	none	Hot	—	—	—	30	—	—	—	—	—	46048.85	46048.85	
56	2	none	Hot	—	—	—	30	—	—	—	—	—	46181.77	46181.77	
57	3	none	Hot	—	—	—	30	—	—	—	—	—	46812.77	46812.77	

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
1																	
2																	
3	Competitive Assay of a known Weak Positive 57 Assay Tubes Please return by eMail to n.a.Holter@pnl.gov																
4																	
5	Provide information in all blue cells in columns O, AE and AI																
6	If the DPM value for a tube was judged unreliable, Include the DPM value in column O																
7																	
8																	
9	Provide a reason in column R The value in column Q will automatically change to FALSE																
10																	
11	Weak Positive, Max Concentration: Weak Positive IC50: RBA:																
12																	
13																	
14																	
15																	
16																	
17																	
18																	

Laboratory Code: B			
Run Identification:	3		
Assay start date:	8/18/2005		
Tracer lot number:	3559-507		
Specific activity on day of assay:	80.48		Ci/mmole
Cytosol vial or lot Identification:	8/15/2005		
Protein (cytosol):	1000		micro gram per tube
Standard Curve IC50:	1.76E-09		M
Weak Positive, Max Concentration:	3.00E-02		M
Weak Positive IC50:	7.47E-05		M
RBA:	0.00236%		

volume of ethanol counted: 2 mL

	A	B	R
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			
18			

protocol calls for counting decanted EtOH su
reflects 100ul of reaction mixture processed

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12														
13														
14														
15														
16														
17														
18														

Summary values			
	n	Mean	SD
EtOH	6	5485.0	369.49
Hot	6	46330.0	580.66
NSB	6	548.7	104.67
Specific EtOH	6	4936.4	369.49

Assay Characterization Values	
EtOH / Hot	0.12 less than 0.1?
NSB / EtOH	0.10 around 0.25 ?

permate

	A	B	AF	AG	AH	AI	AJ	AK	AL	AM	AN
1											
2											
3											
4											
5											
6											
7											
8											
9											
10											
11											
12											
13											
14											
15											
16											
17											
18											

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19	Column O, Rows 10 through 13 will contain output parameters from the nonlinear regression software. and the maximum concentration for the weak positive Competitive Assay Tube Layout - One Test Chemical (Weak Positive)																
20	total volume in tubes																
21	3.1E+00 uL																
22	Check the 10% rule: 11.84%																
23	Use this value?																
24	1	1	ethanol	EtOH	0	—	—	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1583.21
25	2	2	ethanol	EtOH	0	—	—	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1889.10
26	3	3	ethanol	EtOH	0	—	—	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1784.18
27	4	1	Inert R1881	NSB	0	B-1-S0	1.00E-05	300	Tracer (Hot R1881) Volume (uL)	30	30	50	300	Final Volume (uL)	Competitor Final Concentration (M)	100	137.68
28	5	2	Inert R1881	NSB	0	B-1-S0	1.00E-05	300	Tracer (Hot R1881) Volume (uL)	30	30	50	300	Final Volume (uL)	Competitor Final Concentration (M)	100	178.15
29	6	3	Inert R1881	NSB	0	B-1-S0	1.00E-05	300	Tracer (Hot R1881) Volume (uL)	30	30	50	300	Final Volume (uL)	Competitor Final Concentration (M)	100	207.83
30	7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	120.06
31	8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	206.11
32	9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	210.21
33	10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	334.54
34	11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	412.58
35	12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	352.43
36	13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1232.37
37	14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1221.59
38	15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1183.33
39	16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1732.22
40	17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1678.17
41	18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1754.58
42	19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1792.34
43	20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1730.05
44	21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	Tracer (Hot R1881) Volume (uL)	30	10	50	310	Final Volume (uL)	Competitor Final Concentration (M)	100	1888.09

	A	B	R
19			
20			
21			
22			
23			
24			
25			
26			
27			
28			
29			
30			
31			
32			
33			
34			
35			
36			
37			
38			
39			
40			
41			
42			
43			
44			

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
19														
20														
21														
22														
23														
24														
25														
26														
27														
28														
29														
30														
31														
32														
33														
34														
35														
36														
37														
38														
39														
40														
41														
42														
43														
44														

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	Y1-SC	Y2-SC
24	1	-6.0	88.3	4907.951	4359.3	28.4	88.3	10.59347	-6.0	-2.74746
25	2	-6.0	107.5	5956.21	5307.5	-919.8	107.5	12.64022	-6.0	3.30374
26	3	-6.0	100.9	5530.958	4982.3	-594.6	100.9	11.93818	-7.0	-3.57507
27	4	-6.0	-2.7	413.04	-135.6	4523.3	-2.7	0.891518	-8.0	9.89408
28	5	-6.0	-0.3	534.45	-14.2	4401.9	-0.3	1.153573	-9.0	66.27699
29	6	-6.0	0.0	623.49	74.8	4312.9	1.5	1.345759	-10.0	97.66712
30	7	-7.0	-3.6	372.186	-176.5	4564.2	-3.6	0.803337	-11.0	101.44260
31	8	-7.0	1.8	638.941	90.3	4297.4	1.8	1.379109		
32	9	-7.0	2.1	651.651	103.0	4284.7	2.1	1.406543		
33	10	-8.0	9.9	1037.074	488.4	3899.3	9.9	2.238451		
34	11	-8.0	14.8	1278.998	730.3	3657.4	14.8	2.760627		
35	12	-8.0	11.0	1092.533	543.9	3843.8	11.0	2.358155		
36	13	-9.0	66.3	3820.347	3271.7	1116.0	66.3	8.245949		
37	14	-9.0	65.6	3786.929	3238.3	1149.4	65.6	8.173819		
38	15	-9.0	63.2	3668.323	3119.7	1268.1	63.2	7.917816		
39	16	-10.0	97.7	5369.882	4821.2	-433.5	97.7	11.59051		
40	17	-10.0	94.3	5202.327	4653.7	-266.0	94.3	11.22886		
41	18	-10.0	99.1	5439.198	4890.5	-502.8	99.1	11.74013		
42	19	-11.0	101.4	5556.254	5007.6	-619.9	101.4	11.99278		
43	20	-11.0	97.5	5363.155	4814.5	-426.8	97.5	11.57599		
44	21	-11.0	108.1	5884.079	5335.4	-947.7	108.1	12.70037		

Standard Curve B-3-8/18/06

One site competition

Best-fit values

BOTTOM

TOP

LOGEC50

EC50

Std. Error

LOGEC50

95% Confidence Intervals

LOGEC50

EC50

[illegible]

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	
19	Column O, Rows 10 through 13 will contain output parameters																	3.1E+00 uL
20	from the nonlinear regression software.																	
21	and the maximum concentration for the weak positive																	
22	Competitive Assay Tube Layout - One Test Chemical (Weak Positive)																	11.84%
23	Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcetonone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?	
45	22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	241.40	748.34	TRUE	
46	23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	269.61	835.791	TRUE	
47	24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	296.19	918.189	TRUE	
48	25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	826.91	2563.421	TRUE	
49	26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	891.89	2764.859	TRUE	
50	27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	820.05	2542.155	TRUE	
51	28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1522.85	4720.835	TRUE	
52	29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1574.66	4881.446	TRUE	
53	30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	1739.07	5391.117	TRUE	
54	31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1790.83	5551.573	TRUE	
55	32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1694.15	5251.865	TRUE	
56	33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	1955.47	6061.957	TRUE	
57	34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1788.44	5537.964	TRUE	
58	35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1780.82	5520.542	TRUE	
59	36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	1782.68	5526.308	TRUE	
60	37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1861.32	5770.092	TRUE	
61	38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1887.15	5230.165	TRUE	
62	39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	1872.86	5805.866	TRUE	
63	40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1791.89	5554.859	TRUE	
64	41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1963.38	6086.478	TRUE	
65	42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	1826.62	5662.522	TRUE	
66	43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1874.73	5811.663	TRUE	
67	44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1609.63	4989.853	TRUE	
68	45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	1748.44	5420.164	TRUE	
69	46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1718.28	5326.668	TRUE	
70	47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	1904.20	5903.02	TRUE	
71	48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	5385.444	5903.02	TRUE	
72	49	1	Inert R1881	NSB	0	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	1737.24	711.75	TRUE	
73	50	2	Inert R1881	NSB	0	B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	237.25	512.04	TRUE	

	A	B	R
19			
20			
21			
22			
If the ratio of ELOH / HOT is > 10% then there are problems with the assay			
Notes to explain why "Use this value" is set to "FALSE"			
23	Position	Replicate	
45	22	1	
46	23	2	
47	24	3	
48	25	1	
49	26	2	
50	27	3	
51	28	1	
52	29	2	
53	30	3	
54	31	1	
55	32	2	
56	33	3	
57	34	1	
58	35	2	
59	36	3	
60	37	1	
61	38	2	
62	39	3	
63	40	1	
64	41	2	
65	42	3	
66	43	1	
67	44	2	
68	45	3	
69	46	1	
70	47	2	
71	48	3	
72	49	1	
73	50	2	

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
19														
20														
21														
22														
23														
45	Position	Replicate												
46	22	1	Weak Positive											
47	23	2	Weak Positive											
48	24	3	Weak Positive											
49	25	1	Weak Positive											
50	26	2	Weak Positive											
51	27	3	Weak Positive											
52	28	1	Weak Positive											
53	29	2	Weak Positive											
54	30	3	Weak Positive											
55	31	1	Weak Positive											
56	32	2	Weak Positive											
57	33	3	Weak Positive											
58	34	1	Weak Positive											
59	35	2	Weak Positive											
60	36	3	Weak Positive											
61	37	1	Weak Positive											
62	38	2	Weak Positive											
63	39	3	Weak Positive											
64	40	1	Weak Positive											
65	41	2	Weak Positive											
66	42	3	Weak Positive											
67	43	1	Weak Positive											
68	44	2	Weak Positive											
69	45	3	Weak Positive											
70	46	1												
71	47	2												
72	48	3												
73	49	1												
74	50	2												

Values for analysis by nonlinear regression

Standard Curve

Weak Positive

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 Bnd)

Ratio Total binding/ Hot

concentration (log)

standard curve

Goodness of Fit
Degrees of Freedom 20
R² 0.9947
Absolute Sum of Squares 213.9
Sy.x 3.27
Constraints
BOTTOM BOTTOM =
TOP TOP = 100
Data
Number of X values 7
Number of Y replica 3
Total number of values 21
Number of missing values 0

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19	Column O, Rows 10 through 13 will contain output parameters from the nonlinear regression software. and the maximum concentration for the weak positive																3.1E+00 uL
20																	
21																	
22																	
23																	
74	Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on Vials in set 1-1-A	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	Triamcortolone Volume (uL)	Final Volume (uL)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?
75	51	3	Inert R1881	NSB	B-1-S0		1.00E-05	300	30	30	50	300	1.0E-06	100	165.74	497.22	TRUE
76	52	1	none	Hot					30						45896.78	45896.78	TRUE
77	53	2	none	Hot					30						47263.98	47263.98	TRUE
78	54	3	none	Hot					30						45795.75	45795.75	TRUE
79	55	1	none	Hot					30						46048.85	46048.85	TRUE
80	56	2	none	Hot					30						46161.77	46161.77	TRUE
	57	3	none	Hot					30						46812.77	46812.77	TRUE

	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23			
74	Position	Replicate	Notes to explain why "Use this value" is set to "FALSE"
75	51	3	
76	52	1	
77	53	2	
78	54	3	
79	55	1	
80	56	2	
	57	3	

	A	B	S	T	U	V	W	X	Y	AA	AB	AC	AD	AE
19														
20														
21														
22														
23														
74														
75														
76														
77														
78														
79														
80														

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	Y1-SC	Y2-SC
74	51	-6.0	-1.0	497.22	-51.4	4439.2	-1.0	1.073214		
75	52			45896.78	45348.1					
76	53			47263.98	46715.3					
77	54			45795.75	45247.1					
78	55			46048.85	45500.2					
79	56			46161.77	45613.1					
80	57			46812.77	46264.1					

Values for analysis by nonlinear regression
Standard Curve
Weak Positive

standard curve
concentration (log)
Y1-SC
Y2-SC

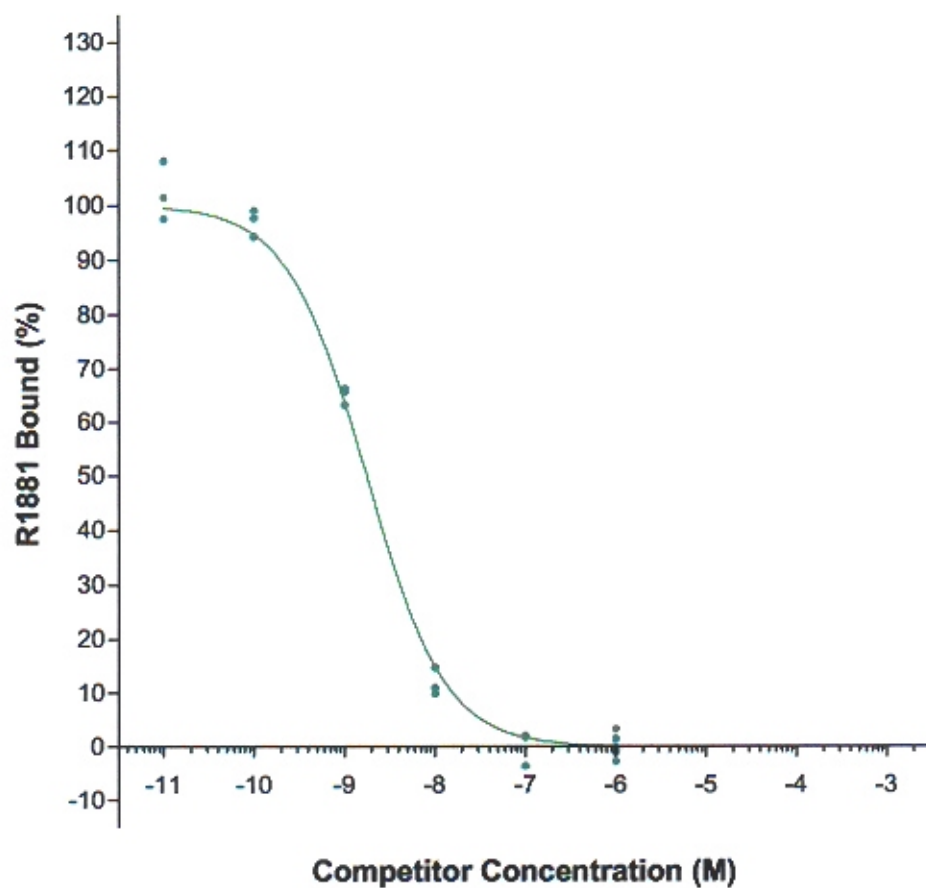
	A	B	AF	AG	AH	AI	AJ	AK	AL	AM	AN
19											
20											
21											
22											
23											
74											
75											
76											
77											
78											
79											
80											

Position	Y3-SC	Y1-PC	Y2-PC	Y3-PC
Replicate				
51				
52				
53				
54				
55				
56				
57				

Prism data
weak positive
concentration (log)

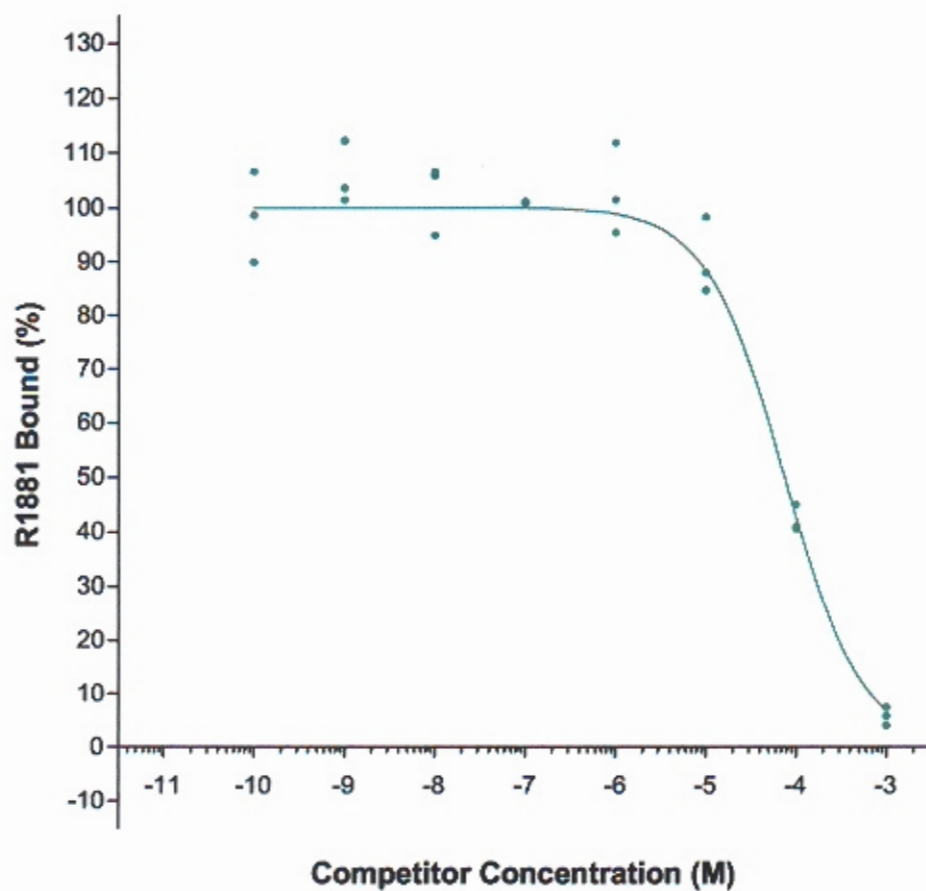
Lab B Run 3 Standard Curve each Replicate

• B-3-8/18/05



**Lab B Run 3
Dexamethasone
'Weak Positive' Data
each Replicate**

• B-3-8/18/05



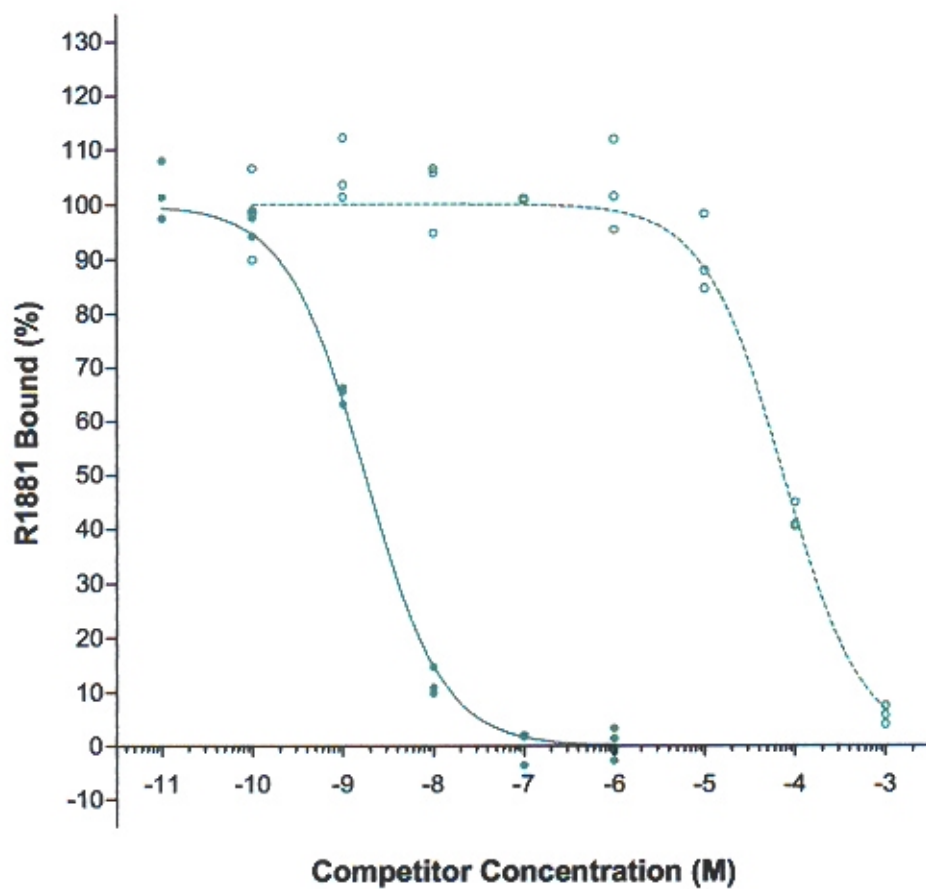
Lab B Run 3 **Standard Curve and 'Weak Positive'**

Standard
Curve

Weak
Positive

• B-3-8/18/05

◊ B-3-8/18/05



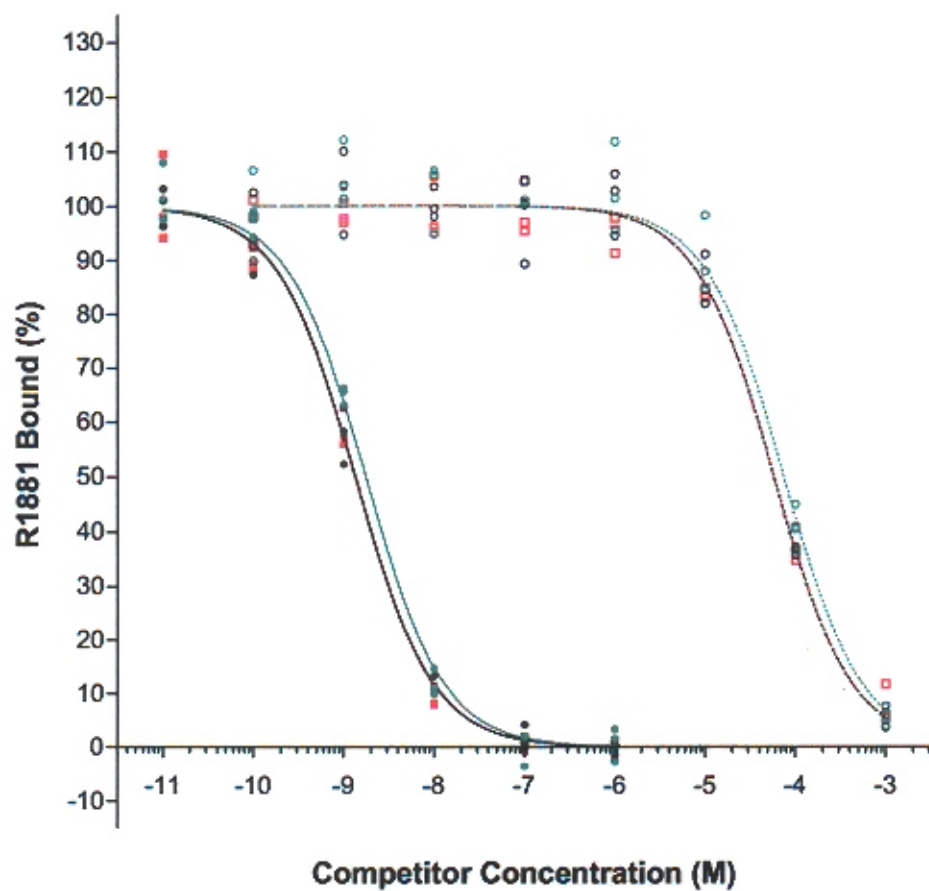
Standard Curve and 'Weak Positive'

Standard
Curve

Weak
Positive

- B-1- 8/10/05
- B-2-8/16/05
- B-3-8/18/05

- B-1- 8/10/05
- B-2-8/16/05
- B-3-8/18/05



WA4-11-3 AR Lab B.xls

IC50 - RBA

run	1	2	3
St Crv IC50	1.32E-09	1.36E-09	1.76E-09
WP IC50	5.73E-05	5.83E-05	7.47E-05
RBA	0.0023%	0.0023%	0.0024%