Audited Draft Final Report

Validation of an Androgen Receptor (AR) Binding Assay: Task 2 – Establish Inter-Laboratory Variability Using a Standard Preparation of Rat Prostate Cytosol

SRI Protocol No. 11232.01

Submitted to:

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Study Initiation: 10/20/04
Study Completion: TBD

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Project 11232.01
Submitted: TBD

TABLE OF CONTENTS

Page

TABLES

FIGURES

APPENDICES

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. However, as noted on page 2 of this report, the 4th competitive assay used some expired materials; this appeared to have no effect on the study. In addition, the dose solutions used in the study were not analyzed; however, the stability of the methyltrienolone and dexamethasone in ethanol were evaluated and will be reported to the EPA separately. Furthermore, the graphing and data analysis to determine Bmax, KD, IC_{50}, 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. Date
Study Director

Quality Assurance Statement

DRAFT

Draft Report

On

Protocol 11232.01

Validation of an Androgen Receptor (AR) Binding Assay:
Task 2 – Establish Inter-Laboratory Variability Using a Standard Preparation of Rat Prostate Cytosol

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.

Phases/Procedures	*Inspection/ Audit Date*	*Date Study Director/ Management Notified*
Final Protocol Review	1/27/05	1/27/05
Saturation Radioligand Binding Assay	1/24-25/05	1/28/05
Protocol Amendment 11232.01A1 Review	2/15/05	4/11/05
Protocol Amendment 11232.01A2 Review	3/24/05	4/11/05
Study File Data and Draft Report Review	3/24-29/05	3/29/05

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

Margery 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 "^{3}H-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 ^{3}H-R1881, whereas dexamethasone has been shown to compete weakly with ^{3}H-R1881 for binding in the assay.

MATERIALS AND METHODS

Unlabeled and radiolabeled R1881 (lots 3411228 and 3538497, respectively), dexamethasone (lot 414045/1), and rat prostate cytosol (batch AR-10/27/04, vials 61-155) were supplied by Battelle for these assays. 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 in 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.

Androgen Receptor Saturation Assay

The assay was performed in siliconized glass tubes. ^{3}H-R1881 and cold R1881 were added from the appropriate stock solutions to the appropriate tubes. Triamcinolone acetonide was next added and tubes were placed in a speed-vac to dry. 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 uL 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.

Androgen Receptor Competitive Assay

The assay was performed in siliconized glass tubes. ^{3}H-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 mg per 300 uL 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 four competitive assays were conducted. The processed raw data from these assays are found in Appendices B-G. Several samples were not included in the data analyses; these are noted on the title pages of the appendices. 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 variation in the three assays conducted. From these data, the Kd and Bmax 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 chosen by Battelle for the competitive assays. Results from competitive assay 2 were considered unacceptable (the standard curve was shifted, most likely due to pipetting technique) and are not shown. Thus, data from competitive assays 1, 3, and 4 are included. Figure 5 shows the results of these assays 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.

CONCLUSION

In terms of accomplishing our purpose of establishing the sensitivity and specificity of the AR binding assay at Southern Research Institute and validating the assay, it seems that we have been

successful and are now prepared to execute Task 3 of this program. There were no ambiguities or insufficiencies in the results from this study.

PARTICIPATING PERSONNEL

Richard D. May, Ph.D.	Study Director, Senior Project Leader
Angela Y. Jones, M.S.	Advanced Biologist
Parrish L. Payne, B.S.	Biologist
Jennifer B. Davis, B.S.	Auditor, Quality Assurance
Margery J. Wirth, B.S.	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

Other than indicated below, there were no significant deviations to the Protocol or circumstances that may have comprised the study.

The standards, inert R1881, radiolabeled R1881, and dexamethasone preparations supplied to Southern Research by Battelle and used in this study expired on 3/10/05, 3/10/05, 3/11/05, 3/11/05, respectively. While all saturation and the first three competitive assays were performed prior to these dates, the fourth competitive assay was performed from 3/14/05 to 3/15/05.

Table 1

Dissociation Constant (Kd) Values

Saturation Assay	Kd Value (M)
1	9.2×10^{-10}
2	9.9×10^{-10}
3	8.9×10^{-10}
Mean	$\mathbf{9.3 \times 10^{-10}}$
SD	$\mathbf{0.5 \times 10^{-10}}$

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Table 2

Maximum Binding Capacity (Bmax) Values

Saturation Assay	Bmax Value (fmole/100 µg)
1	10.45
2	12.03
3	11.08
Mean	**11.19**
SD	**0.80**

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Table 3

IC_{50} and Relative Binding Affinity (RBA) Values

Competitive Assay	IC_{50} Values (M)		RBA Values (%)*
	R1881 Standard	Dexamethasone**	
1	1.35×10^{-9}	3.51×10^{-5}	0.0038
3	1.55×10^{-9}	3.88×10^{-5}	0.0040
4	1.50×10^{-9}	3.68×10^{-5}	0.0041
Mean	**1.46×10^{-9}**	**3.69×10^{-5}**	**0.0040**
SD	**0.10×10^{-9}**	**0.18×10^{-5}**	**0.0001**

* The IC_{50} value of R1881 ÷ the IC_{50} of the weak positive (or competitor), expressed as a percentage.

** The weak positive.

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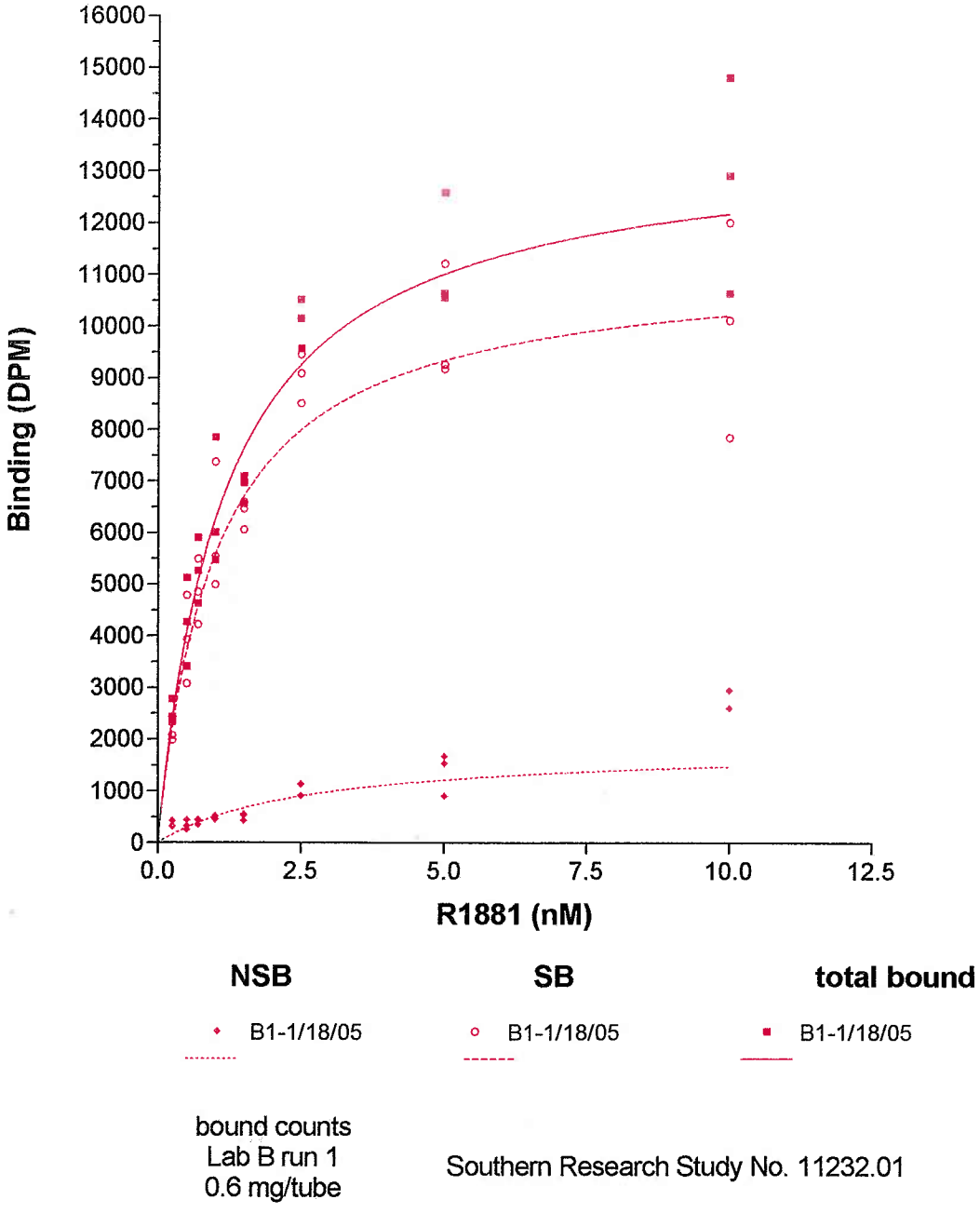
Figure 1

Saturation Assay 1

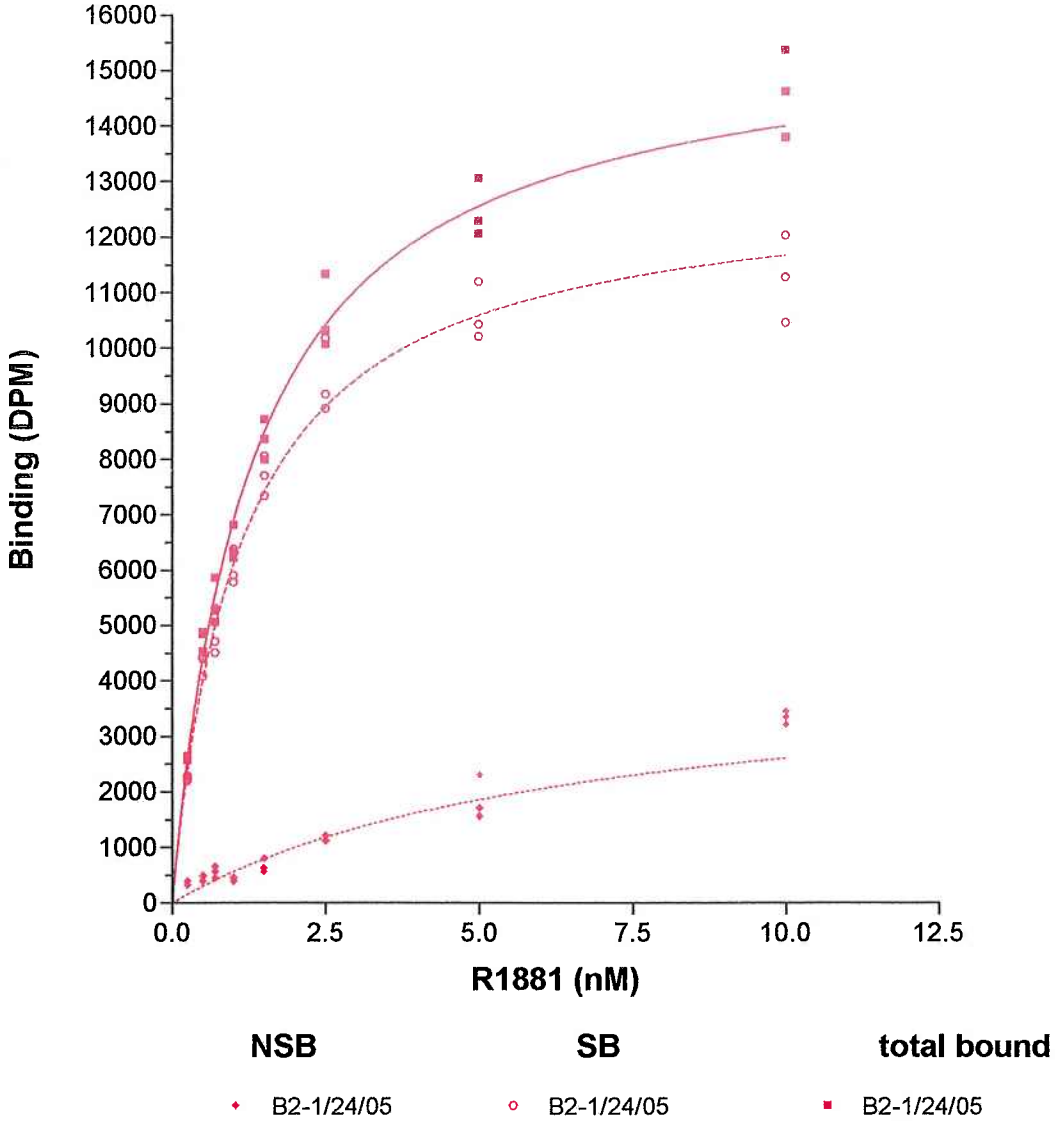


bound counts
Lab B run 1
0.6 mg/tube

Southern Research Study No. 11232.01

Figure 2

Saturation Assay 2



bound counts
Lab B run 2
0.6 mg/tube

Southern Research Study No. 11232.01

Figure 3

Saturation Assay 3

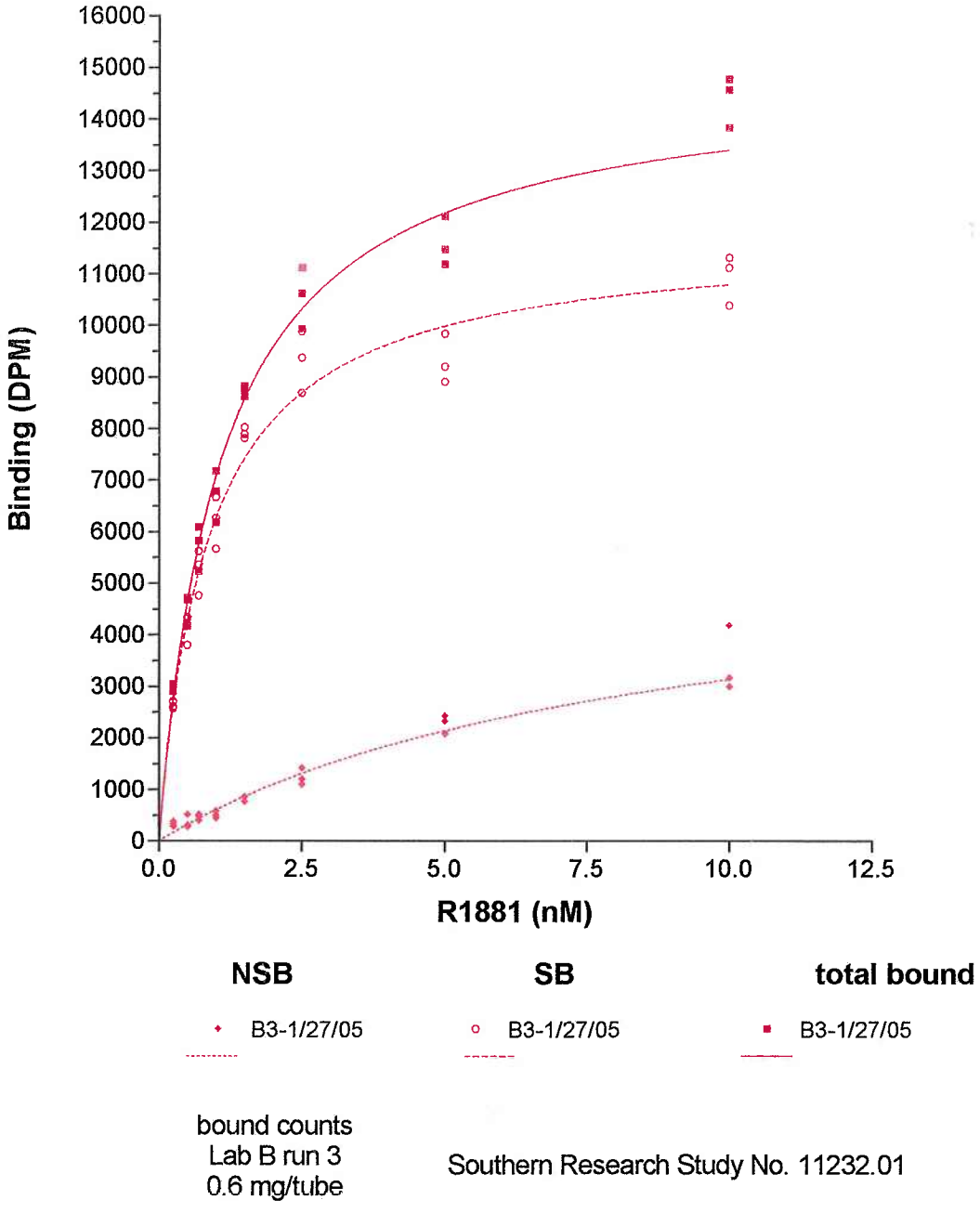
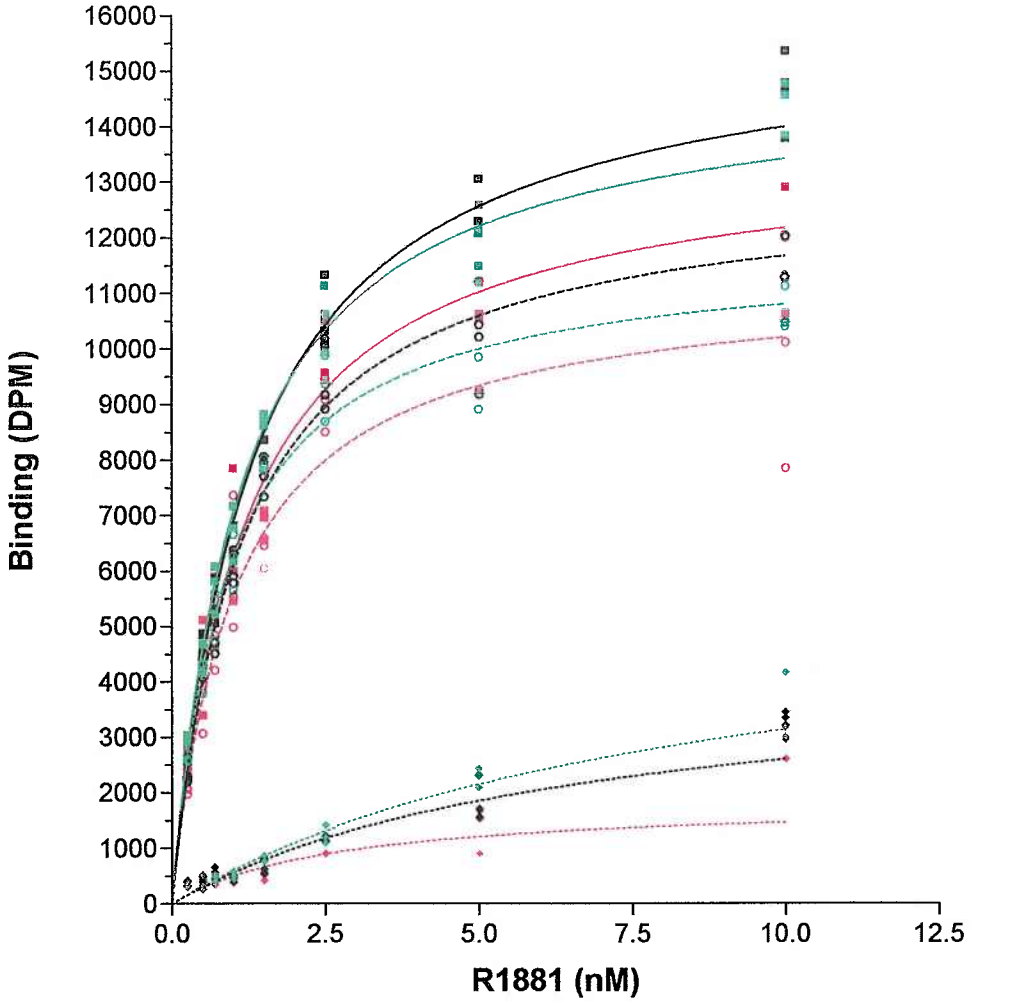


Figure 4

Saturation Assays 1-3



NSB
dotted line

- B1-1/18/05
- B2-1/24/05
- B3-1/27/05

SB
dashed line

- B1-1/18/05
- B2-1/24/05
- B3-1/27/05

total bound
solid line

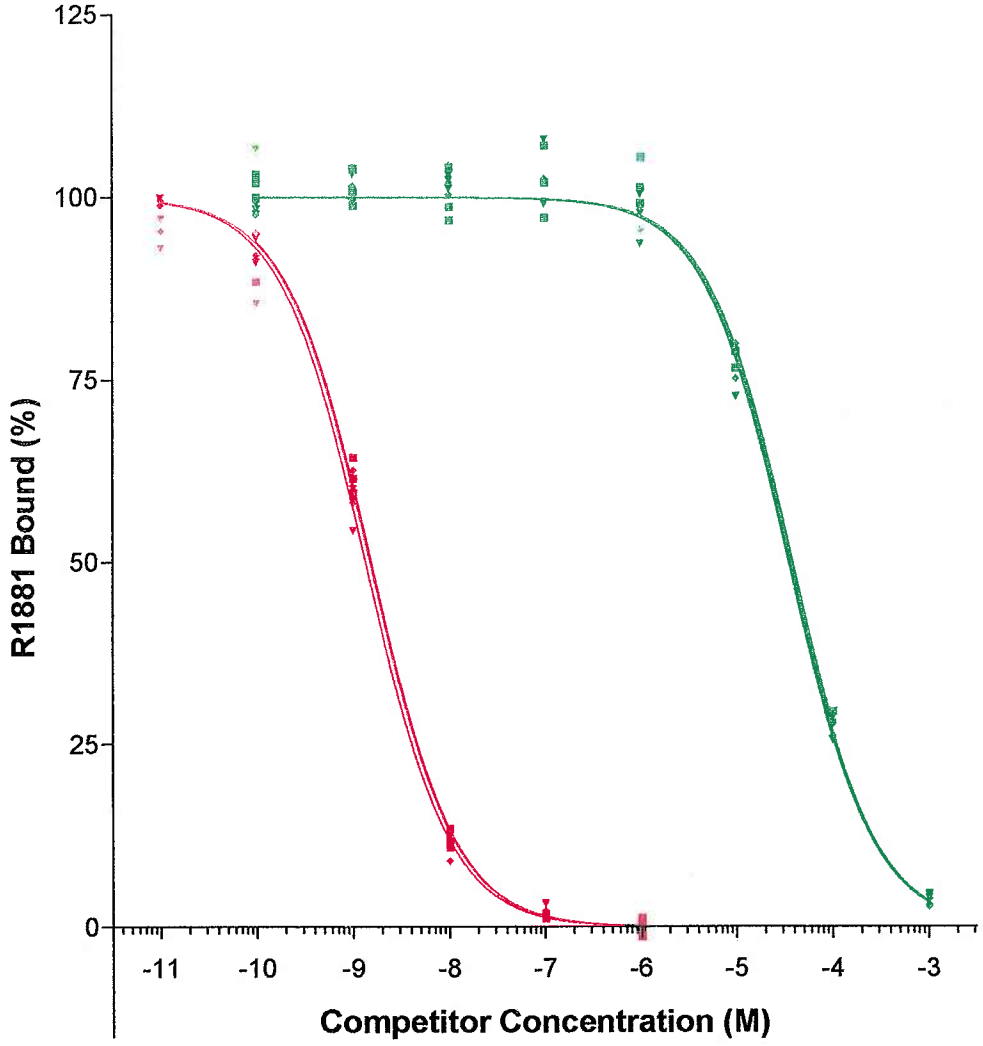
- B1-1/18/05
- B2-1/24/05
- B3-1/27/05

bound counts
Lab B
0.6 mg/tube

Southern Research Study No. 11232.01

Figure 5

Competitive Assays 1, 3, and 4



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

Southern Research Study No. 11232.01

APPENDIX A

Study Protocol and Amendments

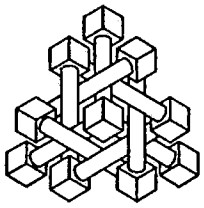
Study Protocol:

Validation of an Androgen Receptor (AR) Binding Assay: Task 2 – Establish Inter-Laboratory Variability Using a Standard Preparation of Rat Prostate Cytosol

Southern Research Institute Study No. : 11232.01

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

Lab Study Code. : 182028-1



SOUTHERN RESEARCH
INSTITUTE

1.0 SPONSOR REPRESENTATIVE AND CONTACTS

Sponsor:

Battelle Memorial Institute
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Sponsor's Representatives & Study Monitor:

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David P. Houchens, Ph.D.
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Protocol Approval:
(Initial last page also)

James E. Morris 12-22-04
Dr. James Morris Date

David P. Houchens 12/21/04
Dr. David P. Houchens Date

Test, Control and Reference Substances:

965-93-5 (1)
Marker substance – ^{3}H-R1881(Methyltrienolone) (CAS ~~68-23-5~~)
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

(1) RE RM 4-7-05

2.0 TITLE:

Validation of an Androgen Receptor (AR) Binding Assay: Task 2 – Establish Inter-Laboratory Variability Using a Standard Preparation of Rat Prostate Cytosol

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.

4.0 TESTING LABORATORY:

Southern Research Institute (Southern Research)
Cell Biology and Immunology Group
2000 Ninth Avenue South • 35205
P.O. Box 55355
Birmingham, AL 35255-5305

(205) 581-2731; FAX (205) 581-2711

5.0 KEY STUDY DATES:

Event	Schedule	Date(s)
Receipt of all reagents	Week 1	January 3 - 14, 2005
Saturation assays	Week 2 - 4	January 10 -21
Competitive assays	Weeks 5 - 6	January 24 – February 4, 2005
QAU audit and management review	Week 7	February 7 -11, 2005
Data submitted to Battelle	Week 8	February 15, 2005

6.0 STUDY PERSONNEL:

Study Director: Gary A. Piazza, Ph.D

Team Leader: Angela Y. Jones, M.S.

Laboratory Technician: Parrish Payne, B.S.

Quality Assurance Manager: Marge 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 by the Sponsor. No laboratory animals are to be used at SRI for this task. Records relating to the number, body weight range, sex, source of supply, species, strain, substrain, and age of the test system will be maintained by the Sponsor.

7.1 SOURCE

Rat prostate cytosol will be supplied by the Sponsor.

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 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:	**^{3}H-R1881 (Methyltrienolone)**
CAS No.:	~~68-23-05~~ 965-93-5 ①
Supplier:	Sponsor (Manufacturer, TBD)
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 in a freezer at -20 degrees C.

8.2 IDENTITY OF THE REFERENCE SUBSTANCE

Name:	**Radioinert Methyltrienolone (R1881)**
CAS No.:	965-93-5
Supplier:	Sponsor (Manufacturer, Perkin Elmer)
Lot Number(s):	3411228

① RE RM 4/11/05

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 duration of the stability study was 56 days at a concentration of 30 mM in 100% ethanol (EDSP.H4-014). The compound will be stored in a freezer at -20 degrees C.

8.3 IDENTITY OF THE TEST SUBSTANCE

Name:	Dexamethasone
CAS No.:	50-02-2
Supplier:	Sponsor (Manufacturer, Biomol)
Lot Number(s):	414045/1

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: The duration of the stability study was 56 days at a concentration of 30 mM in 100% ethanol (EDSP.H4-013). The compound will be stored at room temperature (20-25 degrees C).

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 [^{3}H] ligand (R1881) for binding in rat ventral prostate tissue homogenate which will be supplied by the Sponsor 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 ASSAYS

AR saturation binding experiments will measure total, non-specific, and specific

binding of increasing concentrations of ^{3}H-R1881 under conditions of equilibrium. A graph of specific ^{3}H-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 ^{3}H-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 50 to 100 µg protein per assay tube. The concentration for ^{3}H-R1881 will range from 0.03 to 3.0 nM in a total assay volume of 0.5 mL. Non-specific binding will be determined by adding unlabeled R1881 at 100x the concentration of radiolabeled R1881. Battelle will analyze the data using a non-linear regression analysis (e.g., McPherson, 1985; 1997; Motulsky, 1995) 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 general protocol to conduct the saturation assay is as follows:

Day 1

Set up tubes: 12x75 siliconized glass tubes and label for 8 concentrations in duplicate each with and without 100x inert (48 tubes total, 1 through 48 below as listed in Appendix A).

Add [^{3}H] R1881 from the appropriate stock solutions to tubes as listed in Appendix A.

Place 50 µL of 60 mM stock triamcinolone acetonide to ALL tubes.

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

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

Cytosol should be diluted with the low salt TEDG buffer to a protein concentration of 1.2 mg per 300 µl assay. Add 300 ul of diluted prostate cytosol to all tubes (1-48). Keep tubes and cytosol on ice at all times during this

procedure. Gently vortex and place tubes in refrigerator overnight in rotor (18-20hr).

Before leaving for the day, prepare the first wash of the HAP slurry. If desired, label the HAP tubes and the scintillation vials to be used the following day.

Day 2

Continue as with Day 2 protocol for saturation binding assay as described below for the competitive binding assay.

9.2 AR COMPETITIVE BINDING ASSAY

AR competitive binding assays will measure the binding of a single concentration of ^{3}H-R1881 in the presence of increasing concentrations of a weak binder (test substance) (dexamethasone). The sponsor will recommend an optimal concentration of rat prostate cytosol which is generally from 50 to 100 µg protein per assay tube. The competitive binding curve will be plotted by Battelle as specific ^{3}H-R1881 binding versus the concentration (log units) of the competitor. The concentration of the test substance that inhibits 50% of the maximum specific ^{3}H-R1881 binding is the IC_{50} value. At least three competitive assays will be performed to generate IC_{50} values. The general protocol to conduct the saturation assay is as follows:

Day 1

Set up tubes: Label 12x75 mm siliconized glass tubes as described in Appendix B.

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

For 3 tubes at beginning of assay and at end of assay, also add 100x inert R1881 (30µl of 1.0 µM, i.e. 1 x 10^{-6}M). These tubes are for determining nonspecific binding.

Place tubes in speed-vac and dry the tubes according to instructions. Remove when dry and placed on ice.

Add 10 µL of compound stocks (see Appendix B in triplicate)

Remove aliquot of prostate cytosol and thaw on ice. Cytosol should be diluted with ice-cold low-salt TEDG buffer to give a protein concentration of 1.2 mg per 300 µL assay tube. (Battelle recommends a 1:1 dilution or 150 µL cytosol:150 µL TEDG buffer)

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

Before leaving for the day, prepare the first wash of the HAP slurry.

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

Day 2

The following morning, wash the HAP as described above, dilute with 50 mM TRIS to yield a 60% slurry, and transfer contents to a 100 mL Erlenmeyer flask. Place a stir bar in the flask and place the flask into a beaker containing ice-water; stir the HAP slurry by placing the beaker on a magnetic stir plate.

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

One HAP tube should be prepared for each incubation tube.

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

Centrifuge the HAP tubes for 3 minutes at 4°C and 600 x g. Decant the supernatant as per radiation safety protocol. Then place the tubes back into the rack and into the ice-water bath.

Add 2 mL of 50 mM TRIS to each tube, vortex briefly then centrifuge for another 3 minutes at 4°C and 600 x g. Place the tubes into decanting racks in an ice-water bath and decant the supernatant TRIS wash into the radiation safety container. Gently tap the tube openings on a clean adsorbent towel, place the rack back in the ice-water bath and add 2 mL of 50 mM TRIS.

Repeat the TRIS washing procedure 3 times keeping the tubes at 4°C at all times possible.

Following the last wash and decanting, add 2 mLs of ethanol to each tube, vortex 3 times at 5 minute intervals and centrifuge the tubes at 600 x g for 10 minutes (4°C). Decant the supernatants into pre-labelled 20 mL scintillation vials. Add 14 mL of scintillation cocktail and count samples using the single label DPM program with quench correction.

9.3 Detailed Assay Procedures

Detailed assay procedures will be contained in SOPs for the saturation (SOP number: SRI 40-34-1) and competitive assays (SOP Number: SRI 40-35-1).

10.0 DATA PROCESSING:

10.1 Free Concentration of [^{3}H]-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 [^{3}H]-R1881 initially present in each incubation tube.

Calculation Check -

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

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

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

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

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

10.2.2. Nonspecific binding is calculated by multiplying the DPM from the tubes containing radiolabelled R1881 + 100-fold molar excess of radioinert R1881 x (1.6242×10^{-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 sent back 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 ^{3}H-R1881 bound versus the molar concentration. Estimates of the IC_{50} values 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 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 (Bmax) and association/dissociation constants (Ka / Kd) can be estimated using a number of commercially available iterative nonlinear regression analysis programs. One of the better programs was developed by Munson and Rodbard and is called LIGAND (Munson, P.J., and Rodbard, D. (1980) Anal. Biochem. 107, 220-239).

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:

Data will be entered into template Excel spreadsheets provided by Battelle, and will undergo a 100% review for accuracy and traceability prior to submission to the Southern Research Quality Assurance Unit (QAU). The final data will be audited by the QAU, then will be reviewed by the Project Manager and submitted to Battelle.

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. Food and Drug Administration's Good Laboratory Practice Regulation (21 CFR Part 58).

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 FDA GLPs. The QAU will audit the final report to assure that it accurately represents the raw data collected during the study. Quality Assurance inspection records will be made available to the Sponsor during Sponsor's visits to Southern Research.

14.0 REFERENCES

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

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

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

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

5) McPherson, 1985; 1997

6) Motulsky, 1995

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

15.0 PROTOCOL APPROVALS

This protocol has been reviewed and approved.

Study Director: 12/20/04
Gary A. Piazza, Ph.D. Date

Sponsor's Representative 1: 12/22/04
INITIALS ONLY (See pg. 2) Date

Sponsor's Representative 2: 12/21/04
INITIALS ONLY (See pg. 2) Date

This protocol has been reviewed.

Southern Research Quality Assurance: 12/20/04
Marge Wirth 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 ~~(nM)~~ (μM) (1)	Cold Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)
1	1	H	10.0	7.5	0.25	—	—	—	50	300
2	2	H	10.0	7.5	0.25	—	—	—	50	300
3	3	H	10.0	7.5	0.25	—	—	—	50	300
4	1	H	10.0	15	0.50	—	—	—	50	300
5	2	H	10.0	15	0.50	—	—	—	50	300
6	3	H	10.0	15	0.50	—	—	—	50	300
7	1	H	10.0	21	0.70	—	—	—	50	300
8	2	H	10.0	21	0.70	—	—	—	50	300
9	3	H	10.0	21	0.70	—	—	—	50	300
10	1	H	10.0	30	1.00	—	—	—	50	300
11	2	H	10.0	30	1.00	—	—	—	50	300
12	3	H	10.0	30	1.00	—	—	—	50	300
13	1	H	10.0	45	1.50	—	—	—	50	300
14	2	H	10.0	45	1.50	—	—	—	50	300
15	3	H	10.0	45	1.50	—	—	—	50	300
16	1	H	100.0	7.5	2.50	—	—	—	50	300
17	2	H	100.0	7.5	2.50	—	—	—	50	300
18	3	H	100.0	7.5	2.50	—	—	—	50	300
19	1	H	100.0	15	5.00	—	—	—	50	300
20	2	H	100.0	15	5.00	—	—	—	50	300
21	3	H	100.0	15	5.00	—	—	—	50	300
22	1	H	100.0	30	10.00	—	—	—	50	300
23	2	H	100.0	30	10.00	—	—	—	50	300
24	3	H	100.0	30	10.00	—	—	—	50	300
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300
28	1	HC	10.0	15	0.5	1.00	15	50	50	300
29	2	HC	10.0	15	0.5	1.00	15	50	50	300

(1) RE RM 4/7/05

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

41	2	Weak Positive	P	5	**E-1-P5**	5.00E-06	300	30	10	50	1.7E-07	100	500
42	3	Weak Positive	P	5	**E-1-P5**	5.00E-06	300	30	10	50	1.7E-07	100	500
43	1	Weak Positive	P	6	**E-1-P6**	5.00E-07	300	30	10	50	1.7E-08	100	500
44	2	Weak Positive	P	6	**E-1-P6**	5.00E-07	300	30	10	50	1.7E-08	100	500
45	3	Weak Positive	P	6	**E-1-P6**	5.00E-07	300	30	10	50	1.7E-08	100	500
46	1	Weak Positive	P	7	**E-1-P7**	5.00E-08	300	30	10	50	1.7E-09	100	500
47	2	Weak Positive	P	7	**E-1-P7**	5.00E-08	300	30	10	50	1.7E-09	100	500
48	3	Weak Positive	P	7	**E-1-P7**	5.00E-08	300	30	10	50	1.7E-09	100	500
49	1	Weak Positive	P	8	**E-1-P8**	5.00E-09	300	30	10	50	1.7E-10	100	500
50	2	Weak Positive	P	8	**E-1-P8**	5.00E-09	300	30	10	50	1.7E-10	100	500
51	3	Weak Positive	P	8	**E-1-P8**	5.00E-09	300	30	10	50	1.7E-10	100	500
52	1	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
53	2	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
54	3	ethanol	EtOH	0	—	—	300	30	10	50	—	100	500
55	1	Inert R1881	NSB		**E-1-S0**	3.00E-05	300	30	10	50	1.0E-06	100	500
56	2	Inert R1881	NSB		**E-1-S0**	3.00E-05	300	30	10	50	1.0E-06	100	500
57	3	Inert R1881	NSB		**E-1-S0**	3.00E-05	300	30	10	50	1.0E-06	100	500
58	1	none	Hot		—	—	—	30	—	—	—		
59	2	none	Hot		—	—	—	30	—	—	—		
60	3	none	Hot		—	—	—	30	—	—	—		
61	1	none	Hot		—	—	—	30	—	—	—		
62	2	none	Hot		—	—	—	30	—	—	—		
63	3	none	Hot		—	—	—	30	—	—	—		

PROTOCOL AMENDMENT: 11232.01 A_1

1. Page 4. There is a change in **Section 6.0 STUDY PERSONNEL** as follows:

   Study Director: due to a reassignment of duties, the responsibilities of Study Director have been transferred from Gary A. Piazza, Ph.D. to Richard D. May, Ph.D.

In order to correct some errors in the original protocol issued December 20, 2004, Protocol 11232.01 is amended for items 2-14 as follows:

2. Page 4. The **Key Study Dates** are revised as follows:

Event	Schedule	Date(s)
Receipt of all reagents	Weeks 1-2	January 3-14, 2005
Saturation assays	Weeks 3-5	January 18-February 1, 2005
Competitive assays	Weeks 5-6	January 31-February 11, 2005
QAU audit and management review	Week 7	February 7-11, 2005
Data submitted to Battelle	Week 8	February 15, 2005

3. Page 5. In **Section 8.1 IDENTITY OF THE MARKER SUBSTANCE**, the following is revised to read:

   Supplier: Sponsor (Manufacturer, Perkin-Elmer)
   Lot Number(s): 3538497

   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.

4. Page 6. The last line of the third paragraph of **Section 8.2** is revised to read:

   The compound will be stored at -20°C or lower.

5. Page 7. The second sentence of the first complete paragraph **(Section 9.1)** now reads:

   The sponsor has recommended that the optimal concentration of rat prostate cytosol be 0.6 mg/assay tube.

6. Pages 7-8. In **Section 9.1**, the instructions for **Day 1** are revised to read:

   Add unlabeled (cold) R1881 and ^{3}H-R1881 from the appropriate stock solutions to 12 x 75 mm siliconized glass tubes, as listed in Appendix A.

   Place 50 µL of 60 µM stock triamcinolone acetonide to ALL tubes.

As shown in Appendix A, aliquots of each concentration of ^{3}H-R1881 should also be counted on a scintillation counter to determine total counts added (tubes # 49-72).

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

Cytosol should be diluted with ice-cold TEDG buffer to a protein concentration of 0.6 mg per 300 µL for each assay tube. Add 300 µL of diluted prostate cytosol to all tubes (1-48). Keep tubes and cytosol on ice at all times during this procedure. Gently vortex, then mix tubes on an orbital shaker for 18-20 hours at 2-8°C.

Begin HAP washing procedure.

7. Page 9. In **Section 9.2**, the first and second sentences of the first complete paragraph now read:

AR competitive binding assays will measure the binding of a single concentration of ^{3}H-R1881 in the presence of increasing concentrations of a weak binder (test substance: dexamethasone). The sponsor has recommended that the optimal concentration of rat prostate cytosol be 1.0 mg/assay tube.

8. Page 9. In **Section 9.2**, the instructions for **Day 1** are revised to read:

Set up tubes: Label 12 x 75 mm siliconized glass tubes, as described in Appendix B.

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

For 3 tubes at the beginning and end of the assay, also add 100x inert R1881 [30 µL of 10 µM (*i.e.*, 1 x 10^{-5} M)]. These tubes are for determining nonspecific binding.

Place tubes in a speed-vac and dry the tubes according to the manufacturer's instructions. Remove tubes when dry and place on ice.

Add 10 µL of compound stocks in triplicate, as described in Appendix B.

Remove an aliquot of prostate cytosol and thaw on ice. The cytosol should be diluted with ice-cold TEDG buffer so that there is 1.0 mg of cytosol/tube.

Add 300 µL of diluted cytosol to every tube ON ICE. Gently vortex, then mix tubes on an orbital shaker for 18-20 hours at 2-8°C.

Begin HAP washing procedure.

9. Page 10. In **Section 9.2,** the instructions for **Day 2** are revised to read:

Dilute the HAP with 50 mM TRIS to yield a 60% HAP slurry and transfer contents to an Erlenmeyer flask. Place a stir bar in the flask and place the flask into a beaker containing ice-water; stir the HAP slurry by placing the beaker on a magnetic stir plate.

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

One HAP tube should be prepared for each incubation tube.

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

Centrifuge the HAP tubes for 3 minutes at 2-8°C and 600 x *g*. Then place the tubes into a decanting rack and decant the supernatant into the radiation safety container. Return rack to the ice-water bath.

Add 2 mL of 50 mM TRIS to each tube, vortex briefly, then centrifuge for another 3 minutes at 2-8°C and 600 x *g*. Place the tubes into decanting racks in an ice-water bath and decant the supernatant TRIS wash into the radiation safety container. Gently blot the tubes on a clean absorbent towel, place the rack back in the ice-water bath, and add 2 mL of 50 mM TRIS.

Repeat the TRIS washing procedure 3 times, keeping the tubes at 2-8°C at all times, as much as possible.

Following the last wash and decanting, add 2 mL of ethanol to each tube, vortex 3 times at 5 minute intervals, and centrifuge the tubes at 600 x *g* for 10 minutes (2-8°C). Decant the supernatants into pre-labeled 20 mL scintillation vials containing 14 mL of scintillation cocktail. Count samples using a single label DPM program with quench correction.

10. Pages 11-12. **Section 10.0 DATA PROCESSING** reads as currently worded, with the following sentence added just before **Section 10.1**:

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.

11. Page 13. **Section 12.0 FINAL REPORT** now reads:

As stated above, 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 and approved by personnel at Battelle. These data will then be audited by the Quality Assurance Unit at Southern Research QAU) along with the draft final report. The audited draft final report will 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.

12. Page 13. **Section 13.2 REGULATORY COMPLIANCE** now reads:

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 792).

Quality Assurance Review: Personnel from the Quality Assurance Unit (QAU) at Southern Research will inspect the study in accordance with Southern Research operating procedures and the EPA 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 and/or the EPA, as they request.

13. Pages 18-19. **Appendix B** previously indicated "laboratory E." Since Southern Research is "laboratory B," the 5th column now reads:

Labels on vials in set 1 – supplied by Battelle to laboratory E

In addition, the labels in column 5 now all contain the letter "B" rather than "E" to reflect the correct laboratory.

14. Page 19. In **Appendix B**, the values in the 6th and 9th columns for positions 55-57 now read, "1.00E-05" and "30," respectively.

Effective Date: January 3, 2005

Approval Signatures:

2/1/05

Gary A. Piazza, Ph.D. Date
Current Study Director
Manager and Senior Project Leader
Southern Research Institute

2/1/05

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

2/1/05

Margery J. Wirth Date
Manager, Quality Assurance
Southern Research Institute

2/3/05

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

2/7/05

William R. Waud, Ph.D. Date
Director, Cancer Therapeutics and Immunology Department
Southern Research Institute

PROTOCOL AMENDMENT: 11232.01 A_2

Due to a delay in conducting the competitive assays (as requested by the U.S. EPA) and in order to correct some errors in the original protocol issued December 20, 2004 and protocol amendment 1 (dated February 1, 2005), Protocol 11232.01 is amended as follows:

1. Page 4. The **Key Study Dates** are revised as follows:

Event	Schedule	Date(s)
Receipt of all reagents	Weeks 1-2	January 3-14, 2005
Saturation assays	Weeks 3-5	January 18-February 1, 2005
Competitive assays	Weeks 5-10	January 31-March ~~11~~ ①, 2005
QAU audit and management review	Weeks 11-12	March 14-~~25~~ ②, 2005
Data submitted to Battelle	Week 12	by March 25, 2005

2. Page 13. **Section 12.0 FINAL REPORT** now reads:

As stated above, 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 at Southern Research (QAU). 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.

3. Page 13. **Section 13.2 REGULATORY COMPLIANCE** now reads:

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 Quality Assurance Unit (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.

① Date changed due to a repeat that was requested by Client. RM 4/11/05
② QA delay RM 4/11/05

4. Pages 18-19. **Appendix B** previously referred to our laboratory as "laboratory E." Since Southern Research is "laboratory B," the 6th column (entitled, "Labels on vials in set 1-1-E supplied by Battelle to Laboratory 'E'") now reads:

"Vial ID for laboratory "B" (supplied by Battelle)" as the column heading.

In addition, the individual label IDs on the vials listed in column 6 contain the letter "B" rather than "E" to reflect the correct laboratory.

5. Pages 18-19 In **Appendix B**, the position numbers (column 1) previously skipped from number 21 to 28, excluding numbers 22 to 27. These numbers now read sequentially from 1 to 57 rather than from 1 to 21 and from 28 to 63.

6. Page 19. In **Appendix B**, the values in the 7th and 10th columns [entitled, "Competitor Initial Concentration (M)" and "Competitor Volume (µL)," respectively] for positions 49-51 (as corrected in item 5 above) now read, "1.00E-05" and "30," respectively.

Effective Date: February 24, 2005

Approval Signatures:

2/24/05

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

2/28/05

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

① RE RM 3/11/05

Due to 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). Thus, Protocol 11232.01 is amended as follows:

1. Pages 18-19 (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: February 1, 2005

Approval Signatures:

8-22-05

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

James E. Thomas for David Houchens 8-15-2005

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

Due to an error in the concentration of dexamethasone (Weak Positive) shown in Appendix B that was detected and requires correction (the stock concentration sent to us from Battelle was 30 mM (3.00E-2 M), Protocol 11232.01 is amended as follows:

1. Pages 18-19 (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.

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

2. Page 5. 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.

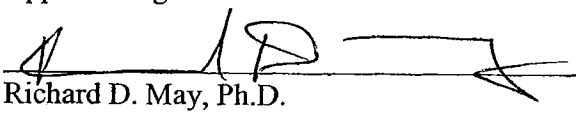
3. Page 13. Section 13.2, second paragraph, now reads:

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.

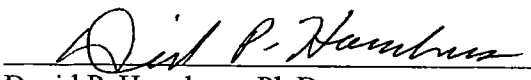
Effective Date: February 1, 2005

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 clearly not consistent with the other two 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)	Triamcelenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-B supplied by Battelle to laboratory "B"	dpm as counted	corrected DPM for 2mL
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—	780.02	2340.06
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—	928.71	2786.13
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—	811.84	2435.52
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—	1423.26	4269.78
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—	1138.93	3416.79
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—	1710.53	5131.59
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—	1755.37	5266.11
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—	1544.69	4634.07
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—	1969.09	5907.27
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—	2617.08	7851.24
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—	2005.51	6016.53
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—	1824.18	5472.54
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—	2187.7	6563.1
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—	2367.13	7101.39
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—	2323.46	6970.38
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3190.5	9571.5
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3506.13	10518.39
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3383.15	10149.45
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—	3547.29	10641.87
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—	4199.34	12598.02
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—	3519.48	10558.44
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—	4931.45	14794.35
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—	4302.5	12907.5
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—	3546.86	10640.58
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8	106.91	320.73
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8	144.47	433.41
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8	105.98	317.94
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7	86.06	258.18
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7	107.24	321.72
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7	145.47	436.41
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6	151.65	454.95
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6	114.75	344.25
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6	146.24	438.72
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5	149.43	448.29
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5	173.21	519.63
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5	153.25	459.75
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4	141.27	423.81
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4	184.26	552.78
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4	177.45	532.35
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3	302.46	907.38
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3	377.77	1133.31
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3	378.2	1134.6
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2	558.84	1676.52
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2	513.57	1540.71
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2	301.89	905.67
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1	2515	7545
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1	985.61	2956.83
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1	871.43	2614.29
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12173.96	12173.96
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12259.42	12259.42
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12369.33	12369.33
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—	23366	23366
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—	24040.47	24040.47
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—	23821.85	23821.85
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—	33436.46	33436.46
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—	34351.67	34351.67
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—	34176.32	34176.32
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—	47641.48	47641.48
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—	48202.02	48202.02
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—	48107.78	48107.78
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—	75345.14	75345.14
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—	72954.4	72954.4
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—	73679.69	73679.69
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	120477.2	120477.2
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	133546.1	133546.1
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	125115.2	125115.2
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—	255445.4	255445.4
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—	243405.4	243405.4
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—	243423.5	243423.5
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—	504179.2	504179.2
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—	500572.8	500572.8
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—	511536.3	511536.3

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1						Laboratory B												
2				AR Saturation Assay (cold R1881 dilutions supplied by Battelle)									*Laboratory Code:*		B			
3				72 assay tubes									*Run identification:*		1			
4				Please return by eMail to n.a.Holter@pnl.gov									*Assay start date:*		1/18/2005			
5				Provide information in all blue cells in column O														
6				If the DPM value for a tube was judged unreliable,														
7				Include the DPM value in column O									*Tracer lot number:*		3538-497			
8				Provide a reason in column R									*Specific activity on day of assay:*		80.45	Ci/mmole		
9				The value in column Q will change to FALSE														
10				For your convenience, data reduction is performed in columns									*Cytosol lot or vial number:*		AR-10/27/04-61 and -62			Receptor_Notes
11				U through BZ, and the values needed for analysis are presented									*protein (cytosol) per tube:*		600	ug		diluted to 2 mg/ml for use (1.5 mg/300 ul)
12				in columns CF through CN									*protein (cytosol) per tube:*		0.6	mg		
13													KD		9.18E-01	nM		
14				Cells in column S are presented with a grey background									Bmax		10.45	fmole/100 ug		
15				if the total binding exceeds 10% of the hot added at that concentration,									*total volume in tubes*		300	uL		
16				the cytosol concentration is probably too high for good competitive assays									volume of ethanol counted:		2	mL		protcol calls for counting decanted EtOH sup
17													mulitply DPM in sample by :		3			reflects 100ul of reaction mixture processed

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
18	Saturation Assay Tube Layout																			
19	Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-B supplied by Battelle to laboratory "B"		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
20																				
21	1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—		780.02	2340.06	TRUE		19.1%	0.25
22	2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—		928.71	2786.13	TRUE		22.7%	0.25
23	3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—		811.84	2435.52	TRUE		19.9%	0.25
24	4	1	H	10.0	15	0.50	—	—	—	50	300	—	—		1423.26	4269.78	TRUE		18.0%	0.5
25	5	2	H	10.0	15	0.50	—	—	—	50	300	—	—		1138.93	3416.79	TRUE		14.4%	0.5
26	6	3	H	10.0	15	0.50	—	—	—	50	300	—	—		1710.53	5131.59	TRUE		21.6%	0.5
27	7	1	H	10.0	21	0.70	—	—	—	50	300	—	—		1755.37	5266.11	TRUE		15.5%	0.7
28	8	2	H	10.0	21	0.70	—	—	—	50	300	—	—		1544.69	4634.07	TRUE		13.6%	0.7
29	9	3	H	10.0	21	0.70	—	—	—	50	300	—	—		1969.09	5907.27	TRUE		17.4%	0.7
30	10	1	H	10.0	30	1.00	—	—	—	50	300	—	—		2617.08	7851.24	TRUE		16.4%	1
31	11	2	H	10.0	30	1.00	—	—	—	50	300	—	—		2005.51	6016.53	TRUE		12.5%	1
32	12	3	H	10.0	30	1.00	—	—	—	50	300	—	—		1824.18	5472.54	TRUE		11.4%	1
33	13	1	H	10.0	45	1.50	—	—	—	50	300	—	—		2187.7	6563.1	TRUE		8.9%	1.5
34	14	2	H	10.0	45	1.50	—	—	—	50	300	—	—		2367.13	7101.39	TRUE		9.6%	1.5
35	15	3	H	10.0	45	1.50	—	—	—	50	300	—	—		2323.46	6970.38	TRUE		9.4%	1.5
36	16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3190.5	9571.5	TRUE		7.6%	2.5
37	17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3506.13	10518.39	TRUE		8.3%	2.5
38	18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3383.15	10149.45	TRUE		8.0%	2.5
39	19	1	H	100.0	15	5.00	—	—	—	50	300	—	—		3547.29	10641.87	TRUE		4.3%	5
40	20	2	H	100.0	15	5.00	—	—	—	50	300	—	—		4199.34	12598.02	TRUE		5.1%	5
41	21	3	H	100.0	15	5.00	—	—	—	50	300	—	—		3519.48	10558.44	TRUE		4.3%	5
42	22	1	H	100.0	30	10.00	—	—	—	50	300	—	—		4931.45	14794.35	TRUE		2.9%	10
43	23	2	H	100.0	30	10.00	—	—	—	50	300	—	—		4302.5	12907.5	TRUE		2.6%	10
44	24	3	H	100.0	30	10.00	—	—	—	50	300	—	—		3546.86	10640.58	TRUE		2.1%	10

	U	V
18		
19	Bound y values	NSB y values
20		
21	2340.1	357.4
22	2786.1	357.4
23	2435.5	357.4
24	4269.8	338.8
25	3416.8	338.8
26	5131.6	338.8
27	5266.1	412.6
28	4634.1	412.6
29	5907.3	412.6
30	7851.2	475.9
31	6016.5	475.9
32	5472.5	475.9
33	6563.1	503.0
34	7101.4	503.0
35	6970.4	503.0
36	9571.5	1058.4
37	10518.4	1058.4
38	10149.5	1058.4
39	10641.9	1374.3
40	12598.0	1374.3
41	10558.4	1374.3
42	14794.4	2785.6
43	12907.5	2785.6
44	10640.6	2785.6

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
45	25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		106.91	320.73	TRUE			
46	26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		144.47	433.41	TRUE			
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		105.98	317.94	TRUE			
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		86.06	258.18	TRUE			
49	29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		107.24	321.72	TRUE			
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		145.47	436.41	TRUE			
51	31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		151.65	454.95	TRUE			
52	32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		114.75	344.25	TRUE			
53	33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		146.24	438.72	TRUE			
54	34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		149.43	448.29	TRUE			
55	35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		173.21	519.63	TRUE			
56	36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		153.25	459.75	TRUE			
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		141.27	423.81	TRUE			
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		184.26	552.78	TRUE			
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		177.45	532.35	TRUE			
60	40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		302.46	907.38	TRUE			
61	41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		377.77	1133.31	TRUE			
62	42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		378.2	1134.6	TRUE			
63	43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		558.84	1676.52	TRUE			
64	44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		513.57	1540.71	TRUE			
65	45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		301.89	905.67	TRUE			
66	46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		2515	7545	FALSE	outlier		
67	47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		985.61	2956.83	TRUE			
68	48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		871.43	2614.29	TRUE			
69	49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		12173.96	12173.96	TRUE			
70	50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		12259.42	12259.42	TRUE			
71	51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		12369.33	12369.33	TRUE			
72	52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—		23366	23366	TRUE			
73	53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—		24040.47	24040.47	TRUE			
74	54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—		23821.85	23821.85	TRUE			
75	55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—		33436.46	33436.46	TRUE			
76	56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—		34351.67	34351.67	TRUE			
77	57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—		34176.32	34176.32	TRUE			
78	58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—		47641.48	47641.48	TRUE			
79	59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—		48202.02	48202.02	TRUE			
80	60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—		48107.78	48107.78	TRUE			
81	61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—		75345.14	75345.14	TRUE			
82	62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—		72954.4	72954.4	TRUE			
83	63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—		73679.69	73679.69	TRUE			
84	64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		120477.2	120477.2	TRUE			
85	65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		133546.1	133546.1	TRUE			
86	66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		125115.2	125115.2	TRUE			
87	67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—		255445.4	255445.4	TRUE			
88	68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—		243405.4	243405.4	TRUE			
89	69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—		243423.5	243423.5	TRUE			
90	70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—		504179.2	504179.2	TRUE			
91	71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—		500572.8	500572.8	TRUE			
92	72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—		511536.3	511536.3	TRUE			

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	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
17			Total Binding – Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)											
18			Tube Identification			Assay tube contents								
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
21	1	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
22	1	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
23	1	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
24	1	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
25	1	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
26	1	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
27	1	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
28	1	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
29	1	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
30	1	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
31	1	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
32	1	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
33	1	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
34	1	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
35	1	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
36	1	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
37	1	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
38	1	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
39	1	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
40	1	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
41	1	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
42	1	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
43	1	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
44	1	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300
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18										Number of molecules					Ratio
19	Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
20			(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
21	1	1	2340.1	357.4	1982.7	15.3%	19.1%	12267.6	9927.5	14	2	12	73	59	0.20
22	1	2	2786.1	357.4	2428.8	12.8%	22.7%	12267.6	9481.4	17	2	14	73	57	0.26
23	1	3	2435.5	357.4	2078.2	14.7%	19.9%	12267.6	9832.1	15	2	12	73	59	0.21
24	1	4	4269.8	338.8	3931.0	7.9%	18.0%	23742.8	19473.0	25	2	23	142	116	0.20
25	1	5	3416.8	338.8	3078.0	9.9%	14.4%	23742.8	20326.0	20	2	18	142	121	0.15
26	1	6	5131.6	338.8	4792.8	6.6%	21.6%	23742.8	18611.2	31	2	29	142	111	0.26
27	1	7	5266.1	412.6	4853.5	7.8%	15.5%	33988.2	28722.0	31	2	29	203	171	0.17
28	1	8	4634.1	412.6	4221.4	8.9%	13.6%	33988.2	29354.1	28	2	25	203	175	0.14
29	1	9	5907.3	412.6	5494.6	7.0%	17.4%	33988.2	28080.9	35	2	33	203	167	0.20
30	1	10	7851.2	475.9	7375.4	6.1%	16.4%	47983.8	40132.5	47	3	44	286	239	0.18
31	1	11	6016.5	475.9	5540.6	7.9%	12.5%	47983.8	41967.2	36	3	33	286	250	0.13
32	1	12	5472.5	475.9	4996.7	8.7%	11.4%	47983.8	42511.2	33	3	30	286	254	0.12
33	1	13	6563.1	503.0	6060.1	7.7%	8.9%	73993.1	67430.0	39	3	36	441	402	0.09
34	1	14	7101.4	503.0	6598.4	7.1%	9.6%	73993.1	66891.7	42	3	39	441	399	0.10
35	1	15	6970.4	503.0	6467.4	7.2%	9.4%	73993.1	67022.7	42	3	39	441	400	0.10
36	1	16	9571.5	1058.4	8513.1	11.1%	7.6%	126379.5	116808.0	57	6	51	754	697	0.07
37	1	17	10518.4	1058.4	9460.0	10.1%	8.3%	126379.5	115861.1	63	6	56	754	691	0.08
38	1	18	10149.5	1058.4	9091.0	10.4%	8.0%	126379.5	116230.1	61	6	54	754	693	0.08
39	1	19	10641.9	1374.3	9267.6	12.9%	4.3%	247424.8	236782.9	63	8	55	1476	1412	0.04
40	1	20	12598.0	1374.3	11223.7	10.9%	5.1%	247424.8	234826.7	75	8	67	1476	1401	0.05
41	1	21	10558.4	1374.3	9184.1	13.0%	4.3%	247424.8	236866.3	63	8	55	1476	1413	0.04
42	1	22	14794.4	2785.6	12008.8	18.8%	2.9%	505429.4	490635.1	88	17	72	3015	2927	0.02
43	1	23	12907.5	2785.6	10121.9	21.6%	2.6%	505429.4	492521.9	77	17	60	3015	2938	0.02
44	1	24	10640.6	2785.6	7855.0	26.2%	2.1%	505429.4	494788.9	63	17	47	3015	2951	0.02
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	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
17			Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X Inert R1881 plus cytosol												
18			Tube Identification			Assay tube contents								Scintillation Results	
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
21	1	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	320.7	357.4
22	1	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	433.4	357.4
23	1	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	317.9	357.4
24	1	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	258.2	338.8
25	1	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	321.7	338.8
26	1	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	436.4	338.8
27	1	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	455.0	412.6
28	1	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	344.3	412.6
29	1	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	438.7	412.6
30	1	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	448.3	475.9
31	1	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	519.6	475.9
32	1	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	459.8	475.9
33	1	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	423.8	503.0
34	1	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	552.8	503.0
35	1	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	532.4	503.0
36	1	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	907.4	1058.4
37	1	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1133.3	1058.4
38	1	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1134.6	1058.4
39	1	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	1676.5	1374.3
40	1	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	1540.7	1374.3
41	1	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	905.7	1374.3
42	1	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000		2785.6
43	1	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	2956.8	2785.6
44	1	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	2614.3	2785.6
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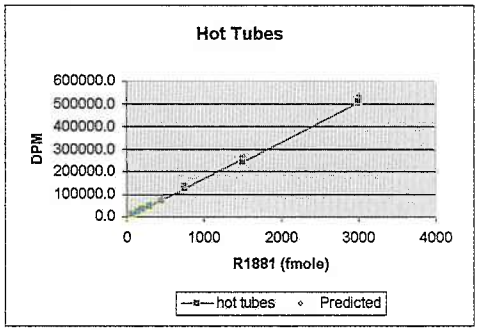
Free -- Positions 49-72, radiolabeled R1881 without cytosol

Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial (nM)	Hot R1881 Volume (ul)	Molecules of R1881 (fmole)	Counts per Scintillation Vial (dpm)	Experimental number of molecules (fmole)	Total Added (Mean of reps in pos. 49-72) (dpm)	predicted dpm
1	49	1	Hot	c1	10	7.5	75	12174.0	73	12267.6	13395
1	50	2	Hot	c1	10	7.5	75	12259.4	73	12267.6	13395
1	51	3	Hot	c1	10	7.5	75	12369.3	74	12267.6	13395
1	52	1	Hot	c2	10	15	150	23366.0	139	23742.8	26791
1	53	2	Hot	c2	10	15	150	24040.5	143	23742.8	26791
1	54	3	Hot	c2	10	15	150	23821.9	142	23742.8	26791
1	55	1	Hot	c3	10	21	210	33436.5	199	33988.2	37507
1	56	2	Hot	c3	10	21	210	34351.7	205	33988.2	37507
1	57	3	Hot	c3	10	21	210	34176.3	204	33988.2	37507
1	58	1	Hot	c4	10	30	300	47641.5	284	47983.8	53581
1	59	2	Hot	c4	10	30	300	48202.0	288	47983.8	53581
1	60	3	Hot	c4	10	30	300	48107.8	287	47983.8	53581
1	61	1	Hot	c5	10	45	450	75345.1	449	73993.1	80372
1	62	2	Hot	c5	10	45	450	72954.4	435	73993.1	80372
1	63	3	Hot	c5	10	45	450	73679.7	439	73993.1	80372
1	64	1	Hot	c6	100	7.5	750	120477.2	719	126379.5	133953
1	65	2	Hot	c6	100	7.5	750	133546.1	797	126379.5	133953
1	66	3	Hot	c6	100	7.5	750	125115.2	746	126379.5	133953
1	67	1	Hot	c7	100	15	1500	255445.4	1524	247424.8	267906
1	68	2	Hot	c7	100	15	1500	243405.4	1452	247424.8	267906
1	69	3	Hot	c7	100	15	1500	243423.5	1452	247424.8	267906
1	70	1	Hot	c8	100	30	3000	504179.2	3007	505429.4	535813
1	71	2	Hot	c8	100	30	3000	500572.8	2986	505429.4	535813
1	72	3	Hot	c8	100	30	3000	511536.3	3051	505429.4	535813

Computation Check

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



Linear regression results (LINEST function)

(Regression line forced through 0,0)

Slope	167.650765	dpm/fmole
1/slope	0.00596478	fmole/dpm
	x	y
origin	0	0
end point	3051.2	511536.3

SLOPE function, used if missing HOT tubes

Slope	168.5	dpm/fmole
1/slope	0.005934	fmole/dpm
	x	y
origin	0	0
end point	3035.3	511536.3

	CF	CG	CH	CI
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
21	3.70036E-11	0.19972	2.28953E-10	3.70036E-11
22	4.53287E-11	0.25616	2.28953E-10	4.53287E-11
23	3.87852E-11	0.21137	2.28953E-10	3.87852E-11
24	7.33654E-11	0.20187	4.43117E-10	7.33654E-11
25	5.74458E-11	0.15143	4.43117E-10	5.74458E-11
26	8.94496E-11	0.25752	4.43117E-10	8.94496E-11
27	9.05815E-11	0.16898	6.34329E-10	9.05815E-11
28	7.87856E-11	0.14381	6.34329E-10	7.87856E-11
29	1.02548E-10	0.19567	6.34329E-10	1.02548E-10
30	1.37648E-10	0.18377	8.95532E-10	1.37648E-10
31	1.03406E-10	0.13202	8.95532E-10	1.03406E-10
32	9.32537E-11	0.11754	8.95532E-10	9.32537E-11
33	1.13101E-10	0.08987	1.38095E-09	1.13101E-10
34	1.23148E-10	0.09864	1.38095E-09	1.23148E-10
35	1.20703E-10	0.09650	1.38095E-09	1.20703E-10
36	1.58881E-10	0.07288	2.35865E-09	1.58881E-10
37	1.76554E-10	0.08165	2.35865E-09	1.76554E-10
38	1.69668E-10	0.07822	2.35865E-09	1.69668E-10
39	1.72963E-10	0.03914	4.61775E-09	1.72963E-10
40	2.09471E-10	0.04780	4.61775E-09	2.09471E-10
41	1.71406E-10	0.03877	4.61775E-09	1.71406E-10
42	2.24123E-10	0.02448	9.43295E-09	2.24123E-10
43	1.88908E-10	0.02055	9.43295E-09	1.88908E-10
44	1.466E-10	0.01588	9.43295E-09	1.466E-10
45				
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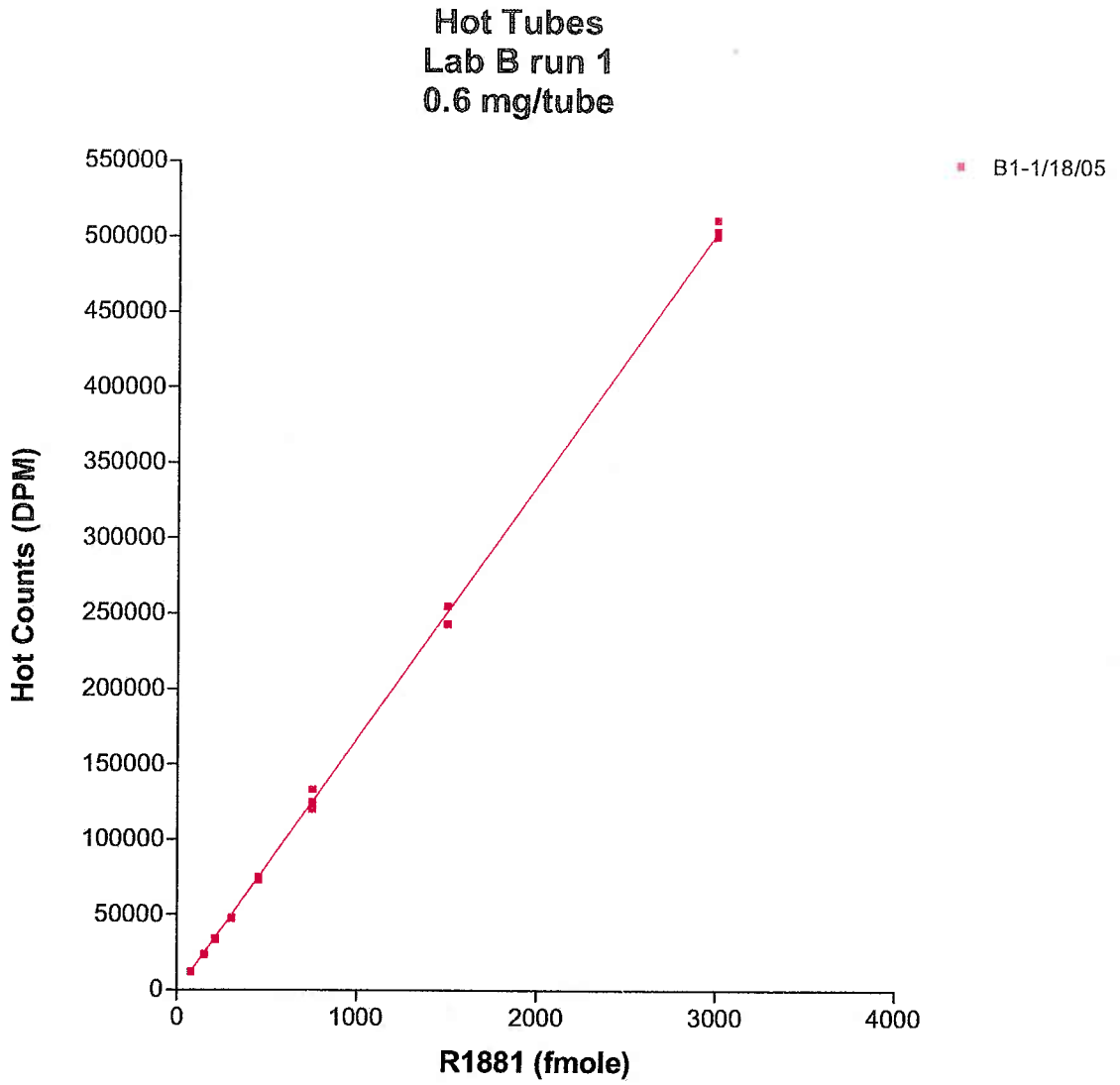
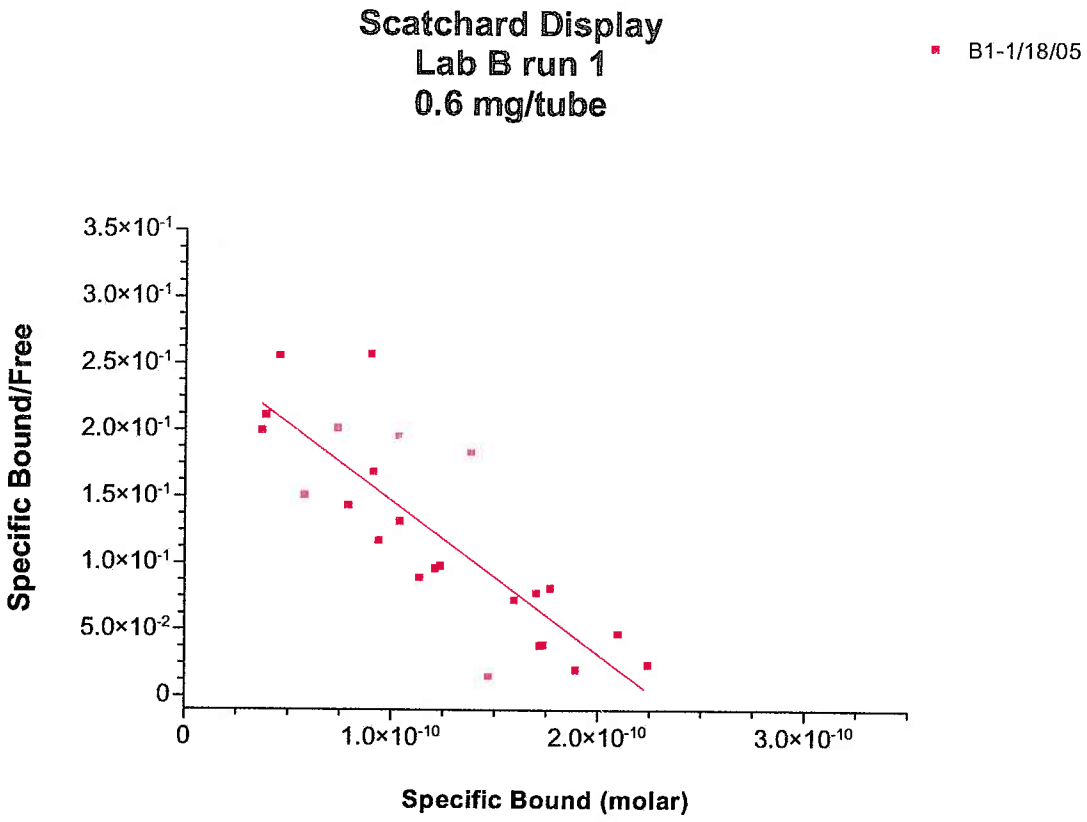
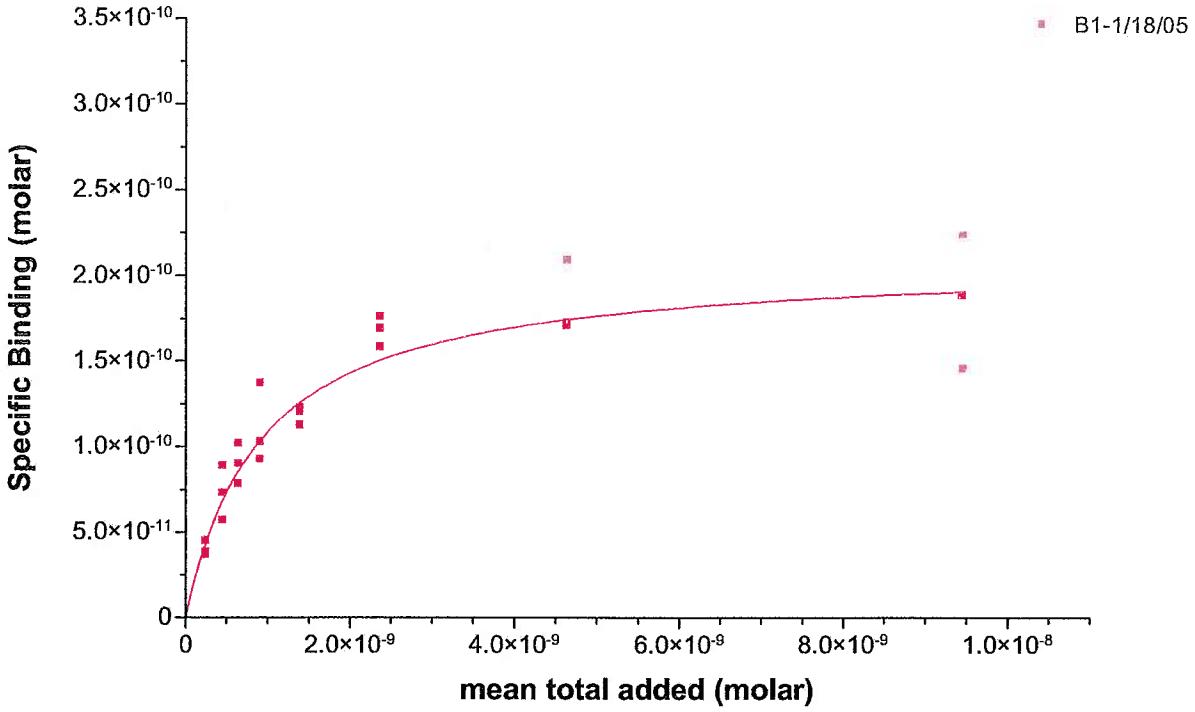
58				

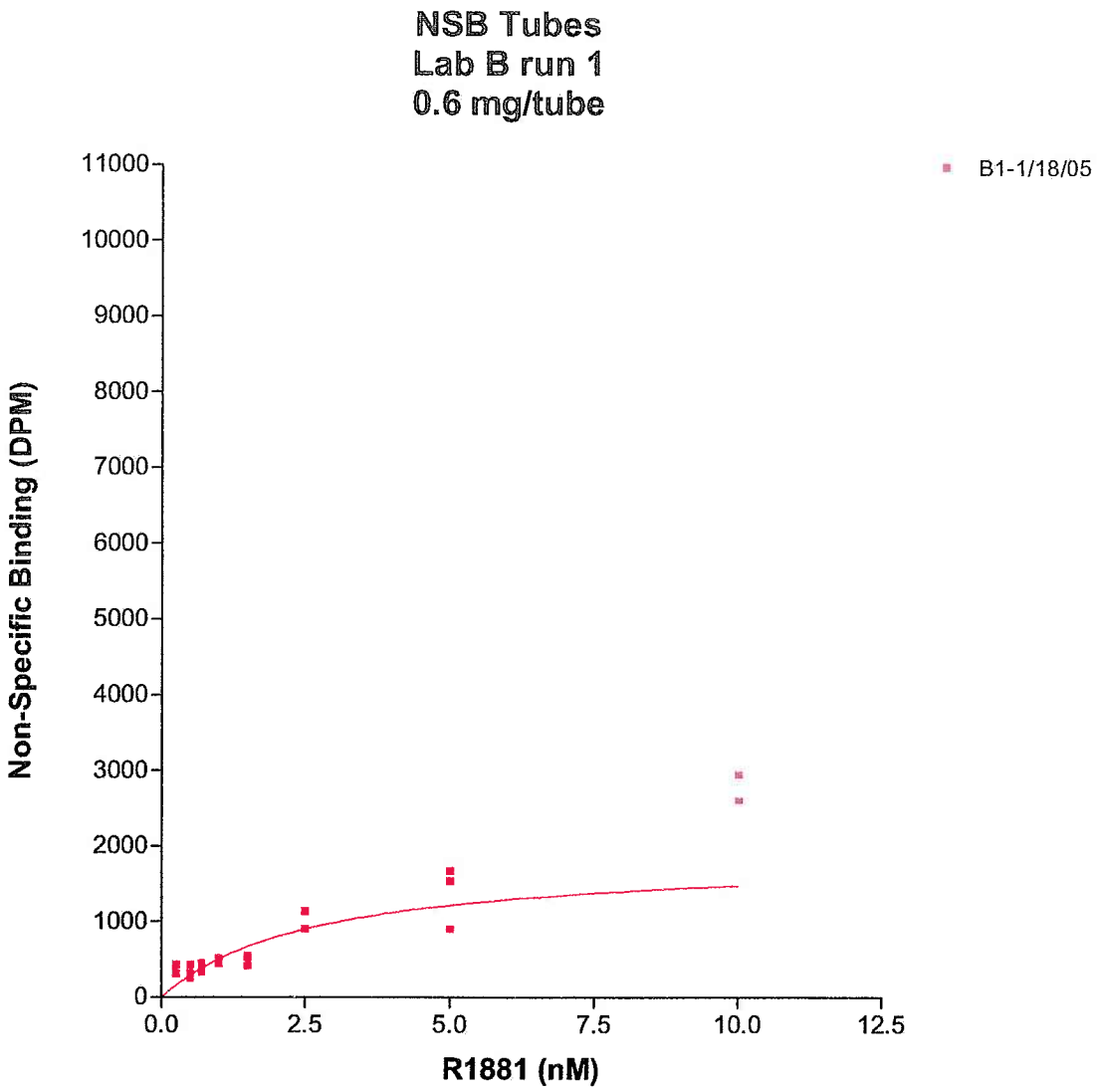

Hot Tubes
Lab B run 1
0.6 mg/tube
B1-1/18/05
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
1000
2000
3000
4000
R1881 (fmole)

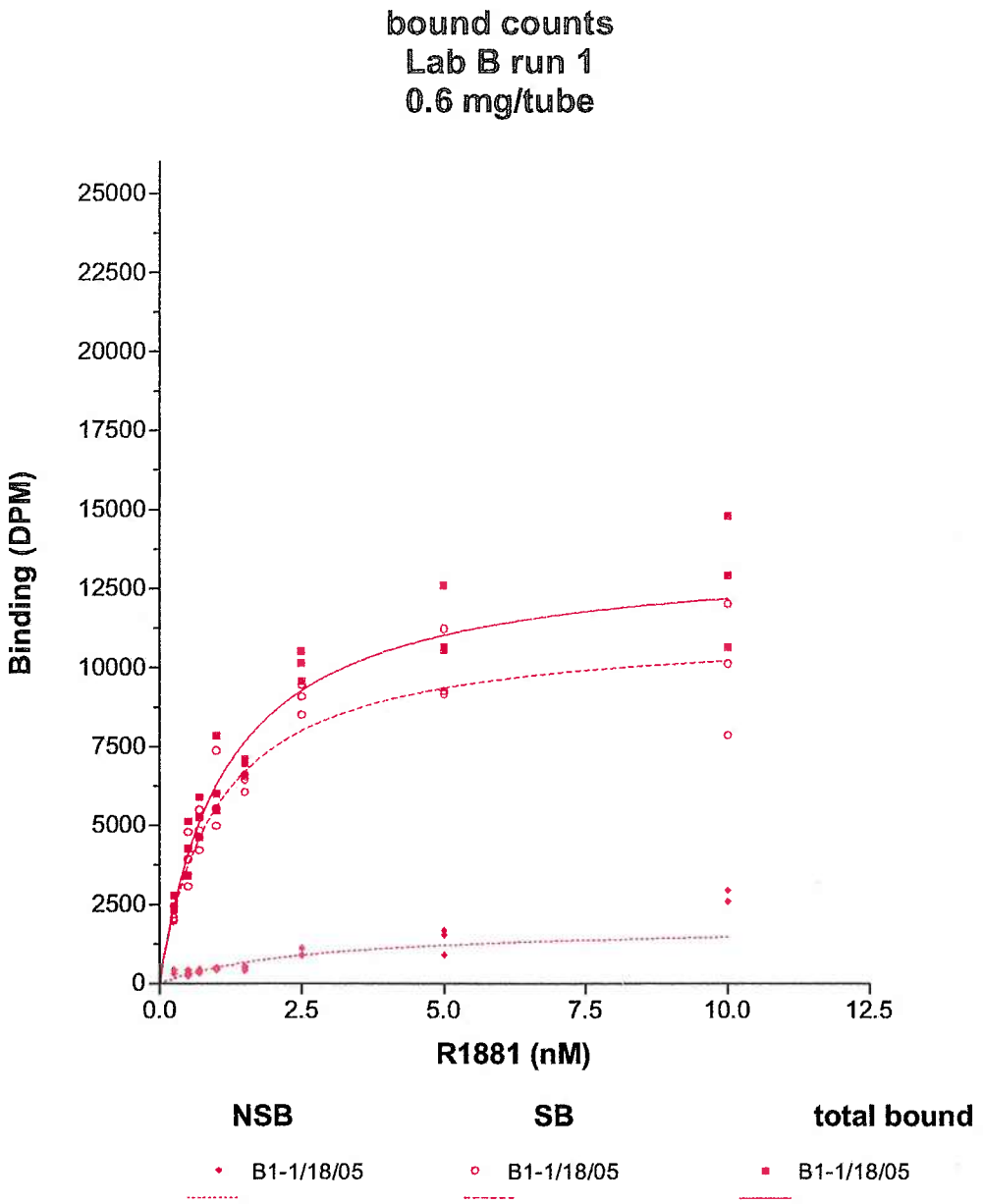

Scatchard Display
Lab B run 1
0.6 mg/tube
B1-1/18/05
Specific Bound/Free
3.5×10-1
3.0×10-1
2.5×10-1
2.0×10-1
1.5×10-1
1.0×10-1
5.0×10-2
0
0
1.0×10-10
2.0×10-10
3.0×10-10
Specific Bound (molar)

Lab B run 1
0.6 mg/tube



specific bound	B1-1/18/05
BMAX	2.0896e-010
KD	9.1846e-010
Std. Error	
BMAX	1.2550e-011
KD	1.1034e-010
95% Confidence Intervals	
BMAX	1.8293e-010 to 2.3499e-010
KD	6.8961e-010 to 1.1473e-009
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.8822
Weighted Sum of Squares ($1/Y^2$)	0.4294
Absolute Sum of Squares	7.9129e-021
Sy.x	1.8965e-011
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0


NSB Tubes
Lab B run 1
0.6 mg/tube
B1-1/18/05
Non-Specific Binding (DPM)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)


bound counts
Lab B run 1
0.6 mg/tube
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
SB
total bound
B1-1/18/05
B1-1/18/05
B1-1/18/05

APPENDIX C

Processed Raw Data from Saturation Assay 2 and Associated Figures

Counts from Saturation Assay Number 2

Saturation Assay Tube Layout

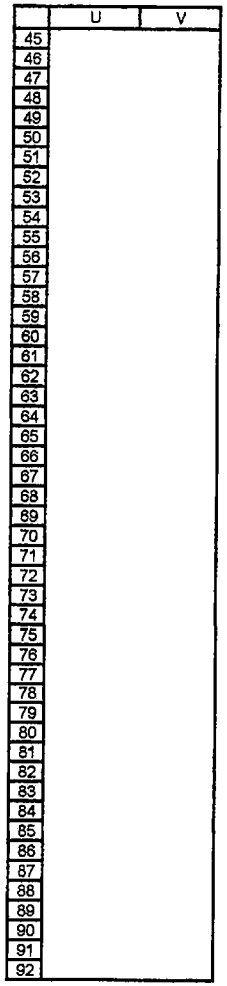
Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-B supplied by Battelle to laboratory "B"	dpm as counted	corrected DPM for 2mL
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—	887.56	2662.68
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—	872.43	2617.29
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—	858.7	2576.1
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—	1631.36	4894.08
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—	1515.5	4546.5
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—	1619.07	4857.21
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—	1692.28	5076.84
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—	1758.73	5276.19
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—	1958.91	5876.73
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—	2117.11	6351.33
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—	2274.88	6824.64
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—	2075.45	6226.35
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—	2789.55	8368.65
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—	2668.26	8004.78
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—	2908.16	8724.48
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3358.54	10075.62
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3779.12	11337.36
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3444.09	10332.27
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—	4025.64	12076.92
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—	4098.96	12296.88
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—	4355.12	13065.36
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—	4601.55	13804.65
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—	5125.22	15375.66
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—	4875.01	14625.03
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8	107.31	321.93
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8	130.77	392.31
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8	133.03	399.09
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7	165.24	495.72
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7	130.1	390.3
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7	162.93	488.79
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6	188.17	564.51
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6	152.25	456.75
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6	218.95	656.85
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5	153.77	461.31
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5	130.9	392.7
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5	151.81	455.43
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4	267.7	803.1
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4	184.18	552.54
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4	207.65	622.95
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3	371.59	1114.77
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3	375.8	1127.4
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3	407.58	1222.74
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2	519.66	1558.98
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2	768.21	2304.63
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2	570.73	1712.19
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1	1117.5	3352.5
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1	1070.73	3212.19
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1	1151.25	3453.75
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	11982.99	11982.99
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	11736.77	11736.77
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	12635.99	12635.99
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—	22571.55	22571.55
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—	22537.46	22537.46
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—	23925.38	23925.38
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—	30954.94	30954.94
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—	32008.07	32008.07
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—	32788.39	32788.39
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—	45810.08	45810.08
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—	49800.71	49800.71
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—	48652.25	48652.25
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—	74590.42	74590.42
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—	70393.89	70393.89
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—	71870.12	71870.12
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	127392.5	127392.5
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	125390.6	125390.6
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	130942.7	130942.7
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—	231220.3	231220.3
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—	239984.9	239984.9
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—	245186.3	245186.3
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—	505751.2	505751.2
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—	514635.1	514635.1
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—	476717.4	476717.4

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1						Laboratory B												
2				AR Saturation Assay (cold R1881 dilutions supplied by Battelle)											Laboratory Code:	B		
3				72 assay tubes											Run identification:	2		
4				Please return by eMail to n.a.Holter@pnl.gov											Assay start date:	1/24/2005		
5				Provide information in all blue cells in column O														
6				If the DPM value for a tube was judged unreliable,														
7				Include the DPM value in column O											Tracer lot number:	3538-497		
8				Provide a reason in column R											Specific activity on day of assay:	80.38	Ci/mmole	
9				The value in column Q will change to FALSE														
10				For your convenience, data reduction is performed in columns											Cytosol lot or vial number:	AR-10/27/04-63 and -64		Receptor_Notes
11				U through BZ, and the values needed for analysis are presented											protein (cytosol) per tube:	600	ug	diluted to 2 mg/ml for use (0.6 mg/300 ul)
12				in columns CF through CN											protein (cytosol) per tube:	0.6	mg	
13															KD	9.89E-01	nM	
14				Cells in column S are presented with a grey background											Bmax	12.03	fmole/100 ug	
15				if the total binding exceeds 10% of the hot added at that concentration,											total volume in tubes	300	uL	
16				the cytosol concentration is probably too high for good competitive assays											volume of ethanol counted:	2	mL	protcol calls for counting decanted EtOH sup
17															mulitply DPM in sample by :	3		reflects 100ul of reaction mixture processed

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
18	Saturation Assay Tube Layout																			
19	Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-B supplied by Battelle to laboratory "B"		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
20																				
21	1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—		887.56	2662.68	TRUE		22.0%	0.25
22	2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—		872.43	2617.29	TRUE		21.6%	0.25
23	3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—		858.7	2576.1	TRUE		21.3%	0.25
24	4	1	H	10.0	15	0.50	—	—	—	50	300	—	—		1631.36	4894.08	TRUE		21.3%	0.5
25	5	2	H	10.0	15	0.50	—	—	—	50	300	—	—		1515.5	4546.5	TRUE		19.8%	0.5
26	6	3	H	10.0	15	0.50	—	—	—	50	300	—	—		1619.07	4857.21	TRUE		21.1%	0.5
27	7	1	H	10.0	21	0.70	—	—	—	50	300	—	—		1692.28	5076.84	TRUE		15.9%	0.7
28	8	2	H	10.0	21	0.70	—	—	—	50	300	—	—		1758.73	5276.19	TRUE		16.5%	0.7
29	9	3	H	10.0	21	0.70	—	—	—	50	300	—	—		1958.91	5876.73	TRUE		18.4%	0.7
30	10	1	H	10.0	30	1.00	—	—	—	50	300	—	—		2117.11	6351.33	TRUE		13.2%	1
31	11	2	H	10.0	30	1.00	—	—	—	50	300	—	—		2274.88	6824.64	TRUE		14.2%	1
32	12	3	H	10.0	30	1.00	—	—	—	50	300	—	—		2075.45	6226.35	TRUE		12.9%	1
33	13	1	H	10.0	45	1.50	—	—	—	50	300	—	—		2789.55	8368.65	TRUE		11.6%	1.5
34	14	2	H	10.0	45	1.50	—	—	—	50	300	—	—		2668.26	8004.78	TRUE		11.1%	1.5
35	15	3	H	10.0	45	1.50	—	—	—	50	300	—	—		2908.16	8724.48	TRUE		12.1%	1.5
36	16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3358.54	10075.62	TRUE		7.9%	2.5
37	17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3779.12	11337.36	TRUE		8.9%	2.5
38	18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3444.09	10332.27	TRUE		8.1%	2.5
39	19	1	H	100.0	15	5.00	—	—	—	50	300	—	—		4025.64	12076.92	TRUE		5.1%	5
40	20	2	H	100.0	15	5.00	—	—	—	50	300	—	—		4098.96	12296.88	TRUE		5.1%	5
41	21	3	H	100.0	15	5.00	—	—	—	50	300	—	—		4355.12	13065.36	TRUE		5.5%	5
42	22	1	H	100.0	30	10.00	—	—	—	50	300	—	—		4601.55	13804.65	TRUE		2.8%	10
43	23	2	H	100.0	30	10.00	—	—	—	50	300	—	—		5125.22	15375.66	TRUE		3.1%	10
44	24	3	H	100.0	30	10.00	—	—	—	50	300	—	—		4875.01	14625.03	TRUE		2.9%	10

	U	V
18		
19	Bound y values	NSB y values
20		
21	2862.7	371.1
22	2617.3	371.1
23	2576.1	371.1
24	4894.1	458.3
25	4546.5	458.3
26	4857.2	458.3
27	5076.8	559.4
28	5276.2	559.4
29	5876.7	559.4
30	6351.3	436.5
31	6824.6	436.5
32	6226.4	436.5
33	8368.7	659.5
34	8004.8	659.5
35	8724.5	659.5
36	10075.6	1155.0
37	11337.4	1155.0
38	10332.3	1155.0
39	12076.9	1858.6
40	12296.9	1858.6
41	13065.4	1858.6
42	13804.7	3339.5
43	15375.7	3339.5
44	14625.0	3339.5

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T
45	25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		107.31	321.93	TRUE			
46	26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		130.77	392.31	TRUE			
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		133.03	399.09	TRUE			
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		165.24	495.72	TRUE			
49	29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		130.1	390.3	TRUE			
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		162.93	488.79	TRUE			
51	31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		188.17	564.51	TRUE			
52	32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		152.25	456.75	TRUE			
53	33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		218.95	656.85	TRUE			
54	34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		153.77	461.31	TRUE			
55	35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		130.9	392.7	TRUE			
56	36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		151.81	455.43	TRUE			
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		267.7	803.1	TRUE			
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		184.18	552.54	TRUE			
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		207.65	622.95	TRUE			
60	40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		371.59	1114.77	TRUE			
61	41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		375.8	1127.4	TRUE			
62	42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		407.58	1222.74	TRUE			
63	43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		519.66	1558.98	TRUE			
64	44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		768.21	2304.63	TRUE			
65	45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		570.73	1712.19	TRUE			
66	46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		1117.5	3352.5	TRUE			
67	47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		1070.73	3212.19	TRUE			
68	48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		1151.25	3453.75	TRUE			
69	49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		11982.99	11982.99	TRUE			
70	50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		11736.77	11736.77	TRUE			
71	51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		12635.99	12635.99	TRUE			
72	52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—		22571.55	22571.55	TRUE			
73	53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—		22537.46	22537.46	TRUE			
74	54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—		23925.38	23925.38	TRUE			
75	55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—		30954.94	30954.94	TRUE			
76	56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—		32008.07	32008.07	TRUE			
77	57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—		32788.39	32788.39	TRUE			
78	58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—		45810.08	45810.08	TRUE			
79	59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—		49800.71	49800.71	TRUE			
80	60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—		48652.25	48652.25	TRUE			
81	61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—		74590.42	74590.42	TRUE			
82	62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—		70393.89	70393.89	TRUE			
83	63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—		71870.12	71870.12	TRUE			
84	64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		127392.5	127392.5	TRUE			
85	65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		125390.6	125390.6	TRUE			
86	66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		130942.7	130942.7	TRUE			
87	67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—		231220.3	231220.3	TRUE			
88	68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—		239984.9	239984.9	TRUE			
89	69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—		245186.3	245186.3	TRUE			
90	70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—		505751.2	505751.2	TRUE			
91	71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—		514635.1	514635.1	TRUE			
92	72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—		476717.4	476717.4	TRUE			



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	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
17			Total Binding -- Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)											
18			Tube Identification			Assay tube contents								
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
21	2	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
22	2	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
23	2	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
24	2	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
25	2	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
26	2	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
27	2	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
28	2	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
29	2	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
30	2	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
31	2	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
32	2	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
33	2	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
34	2	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
35	2	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
36	2	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
37	2	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
38	2	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
39	2	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
40	2	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
41	2	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
42	2	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
43	2	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
44	2	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300
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	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY
17											Number of molecules				Ratio
18															
19	Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 25 48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Hot	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
20			(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
21	2	1	2662.7	371.1	2291.6	13.9%	22.0%	12118.6	9455.9	16	2	14	73	57	0.24
22	2	2	2617.3	371.1	2246.2	14.2%	21.6%	12118.6	9501.3	16	2	14	73	58	0.24
23	2	3	2576.1	371.1	2205.0	14.4%	21.3%	12118.6	9542.5	16	2	13	73	58	0.23
24	2	4	4894.1	458.3	4435.8	9.4%	21.3%	23011.5	18117.4	30	3	27	139	110	0.24
25	2	5	4546.5	458.3	4088.2	10.1%	19.8%	23011.5	18465.0	28	3	25	139	112	0.22
26	2	6	4857.2	458.3	4398.9	9.4%	21.1%	23011.5	18154.3	29	3	27	139	110	0.24
27	2	7	5076.8	559.4	4517.5	11.0%	15.9%	31917.1	26840.3	31	3	27	193	163	0.17
28	2	8	5276.2	559.4	4716.8	10.6%	16.5%	31917.1	26640.9	32	3	29	193	161	0.18
29	2	9	5876.7	559.4	5317.4	9.5%	18.4%	31917.1	26040.4	36	3	32	193	158	0.20
30	2	10	6351.3	436.5	5914.9	6.9%	13.2%	48087.7	41736.4	38	3	36	291	253	0.14
31	2	11	6824.6	436.5	6388.2	6.4%	14.2%	48087.7	41263.0	41	3	39	291	250	0.15
32	2	12	6226.4	436.5	5789.9	7.0%	12.9%	48087.7	41861.3	38	3	35	291	254	0.14
33	2	13	8368.7	659.5	7709.1	7.9%	11.6%	72284.8	63916.2	51	4	47	438	387	0.12
34	2	14	8004.8	659.5	7345.3	8.2%	11.1%	72284.8	64280.0	49	4	45	438	390	0.11
35	2	15	8724.5	659.5	8065.0	7.6%	12.1%	72284.8	63560.3	53	4	49	438	385	0.13
36	2	16	10075.6	1155.0	8920.7	11.5%	7.9%	127908.6	117833.0	61	7	54	775	714	0.08
37	2	17	11337.4	1155.0	10182.4	10.2%	8.9%	127908.6	116571.2	69	7	62	775	706	0.09
38	2	18	10332.3	1155.0	9177.3	11.2%	8.1%	127908.6	117576.3	63	7	56	775	713	0.08
39	2	19	12076.9	1858.6	10218.3	15.4%	5.1%	238797.2	226720.2	73	11	62	1447	1374	0.05
40	2	20	12296.9	1858.6	10438.3	15.1%	5.1%	238797.2	226500.3	75	11	63	1447	1373	0.05
41	2	21	13065.4	1858.6	11206.8	14.2%	5.5%	238797.2	225731.8	79	11	68	1447	1368	0.05
42	2	22	13804.7	3339.5	10465.2	24.2%	2.8%	499034.6	485229.9	84	20	63	3024	2941	0.02
43	2	23	15375.7	3339.5	12036.2	21.7%	3.1%	499034.6	483658.9	93	20	73	3024	2931	0.02
44	2	24	14625.0	3339.5	11285.6	22.8%	2.9%	499034.6	484409.5	89	20	68	3024	2936	0.02
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	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
17			Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
18			Tube Identification			Assay tube contents								Scintillation Results	
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
21	2	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	321.9	371.1
22	2	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	392.3	371.1
23	2	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	399.1	371.1
24	2	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	495.7	458.3
25	2	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	390.3	458.3
26	2	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	488.8	458.3
27	2	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	564.5	559.4
28	2	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	456.8	559.4
29	2	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	656.9	559.4
30	2	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	461.3	436.5
31	2	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	392.7	436.5
32	2	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	455.4	436.5
33	2	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	803.1	659.5
34	2	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	552.5	659.5
35	2	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	623.0	659.5
36	2	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1114.8	1155.0
37	2	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1127.4	1155.0
38	2	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1222.7	1155.0
39	2	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	1559.0	1858.6
40	2	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	2304.6	1858.6
41	2	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	1712.2	1858.6
42	2	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	3352.5	3339.5
43	2	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	3212.2	3339.5
44	2	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	3453.8	3339.5
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	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
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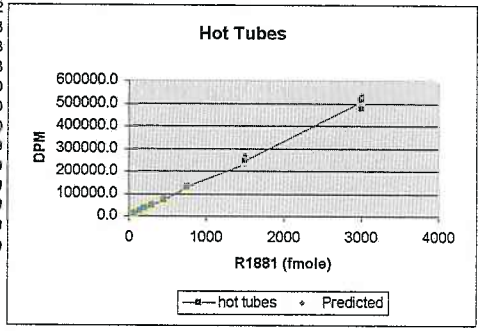
18			Free -- Positions 49-72, radiolabeled R1881 without cytosol														
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial	Hot R1881 Volume	Molecules of R1881	Counts per Scintillation Vial	Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)	predicted dpm					
20						(nM)	(ul)	(fmole)	(dpm)	(fmole)	(dpm)						
21	2	49	1	Hot	c1	10	7.5	75	11983.0	73	12118.6	13383					
22	2	50	2	Hot	c1	10	7.5	75	11736.8	71	12118.6	13383					
23	2	51	3	Hot	c1	10	7.5	75	12636.0	77	12118.6	13383					
24	2	52	1	Hot	c2	10	15	150	22571.6	137	23011.5	26766					
25	2	53	2	Hot	c2	10	15	150	22537.5	137	23011.5	26766					
26	2	54	3	Hot	c2	10	15	150	23925.4	145	23011.5	26766					
27	2	55	1	Hot	c3	10	21	210	30954.9	188	31917.1	37472					
28	2	56	2	Hot	c3	10	21	210	32008.1	194	31917.1	37472					
29	2	57	3	Hot	c3	10	21	210	32788.4	199	31917.1	37472					
30	2	58	1	Hot	c4	10	30	300	45810.1	278	48087.7	53532					
31	2	59	2	Hot	c4	10	30	300	49800.7	302	48087.7	53532					
32	2	60	3	Hot	c4	10	30	300	48652.3	295	48087.7	53532					
33	2	61	1	Hot	c5	10	45	450	74590.4	452	72284.8	80298					
34	2	62	2	Hot	c5	10	45	450	70393.9	427	72284.8	80298					
35	2	63	3	Hot	c5	10	45	450	71870.1	436	72284.8	80298					
36	2	64	1	Hot	c6	100	7.5	750	127392.5	772	127908.6	133830					
37	2	65	2	Hot	c6	100	7.5	750	125390.6	760	127908.6	133830					
38	2	66	3	Hot	c6	100	7.5	750	130942.7	794	127908.6	133830					
39	2	67	1	Hot	c7	100	15	1500	231220.3	1401	238797.2	267659					
40	2	68	2	Hot	c7	100	15	1500	239984.9	1454	238797.2	267659					
41	2	69	3	Hot	c7	100	15	1500	245186.3	1486	238797.2	267659					
42	2	70	1	Hot	c8	100	30	3000	505751.2	3065	499034.6	535319					
43	2	71	2	Hot	c8	100	30	3000	514635.1	3119	499034.6	535319					
44	2	72	3	Hot	c8	100	30	3000	476717.4	2889	499034.6	535319					
45																	
46																	
47																	
48																	
49																	
50																	
51																	
52																	
53																	
54																	
55																	
56																	
57																	
58																	

Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	165.00053 dpm/fmole	
1/slope	0.00606059 fmole/dpm	
	x	y
origin	0	0
end point	3119.0	514635.1
SLOPE function, used if missing HOT tubes		
Slope	166.0 dpm/fmole	
1/slope	0.006024 fmole/dpm	
	x	y
origin	0	0
end point	3100.4	514635.1

Computation Check	
1/24/05	specific activity date
80.38	Ci/mMole 3H R1881
2.22E+12	DPM/Ci (definition)
1.7844E+14	DPM/mmole
1.7844E+11	DPM/nmole
178.4	DPM/fmole
0.005604	fmole/DPM



	CF	CG	CH	CI
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
21	4.28076E-11	0.24234	2.26381E-10	4.28076E-11
22	4.19597E-11	0.23641	2.26381E-10	4.19597E-11
23	4.11902E-11	0.23107	2.26381E-10	4.11902E-11
24	8.2863E-11	0.24484	4.29865E-10	8.2863E-11
25	7.637E-11	0.22140	4.29865E-10	7.637E-11
26	8.21742E-11	0.24231	4.29865E-10	8.21742E-11
27	8.43884E-11	0.16831	5.96227E-10	8.43884E-11
28	8.81124E-11	0.17705	5.96227E-10	8.81124E-11
29	9.93307E-11	0.20420	5.96227E-10	9.93307E-11
30	1.10492E-10	0.14172	8.983E-10	1.10492E-10
31	1.19334E-10	0.15482	8.983E-10	1.19334E-10
32	1.08157E-10	0.13831	8.983E-10	1.08157E-10
33	1.4401E-10	0.12061	1.35031E-09	1.4401E-10
34	1.37213E-10	0.11427	1.35031E-09	1.37213E-10
35	1.50657E-10	0.12689	1.35031E-09	1.50657E-10
36	1.66642E-10	0.07571	2.38939E-09	1.66642E-10
37	1.90212E-10	0.08735	2.38939E-09	1.90212E-10
38	1.71436E-10	0.07805	2.38939E-09	1.71436E-10
39	1.90883E-10	0.04507	4.46084E-09	1.90883E-10
40	1.94992E-10	0.04609	4.46084E-09	1.94992E-10
41	2.09347E-10	0.04965	4.46084E-09	2.09347E-10
42	1.95494E-10	0.02157	9.32219E-09	1.95494E-10
43	2.24841E-10	0.02489	9.32219E-09	2.24841E-10
44	2.10819E-10	0.02330	9.32219E-09	2.10819E-10
45				
46				
47				
48				
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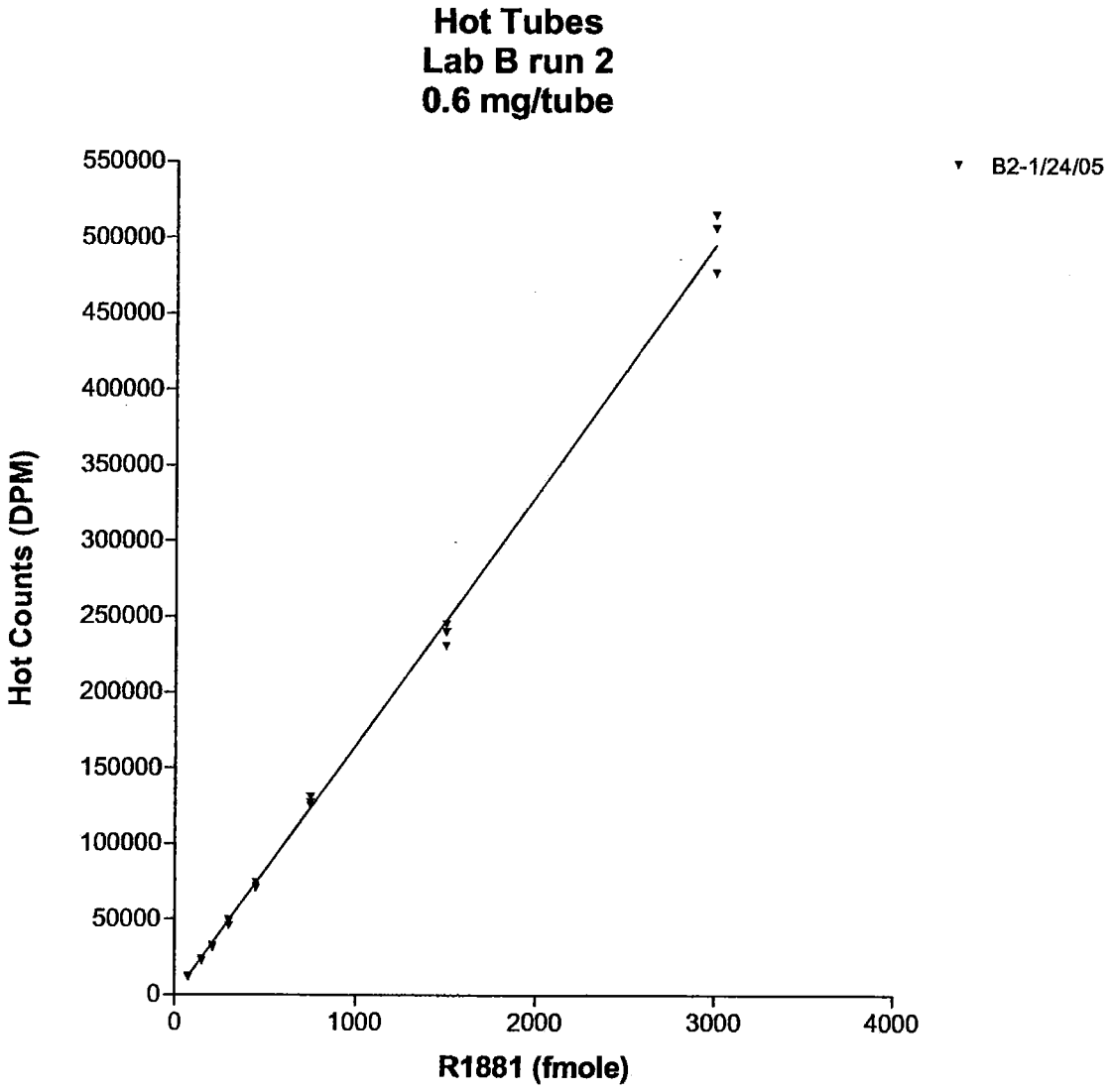
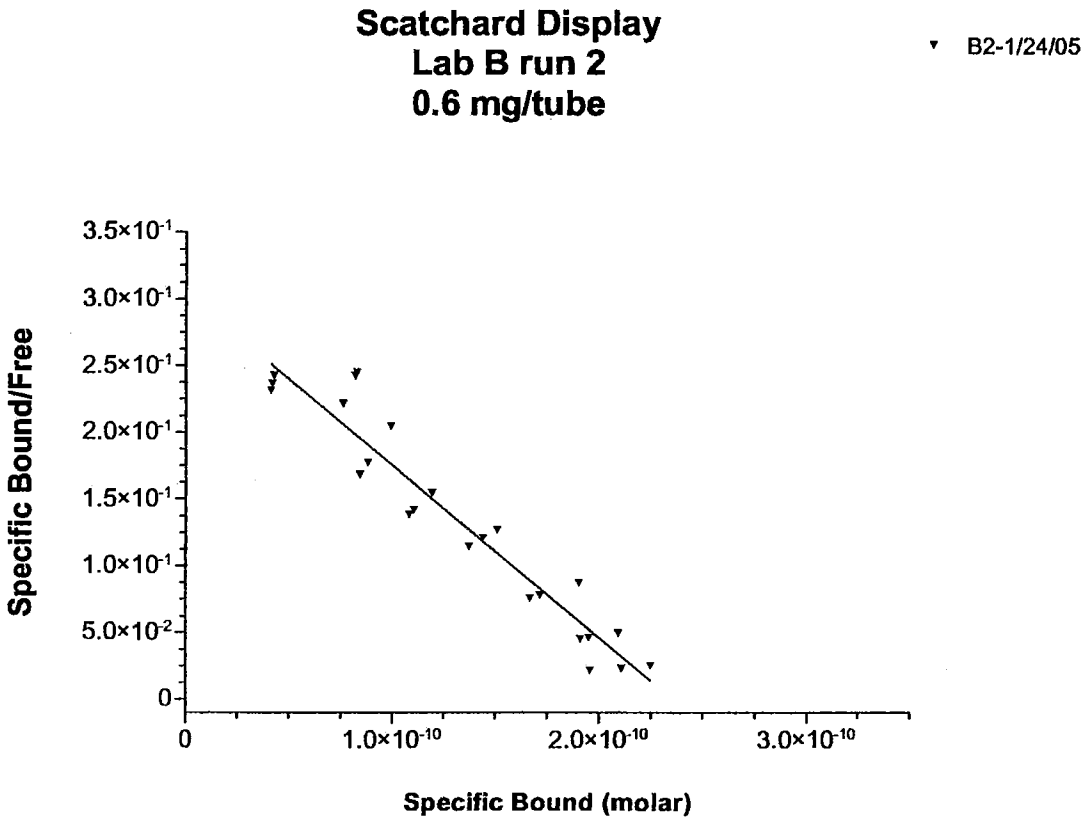
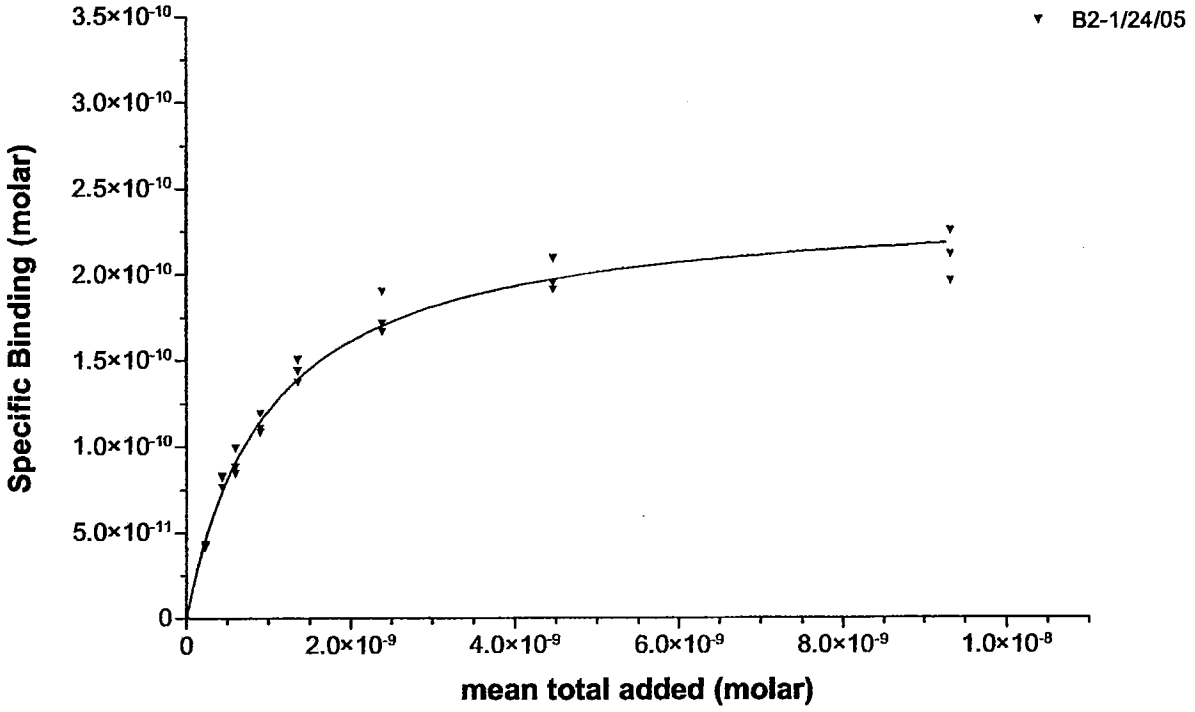
54				
55				
56				
57				
58				


Hot Tubes
Lab B run 2
0.6 mg/tube
B2-1/24/05
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
1000
2000
3000
4000
R1881 (fmole)

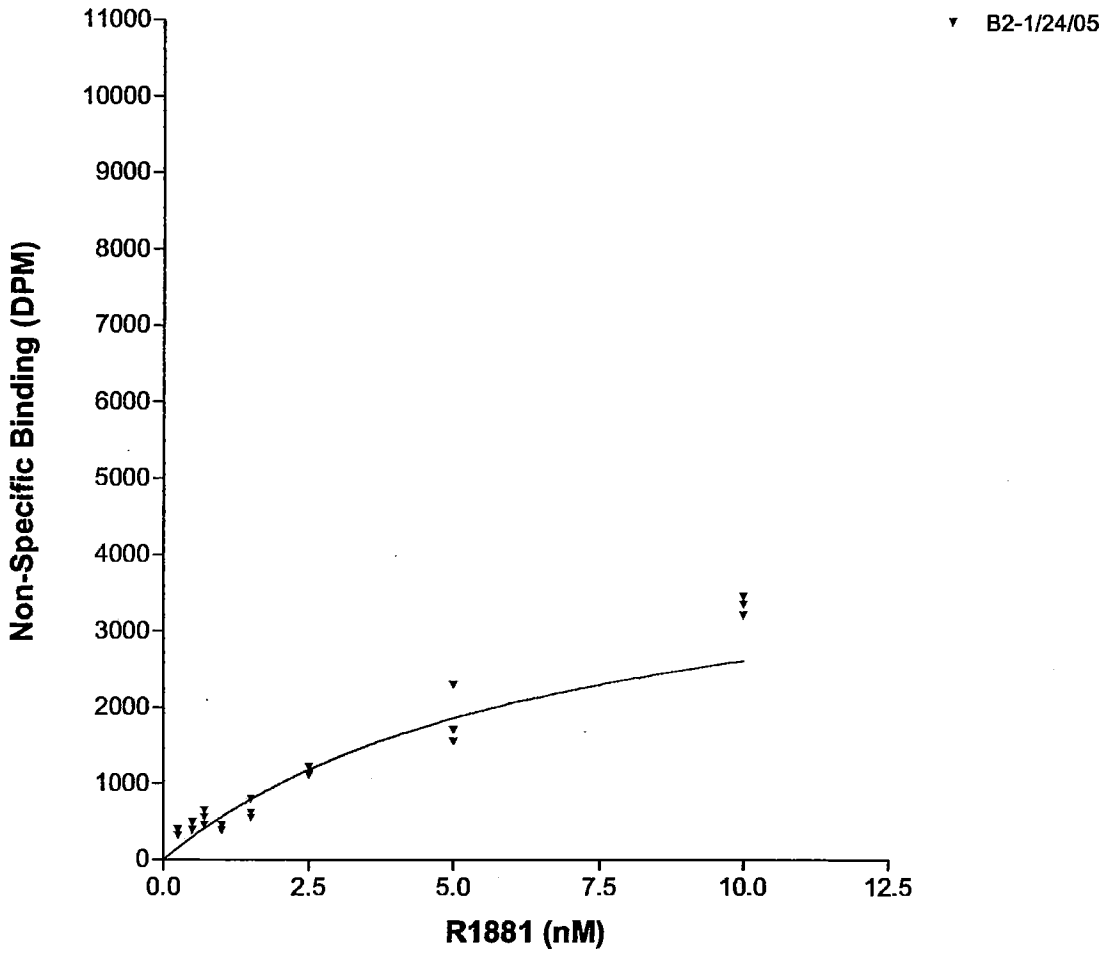

Scatchard Display
Lab B run 2
0.6 mg/tube
B2-1/24/05
Specific Bound/Free
3.5×10-1
3.0×10-1
2.5×10-1
2.0×10-1
1.5×10-1
1.0×10-1
5.0×10-2
0
0
1.0×10-10
2.0×10-10
3.0×10-10
Specific Bound (molar)

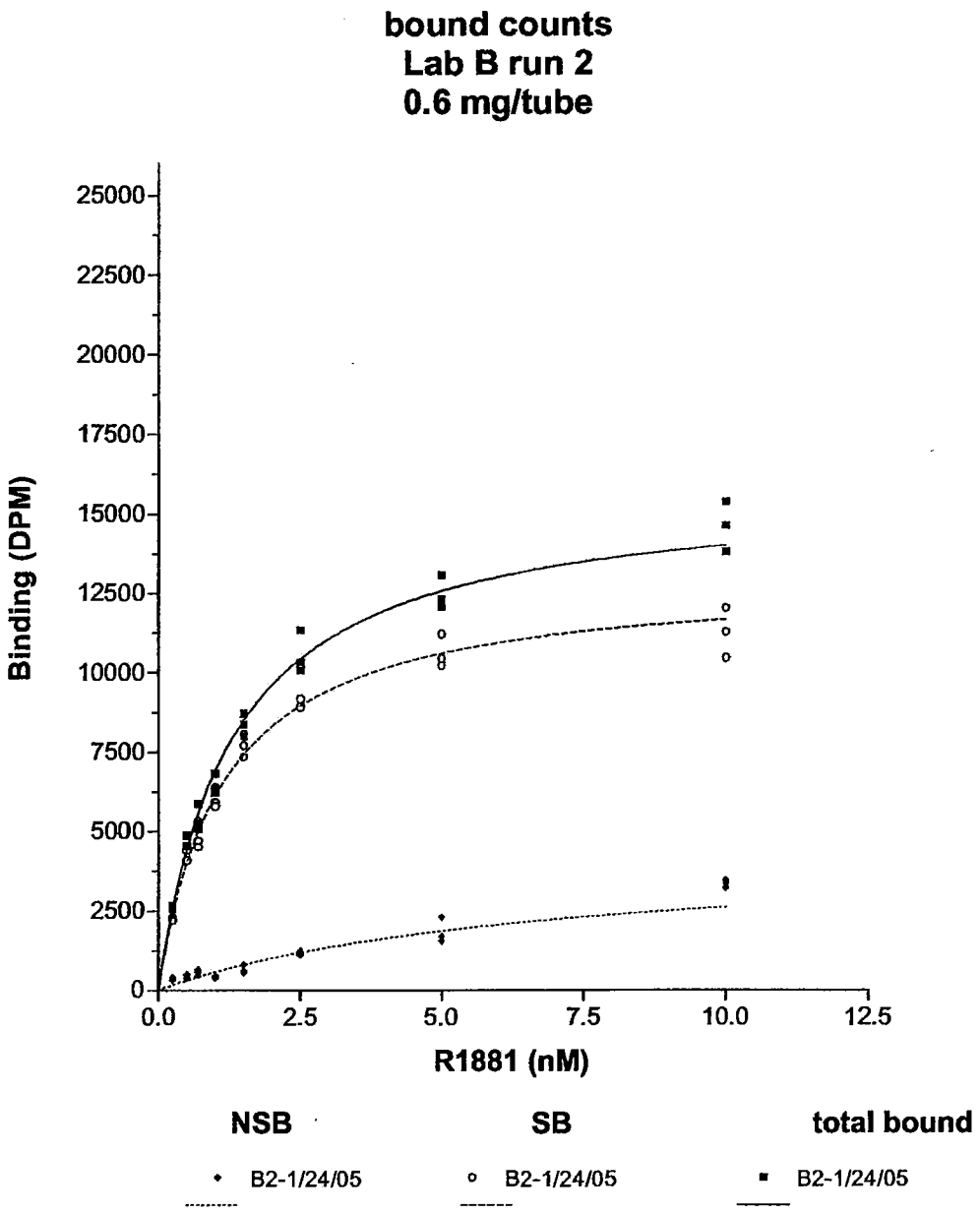
Lab B run 2
0.6 mg/tube



specific bound	B2-1/24/05
BMAX	2.4067e-010
KD	9.8904e-010
Std. Error	
BMAX	7.1682e-012
KD	5.7022e-011
95% Confidence Intervals	
BMAX	2.2581e-010 to 2.5554e-010
KD	8.7077e-010 to 1.1073e-009
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.9770
Weighted Sum of Squares ($1/Y^2$)	0.1027
Absolute Sum of Squares	1.7847e-021
Sy.x	9.0068e-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
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
SB
total bound
B2-1/24/05
B2-1/24/05
B2-1/24/05

APPENDIX D

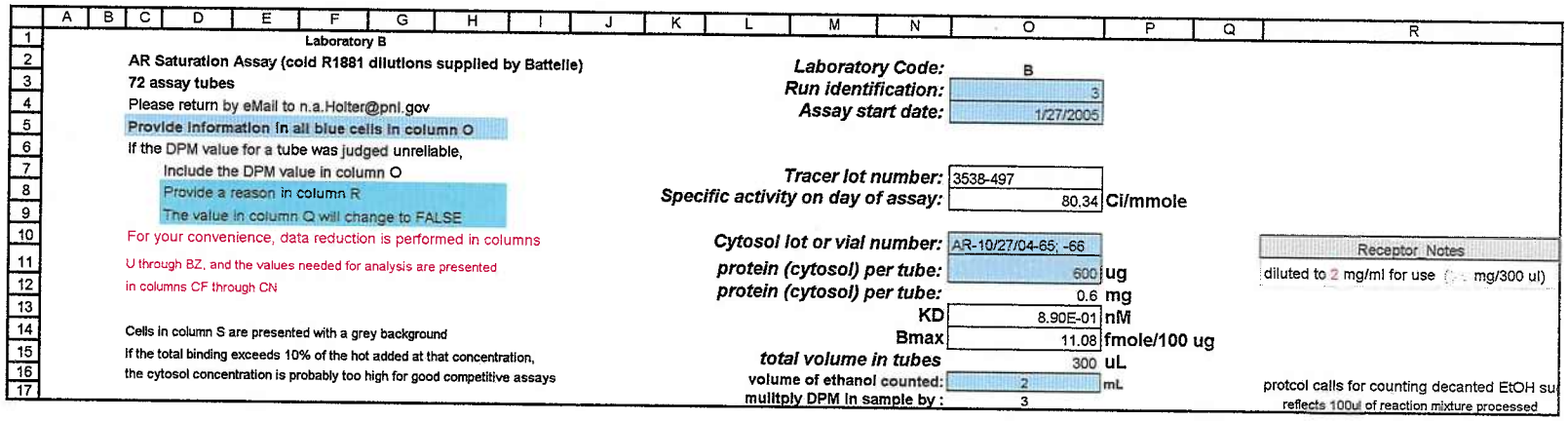
Processed Raw Data from Saturation Assay 3 and Associated Figures

Note: Due to insufficient volume in the tube in position 72, this tube was not included in the data analysis.

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)	Triamcelenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-B supplied by Battelle to laboratory "B"	dpm as counted	corrected DPM for 2mL
1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—	970.26	2910.78
2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—	1013.63	3040.89
3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—	982.14	2946.42
4	1	H	10.0	15	0.50	—	—	—	50	300	—	—	1572.24	4716.72
5	2	H	10.0	15	0.50	—	—	—	50	300	—	—	1562.98	4688.94
6	3	H	10.0	15	0.50	—	—	—	50	300	—	—	1390.43	4171.29
7	1	H	10.0	21	0.70	—	—	—	50	300	—	—	2030.02	6090.06
8	2	H	10.0	21	0.70	—	—	—	50	300	—	—	1744.04	5232.12
9	3	H	10.0	21	0.70	—	—	—	50	300	—	—	1943.86	5831.58
10	1	H	10.0	30	1.00	—	—	—	50	300	—	—	2395.24	7185.72
11	2	H	10.0	30	1.00	—	—	—	50	300	—	—	2262.26	6786.78
12	3	H	10.0	30	1.00	—	—	—	50	300	—	—	2062.89	6188.67
13	1	H	10.0	45	1.50	—	—	—	50	300	—	—	2900.19	8700.57
14	2	H	10.0	45	1.50	—	—	—	50	300	—	—	2944.18	8832.54
15	3	H	10.0	45	1.50	—	—	—	50	300	—	—	2874.3	8622.9
16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3313.57	9940.71
17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3712.32	11136.96
18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—	3542.19	10626.57
19	1	H	100.0	15	5.00	—	—	—	50	300	—	—	4042.82	12128.46
20	2	H	100.0	15	5.00	—	—	—	50	300	—	—	3829.4	11488.2
21	3	H	100.0	15	5.00	—	—	—	50	300	—	—	3731.99	11195.97
22	1	H	100.0	30	10.00	—	—	—	50	300	—	—	4927.03	14781.09
23	2	H	100.0	30	10.00	—	—	—	50	300	—	—	4617.11	13851.33
24	3	H	100.0	30	10.00	—	—	—	50	300	—	—	4861.25	14583.75
25	1	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	**B-1-C8**	113.47	340.41
26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	**B-1-C8**	95.6	286.8
27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	**B-1-C8**	128.61	385.83
28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	**B-1-C7**	175.34	526.02
29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	**B-1-C7**	92.56	277.68
30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	**B-1-C7**	105.38	316.14
31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	**B-1-C6**	172.52	517.56
32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	**B-1-C6**	161.82	485.46
33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	**B-1-C6**	135.02	405.06
34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	**B-1-C5**	152.16	456.48
35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	**B-1-C5**	197.66	592.98
36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	**B-1-C5**	170.12	510.36
37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	**B-1-C4**	289.78	869.34
38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	**B-1-C4**	257.65	772.95
39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	**B-1-C4**	259.45	778.35
40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	**B-1-C3**	403	1209
41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	**B-1-C3**	476.61	1429.83
42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	**B-1-C3**	367.89	1103.67
43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	**B-1-C2**	775.68	2327.04
44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	**B-1-C2**	697.17	2091.51
45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	**B-1-C2**	811.36	2434.08
46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	**B-1-C1**	1394.74	4184.22
47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	**B-1-C1**	1058.93	3176.79
48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	**B-1-C1**	1000.66	3001.98
49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	14082.24	14082.24
50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	13714.37	13714.37
51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—	13607.41	13607.41
52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—	25371.18	25371.18
53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—	26766.36	26766.36
54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—	26160.93	26160.93
55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—	36203.26	36203.26
56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—	36518.61	36518.61
57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—	36514.78	36514.78
58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—	53050.72	53050.72
59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—	53415.62	53415.62
60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—	51240.89	51240.89
61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—	80188.6	80188.6
62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—	81014.93	81014.93
63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—	81941.27	81941.27
64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	132848.5	132848.5
65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	132501.1	132501.1
66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—	130635	130635
67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—	255798.9	255798.9
68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—	257788.9	257788.9
69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—	252594.7	252594.7
70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—	523577.8	523577.8
71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—	524434.2	524434.2
72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—	468171.6	468171.6



Laboratory B

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

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

Laboratory Code:	B	
Run identification:	3	
Assay start date:	1/27/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.34	Ci/mmole
Cytosol lot or vial number:	AR-10/27/04-65; -66	
protein (cytosol) per tube:	600	ug
protein (cytosol) per tube:	0.6	mg
KD	8.90E-01	nM
Bmax	11.08	fmole/100 ug
total volume in tubes	300	uL
volume of ethanol counted:	2	mL
muiltply DPM in sample by :	3	

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

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

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
18	Saturation Assay Tube Layout																				
19	Position	Replicate	Tube Type Code	Hot Initial Concentration (nM)	Hot R1881 Volume (uL)	Hot Final Concentration (nM)	Cold Initial Concentration (uM)	Cold R1881 Volume (uL)	Cold Final Concentration (nM)	Triamcelenone Acetate (uL)	Cytosol (ul)	Significant portion of label on Vial supplied by Battelle	Full on vials in set 1-1-B supplied by Battelle to laboratory "g"		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																					
21	1	1	H	10.0	7.5	0.25	—	—	—	50	300	—	—		970.26	2910.78	TRUE		21.1%	0.25	2910.8
22	2	2	H	10.0	7.5	0.25	—	—	—	50	300	—	—		1013.63	3040.89	TRUE		22.0%	0.25	3040.9
23	3	3	H	10.0	7.5	0.25	—	—	—	50	300	—	—		982.14	2946.42	TRUE		21.3%	0.25	2946.4
24	4	1	H	10.0	15	0.50	—	—	—	50	300	—	—		1572.24	4716.72	TRUE		18.1%	0.5	4716.7
25	5	2	H	10.0	15	0.50	—	—	—	50	300	—	—		1562.98	4688.94	TRUE		18.0%	0.5	4688.9
26	6	3	H	10.0	15	0.50	—	—	—	50	300	—	—		1390.43	4171.29	TRUE		16.0%	0.5	4171.3
27	7	1	H	10.0	21	0.70	—	—	—	50	300	—	—		2030.02	6090.06	TRUE		16.7%	0.7	6090.1
28	8	2	H	10.0	21	0.70	—	—	—	50	300	—	—		1744.04	5232.12	TRUE		14.4%	0.7	5232.1
29	9	3	H	10.0	21	0.70	—	—	—	50	300	—	—		1943.86	5831.58	TRUE		16.0%	0.7	5831.6
30	10	1	H	10.0	30	1.00	—	—	—	50	300	—	—		2395.24	7185.72	TRUE		13.7%	1	7185.7
31	11	2	H	10.0	30	1.00	—	—	—	50	300	—	—		2262.26	6786.78	TRUE		12.9%	1	6786.8
32	12	3	H	10.0	30	1.00	—	—	—	50	300	—	—		2062.89	6188.67	TRUE		11.8%	1	6188.7
33	13	1	H	10.0	45	1.50	—	—	—	50	300	—	—		2900.19	8700.57	TRUE		10.7%	1.5	8700.6
34	14	2	H	10.0	45	1.50	—	—	—	50	300	—	—		2944.18	8832.54	TRUE		10.9%	1.5	8832.5
35	15	3	H	10.0	45	1.50	—	—	—	50	300	—	—		2874.3	8622.9	TRUE		10.6%	1.5	8622.9
36	16	1	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3313.57	9940.71	TRUE		7.5%	2.5	9940.7
37	17	2	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3712.32	11136.96	TRUE		8.4%	2.5	11137.0
38	18	3	H	100.0	7.5	2.50	—	—	—	50	300	—	—		3542.19	10626.57	TRUE		8.1%	2.5	10626.6
39	19	1	H	100.0	15	5.00	—	—	—	50	300	—	—		4042.82	12128.46	TRUE		4.7%	5	12128.5
40	20	2	H	100.0	15	5.00	—	—	—	50	300	—	—		3829.4	11488.2	TRUE		4.5%	5	11488.2
41	21	3	H	100.0	15	5.00	—	—	—	50	300	—	—		3731.99	11195.97	TRUE		4.4%	5	11196.0
42	22	1	H	100.0	30	10.00	—	—	—	50	300	—	—		4927.03	14781.09	TRUE		2.8%	10	14781.1
43	23	2	H	100.0	30	10.00	—	—	—	50	300	—	—		4617.11	13851.33	TRUE		2.6%	10	13851.3
44	24	3	H	100.0	30	10.00	—	—	—	50	300	—	—		4861.25	14583.75	TRUE		2.8%	10	14583.8

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18	
19	NSB y values
20	
21	337.7
22	337.7
23	337.7
24	373.3
25	373.3
26	373.3
27	469.4
28	469.4
29	469.4
30	519.9
31	519.9
32	519.9
33	806.9
34	806.9
35	806.9
36	1247.5
37	1247.5
38	1247.5
39	2284.2
40	2284.2
41	2284.2
42	3454.3
43	3454.3
44	3454.3

	A	B	C	D	E	F	G	H	I	J	K	L	M	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	B-1-C8		113.47	340.41	TRUE				
46	26	2	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		95.6	286.8	TRUE				
47	27	3	HC	10.0	7.5	0.25	1.00	7.5	25	50	300	C8	B-1-C8		128.61	385.83	TRUE				
48	28	1	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		175.34	526.02	TRUE				
49	29	2	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		92.56	277.68	TRUE				
50	30	3	HC	10.0	15	0.5	1.00	15	50	50	300	C7	B-1-C7		105.38	316.14	TRUE				
51	31	1	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		172.52	517.56	TRUE				
52	32	2	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		161.82	485.46	TRUE				
53	33	3	HC	10.0	21	0.7	1.00	21	70	50	300	C6	B-1-C6		135.02	405.06	TRUE				
54	34	1	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		152.16	456.48	TRUE				
55	35	2	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		197.66	592.98	TRUE				
56	36	3	HC	10.0	30	1	1.00	30	100	50	300	C5	B-1-C5		170.12	510.36	TRUE				
57	37	1	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		289.78	869.34	TRUE				
58	38	2	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		257.65	772.95	TRUE				
59	39	3	HC	10.0	45	1.5	1.00	45	150	50	300	C4	B-1-C4		259.45	778.35	TRUE				
60	40	1	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		403	1209	TRUE				
61	41	2	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		476.61	1429.83	TRUE				
62	42	3	HC	100.0	7.5	2.5	10.00	7.5	250	50	300	C3	B-1-C3		367.89	1103.67	TRUE				
63	43	1	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		775.68	2327.04	TRUE				
64	44	2	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		697.17	2091.51	TRUE				
65	45	3	HC	100.0	15	5	10.00	15	500	50	300	C2	B-1-C2		811.36	2434.08	TRUE				
66	46	1	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		1394.74	4184.22	TRUE				
67	47	2	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		1058.93	3176.79	TRUE				
68	48	3	HC	100.0	30	10	10.00	30	1000	50	300	C1	B-1-C1		1000.66	3001.98	TRUE				
69	49	1	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		14082.24	14082.24	TRUE				
70	50	2	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		13714.37	13714.37	TRUE				
71	51	3	Hot	10.0	7.5	0.03	—	—	—	—	—	—	—		13607.41	13607.41	TRUE				
72	52	1	Hot	10.0	15	0.06	—	—	—	—	—	—	—		25371.18	25371.18	TRUE				
73	53	2	Hot	10.0	15	0.06	—	—	—	—	—	—	—		26766.36	26766.36	TRUE				
74	54	3	Hot	10.0	15	0.06	—	—	—	—	—	—	—		26160.93	26160.93	TRUE				
75	55	1	Hot	10.0	21	0.08	—	—	—	—	—	—	—		36203.26	36203.26	TRUE				
76	56	2	Hot	10.0	21	0.08	—	—	—	—	—	—	—		36518.61	36518.61	TRUE				
77	57	3	Hot	10.0	21	0.08	—	—	—	—	—	—	—		36514.78	36514.78	TRUE				
78	58	1	Hot	10.0	30	0.10	—	—	—	—	—	—	—		53050.72	53050.72	TRUE				
79	59	2	Hot	10.0	30	0.10	—	—	—	—	—	—	—		53415.62	53415.62	TRUE				
80	60	3	Hot	10.0	30	0.10	—	—	—	—	—	—	—		51240.89	51240.89	TRUE				
81	61	1	Hot	10.0	45	0.30	—	—	—	—	—	—	—		80188.6	80188.6	TRUE				
82	62	2	Hot	10.0	45	0.30	—	—	—	—	—	—	—		81014.93	81014.93	TRUE				
83	63	3	Hot	10.0	45	0.30	—	—	—	—	—	—	—		81941.27	81941.27	TRUE				
84	64	1	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		132848.5	132848.5	TRUE				
85	65	2	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		132501.1	132501.1	TRUE				
86	66	3	Hot	100.0	7.5	0.60	—	—	—	—	—	—	—		130635	130635	TRUE				
87	67	1	Hot	100.0	15	1.00	—	—	—	—	—	—	—		255798.9	255798.9	TRUE				
88	68	2	Hot	100.0	15	1.00	—	—	—	—	—	—	—		257788.9	257788.9	TRUE				
89	69	3	Hot	100.0	15	1.00	—	—	—	—	—	—	—		252594.7	252594.7	TRUE				
90	70	1	Hot	100.0	30	3.00	—	—	—	—	—	—	—		523577.8	523577.8	TRUE				
91	71	2	Hot	100.0	30	3.00	—	—	—	—	—	—	—		524434.2	524434.2	TRUE				
92	72	3	Hot	100.0	30	3.00	—	—	—	—	—	—	—		468171.6	468171.6	FALSE	insufficient volume			

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	W	X	Y	Z	AA	AB	AC	AD	AE	AF	AG	AH	AI	AJ
17			Total Binding – Positions 1-24 radiolabeled R1881 plus cytosol (Panel A)											
18			Tube Identification			Assay tube contents								
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. Initial	Hot R1881 Volume	Cold R1881 Conc. Initial	Cold R1881 volume	Triamcelenone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Total Volume
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(ul)
21	3	1	1	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
22	3	2	2	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
23	3	3	3	H	c1	10.0	7.5	—	—	—	300	0.25	—	300
24	3	4	1	H	c2	10.0	15	—	—	—	300	0.50	—	300
25	3	5	2	H	c2	10.0	15	—	—	—	300	0.50	—	300
26	3	6	3	H	c2	10.0	15	—	—	—	300	0.50	—	300
27	3	7	1	H	c3	10.0	21	—	—	—	300	0.70	—	300
28	3	8	2	H	c3	10.0	21	—	—	—	300	0.70	—	300
29	3	9	3	H	c3	10.0	21	—	—	—	300	0.70	—	300
30	3	10	1	H	c4	10.0	30	—	—	—	300	1.00	—	300
31	3	11	2	H	c4	10.0	30	—	—	—	300	1.00	—	300
32	3	12	3	H	c4	10.0	30	—	—	—	300	1.00	—	300
33	3	13	1	H	c5	10.0	45	—	—	—	300	1.50	—	300
34	3	14	2	H	c5	10.0	45	—	—	—	300	1.50	—	300
35	3	15	3	H	c5	10.0	45	—	—	—	300	1.50	—	300
36	3	16	1	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
37	3	17	2	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
38	3	18	3	H	c6	100.0	7.5	—	—	—	300	2.50	—	300
39	3	19	1	H	c7	100.0	15	—	—	—	300	5.00	—	300
40	3	20	2	H	c7	100.0	15	—	—	—	300	5.00	—	300
41	3	21	3	H	c7	100.0	15	—	—	—	300	5.00	—	300
42	3	22	1	H	c8	100.0	30	—	—	—	300	10.00	—	300
43	3	23	2	H	c8	100.0	30	—	—	—	300	10.00	—	300
44	3	24	3	H	c8	100.0	30	—	—	—	300	10.00	—	300
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	AK	AL	AM	AN	AO	AP	AQ	AR	AS	AT	AU	AV	AW	AX	AY
17															
18										Number of molecules					Ratio
19	Run	Position	Total Counts	Non Specific Binding (Mean of reps in pos. 23-48)	Specific Binding (Total - Non Specific)	Ratio of NSB/ total binding	Ratio Total binding/ Not	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Total Binding molecules	Non Specific Binding molecules	Specific Binding molecules	Total Added (Mean of reps in pos. 49-72)	Free (total added - bound)	Specific Bound / Free
20			(dpm)	(dpm)	(dpm)			(dpm)	(dpm)	(fmole)	(fmole)	(fmole)	(fmole)	(fmole)	
21	3	1	2910.8	337.7	2573.1	11.6%	21.1%	13801.3	10890.6	17	2	15	80	63	0.24
22	3	2	3040.9	337.7	2703.2	11.1%	22.0%	13801.3	10760.5	18	2	16	80	62	0.25
23	3	3	2946.4	337.7	2608.7	11.5%	21.3%	13801.3	10854.9	17	2	15	80	63	0.24
24	3	4	4716.7	373.3	4343.4	7.9%	18.1%	26099.5	21382.8	27	2	25	150	123	0.20
25	3	5	4688.9	373.3	4315.7	8.0%	18.0%	26099.5	21410.6	27	2	25	150	123	0.20
26	3	6	4171.3	373.3	3798.0	8.9%	16.0%	26099.5	21928.2	24	2	22	150	126	0.17
27	3	7	6090.1	469.4	5620.7	7.7%	16.7%	36412.2	30322.2	35	3	32	210	175	0.19
28	3	8	5232.1	469.4	4762.8	9.0%	14.4%	36412.2	31180.1	30	3	27	210	180	0.15
29	3	9	5831.6	469.4	5362.2	8.0%	16.0%	36412.2	30580.6	34	3	31	210	176	0.18
30	3	10	7185.7	519.9	6665.8	7.2%	13.7%	52569.1	45383.4	41	3	38	303	262	0.15
31	3	11	6786.8	519.9	6266.8	7.7%	12.9%	52569.1	45782.3	39	3	36	303	264	0.14
32	3	12	6188.7	519.9	5668.7	8.4%	11.8%	52569.1	46380.4	36	3	33	303	267	0.12
33	3	13	8700.6	806.9	7893.7	9.3%	10.7%	81048.3	72347.7	50	5	45	467	417	0.11
34	3	14	8832.5	806.9	8025.7	9.1%	10.9%	81048.3	72215.7	51	5	46	467	416	0.11
35	3	15	8622.9	806.9	7816.0	9.4%	10.6%	81048.3	72425.4	50	5	45	467	417	0.11
36	3	16	9940.7	1247.5	8693.2	12.5%	7.5%	131994.9	122054.2	57	7	50	761	703	0.07
37	3	17	11137.0	1247.5	9889.5	11.2%	8.4%	131994.9	120857.9	64	7	57	761	697	0.08
38	3	18	10626.6	1247.5	9379.1	11.7%	8.1%	131994.9	121368.3	61	7	54	761	699	0.08
39	3	19	12128.5	2284.2	9844.3	18.8%	4.7%	255394.2	243265.7	70	13	57	1472	1402	0.04
40	3	20	11488.2	2284.2	9204.0	19.9%	4.5%	255394.2	243906.0	66	13	53	1472	1406	0.04
41	3	21	11196.0	2284.2	8911.8	20.4%	4.4%	255394.2	244198.2	65	13	51	1472	1407	0.04
42	3	22	14781.1	3454.3	11326.8	23.4%	2.8%	524006.0	509224.9	85	20	65	3020	2935	0.02
43	3	23	13851.3	3454.3	10397.0	24.9%	2.6%	524006.0	510154.7	80	20	60	3020	2940	0.02
44	3	24	14583.8	3454.3	11129.4	23.7%	2.8%	524006.0	509422.3	84	20	64	3020	2936	0.02
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	AZ	BA	BB	BC	BD	BE	BF	BG	BH	BI	BJ	BK	BL	BM	BN
17			Non Specific Binding -- Positions 25-48 radiolabeled R1881 plus 100 X inert R1881 plus cytosol												
18			Tube Identification			Assay tube contents								Scintillation Results	
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot Conc. R1881 Initial	Hot	Cold R1881 Conc. Initial	Cold	Triamcelonone Acetate	Cytosol	Hot Conc. Final	Cold Conc. Final	Counts per Scintillation Vial (Total Binding)	Non Specific Binding (Mean of reps in pos. 25-48)
20						(nM)	(ul)	(mM)	(ul)	(ul)	(ul)	(nM)	(nM)	(dpm)	(dpm)
21	3	25	1	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	340.4	337.7
22	3	26	2	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	286.8	337.7
23	3	27	3	HC	c1	10.0	7.5	1.00	7.5	50	300	0.25	25	385.8	337.7
24	3	28	1	HC	c2	10.0	15	1.00	15	50	300	0.5	50	526.0	373.3
25	3	29	2	HC	c2	10.0	15	1.00	15	50	300	0.5	50	277.7	373.3
26	3	30	3	HC	c2	10.0	15	1.00	15	50	300	0.5	50	318.1	373.3
27	3	31	1	HC	c3	10.0	21	1.00	21	50	300	0.7	70	517.8	469.4
28	3	32	2	HC	c3	10.0	21	1.00	21	50	300	0.7	70	485.5	469.4
29	3	33	3	HC	c3	10.0	21	1.00	21	50	300	0.7	70	405.1	469.4
30	3	34	1	HC	c4	10.0	30	1.00	30	50	300	1	100	456.5	519.9
31	3	35	2	HC	c4	10.0	30	1.00	30	50	300	1	100	593.0	519.9
32	3	36	3	HC	c4	10.0	30	1.00	30	50	300	1	100	510.4	519.9
33	3	37	1	HC	c5	10.0	45	1.00	45	50	300	1.5	150	869.3	806.9
34	3	38	2	HC	c5	10.0	45	1.00	45	50	300	1.5	150	773.0	806.9
35	3	39	3	HC	c5	10.0	45	1.00	45	50	300	1.5	150	778.4	806.9
36	3	40	1	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1209.0	1247.5
37	3	41	2	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1429.8	1247.5
38	3	42	3	HC	c6	100.0	7.5	10.00	7.5	50	300	2.5	250	1103.7	1247.5
39	3	43	1	HC	c7	100.0	15	10.00	15	50	300	5	500	2327.0	2284.2
40	3	44	2	HC	c7	100.0	15	10.00	15	50	300	5	500	2091.5	2284.2
41	3	45	3	HC	c7	100.0	15	10.00	15	50	300	5	500	2434.1	2284.2
42	3	46	1	HC	c8	100.0	30	10.00	30	50	300	10	1000	4184.2	3454.3
43	3	47	2	HC	c8	100.0	30	10.00	30	50	300	10	1000	3176.8	3454.3
44	3	48	3	HC	c8	100.0	30	10.00	30	50	300	10	1000	3002.0	3454.3
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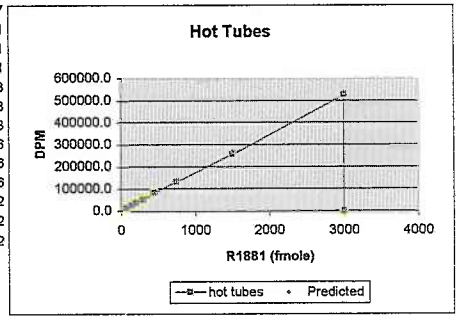
	BO	BP	BQ	BR	BS	BT	BU	BV	BW	BX	BY	BZ	CA	CB	CC	CD	CE
17																	
18			Free -- Positions 49-72, radiolabeled R1881 without cytosol														
19	Run	Position	Rep	Tube Type Code	Conc. Code	Hot R1881 Conc. Initial	Hot R1881 Volume	Molecules of R1881	Counts per Scintillation Vial	Experimental number of molecules	Total Added (Mean of reps in pos. 49-72)	predicted dpm					
20						(nM)	(ul)	(fmole)	(dpm)	(fmole)	(dpm)						
21	3	49	1	Hot	c1	10	7.5	75	14082.2	81	13801.3	13377					
22	3	50	2	Hot	c1	10	7.5	75	13714.4	79	13801.3	13377					
23	3	51	3	Hot	c1	10	7.5	75	13607.4	78	13801.3	13377					
24	3	52	1	Hot	c2	10	15	150	25371.2	146	26099.5	26754					
25	3	53	2	Hot	c2	10	15	150	26766.4	154	26099.5	26754					
26	3	54	3	Hot	c2	10	15	150	26160.9	151	26099.5	26754					
27	3	55	1	Hot	c3	10	21	210	36203.3	209	36412.2	37455					
28	3	56	2	Hot	c3	10	21	210	36518.6	210	36412.2	37455					
29	3	57	3	Hot	c3	10	21	210	36514.8	210	36412.2	37455					
30	3	58	1	Hot	c4	10	30	300	53050.7	306	52569.1	53507					
31	3	59	2	Hot	c4	10	30	300	53415.6	308	52569.1	53507					
32	3	60	3	Hot	c4	10	30	300	51240.9	295	52569.1	53507					
33	3	61	1	Hot	c5	10	45	450	80188.6	462	81048.3	80261					
34	3	62	2	Hot	c5	10	45	450	81014.9	467	81048.3	80261					
35	3	63	3	Hot	c5	10	45	450	81941.3	472	81048.3	80261					
36	3	64	1	Hot	c6	100	7.5	750	132848.5	766	131994.9	133768					
37	3	65	2	Hot	c6	100	7.5	750	132501.1	764	131994.9	133768					
38	3	66	3	Hot	c6	100	7.5	750	130635.0	753	131994.9	133768					
39	3	67	1	Hot	c7	100	15	1500	255798.9	1474	255394.2	267536					
40	3	68	2	Hot	c7	100	15	1500	257788.9	1486	255394.2	267536					
41	3	69	3	Hot	c7	100	15	1500	252594.7	1456	255394.2	267536					
42	3	70	1	Hot	c8	100	30	3000	523577.8	3017	524006.0	535072					
43	3	71	2	Hot	c8	100	30	3000	524434.2	3022	524006.0	535072					
44	3	72	3	Hot	c8	100	30	3000	FALSE		524006.0	535072					
45																	
46																	
47																	
48																	
49																	
50																	
51																	
52																	
53																	
54																	
55																	
56																	
57																	
58																	

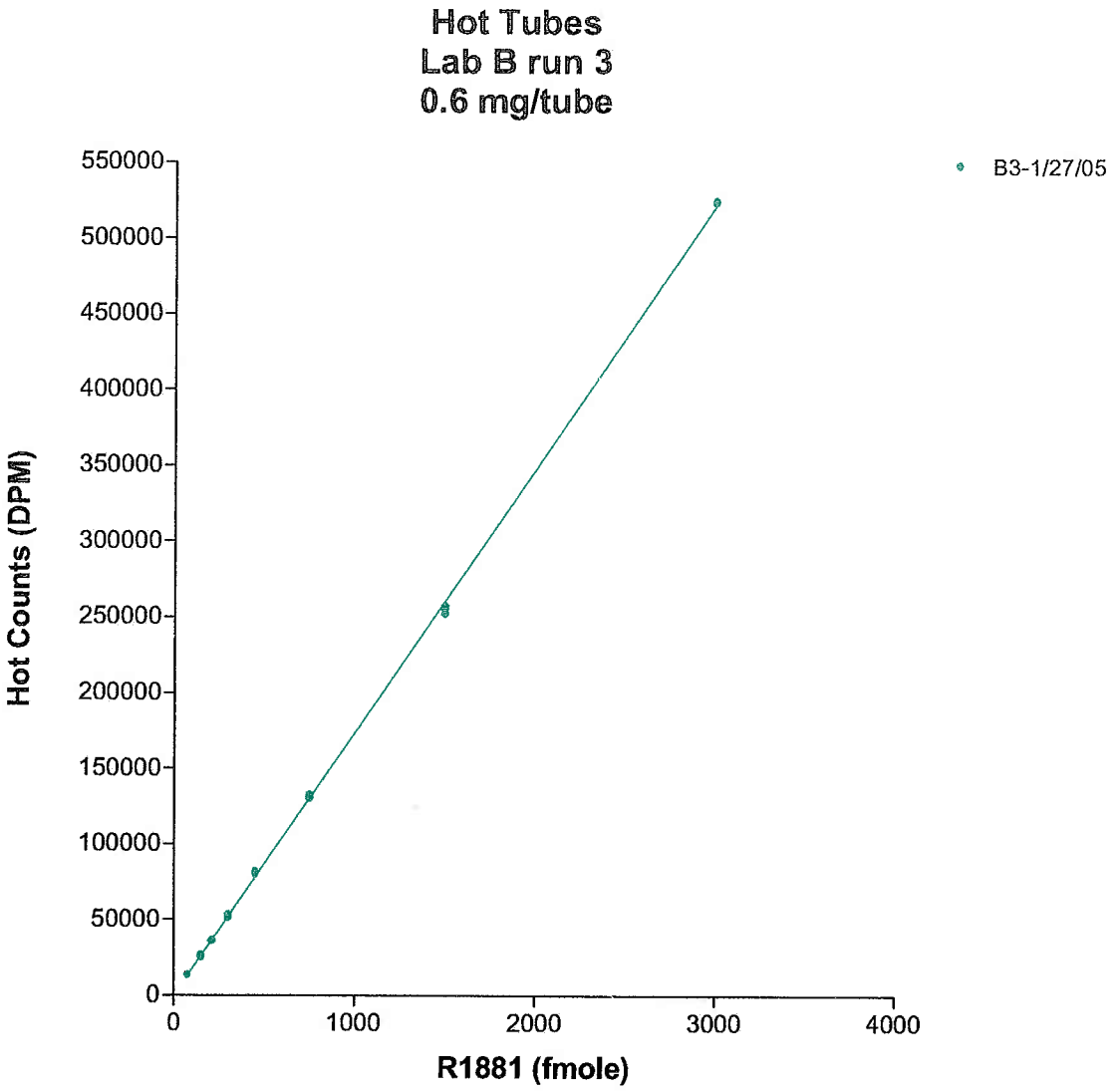
Linear regression results (LINEST function)		
(Regression line forced through 0,0)		
Slope	dpm/fmole	
1/slope	fmole/dpm	
	x	y
origin	0	0
end point	3022.4	FALSE
SLOPE function, used if missing HOT tubes		
Slope	173.5	dpm/fmole
1/slope	0.005763	fmole/dpm
	x	y
origin	0	0
end point	3022.4	524434.2

Computation Check		
1/27/05	specific activity date	
80.34	Ci/mMole 3H R1881	
2.22E+12	DPM/Ci (definition)	
1.7836E+14	DPM/mmole	
1.7836E+11	DPM/nmole	
178.4	DPM/fmole	
0.005607	fmole/DPM	



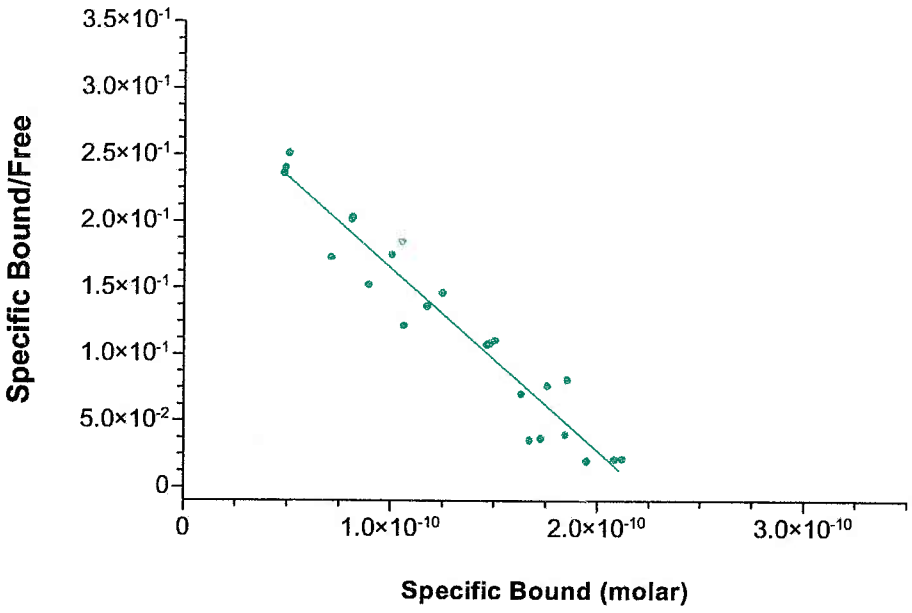
	CF	CG	CH	CI	CJ	CK	CL	CM	CN
17									
18									
19	**Prism input for bound/free**		**Prism input for specific bound**						
20	specific bound/molar	bound/free	average total added molar	specific bound/molar	spcific bound/dpm	total added dpm	total bound dpm	NSB dpm	Hot Final Concentration (nM)
21	4.80888E-11	0.23627	2.57934E-10	4.80888E-11	2573.1	14082.2	2910.8	340.4	0.25
22	5.05205E-11	0.25122	2.57934E-10	5.05205E-11	2703.2	13714.4	3040.9	286.8	0.25
23	4.87549E-11	0.24033	2.57934E-10	4.87549E-11	2608.7	13607.4	2946.4	385.8	0.25
24	8.11749E-11	0.20313	4.87775E-10	8.11749E-11	4343.4	25371.2	4716.7	526.0	0.50
25	8.06557E-11	0.20157	4.87775E-10	8.06557E-11	4315.7	26766.4	4688.9	277.7	0.50
26	7.09813E-11	0.17320	4.87775E-10	7.09813E-11	3798.0	26160.9	4171.3	316.1	0.50
27	1.05046E-10	0.18537	6.8051E-10	1.05046E-10	5620.7	36203.3	6090.1	517.6	0.70
28	8.90116E-11	0.15275	6.8051E-10	8.90116E-11	4762.8	36518.6	5232.1	485.5	0.70
29	1.00215E-10	0.17535	6.8051E-10	1.00215E-10	5362.2	36514.8	5831.6	405.1	0.70
30	1.24577E-10	0.14688	9.82467E-10	1.24577E-10	6665.8	53050.7	7185.7	456.5	1.00
31	1.17121E-10	0.13688	9.82467E-10	1.17121E-10	6266.8	53415.6	6786.8	593.0	1.00
32	1.05943E-10	0.12222	9.82467E-10	1.05943E-10	5668.7	51240.9	6188.7	510.4	1.00
33	1.47526E-10	0.10911	1.51472E-09	1.47526E-10	7893.7	80188.6	8700.6	869.3	1.50
34	1.49992E-10	0.11113	1.51472E-09	1.49992E-10	8025.7	81014.9	8832.5	773.0	1.50
35	1.46074E-10	0.10792	1.51472E-09	1.46074E-10	7816.0	81941.3	8622.9	778.4	1.50
36	1.62468E-10	0.07122	2.46686E-09	1.62468E-10	8693.2	132848.5	9940.7	1209.0	2.50
37	1.84825E-10	0.08183	2.46686E-09	1.84825E-10	9889.5	132501.1	11137.0	1429.8	2.50
38	1.75286E-10	0.07728	2.46686E-09	1.75286E-10	9379.1	130635.0	10626.6	1103.7	2.50
39	1.8398E-10	0.04047	4.77308E-09	1.8398E-10	9844.3	255798.9	12128.5	2327.0	5.00
40	1.72014E-10	0.03774	4.77308E-09	1.72014E-10	9204.0	257788.9	11488.2	2091.5	5.00
41	1.66553E-10	0.03649	4.77308E-09	1.66553E-10	8911.8	252594.7	11196.0	2434.1	5.00
42	2.11687E-10	0.02224	9.79318E-09	2.11687E-10	11326.8	523577.8	14781.1	4184.2	10.00
43	1.9431E-10	0.02038	9.79318E-09	1.9431E-10	10397.0	524434.2	13851.3	3176.8	10.00
44	2.07999E-10	0.02185	9.79318E-09	2.07999E-10	11129.4		14583.8	3002.0	10.00
45									
46									
47									
48									
49									
50									
51									
52									
53									
54									
55									
56									
57									
58									

	DL	DM	DN	DO	DP
21	Bmax molar	2.22E-10		KD molar	8.90E-10
22	mole to molar conversion value	0.0003		molar to nM converison	1.00E+09
23	DPM/mole = (DPM/mmole)*1000	1.78E+17		kd nM =	8.90E-01
24	Bmax molar to Bmax moles	6.6456E-14			
25	= DPM/((DPM/mmole)*1000)	6.6456E-14			
26	=Bmax DPM	11852.91732			
27					
28					
29	assay date	1/27/2005			
30	Bmax(dpm)	11852.91732			
31	DPM/Ci (definition)	2.22E+12			
32	Ci/mmole	80.34			
33	DPM/mmole	1.78E+14			
34	DPM/pmole	1.78E+05			
35	1/(DPM/mmole)	5.61E-15			
36	1/(DPM/pmole)	5.61E-06			
37	SA(dpm/pmole)	1.78E+05			
38	protein/tube (ug)	600			
39	protein./tube(mg)	0.6			
40	bmax pmole	0.066456			
41	bmax pmole/mg	0.11076			
42	Bmax fmole/mg	110.76			
43	Bmax (fmole/100 ug)	11.076			
44	Bmax(fmole/100 ug)/Bmax molar	5.00E+10			

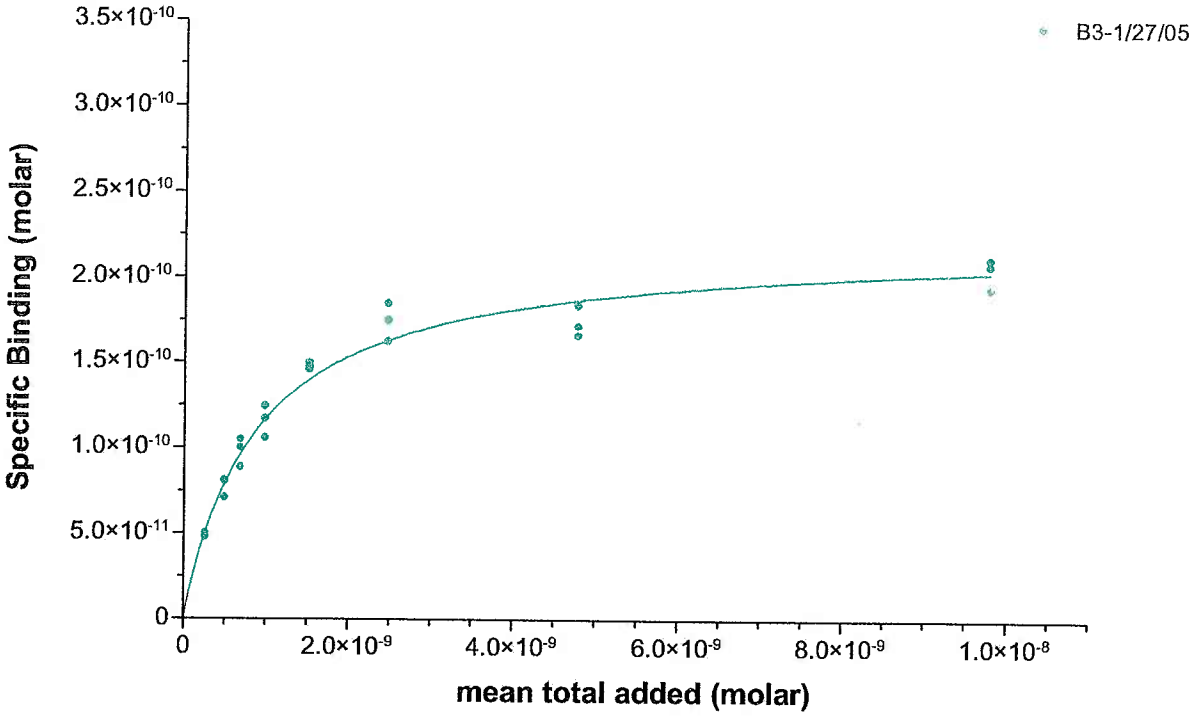

Hot Tubes
Lab B run 3
0.6 mg/tube
B3-1/27/05
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
1000
2000
3000
4000
R1881 (fmole)

Scatchard Display
Lab B run 3
0.6 mg/tube

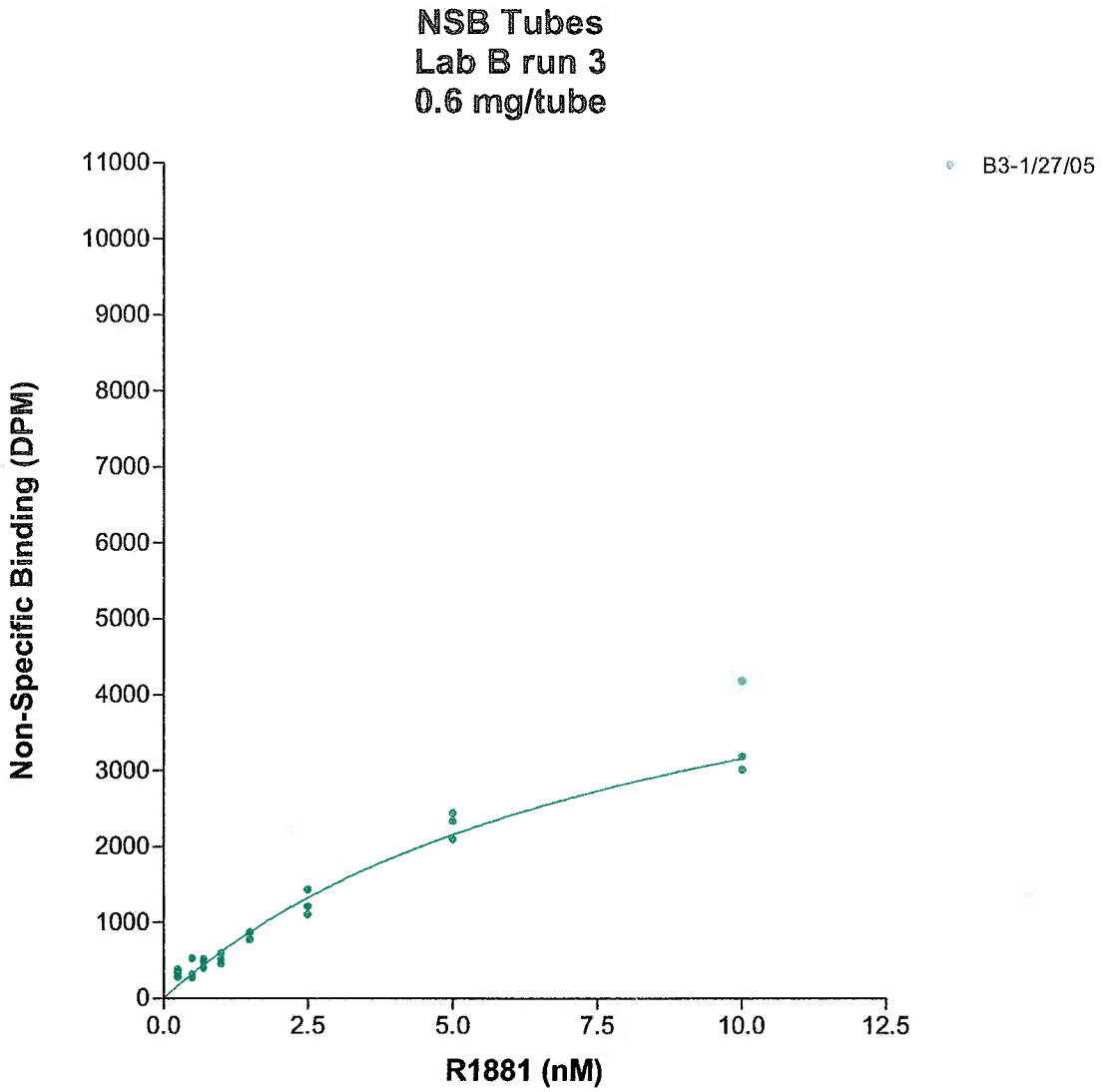
B3-1/27/05

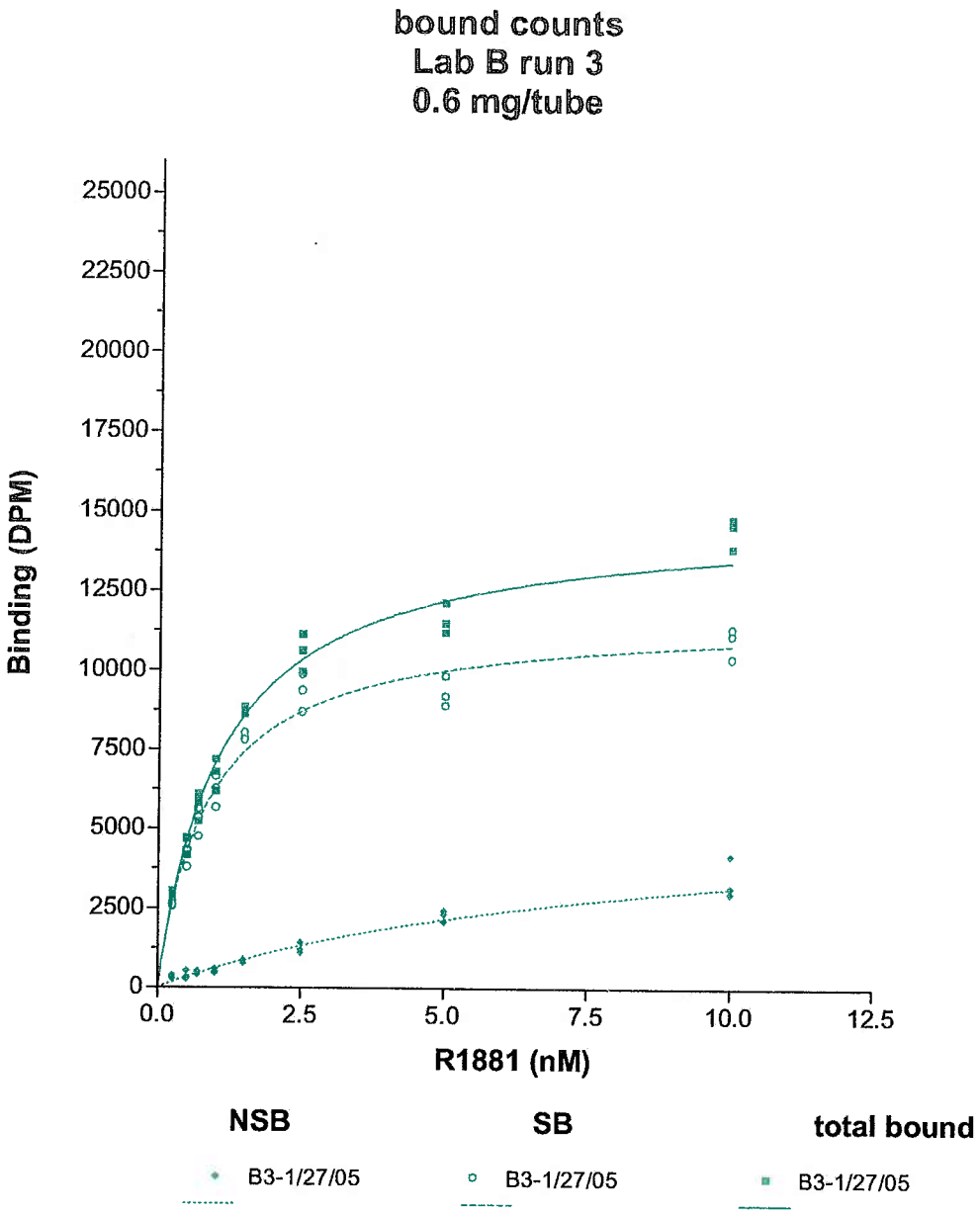


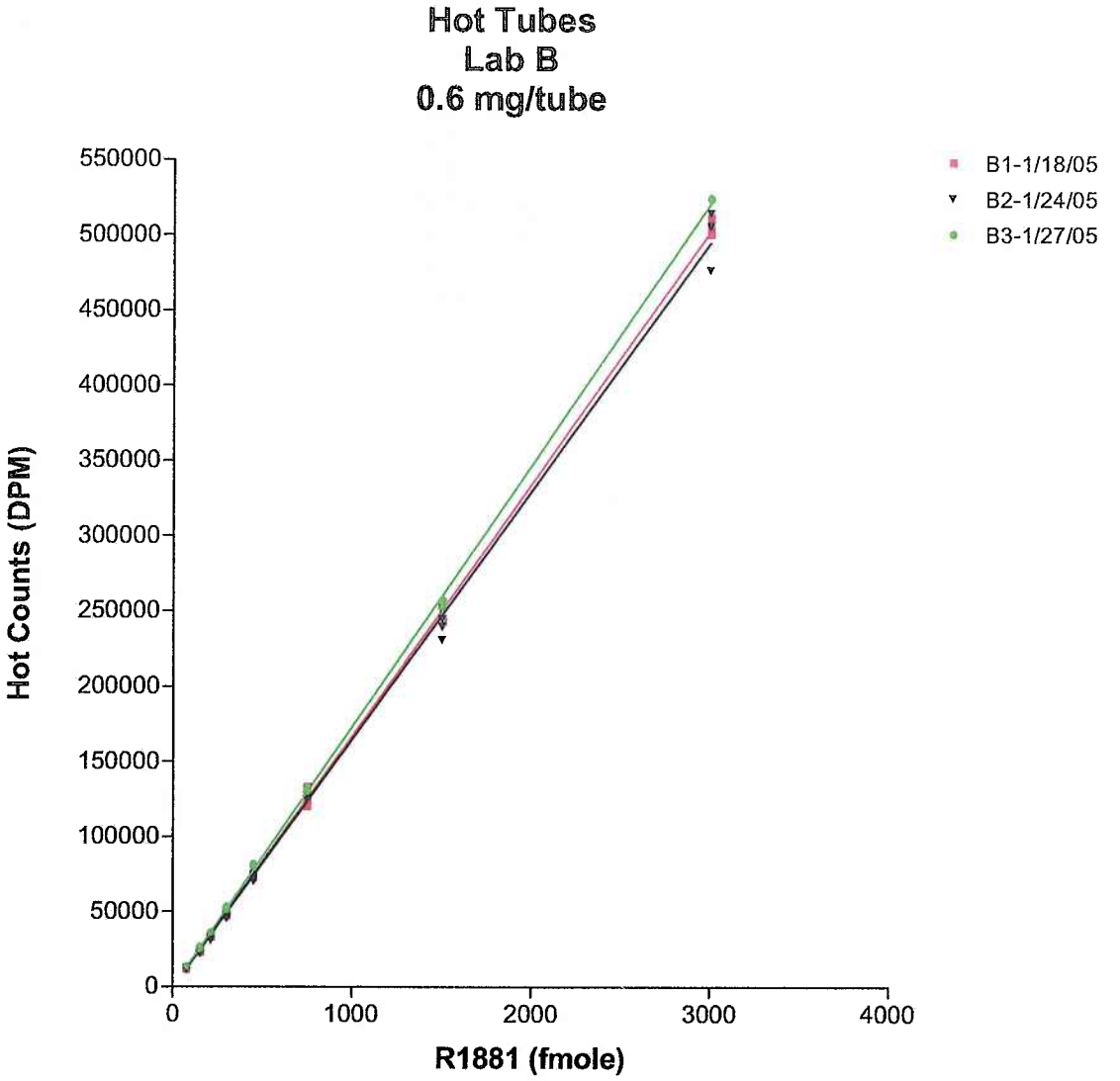
Lab B run 3
0.6 mg/tube

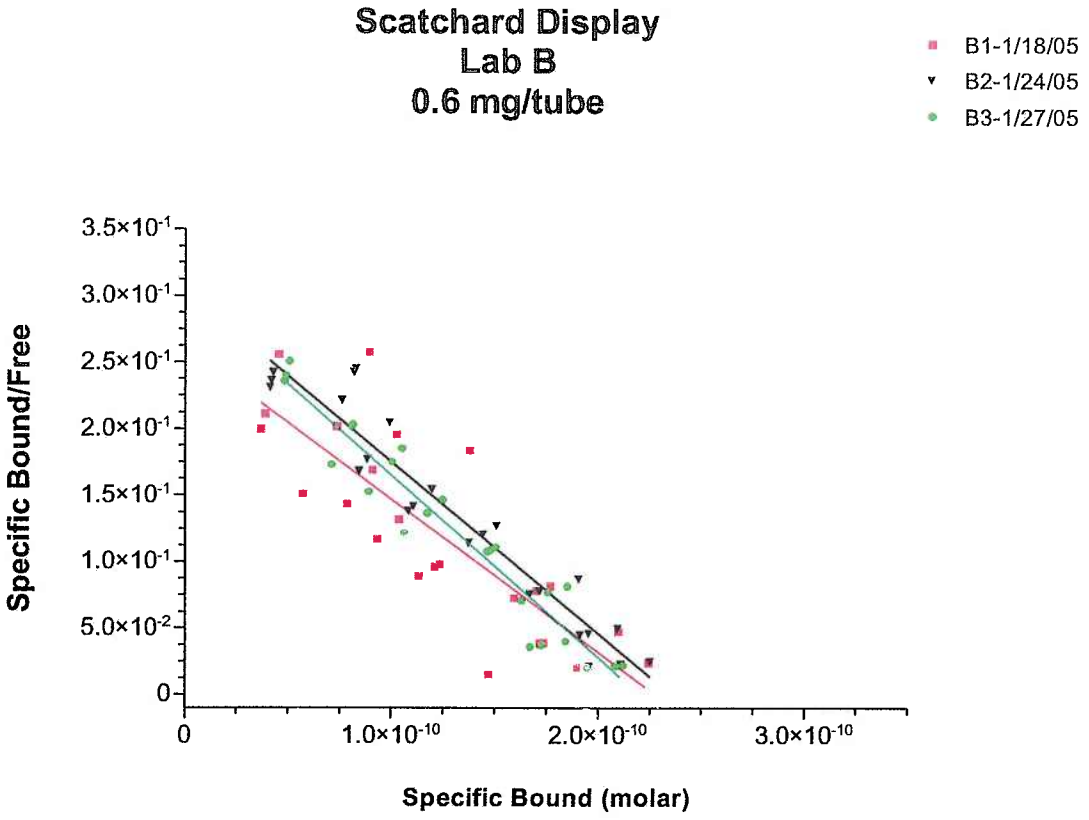


specific bound	B3-1/27/05
BMAX	2.2152e-010
KD	8.9013e-010
Std. Error	
BMAX	6.3191e-012
KD	5.3260e-011
95% Confidence Intervals	
BMAX	2.0842e-010 to 2.3463e-010
KD	7.7967e-010 to 1.0006e-009
Goodness of Fit	
Degrees of Freedom	22
R^2 (unweighted)	0.9667
Weighted Sum of Squares ($1/Y^2$)	0.09964
Absolute Sum of Squares	2.0604e-021
Sy.x	9.6776e-012
Data	
Number of X values	24
Number of Y replicates	1
Total number of values	24
Number of missing values	0

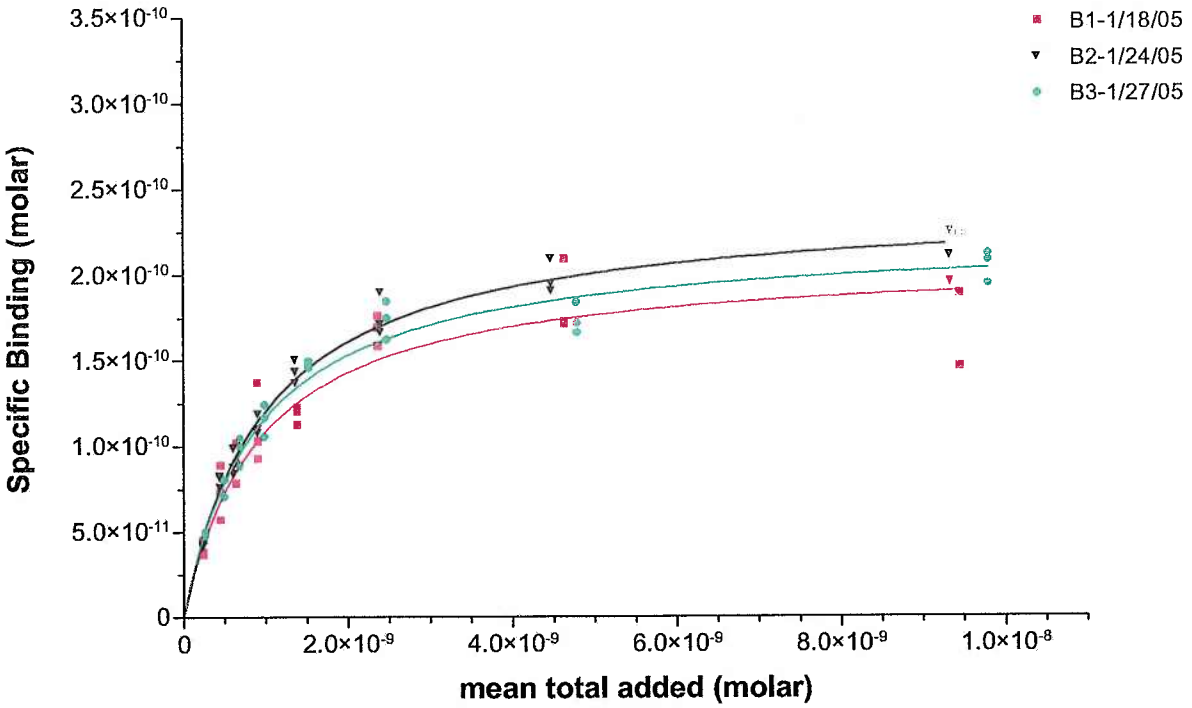

NSB Tubes
Lab B run 3
0.6 mg/tube
B3-1/27/05
Non-Specific Binding (DPM)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)


bound counts
Lab B run 3
0.6 mg/tube
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
SB
total bound
B3-1/27/05
B3-1/27/05
B3-1/27/05

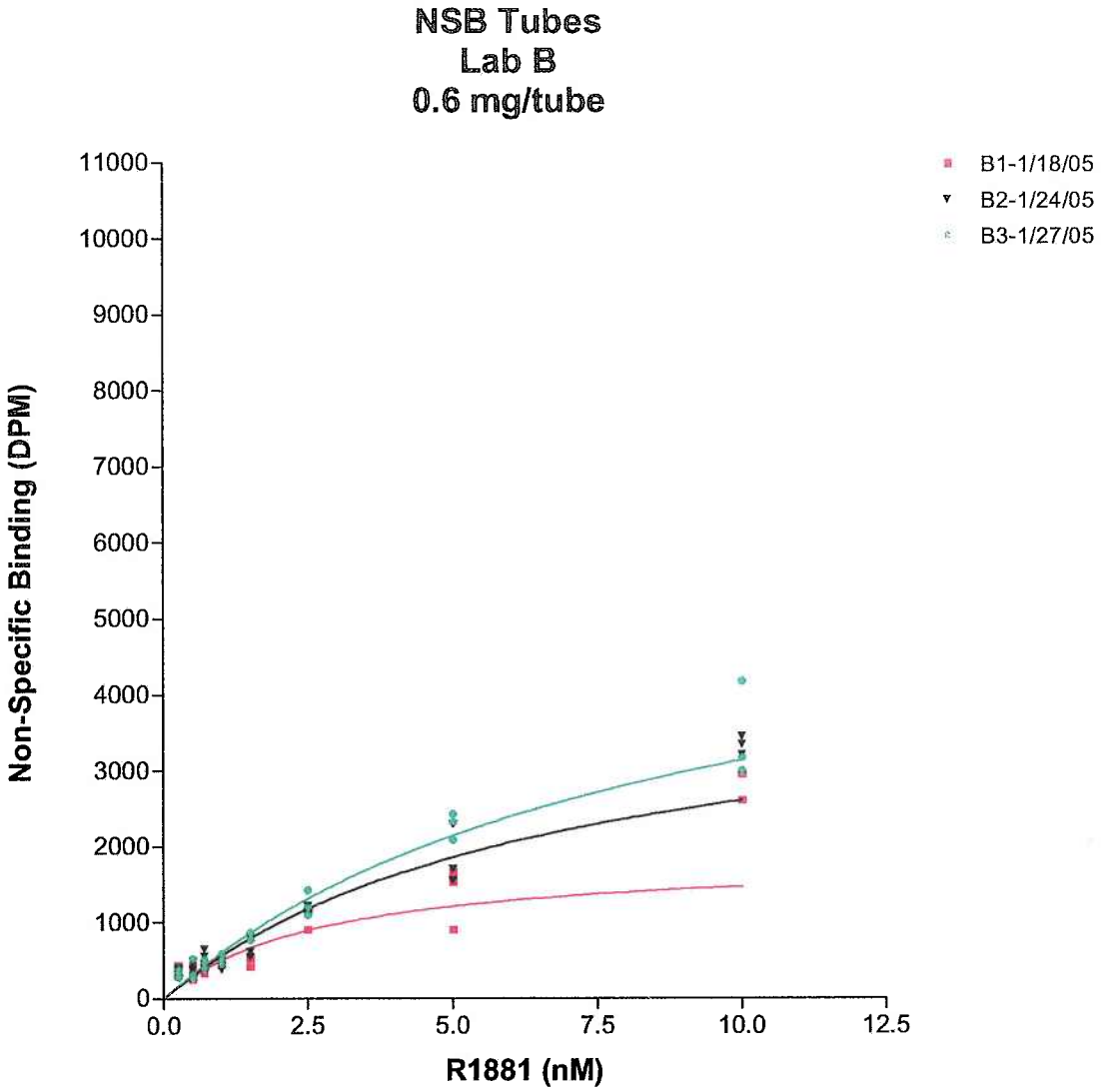

Hot Tubes
Lab B
0.6 mg/tube
Hot Counts (DPM)
550000
500000
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
0
1000
2000
3000
4000
R1881 (fmole)
B1-1/18/05
B2-1/24/05
B3-1/27/05

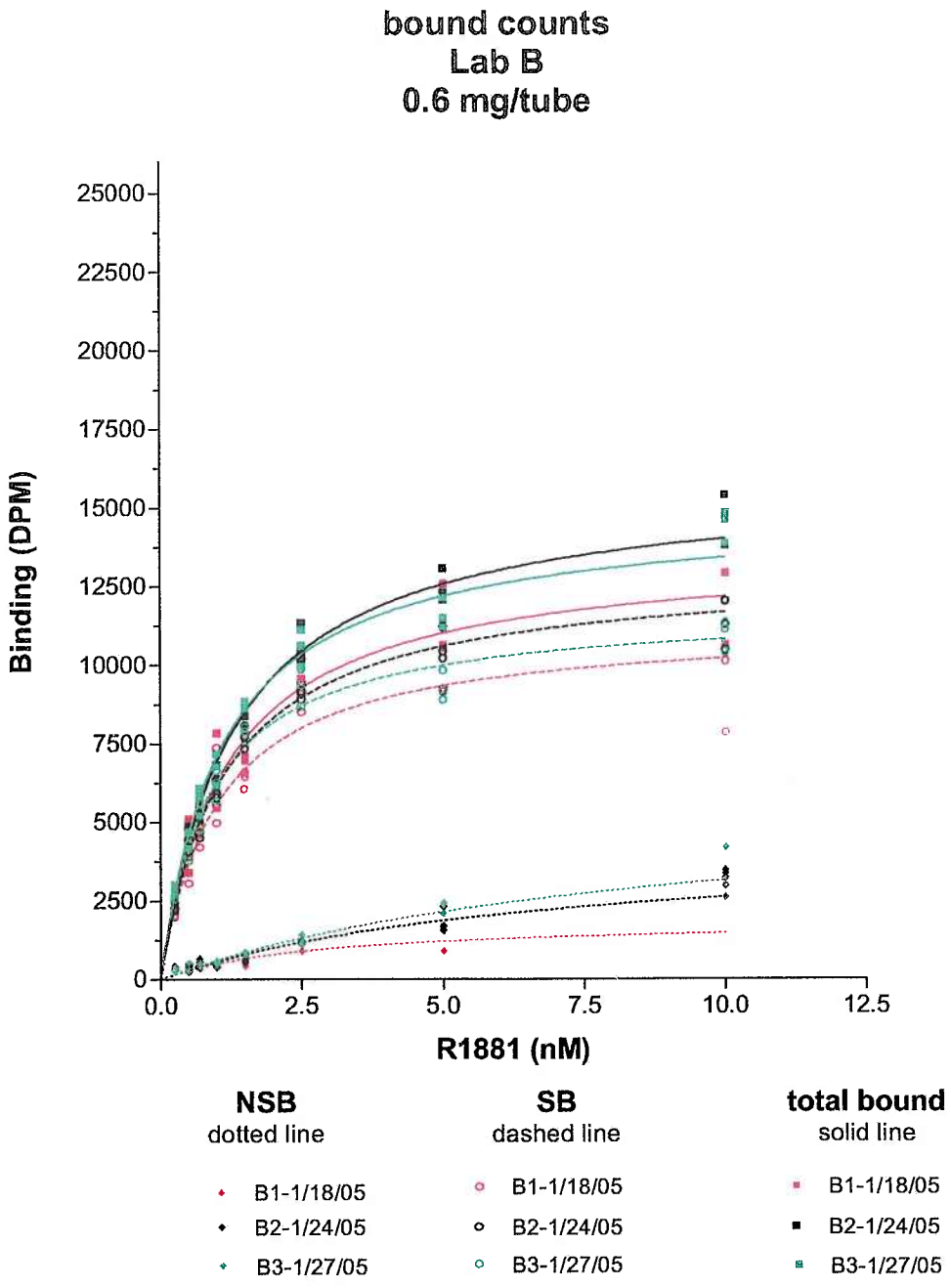

Scatchard Display
Lab B
0.6 mg/tube
B1-1/18/05
B2-1/24/05
B3-1/27/05
Specific Bound/Free
3.5×10-1
3.0×10-1
2.5×10-1
2.0×10-1
1.5×10-1
1.0×10-1
5.0×10-2
0
0
1.0×10-10
2.0×10-10
3.0×10-10
Specific Bound (molar)

Lab B
0.6 mg/tube



specific bound	B1-1/18/05	B2-1/24/05	B3-1/27/05
BMAX	2.0896e-010	2.4067e-010	2.2152e-010
KD	9.1846e-010	9.8904e-010	8.9013e-010
Std. Error			
BMAX	1.2550e-011	7.1682e-012	6.3191e-012
KD	1.1034e-010	5.7022e-011	5.3260e-011
95% Confidence Intervals			
BMAX	1.8293e-010 to 2.3499e-010	2.2581e-010 to 2.5554e-010	2.0842e-010 to 2.3463e-010
KD	6.8961e-010 to 1.1473e-009	8.7077e-010 to 1.1073e-009	7.7967e-010 to 1.0006e-009
Goodness of Fit			
Degrees of Freedom	22	22	22
R^2 (unweighted)	0.8822	0.9770	0.9667
Weighted Sum of Squares ($1/Y^2$)	0.4294	0.1027	0.09964
Absolute Sum of Squares	7.9129e-021	1.7847e-021	2.0604e-021
Sy.x	1.8965e-011	9.0068e-012	9.6776e-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
B1-1/18/05
B2-1/24/05
B3-1/27/05
Non-Specific Binding (DPM)
11000
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)


bound counts
Lab B
0.6 mg/tube
Binding (DPM)
25000
22500
20000
17500
15000
12500
10000
7500
5000
2500
0
0.0
2.5
5.0
7.5
10.0
12.5
R1881 (nM)
NSB
dotted line
B1-1/18/05
B2-1/24/05
B3-1/27/05
SB
dashed line
B1-1/18/05
B2-1/24/05
B3-1/27/05
total bound
solid line
B1-1/18/05
B2-1/24/05
B3-1/27/05

APPENDIX E

Processed Raw Data from Competitive Assay 1 and Associated Figures

Note: Due to pipetting error, the tube in position 52 was not included in the data analysis.

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-E supplied by Battelle to laboratory "B"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4065.63	12603.453
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4092.27	12686.037
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3970.47	12308.457
4	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	150.26	450.78
5	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	163.39	490.17
6	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	144.01	432.03
7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	282.81	876.711
8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	278.04	861.924
9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	219.67	680.977
10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	667.42	2069.002
11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	634.92	1968.252
12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	621.25	1925.875
13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2222.41	6889.471
14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2438.57	7559.567
15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2395.41	7425.771
16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3617.86	11215.366
17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3405.56	10557.236
18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3746.15	11613.065
19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3692.29	11446.099
20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3952.60	12253.06
21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3842.53	11911.843
22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	323.13	1001.703
23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	329.94	1022.814
24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	309.55	959.605
25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1129.88	3502.628
26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1246.52	3864.212
27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1213.58	3762.098
28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3063.31	9496.261
29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	2920.86	9054.666
30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3152.34	9772.254
31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3870.94	11999.914
32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3713.76	11512.656
33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3972.21	12313.851
34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3933.62	12194.222
35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4257.29	13197.599
36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3922.49	12159.719
37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4072.16	12623.696
38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4001.95	12406.045
39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4047.98	12548.738
40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4078.85	12644.435
41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4104.19	12722.989
42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3929.53	12181.543
43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	4207.76	13044.056
44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3868.14	11991.234
45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3894.86	12074.066
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3832.55	11880.905
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4025.52	12479.112
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3747.05	11615.855
49	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	163.50	490.5
50	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	167.98	503.94
51	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	189.80	569.4
52	1	none	Hot		—	—	—	30	—	—	—	—		45014.18	45014.18
53	2	none	Hot		—	—	—	30	—	—	—	—		48343.58	48343.58
54	3	none	Hot		—	—	—	30	—	—	—	—		48830.22	48830.22
55	1	none	Hot		—	—	—	30	—	—	—	—		48022.11	48022.11
56	2	none	Hot		—	—	—	30	—	—	—	—		48260.09	48260.09
57	3	none	Hot		—	—	—	30	—	—	—	—		47688.23	47688.23

Competitive Assay of a known Weak Positive

57 Assay Tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

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

Provide a reason in column R

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

Provide information in all blue cells in this column

Laboratory Code:	B	
Run identification:	1	
Assay start date:	2/1/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.28	Ci/mmole
Cytosol vial or lot identification:	67, 68, 69	
Protein (cytosol):	1000	micro gram per tube
Standard Curve IC50:	1.35E-09	M
Weak Positive, Max Concentration:	3.00E-02	M
Weak Positive IC50:	3.51E-05	M
RBA:	3.84554E-05	
volume of ethanol counted:	2	mL
mulitply DPM in sample by :	3	

	A	B	R
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			protcol calls for counting decanted EtOH su
18			reflects 100ul of reaction mixture processed

	A	B	S	T	U	V	W	X	Y	AA
1										
2				**Summary values**						
3				**n**		**Mean**	**SD**			
4			EtOH	6		12262.3	426.25	Total Binding, solvent control, tubes		
5			Hot	5		48228.8	421.54	Total hot R1881 added to each tube		
6			NSB	6		489.5	47.74	Nonspecific Binding		
7			Specific EtOH	6		11772.8	426.25			
8										
9										
10										
11				**Assay Characterization Values**						
12			EtOH / Hot	0.25	less than 0.1?					
13			NSB / EtOH	0.04	around 0.25 ?					
14										
15										
16										
17			pernate							
18										

Column O, Rows 10 through 13 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 25.43%

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)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4065.63	12603.453	TRUE
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4092.27	12686.037	TRUE
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3970.47	12308.457	TRUE
4	1	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	150.26	450.78	TRUE
5	2	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	163.39	490.17	TRUE
6	3	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	144.01	432.03	TRUE
7	1	Inert R1881	S	1	**B-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	282.81	876.711	TRUE
8	2	Inert R1881	S	1	**B-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	278.04	861.924	TRUE
9	3	Inert R1881	S	1	**B-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	219.67	680.977	TRUE
10	1	Inert R1881	S	2	**B-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	667.42	2069.002	TRUE
11	2	Inert R1881	S	2	**B-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	634.92	1968.252	TRUE
12	3	Inert R1881	S	2	**B-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	621.25	1925.875	TRUE
13	1	Inert R1881	S	3	**B-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2222.41	6889.471	TRUE
14	2	Inert R1881	S	3	**B-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2438.57	7559.567	TRUE
15	3	Inert R1881	S	3	**B-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2395.41	7425.771	TRUE
16	1	Inert R1881	S	4	**B-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3617.86	11215.366	TRUE
17	2	Inert R1881	S	4	**B-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3405.56	10557.236	TRUE
18	3	Inert R1881	S	4	**B-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3746.15	11613.065	TRUE
19	1	Inert R1881	S	5	**B-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3692.29	11446.099	TRUE
20	2	Inert R1881	S	5	**B-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3952.60	12253.06	TRUE
21	3	Inert R1881	S	5	**B-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3842.53	11911.843	TRUE

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

	A	B	S	T	U	V	W	X	Y	AA
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	*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*
24	1	1			102.9	12603.45	12114.0	-830.6	102.9	26.1326
25	2	2			103.6	12686.04	12196.6	-913.2	103.6	26.30384
26	3	3			100.4	12308.46	11819.0	-535.6	100.4	25.52094
27	4	1		-6.00	-0.3	450.78	-38.7	11322.1	-0.3	0.934669
28	5	2		-6.00	0.0	490.17	0.7	11282.7	0.0	1.016342
29	6	3		-6.00	0.0	432.03	-57.4	11340.8	-0.5	0.895792
30	7	1	cold R1881	-7.01	3.3	876.711	387.2	10896.1	3.3	1.817815
31	8	2	cold R1881	-7.01	3.2	861.924	372.5	10910.9	3.2	1.787155
32	9	3	cold R1881	-7.01	1.6	680.977	191.5	11091.9	1.6	1.41197
33	10	1	cold R1881	-8.01	13.4	2069.002	1579.5	9703.8	13.4	4.289968
34	11	2	cold R1881	-8.01	12.6	1968.252	1478.8	9804.6	12.6	4.081068
35	12	3	cold R1881	-8.01	12.2	1925.875	1436.4	9847.0	12.2	3.993201
36	13	1	cold R1881	-9.01	54.4	6889.471	6400.0	4883.4	54.4	14.28496
37	14	2	cold R1881	-9.01	60.1	7559.567	7070.1	4213.3	60.1	15.67437
38	15	3	cold R1881	-9.01	58.9	7425.771	6936.3	4347.1	58.9	15.39695
39	16	1	cold R1881	-10.01	91.1	11215.37	10725.9	557.5	91.1	23.25448
40	17	2	cold R1881	-10.01	85.5	10557.24	10067.8	1215.6	85.5	21.88988
41	18	3	cold R1881	-10.01	94.5	11613.07	11123.6	159.8	94.5	24.07909
42	19	1	cold R1881	-11.01	93.1	11446.1	10956.6	326.7	93.1	23.73289
43	20	2	cold R1881	-11.01	99.9	12253.06	11763.6	-480.2	99.9	25.40608
44	21	3	cold R1881	-11.01	97.0	11911.84	11422.4	-139.0	97.0	24.69859

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

working volume **3.1E+02** uL

and the maximum concentration for the weak positive

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

Check the 10% rule: 25.43%

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)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?
22	1	Weak Positive	P	1	**B-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	323.13	1001.703	TRUE
23	2	Weak Positive	P	1	**B-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	329.94	1022.814	TRUE
24	3	Weak Positive	P	1	**B-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	309.55	959.605	TRUE
25	1	Weak Positive	P	2	**B-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1129.88	3502.628	TRUE
26	2	Weak Positive	P	2	**B-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1246.52	3864.212	TRUE
27	3	Weak Positive	P	2	**B-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1213.58	3762.098	TRUE
28	1	Weak Positive	P	3	**B-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3063.31	9496.261	TRUE
29	2	Weak Positive	P	3	**B-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	2920.86	9054.666	TRUE
30	3	Weak Positive	P	3	**B-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3152.34	9772.254	TRUE
31	1	Weak Positive	P	4	**B-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3870.94	11999.914	TRUE
32	2	Weak Positive	P	4	**B-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3713.76	11512.656	TRUE
33	3	Weak Positive	P	4	**B-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	3972.21	12313.851	TRUE
34	1	Weak Positive	P	5	**B-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3933.62	12194.222	TRUE
35	2	Weak Positive	P	5	**B-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4257.29	13197.599	TRUE
36	3	Weak Positive	P	5	**B-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3922.49	12159.719	TRUE
37	1	Weak Positive	P	6	**B-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4072.16	12623.696	TRUE
38	2	Weak Positive	P	6	**B-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4001.95	12406.045	TRUE
39	3	Weak Positive	P	6	**B-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4047.98	12548.738	TRUE
40	1	Weak Positive	P	7	**B-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4078.85	12644.435	TRUE
41	2	Weak Positive	P	7	**B-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4104.19	12722.989	TRUE
42	3	Weak Positive	P	7	**B-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	3929.53	12181.543	TRUE
43	1	Weak Positive	P	8	**B-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4207.76	13044.056	TRUE
44	2	Weak Positive	P	8	**B-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3868.14	11991.234	TRUE
45	3	Weak Positive	P	8	**B-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	3894.86	12074.066	TRUE
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3832.55	11880.905	TRUE
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4025.52	12479.112	TRUE
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3747.05	11615.855	TRUE
49	1	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	163.50	490.5	TRUE
50	2	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	167.98	503.94	TRUE

	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23	***Position***	***Replicate***	***Notes to explain why "Use this value" is set to "FALSE"***
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	

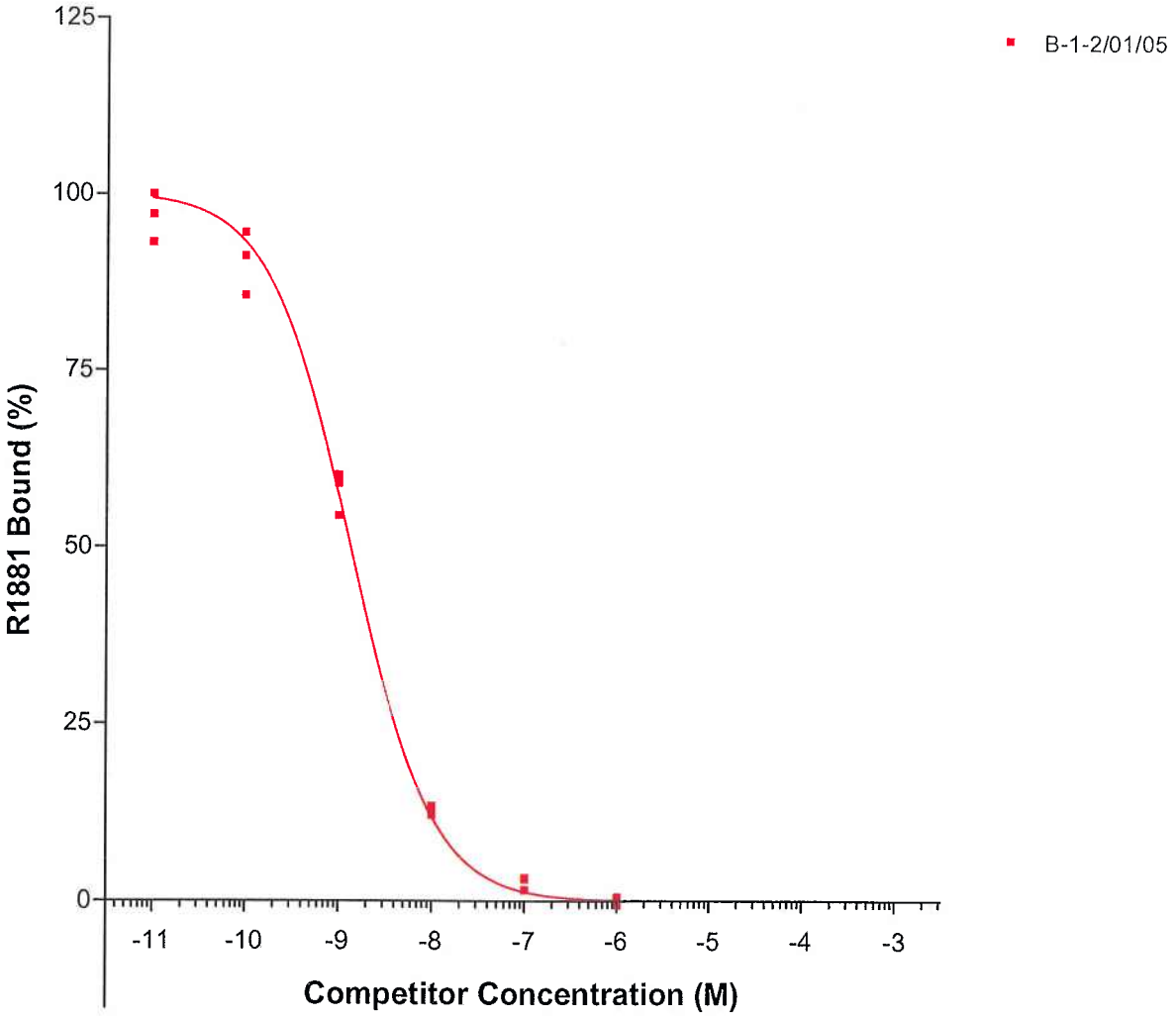
	A	B	S	T	U	V	W	X	Y	AA
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	*Position*	*Replicate*		*concentration (log)*	*percent bound*	*Usable DPM values*	*Specific Binding (Total - mean NSB)*	*Free DPM (mean total add - total bound)*	*Percent Binding (specific bound / mean specific EtOH)*	*Ratio Total binding/ Hot*
45	22	1	Weak Positive	-3.01424	4.4	1001.703	512.2	10771.1	4.4	2.076979
46	23	2	Weak Positive	-3.01424	4.5	1022.814	533.3	10750.0	4.5	2.120752
47	24	3	Weak Positive	-3.01424	4.0	959.605	470.1	10813.2	4.0	1.989691
48	25	1	Weak Positive	-4.01424	25.6	3502.628	3013.2	8270.2	25.6	7.262517
49	26	2	Weak Positive	-4.01424	28.7	3864.212	3374.7	7908.6	28.7	8.012242
50	27	3	Weak Positive	-4.01424	27.8	3762.098	3272.6	8010.7	27.8	7.800514
51	28	1	Weak Positive	-5.01424	76.5	9496.261	9006.8	2276.6	76.5	19.69
52	29	2	Weak Positive	-5.01424	72.8	9054.666	8565.2	2718.2	72.8	18.77438
53	30	3	Weak Positive	-5.01424	78.8	9772.254	9282.8	2000.6	78.8	20.26226
54	31	1	Weak Positive	-6.01424	97.8	11999.91	11510.4	-227.1	97.8	24.8812
55	32	2	Weak Positive	-6.01424	93.6	11512.66	11023.2	260.2	93.6	23.87089
56	33	3	Weak Positive	-6.01424	100.4	12313.85	11824.4	-541.0	100.4	25.53213
57	34	1	Weak Positive	-7.01424	99.4	12194.22	11704.8	-421.4	99.4	25.28408
58	35	2	Weak Positive	-7.01424	107.9	13197.6	12708.1	-1424.8	107.9	27.36453
59	36	3	Weak Positive	-7.01424	99.1	12159.72	11670.2	-386.9	99.1	25.21254
60	37	1	Weak Positive	-8.01424	103.1	12623.7	12134.2	-850.9	103.1	26.17458
61	38	2	Weak Positive	-8.01424	101.2	12406.05	11916.6	-633.2	101.2	25.72329
62	39	3	Weak Positive	-8.01424	102.4	12548.74	12059.3	-775.9	102.4	26.01915
63	40	1	Weak Positive	-9.01424	103.2	12644.44	12155.0	-871.6	103.2	26.21758
64	41	2	Weak Positive	-9.01424	103.9	12722.99	12233.5	-950.2	103.9	26.38045
65	42	3	Weak Positive	-9.01424	99.3	12181.54	11692.1	-408.7	99.3	25.25779
66	43	1	Weak Positive	-10.01424	106.6	13044.06	12554.6	-1271.2	106.6	27.04617
67	44	2	Weak Positive	-10.01424	97.7	11991.23	11501.8	-218.4	97.7	24.8632
68	45	3	Weak Positive	-10.01424	98.4	12074.07	11584.6	-301.2	98.4	25.03495
69	46	1		—	96.8	11880.91	11391.4	-108.1	96.8	24.63444
70	47	2		—	101.8	12479.11	11989.6	-706.3	101.8	25.87479
71	48	3		—	94.5	11615.86	11126.4	157.0	94.5	24.08487
72	49	1		-6.00	0.0	490.5	1.0	11282.3	0.0	1.017026
73	50	2		-6.00	0.1	503.94	14.5	11268.9	0.1	1.044893

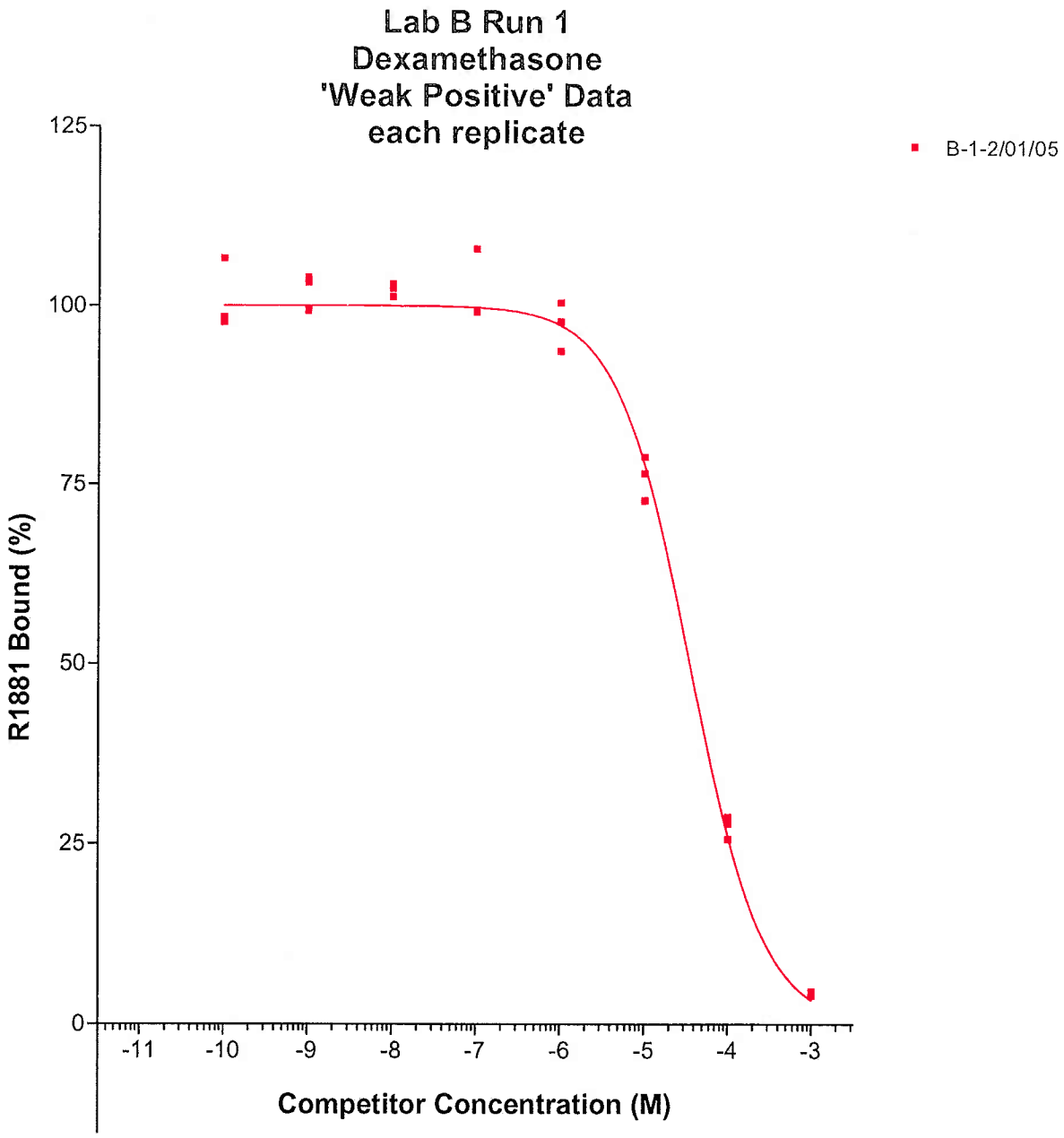
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19				Column O, Rows 10 through 13 will contain output parameters									**working volume**		**3.1E+02** uL		
20				from the nonlinear regression software.													
21				and the maximum concentration for the weak positive													
22				**Competitive Assay Tube Layout - One Test Chemical (Weak Positive)**												Check the 10% rule:	25.43%
23	*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)*	*Final Volume (ul)*	*Competitor Final Concentration (M)*	*Aliquot (uL)*	*DPM as sampled*	*corrected DPM for 2.0 mL*	*Use this value?*
74	51	3	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	189.80	569.4	TRUE
75	52	1	none	Hot		—	—	—	30	—	—	—	—		45014.18	45014.18	FALSE
76	53	2	none	Hot		—	—	—	30	—	—	—	—		48343.58	48343.58	TRUE
77	54	3	none	Hot		—	—	—	30	—	—	—	—		48830.22	48830.22	TRUE
78	55	1	none	Hot		—	—	—	30	—	—	—	—		48022.11	48022.11	TRUE
79	56	2	none	Hot		—	—	—	30	—	—	—	—		48260.09	48260.09	TRUE
80	57	3	none	Hot		—	—	—	30	—	—	—	—		47688.23	47688.23	TRUE

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

	A	B	S	T	U	V	W	X	Y	AA
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	***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***
74	51	3		-6.00	0.7	569.4	79.9	11203.4	0.7	1.180621
75	52	1								
76	53	2				48343.58	47854.1			
77	54	3				48830.22	48340.8			
78	55	1				48022.11	47532.6			
79	56	2				48260.09	47770.6			
80	57	3				47688.23	47198.8			

Lab B
Run 1
Standard Curve
each replicate




Lab B Run 1
Dexamethasone
'Weak Positive' Data
each replicate
B-1-2/01/05
R1881 Bound (%)
125
100
75
50
25
0
-11
-10
-9
-8
-7
-6
-5
-4
-3
Competitor Concentration (M)

Competitive Assay of a known Weak Positive

57 Assay Tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

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

Provide a reason in column R

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

Provide information in all blue cells in this column

Laboratory Code:	B	
Run identification:	1	
Assay start date:	2/1/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	80.28	**Ci/mmole**
Cytosol vial or lot identification:	67, 68, 69	
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.35E-09	**M**
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	3.51E-05	**M**
RBA:	3.84554E-05	

APPENDIX F

Processed Raw Data from Competitive Assay 3 and Associated Figures

Note: Due to spillage, the tubes in position17 through 26 were not included in the data analysis.

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-E supplied by Battelle to laboratory "g"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4052.04	12561.324
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4275.29	13253.399
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3942.02	12220.262
4	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	202.28	606.84
5	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	189.77	569.31
6	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	157.83	473.49
7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	274.05	849.555
8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	249.44	773.264
9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	274.65	851.415
10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	666.70	2066.77
11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	728.61	2258.691
12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	628.13	1947.203
13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2597.97	8053.707
14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2710.11	8401.341
15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2521.06	7815.286
16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3648.17	11309.327
17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3241.53	10048.743
18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3173.82	9838.842
19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3232.46	10020.626
20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3186.07	9876.817
21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	2879.29	8925.799
22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	324.85	1007.035
23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	363.99	1128.369
24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	277.66	860.746
25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	848.73	2631.063
26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	937.19	2905.289
27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1353.52	4195.912
28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3272.46	10144.626
29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3186.06	9876.786
30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3271.68	10142.208
31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	4063.82	12597.842
32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	4147.86	12858.366
33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	4312.92	13370.052
34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4373.39	13557.509
35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	3989.69	12368.039
36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4175.03	12942.593
37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4045.65	12541.515
38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	3973.75	12318.625
39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4257.37	13197.847
40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4054.82	12569.942
41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4129.36	12801.016
42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4247.56	13167.436
43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	4098.50	12705.35
44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	4218.80	13078.28
45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	4174.72	12941.632
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4169.54	12925.574
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4311.52	13365.712
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3826.90	11863.39
49	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	220.70	662.1
50	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	262.41	787.23
51	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	221.05	663.15
52	1	none	Hot		—	—	—	30	—	—	—	—		45965.26	45965.26
53	2	none	Hot		—	—	—	30	—	—	—	—		47010.21	47010.21
54	3	none	Hot		—	—	—	30	—	—	—	—		45851.15	45851.15
55	1	none	Hot		—	—	—	30	—	—	—	—		46137.12	46137.12
56	2	none	Hot		—	—	—	30	—	—	—	—		46722.82	46722.82
57	3	none	Hot		—	—	—	30	—	—	—	—		44737.98	44737.98

	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**											*Provide information in all blue cells in this column*		
2				**57 Assay Tubes**										**Laboratory Code:**	**B**		
3				Please return by eMail to n.a.Holter@pnl.gov										**Run identification:**	3		
4														**Assay start date:**	3/3/2005		
5														**Tracer lot number:**	3538-497		
6				Provide information in all blue cells in column O										**Specific activity on day of assay:**	79.91	**Ci/mmole**	
7				If the DPM value for a tube was judged unreliable,										**Cytosol vial or lot identification:**	73, 74, 75		
8				Include the DPM value in column O										**Protein (cytosol):**	1000	**micro gram per tube**	
9				Provide a reason in column R													
10				the TRUE in column Q will automatically change to FALSE										**Standard Curve IC50:**	1.55E-09		
11														**Weak Positive, Max Concentration:**	**3.00E-02**	**M**	
12														**Weak Positive IC50:**	3.88E-05		
13				Columns T and U contain values to be analyzed										**RBA:**	4.00E-05		
14				by nonlinear regression software													
15																	
16																	
17														**volume of ethanol counted:**	2	**mL**	
18														**mulitply DPM in sample by :**	3		

	A	B	R
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			protcol calls for counting decanted EtOH su
18			reflects 100ul of reaction mixture processed

	A	B	S	T	U	V	W	X	Y	AA
1										
2				**Summary values**						
3				**n**		**Mean**	**SD**			
4			EtOH	6		12698.3	591.54	Total Binding, solvent control, tubes		
5			Hot	6		46070.8	793.45	Total hot R1881 added to each tube		
6			NSB	6		627.0	105.37	Nonspecific Binding		
7			Specific EtOH	6		12071.3	591.54			
8										
9										
10										
11				**Assay Characterization Values**						
12			EtOH / Hot			0.28	less than 0.1?			
13			NSB / EtOH			0.05	around 0.25 ?			
14										
15										
16										
17			pernate							
18										

Column O, Rows 10 through 13 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 27.56%

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory "g"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4052.04	12561.324	TRUE
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4275.29	13253.399	TRUE
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3942.02	12220.262	TRUE
4	1	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	202.28	606.84	TRUE
5	2	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	189.77	569.31	TRUE
6	3	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	157.83	473.49	TRUE
7	1	Inert R1881	S	1	**B-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	274.05	849.555	TRUE
8	2	Inert R1881	S	1	**B-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	249.44	773.264	TRUE
9	3	Inert R1881	S	1	**B-1-S1**	3.00E-06	300	30	10	50	310	9.7E-08	100	274.65	851.415	TRUE
10	1	Inert R1881	S	2	**B-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	666.70	2066.77	TRUE
11	2	Inert R1881	S	2	**B-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	728.61	2258.691	TRUE
12	3	Inert R1881	S	2	**B-1-S2**	3.00E-07	300	30	10	50	310	9.7E-09	100	628.13	1947.203	TRUE
13	1	Inert R1881	S	3	**B-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2597.97	8053.707	TRUE
14	2	Inert R1881	S	3	**B-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2710.11	8401.341	TRUE
15	3	Inert R1881	S	3	**B-1-S3**	3.00E-08	300	30	10	50	310	9.7E-10	100	2521.06	7815.286	TRUE
16	1	Inert R1881	S	4	**B-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3648.17	11309.327	TRUE
17	2	Inert R1881	S	4	**B-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3241.53	10048.743	FALSE
18	3	Inert R1881	S	4	**B-1-S4**	3.00E-09	300	30	10	50	310	9.7E-11	100	3173.82	9838.842	FALSE
19	1	Inert R1881	S	5	**B-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3232.46	10020.626	FALSE
20	2	Inert R1881	S	5	**B-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	3186.07	9876.817	FALSE
21	3	Inert R1881	S	5	**B-1-S5**	3.00E-10	300	30	10	50	310	9.7E-12	100	2879.29	8925.799	FALSE

	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23	***Position***	***Replicate***	***Notes to explain why "Use this value" is set to "FALSE"***
24	1	1	
25	2	2	
26	3	3	
27	4	1	
28	5	2	
29	6	3	
30	7	1	
31	8	2	
32	9	3	
33	10	1	
34	11	2	
35	12	3	
36	13	1	
37	14	2	
38	15	3	
39	16	1	
40	17	2	spilled approx. 0.3 mL of sample
41	18	3	spilled approx. 0.3 mL of sample
42	19	1	spilled approx. 0.4 mL of sample
43	20	2	spilled approx. 0.4 mL of sample
44	21	3	spilled approx. 0.5 mL of sample

	A	B	S	T	U	V	W	X	Y	AA
19										
20				***Values for analysis by nonlinear regression***						
21				Standard Curve						
22				Weak Positive						
23	***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***
24	1	1			98.9	12561.32	11934.3	-490.1	98.9	27.26529
25	2	2			104.6	13253.4	12626.4	-1182.1	104.6	28.76749
26	3	3			96.0	12220.26	11593.2	-149.0	96.0	26.52499
27	4	1		-6.0	-0.2	606.84	-20.2	11464.4	-0.2	1.317191
28	5	2		-6.0	-0.5	569.31	-57.7	11501.9	-0.5	1.23573
29	6	3		-6.0	0.0	473.49	-153.5	11597.8	-1.3	1.027745
30	7	1	cold R1881	-7.0	1.8	849.555	222.5	11221.7	1.8	1.844022
31	8	2	cold R1881	-7.0	1.2	773.264	146.2	11298.0	1.2	1.678427
32	9	3	cold R1881	-7.0	1.9	851.415	224.4	11219.8	1.9	1.84806
33	10	1	cold R1881	-8.0	11.9	2066.77	1439.8	10004.5	11.9	4.486078
34	11	2	cold R1881	-8.0	13.5	2258.691	1631.7	9812.6	13.5	4.902657
35	12	3	cold R1881	-8.0	10.9	1947.203	1320.2	10124.1	10.9	4.226549
36	13	1	cold R1881	-9.0	61.5	8053.707	7426.7	4017.5	61.5	17.48117
37	14	2	cold R1881	-9.0	64.4	8401.341	7774.3	3669.9	64.4	18.23573
38	15	3	cold R1881	-9.0	59.5	7815.286	7188.3	4256.0	59.5	16.96366
39	16	1	cold R1881	-10.0	88.5	11309.33	10682.3	761.9	88.5	24.54773
40	17	2	cold R1881	-10.0						
41	18	3	cold R1881	-10.0						
42	19	1	cold R1881	-11.0						
43	20	2	cold R1881	-11.0						
44	21	3	cold R1881	-11.0						

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

working volume **3.1E+02** uL

and the maximum concentration for the weak positive

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

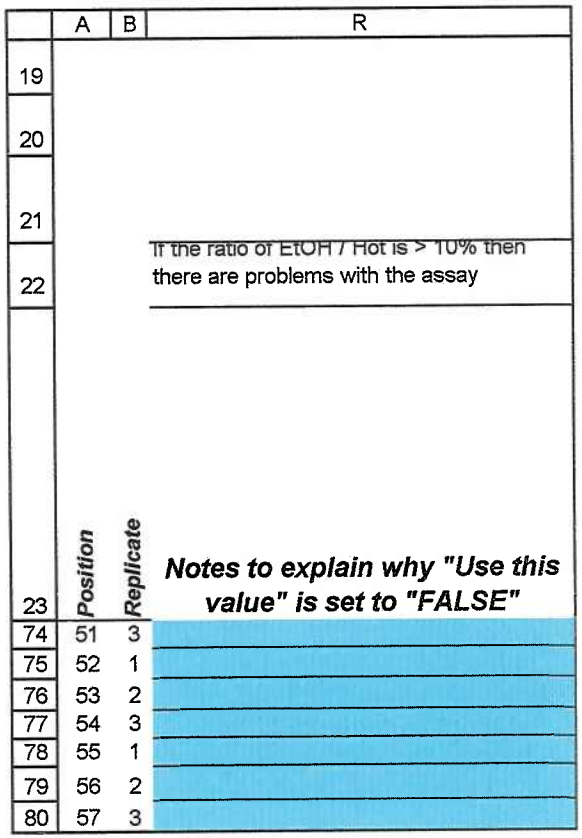
Check the 10% rule: 27 56%

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
23	*Position*	*Replicate*	*Competitor*	*Competitor Code*	*Concentration Code*	*Labels on vials in set 1-1-E supplied by Battelle to laboratory "B"*	*Competitor Initial Concentration (M)*	*Cytosol (uL)*	*Tracer (Hot R1881) Volume (uL)*	*Competitor Volume (uL)*	*triamcelenone Volume (uL)*	*Final Volume (ul)*	*Competitor Final Concentration (M)*	*Aliquot (uL)*	*DPM as sampled*	*corrected DPM for 2.0 mL*	*Use this value?*
45	22	1	Weak Positive	P	1	**B-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	324.85	1007.035	FALSE
46	23	2	Weak Positive	P	1	**B-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	363.99	1128.369	FALSE
47	24	3	Weak Positive	P	1	**B-1-P1**	3.00E-02	300	30	10	50	310	9.7E-04	100	277.66	860.746	FALSE
48	25	1	Weak Positive	P	2	**B-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	848.73	2631.063	FALSE
49	26	2	Weak Positive	P	2	**B-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	937.19	2905.289	FALSE
50	27	3	Weak Positive	P	2	**B-1-P2**	3.00E-03	300	30	10	50	310	9.7E-05	100	1353.52	4195.912	TRUE
51	28	1	Weak Positive	P	3	**B-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3272.46	10144.626	TRUE
52	29	2	Weak Positive	P	3	**B-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3186.06	9876.786	TRUE
53	30	3	Weak Positive	P	3	**B-1-P3**	3.00E-04	300	30	10	50	310	9.7E-06	100	3271.68	10142.208	TRUE
54	31	1	Weak Positive	P	4	**B-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4063.82	12597.842	TRUE
55	32	2	Weak Positive	P	4	**B-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4147.86	12858.366	TRUE
56	33	3	Weak Positive	P	4	**B-1-P4**	3.00E-05	300	30	10	50	310	9.7E-07	100	4312.92	13370.052	TRUE
57	34	1	Weak Positive	P	5	**B-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4373.39	13557.509	TRUE
58	35	2	Weak Positive	P	5	**B-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	3989.69	12368.039	TRUE
59	36	3	Weak Positive	P	5	**B-1-P5**	3.00E-06	300	30	10	50	310	9.7E-08	100	4175.03	12942.593	TRUE
60	37	1	Weak Positive	P	6	**B-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4045.65	12541.515	TRUE
61	38	2	Weak Positive	P	6	**B-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	3973.75	12318.625	TRUE
62	39	3	Weak Positive	P	6	**B-1-P6**	3.00E-07	300	30	10	50	310	9.7E-09	100	4257.37	13197.847	TRUE
63	40	1	Weak Positive	P	7	**B-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4054.82	12569.942	TRUE
64	41	2	Weak Positive	P	7	**B-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4129.36	12801.016	TRUE
65	42	3	Weak Positive	P	7	**B-1-P7**	3.00E-08	300	30	10	50	310	9.7E-10	100	4247.56	13167.436	TRUE
66	43	1	Weak Positive	P	8	**B-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4098.50	12705.35	TRUE
67	44	2	Weak Positive	P	8	**B-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4218.80	13078.28	TRUE
68	45	3	Weak Positive	P	8	**B-1-P8**	3.00E-09	300	30	10	50	310	9.7E-11	100	4174.72	12941.632	TRUE
69	46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4169.54	12925.574	TRUE
70	47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4311.52	13365.712	TRUE
71	48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3826.90	11863.39	TRUE
72	49	1	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	220.70	662.1	TRUE
73	50	2	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	262.41	787.23	TRUE

	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23	*Position*	*Replicate*	***Notes to explain why "Use this value" is set to "FALSE"***
45	22	1	spilled approx. 0.4 mL of sample
46	23	2	spilled approx. 0.3 mL of sample
47	24	3	spilled approx. 0.6 mL of sample
48	25	1	spilled approx. 0.7 mL of sample
49	26	2	spilled approx. 0.6 mL of sample
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	

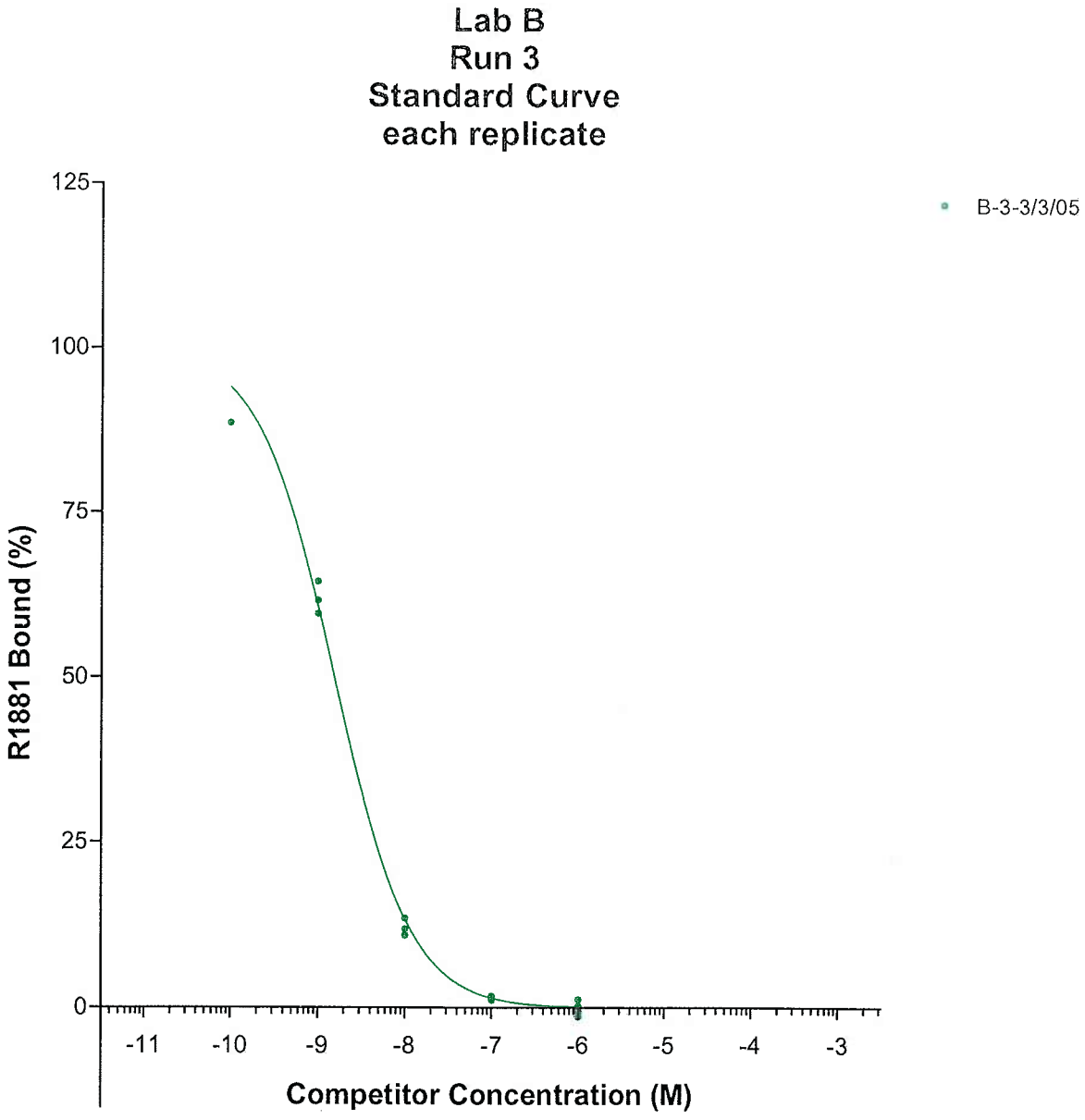
	A	B	S	T	U	V	W	X	Y	AA
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	*Position*	*Replicate*		*concentration (log)*	*percent bound*	*Usable DPM values*	*Specific Binding (Total - mean NSB)*	*Free DPM (mean total add - total bound)*	*Percent Binding (specific bound / mean specific EtOH)*	*Ratio Total binding/ Hot*
45	22	1	Weak Positive	-3.0						
46	23	2	Weak Positive	-3.0						
47	24	3	Weak Positive	-3.0						
48	25	1	Weak Positive	-4.0						
49	26	2	Weak Positive	-4.0						
50	27	3	Weak Positive	-4.0	29.6	4195.912	3568.9	7875.3	29.6	9.107539
51	28	1	Weak Positive	-5.0	78.8	10144.63	9517.6	1926.6	78.8	22.01966
52	29	2	Weak Positive	-5.0	76.6	9876.786	9249.8	2194.5	76.6	21.4383
53	30	3	Weak Positive	-5.0	78.8	10142.21	9515.2	1929.0	78.8	22.01442
54	31	1	Weak Positive	-6.0	99.2	12597.84	11970.8	-526.6	99.2	27.34455
55	32	2	Weak Positive	-6.0	101.3	12858.37	12231.3	-787.1	101.3	27.91004
56	33	3	Weak Positive	-6.0	105.6	13370.05	12743.0	-1298.8	105.6	29.02069
57	34	1	Weak Positive	-7.0	107.1	13557.51	12930.5	-1486.3	107.1	29.42758
58	35	2	Weak Positive	-7.0	97.3	12368.04	11741.0	-296.8	97.3	26.84575
59	36	3	Weak Positive	-7.0	102.0	12942.59	12315.6	-871.3	102.0	28.09286
60	37	1	Weak Positive	-8.0	98.7	12541.52	11914.5	-470.3	98.7	27.22229
61	38	2	Weak Positive	-8.0	96.9	12318.63	11691.6	-247.4	96.9	26.73849
62	39	3	Weak Positive	-8.0	104.1	13197.85	12570.8	-1126.6	104.1	28.64691
63	40	1	Weak Positive	-9.0	98.9	12569.94	11942.9	-498.7	98.9	27.28399
64	41	2	Weak Positive	-9.0	100.9	12801.02	12174.0	-729.8	100.9	27.78556
65	42	3	Weak Positive	-9.0	103.9	13167.44	12540.4	-1096.2	103.9	28.5809
66	43	1	Weak Positive	-10.0	100.1	12705.35	12078.3	-634.1	100.1	27.57791
67	44	2	Weak Positive	-10.0	103.1	13078.28	12451.3	-1007.0	103.1	28.38738
68	45	3	Weak Positive	-10.0	102.0	12941.63	12314.6	-870.4	102.0	28.09077
69	46	1		—	101.9	12925.57	12298.6	-854.3	101.9	28.05592
70	47	2		—	105.5	13365.71	12738.7	-1294.5	105.5	29.01127
71	48	3		—	93.1	11863.39	11236.4	207.9	93.1	25.75037
72	49	1		-6.0	0.3	662.1	35.1	11409.2	0.3	1.437137
73	50	2		-6.0	1.3	787.23	160.2	11284.0	1.3	1.708741

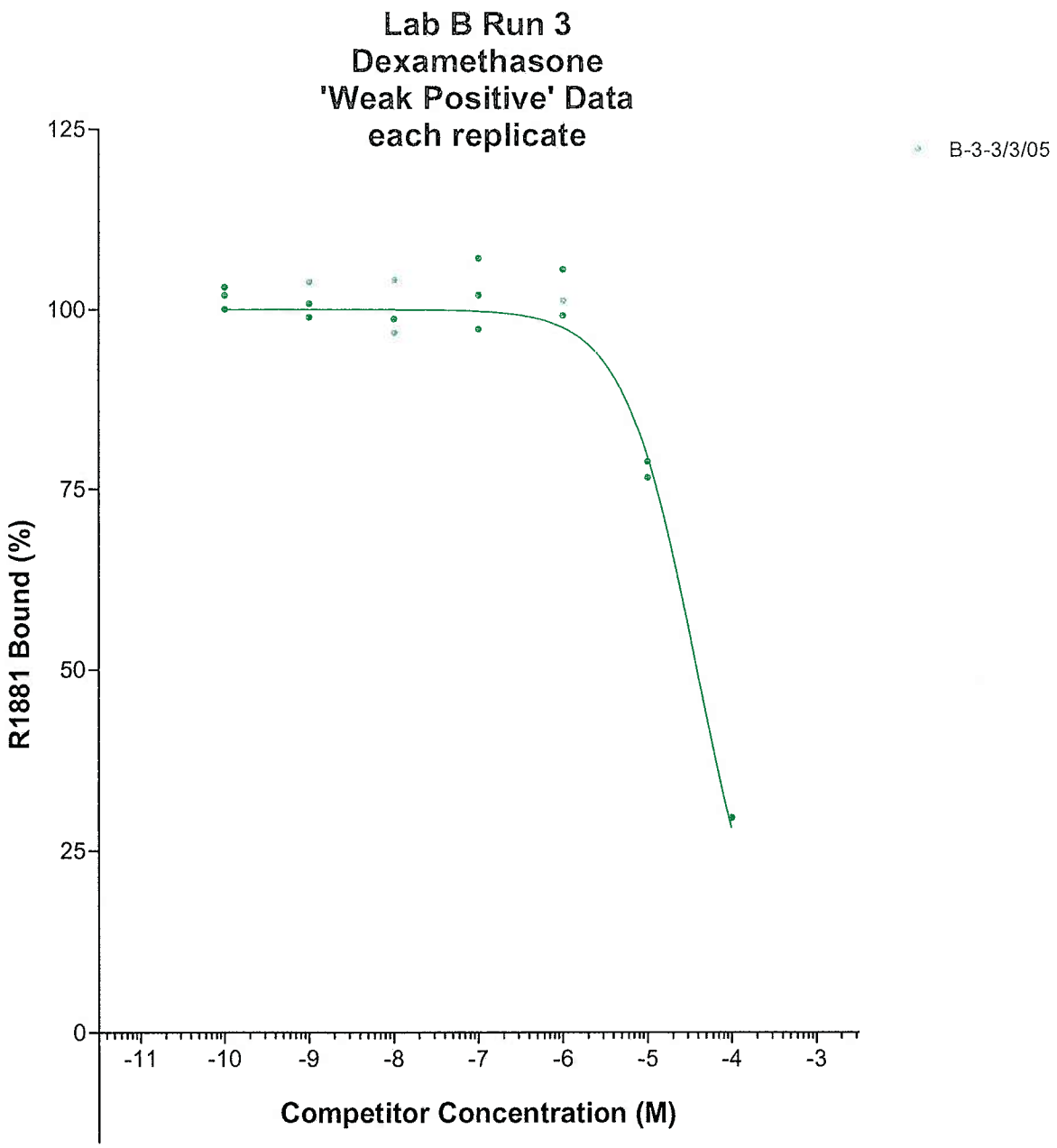
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19				Column O, Rows 10 through 13 will contain output parameters									**working volume**		**3.1E+02** uL		
20				from the nonlinear regression software.													
21				and the maximum concentration for the weak positive													
22				**Competitive Assay Tube Layout - One Test Chemical (Weak Positive)**												Check the 10% rule:	27 56%
23	*Position*	*Replicate*	*Competitor*	*Competitor Code*	*Concentration Code*	*Labels on vials in set 1-1-E supplied by Battelle to laboratory "B"*	*Competitor Initial Concentration (M)*	*Cytosol (uL)*	*Tracer (Hot R1881) Volume (uL)*	*Competitor Volume (uL)*	*triamcelenone Volume (uL)*	*Final Volume (ul)*	*Competitor Final Concentration (M)*	*Aliquot (uL)*	*DPM as sampled*	*corrected DPM for 2.0 mL*	*Use this value?*
74	51	3	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	221.05	663.15	TRUE
75	52	1	none	Hot		—	—	—	30	—	—	—	—		45965.26	45965.26	TRUE
76	53	2	none	Hot		—	—	—	30	—	—	—	—		47010.21	47010.21	TRUE
77	54	3	none	Hot		—	—	—	30	—	—	—	—		45851.15	45851.15	TRUE
78	55	1	none	Hot		—	—	—	30	—	—	—	—		46137.12	46137.12	TRUE
79	56	2	none	Hot		—	—	—	30	—	—	—	—		46722.82	46722.82	TRUE
80	57	3	none	Hot		—	—	—	30	—	—	—	—		44737.98	44737.98	TRUE



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

	A	B	S	T	U	V	W	X	Y	AA
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	***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***
74	51	3		-6.0	0.3	663.15	36.1	11408.1	0.3	1.439416
75	52	1				45965.26	45338.2			
76	53	2				47010.21	46383.2			
77	54	3				45851.15	45224.1			
78	55	1				46137.12	45510.1			
79	56	2				46722.82	46095.8			
80	57	3				44737.98	44111.0			


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


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

Competitive Assay of a known Weak Positive

57 Assay Tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

the TRUE in column Q will automatically change to FALSE

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

Provide information in all blue cells in this column

Laboratory Code:	B	
Run identification:	3	
Assay start date:	3/3/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.91	**Ci/mmole**
Cytosol vial or lot identification:	73, 74, 75	
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.55E-09	
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	3.88E-06	
RBA:	4.00E-05	

APPENDIX G

Processed Raw Data from Competitive Assay 4 and Associated Figures

Counts from Competitive Assay Number 4

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

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory "S"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3918.82	12148.342
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3769.07	11684.117
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3954.49	12258.919
4	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	164.28	492.84
5	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	193.72	581.16
6	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	227.09	681.27
7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	279.33	865.923
8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	264.76	820.756
9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	281.34	872.154
10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	541.43	1678.433
11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	676.98	2098.638
12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	670.18	2077.558
13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2406.30	7459.53
14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2572.14	7973.634
15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2525.24	7828.244
16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3668.91	11373.621
17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3897.57	12082.467
18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3689.13	11436.303
19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3972.14	12313.634
20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3814.27	11824.237
21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3948.43	12240.133
22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	305.05	945.655
23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	342.53	1061.843
24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	313.78	972.718
25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1255.85	3893.135
26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1251.17	3878.627
27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1188.20	3683.42
28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3230.12	10013.372
29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3205.89	9938.259
30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3047.01	9445.731
31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3921.49	12156.619
32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3938.88	12210.528
33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3822.55	11849.905
34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4086.52	12668.212
35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4266.62	13226.522
36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4078.06	12641.986
37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4154.75	12879.725
38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4064.34	12599.454
39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4001.71	12405.301
40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4045.60	12541.36
41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4144.98	12849.438
42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3976.36	12326.716
43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3802.68	11788.308
44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3942.58	12221.998
45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3968.82	12303.342
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3974.91	12322.221
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4343.18	13463.858
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3972.13	12313.603
49	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	248.44	745.32
50	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	178.57	535.71
51	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	223.27	669.81
52	1	none	Hot		—	—	—	30	—	—	—	—		45865.99	45865.99
53	2	none	Hot		—	—	—	30	—	—	—	—		46237.89	46237.89
54	3	none	Hot		—	—	—	30	—	—	—	—		46509.36	46509.36
55	1	none	Hot		—	—	—	30	—	—	—	—		45915.11	45915.11
56	2	none	Hot		—	—	—	30	—	—	—	—		46026.73	46026.73
57	3	none	Hot		—	—	—	30	—	—	—	—		45813.79	45813.79

	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**											*Provide information in all blue cells in this column*		
2				**57 Assay Tubes**										**Laboratory Code:**	**B**		
3				Please return by eMail to n.a.Holter@pnl.gov										**Run identification:**	4		
4														**Assay start date:**	3/14/2005		
5														**Tracer lot number:**	3538-497		
6				Provide information in all blue cells in column O										**Specific activity on day of assay:**	79.78	**Ci/mmole**	
7				If the DPM value for a tube was judged unreliable,										**Cytosol vial or lot identification:**	76, 77, 78		
8				Include the DPM value in column O										**Protein (cytosol):**	1000	**micro gram per tub**	
9				Provide a reason in column R													
10				the TRUE in column Q will automatically change to FALSE										**Standard Curve IC50:**	1.50E-09		
11														**Weak Positive, Max Concentration:**	**3.00E-02**	**M**	
12														**Weak Positive IC50:**	3.68E-05		
13				Columns T and U contain values to be analyzed										**RBA:**	4.07E-05		
14				by nonlinear regression software													
15																	
16																	
17														**volume of ethanol counted:**	2	**mL**	
18														**mulitply DPM in sample by :**	3		

	A	B	R
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			
11			
12			
13			
14			
15			
16			
17			protcol calls for counting decanted EtOH su
18			reflects 100ul of reaction mixture processed

	S	T	U	V	W	X	Y	AA
1								
2		**Summary values**						
3		**n**	**Mean**	**SD**				
4	EtOH	6	12365.2	588.86	Total Binding, solvent control, tubes			
5	Hot	6	46061.5	266.05	Total hot R1881 added to each tube			
6	NSB	6	617.7	96.63	Nonspecific Binding			
7	Specific EtOH	6	11747.5	588.86				
8								
9								
10								
11		**Assay Characterization Values**						
12	EtOH / Hot	0.27	less than 0.1?					
13	NSB / EtOH	0.05	around 0.25 ?					
14								
15								
16								
17	pernate							
18								

Column O, Rows 10 through 13 will contain output parameters **working volume** **3.1E+02** uL

from the nonlinear regression software.

and the maximum concentration for the weak positive

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

Check the 10% rule: 26.84%

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory "B"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?
1	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3918.82	12148.342	TRUE
2	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3769.07	11684.117	TRUE
3	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3954.49	12258.919	TRUE
4	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	164.28	492.84	TRUE
5	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	193.72	581.16	TRUE
6	3	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	227.09	681.27	TRUE
7	1	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	279.33	865.923	TRUE
8	2	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	264.76	820.756	TRUE
9	3	Inert R1881	S	1	B-1-S1	3.00E-06	300	30	10	50	310	9.7E-08	100	281.34	872.154	TRUE
10	1	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	541.43	1678.433	TRUE
11	2	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	676.98	2098.638	TRUE
12	3	Inert R1881	S	2	B-1-S2	3.00E-07	300	30	10	50	310	9.7E-09	100	670.18	2077.558	TRUE
13	1	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2406.30	7459.53	TRUE
14	2	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2572.14	7973.634	TRUE
15	3	Inert R1881	S	3	B-1-S3	3.00E-08	300	30	10	50	310	9.7E-10	100	2525.24	7828.244	TRUE
16	1	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3668.91	11373.621	TRUE
17	2	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3897.57	12082.467	TRUE
18	3	Inert R1881	S	4	B-1-S4	3.00E-09	300	30	10	50	310	9.7E-11	100	3689.13	11436.303	TRUE
19	1	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3972.14	12313.634	TRUE
20	2	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3814.27	11824.237	TRUE
21	3	Inert R1881	S	5	B-1-S5	3.00E-10	300	30	10	50	310	9.7E-12	100	3948.43	12240.133	TRUE

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

	A	B	S	T	U	V	W	X	Y	AA
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	*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*
24	1	1			98.2	12148.34	11530.7	-400.9	98.2	26.37419
25	2	2			94.2	11684.12	11066.4	63.4	94.2	25.36635
26	3	3			99.1	12258.92	11641.2	-511.4	99.1	26.61425
27	4	1		-6.0	-1.1	492.84	-124.8	11254.7	-1.1	1.069961
28	5	2		-6.0	-0.3	581.16	-36.5	11166.3	-0.3	1.261705
29	6	3		-6.0	0.0	681.27	63.6	11066.2	0.5	1.479045
30	7	1	cold R1881	-7.0	2.1	865.923	248.2	10881.6	2.1	1.879929
31	8	2	cold R1881	-7.0	1.7	820.756	203.1	10926.7	1.7	1.781871
32	9	3	cold R1881	-7.0	2.2	872.154	254.5	10875.3	2.2	1.893456
33	10	1	cold R1881	-8.0	9.0	1678.433	1060.7	10069.1	9.0	3.643897
34	11	2	cold R1881	-8.0	12.6	2098.638	1481.0	9648.9	12.6	4.556167
35	12	3	cold R1881	-8.0	12.4	2077.558	1459.9	9669.9	12.4	4.510402
36	13	1	cold R1881	-9.0	58.2	7459.53	6841.8	4288.0	58.2	16.19473
37	14	2	cold R1881	-9.0	62.6	7973.634	7355.9	3773.9	62.6	17.31085
38	15	3	cold R1881	-9.0	61.4	7828.244	7210.6	3919.2	61.4	16.99521
39	16	1	cold R1881	-10.0	91.6	11373.62	10755.9	373.9	91.6	24.69226
40	17	2	cold R1881	-10.0	97.6	12082.47	11464.8	-335.0	97.6	26.23118
41	18	3	cold R1881	-10.0	92.1	11436.3	10818.6	311.2	92.1	24.82835
42	19	1	cold R1881	-11.0	99.6	12313.63	11695.9	-566.1	99.6	26.73304
43	20	2	cold R1881	-11.0	95.4	11824.24	11206.6	-76.7	95.4	25.67055
44	21	3	cold R1881	-11.0	98.9	12240.13	11622.4	-492.6	98.9	26.57347

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

working volume **3.1E+02** uL

and the maximum concentration for the weak positive

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

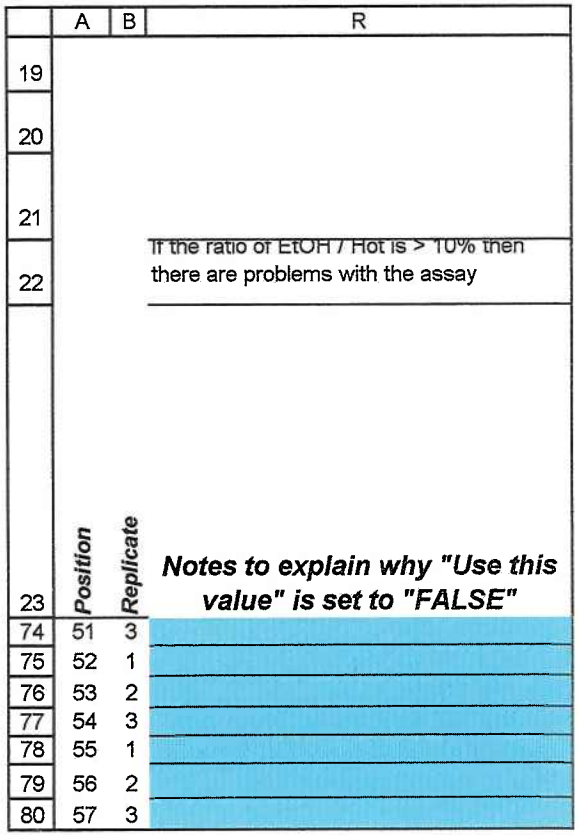
Check the 10% rule: 26.84%

Position	Replicate	Competitor	Competitor Code	Concentration Code	Labels on vials in set 1-1-E supplied by Battelle to laboratory "g"	Competitor Initial Concentration (M)	Cytosol (uL)	Tracer (Hot R1881) Volume (uL)	Competitor Volume (uL)	triamcelenone Volume (uL)	Final Volume (ul)	Competitor Final Concentration (M)	Aliquot (uL)	DPM as sampled	corrected DPM for 2.0 mL	Use this value?
22	1	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	305.05	945.655	TRUE
23	2	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	342.53	1061.843	TRUE
24	3	Weak Positive	P	1	B-1-P1	3.00E-02	300	30	10	50	310	9.7E-04	100	313.78	972.718	TRUE
25	1	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1255.85	3893.135	TRUE
26	2	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1251.17	3878.627	TRUE
27	3	Weak Positive	P	2	B-1-P2	3.00E-03	300	30	10	50	310	9.7E-05	100	1188.20	3683.42	TRUE
28	1	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3230.12	10013.372	TRUE
29	2	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3205.89	9938.259	TRUE
30	3	Weak Positive	P	3	B-1-P3	3.00E-04	300	30	10	50	310	9.7E-06	100	3047.01	9445.731	TRUE
31	1	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3921.49	12156.619	TRUE
32	2	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3938.88	12210.528	TRUE
33	3	Weak Positive	P	4	B-1-P4	3.00E-05	300	30	10	50	310	9.7E-07	100	3822.55	11849.905	TRUE
34	1	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4086.52	12668.212	TRUE
35	2	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4266.62	13226.522	TRUE
36	3	Weak Positive	P	5	B-1-P5	3.00E-06	300	30	10	50	310	9.7E-08	100	4078.06	12641.986	TRUE
37	1	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4154.75	12879.725	TRUE
38	2	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4064.34	12599.454	TRUE
39	3	Weak Positive	P	6	B-1-P6	3.00E-07	300	30	10	50	310	9.7E-09	100	4001.71	12405.301	TRUE
40	1	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4045.60	12541.36	TRUE
41	2	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	4144.98	12849.438	TRUE
42	3	Weak Positive	P	7	B-1-P7	3.00E-08	300	30	10	50	310	9.7E-10	100	3976.36	12326.716	TRUE
43	1	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3802.68	11788.308	TRUE
44	2	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3942.58	12221.998	TRUE
45	3	Weak Positive	P	8	B-1-P8	3.00E-09	300	30	10	50	310	9.7E-11	100	3968.82	12303.342	TRUE
46	1	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3974.91	12322.221	TRUE
47	2	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	4343.18	13463.858	TRUE
48	3	ethanol	EtOH	0	—	—	300	30	10	50	310	—	100	3972.13	12313.603	TRUE
49	1	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	248.44	745.32	TRUE
50	2	Inert R1881	NSB		B-1-S0	1.00E-05	300	30	30	50	300	1.0E-06	100	178.57	535.71	TRUE

	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23	***Position***	***Replicate***	***Notes to explain why "Use this value" is set to "FALSE"***
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
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	*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*
45	22	1	Weak Positive	-3.0	2.8	945.655	328.0	10801.8	2.8	2.053028
46	23	2	Weak Positive	-3.0	3.8	1061.843	444.2	10685.6	3.8	2.305273
47	24	3	Weak Positive	-3.0	3.0	972.718	355.0	10774.8	3.0	2.111782
48	25	1	Weak Positive	-4.0	27.9	3893.135	3275.5	7854.4	27.9	8.452041
49	26	2	Weak Positive	-4.0	27.8	3878.627	3260.9	7868.9	27.8	8.420544
50	27	3	Weak Positive	-4.0	26.1	3683.42	3065.7	8064.1	26.1	7.996747
51	28	1	Weak Positive	-5.0	80.0	10013.37	9395.7	1734.1	80.0	21.73915
52	29	2	Weak Positive	-5.0	79.3	9938.259	9320.6	1809.2	79.3	21.57607
53	30	3	Weak Positive	-5.0	75.1	9445.731	8828.0	2301.8	75.1	20.50679
54	31	1	Weak Positive	-6.0	98.2	12156.62	11538.9	-409.1	98.2	26.39216
55	32	2	Weak Positive	-6.0	98.7	12210.53	11592.8	-463.0	98.7	26.5092
56	33	3	Weak Positive	-6.0	95.6	11849.91	11232.2	-102.4	95.6	25.72628
57	34	1	Weak Positive	-7.0	102.6	12668.21	12050.5	-920.7	102.6	27.50283
58	35	2	Weak Positive	-7.0	107.3	13226.52	12608.8	-1479.0	107.3	28.71493
59	36	3	Weak Positive	-7.0	102.4	12641.99	12024.3	-894.5	102.4	27.4459
60	37	1	Weak Positive	-8.0	104.4	12879.73	12262.0	-1132.2	104.4	27.96203
61	38	2	Weak Positive	-8.0	102.0	12599.45	11981.8	-852.0	102.0	27.35356
62	39	3	Weak Positive	-8.0	100.3	12405.3	11787.6	-657.8	100.3	26.93205
63	40	1	Weak Positive	-9.0	101.5	12541.36	11923.7	-793.9	101.5	27.22744
64	41	2	Weak Positive	-9.0	104.1	12849.44	12231.8	-1101.9	104.1	27.89628
65	42	3	Weak Positive	-9.0	99.7	12326.72	11709.0	-579.2	99.7	26.76144
66	43	1	Weak Positive	-10.0	95.1	11788.31	11170.6	-40.8	95.1	25.59255
67	44	2	Weak Positive	-10.0	98.8	12222	11604.3	-474.5	98.8	26.5341
68	45	3	Weak Positive	-10.0	99.5	12303.34	11685.7	-555.9	99.5	26.7107
69	46	1		—	99.6	12322.22	11704.5	-574.7	99.6	26.75168
70	47	2		—	109.4	13463.86	12846.2	-1716.4	109.4	29.23019
71	48	3		—	99.6	12313.6	11695.9	-566.1	99.6	26.73297
72	49	1		-6.0	1.1	745.32	127.6	11002.2	1.1	1.618098
73	50	2		-6.0	-0.7	535.71	-82.0	11211.8	-0.7	1.163033

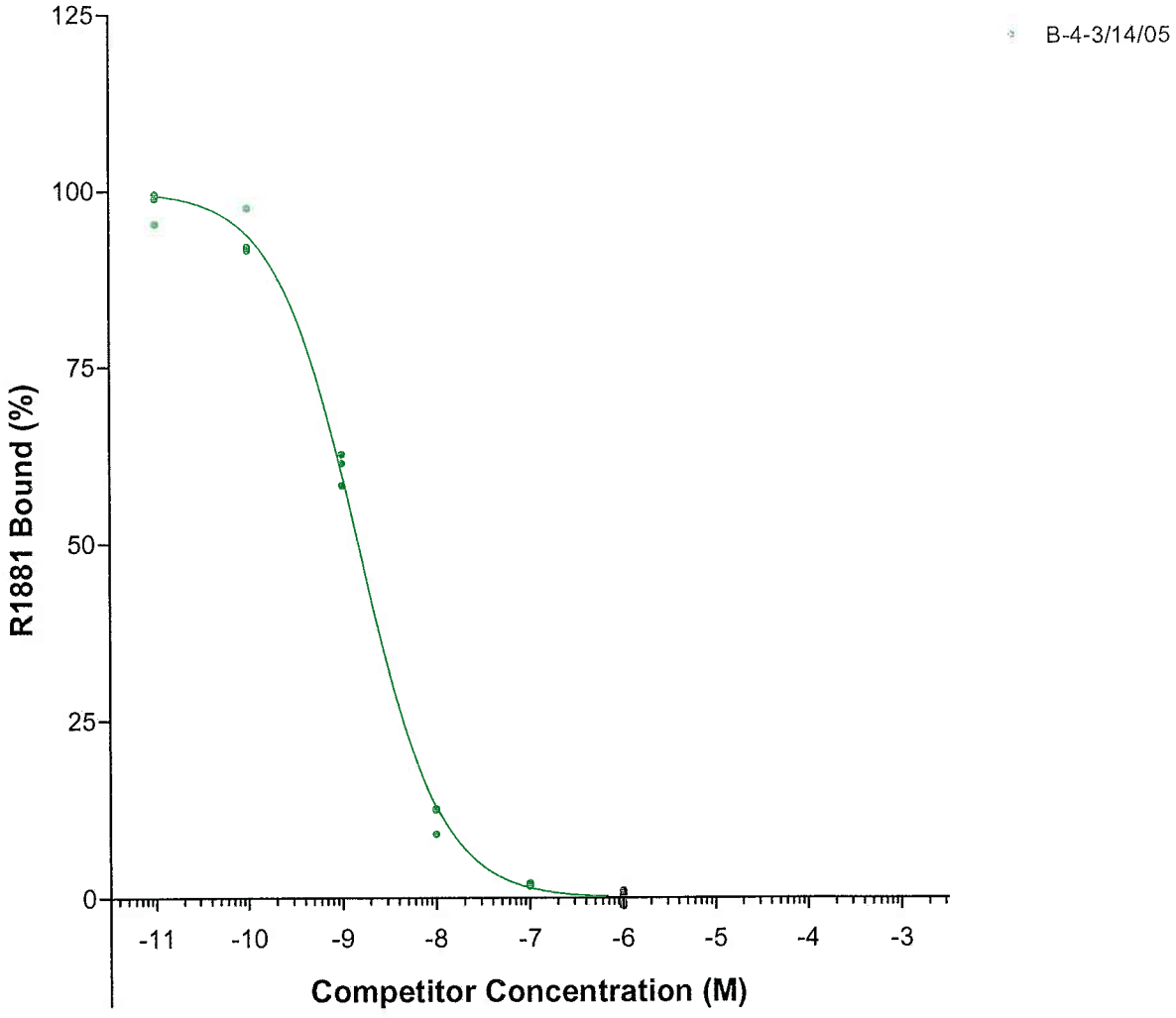
	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q
19				Column O, Rows 10 through 13 will contain output parameters									**working volume**		**3.1E+02** uL		
20				from the nonlinear regression software.													
21				and the maximum concentration for the weak positive													
22				**Competitive Assay Tube Layout - One Test Chemical (Weak Positive)**												Check the 10% rule:	26.84%
23	***Position***	***Replicate***	***Competitor***	***Competitor Code***	***Concentration Code***	***Labels on vials in set 1-1-E supplied by Battelle to laboratory "B"***	***Competitor Initial Concentration (M)***	***Cytosol (uL)***	***Tracer (Hot R1881) Volume (uL)***	***Competitor Volume (uL)***	***triamcelenone Volume (uL)***	***Final Volume (ul)***	***Competitor Final Concentration (M)***	***Aliquot (uL)***	***DPM as sampled***	***corrected DPM for 2.0 mL***	***Use this value?***
74	51	3	Inert R1881	NSB		**B-1-S0**	1.00E-05	300	30	30	50	300	1.0E-06	100	223.27	669.81	TRUE
75	52	1	none	Hot		—	—	—	30	—	—	—	—		45865.99	45865.99	TRUE
76	53	2	none	Hot		—	—	—	30	—	—	—	—		46237.89	46237.89	TRUE
77	54	3	none	Hot		—	—	—	30	—	—	—	—		46509.36	46509.36	TRUE
78	55	1	none	Hot		—	—	—	30	—	—	—	—		45915.11	45915.11	TRUE
79	56	2	none	Hot		—	—	—	30	—	—	—	—		46026.73	46026.73	TRUE
80	57	3	none	Hot		—	—	—	30	—	—	—	—		45813.79	45813.79	TRUE

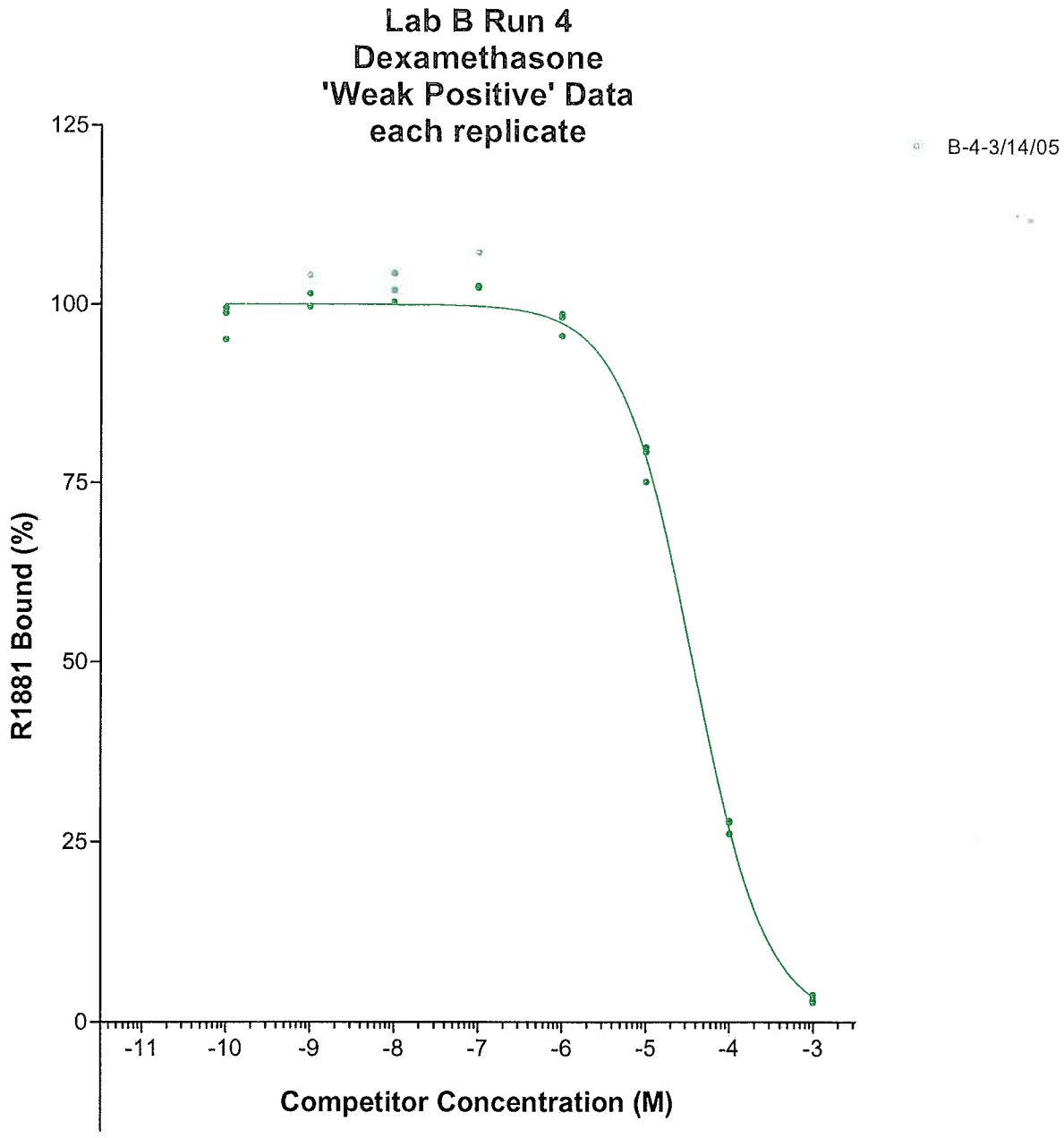


	A	B	R
19			
20			
21			
22			If the ratio of EtOH / Hot is > 10% then there are problems with the assay
23	*Position*	*Replicate*	***Notes to explain why "Use this value" is set to "FALSE"***
74	51	3	
75	52	1	
76	53	2	
77	54	3	
78	55	1	
79	56	2	
80	57	3	

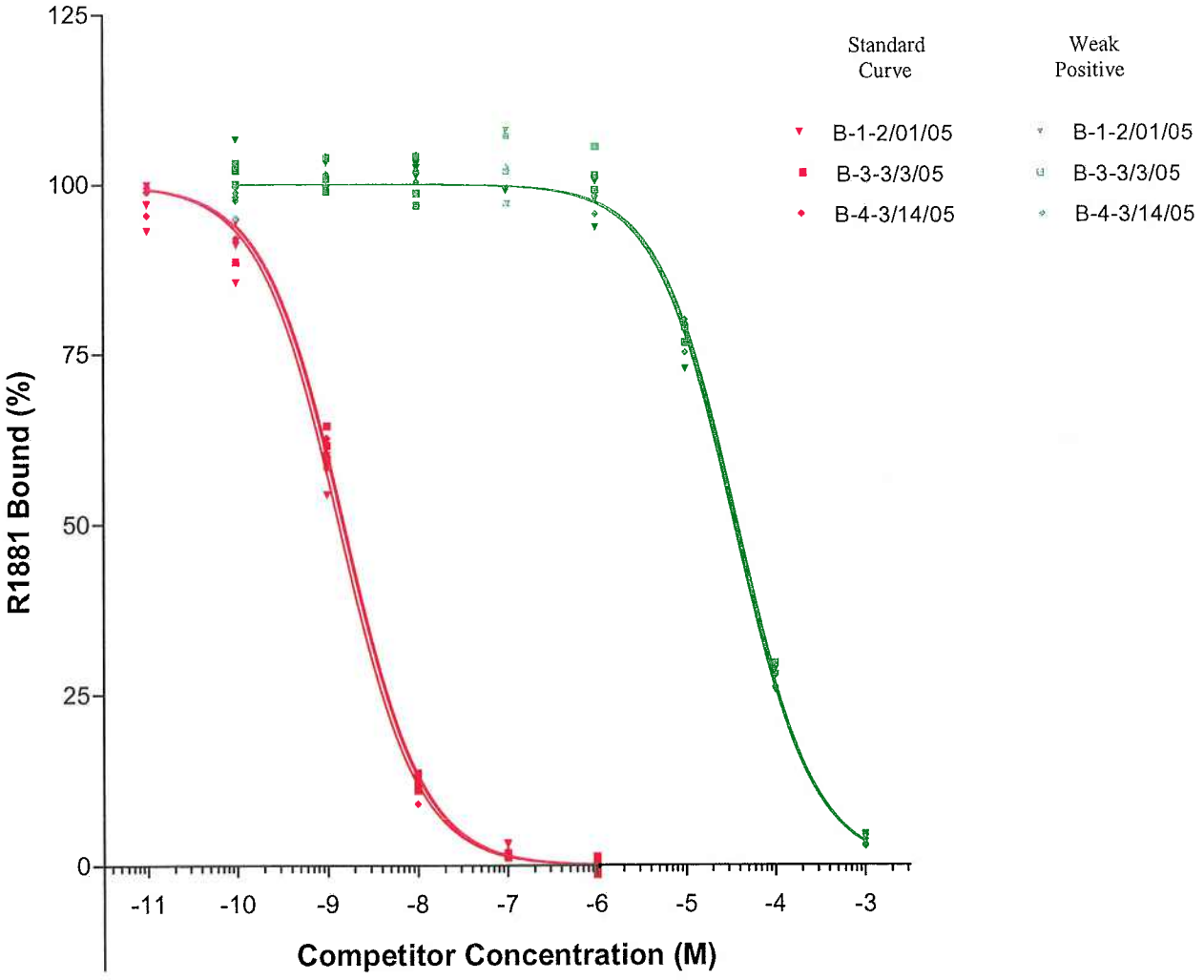
	A	B	S	T	U	V	W	X	Y	AA
19										
20				*Values for analysis by nonlinear regression*						
21				Standard Curve						
22				Weak Positive						
23	***Position***	***Replicate***		***concentration (log)***	***percent bound***	***Usable DPM values***	***Specific Binding (Total - mean NSB)***	***Free DPM (mean total add - total bound)***	***Percent Binding (specific bound / mean specific EtOH)***	***Ratio Total binding/ Hot***
74	51	3		-6.0	0.4	669.81	52.1	11077.7	0.4	1.454165
75	52	1				45865.99	45248.3			
76	53	2				46237.89	45620.2			
77	54	3				46509.36	45891.7			
78	55	1				45915.11	45297.4			
79	56	2				46026.73	45409.0			
80	57	3				45813.79	45196.1			

Lab B
Run 4
Standard Curve
each replicate




Lab B Run 4
Dexamethasone
'Weak Positive' Data
each replicate
B-4-3/14/05
125
100
75
50
25
0
R1881 Bound (%)
-11
-10
-9
-8
-7
-6
-5
-4
-3
Competitor Concentration (M)

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



Competitive Assay of a known Weak Positive

57 Assay Tubes

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

Provide information in all blue cells in column O

If the DPM value for a tube was judged unreliable,

Include the DPM value in column O

Provide a reason in column R

the TRUE in column Q will automatically change to FALSE

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

Provide information in all blue cells in this column

Laboratory Code:	**B**	
Run identification:	4	
Assay start date:	3/14/2005	
Tracer lot number:	3538-497	
Specific activity on day of assay:	79.78	**Ci/mmole**
Cytosol vial or lot identification:	76-77-78	
Protein (cytosol):	1000	**micro gram per tube**
Standard Curve IC50:	1.50E-09	
Weak Positive, Max Concentration:	**3.00E-02**	**M**
Weak Positive IC50:	3.68E-05	
RBA:	4.07E-05	