



## **STUDY REPORT**

### **Example Final Report for Nuclear Receptor Screening Studies**

# **Company X**

## **STUDY SPONSOR**

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### **AUTHORS**

Study Manager  
INDIGO Biosciences

Study Director  
INDIGO Biosciences

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## **DATA FILES ACCOMPANYING THIS STUDY REPORT**

- 1.) CompanyX\_Data Compilation.xls (Microsoft Excel file)
- 2.) Company X\_Test & Ref Cmpd Data.pzf (GraphPad Prism file)

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## **INDIGO PROJECT IDENTIFICATION**

INDIGO Contract #xxxxxxx

1981 Pine Hall Road, State College, PA, 16801, USA  
[www.indigobiosciences.com](http://www.indigobiosciences.com)

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( End of Report )

## Statement of Quality Assurance

This final report accurately provides the primary data generated during this study, and includes INDIGO Biosciences' basic interpretation of the of the results.

Approved:

Study Manager \_\_\_\_\_

Study Director \_\_\_\_\_

### Summary of Receptor Assays Performed (*Example 1*)

**Table 1.** Reporter Cells used in these assays express a receptor hybrid in which the native N-terminal DNA binding domain (DBD) has been replaced with that of the yeast Gal4 DBD. The reporter gene, firefly luciferase, is functionally linked to the Gal4 upstream activation sequence (UAS). The cell line background of respective Reporter Cells is indicated, as are the reference compounds (when available) used to confirm the performance of the respective assays in this study; *na* = not available.

<b>Receptor</b> (gene symbol)	<b>Receptor form</b> ... <b>Reporter Vector</b>	<b>Host Cell Line</b>	<b>INDIGO Reference Agonist</b>	<b>INDIGO Reference Antagonist</b>
<b>Nuclear Receptor Assays</b>				
<b>LXR<math>\alpha</math></b> (NR1H3)	Gal4 DBD hybrid Receptor ... Gal4 UAS-Luciferase	CHO	T0901317	GSK 2033
<b>PPAR<math>\gamma</math></b> (NR1C3)	Gal4 DBD hybrid Receptor ... Gal4 UAS-Luciferase	CHO	Rosiglitazone	T0070907
<b>PPAR<math>\alpha</math></b> (NR1C1)	Gal4 DBD hybrid Receptor ... Gal4 UAS-Luciferase	CHO	GW590735	<i>na</i>

## SUMMARY of the Study Findings

(*Example 2: Exact format will vary depending on study data*)

**Table1. SUMMARY of the Study Findings**

		IB58			LM32			Vehicle
		10 nM	100 nM	1,000 nM	10 nM	100 nM	1,000 nM	0.10% DMSO
<b>PPAR-</b>	Agonist, fold-increase	2.0	8.0	11.8	0.67	0.66	0.60	1.0
	Antagonist, fold-reduction	1.2	1.2	1.4	1.1	1.2	1.2	
<b>ROR-</b>	Inverse-Agonist fold-reduction	1.0	1.0	1.1	1.4	2.3	3.9	1.0

## INDIGO's Assessment of the Study Results

Test Compound IB58: pronounced agonist activity is evident in the PPAR delta assay; no significant antagonist activity is revealed. IB58 shows no activity in the ROR gamma assay.

Test Compound LM32: No activity, either agonist or antagonist, is evident in the PPAR delta assay. LM32 shows a significant, dose-dependent inverse agonist (or antagonist) activity to ROR gamma.

## Study Design

Each Nuclear Receptor *agonist* assay is validated by performing a Reference Agonist dose-response assay. In addition, *antagonist* assay setups are validated by performing a Reference Antagonist dose-response assay *when a validated antagonist is available* (see Assay Validation, below).

## Assay Validation

Reference compounds are utilized to confirm the performance of the specific lot of Nuclear Receptor Reporter Cells treated with the Sponsor's test compounds. Reference Compound and Test Compound assays are performed at the same time and, hence, are exposed to the same assay reagents and environmental conditions. For each Nuclear Receptor Assay a validated reference *agonist* dose-response analysis is performed. For those Nuclear Receptor Assays in which test compounds are analyzed for *antagonist* activities, dose-response analysis is also performed *if* a commercially available validated antagonist is available. NOTE: validated reference antagonists are not commercially available for all nuclear receptors. Reference groups always include a 'Vehicle' control group (DMSO) to determine background activity in the assay and to calculate fold-activation and percent inhibition.

## Assay Methods

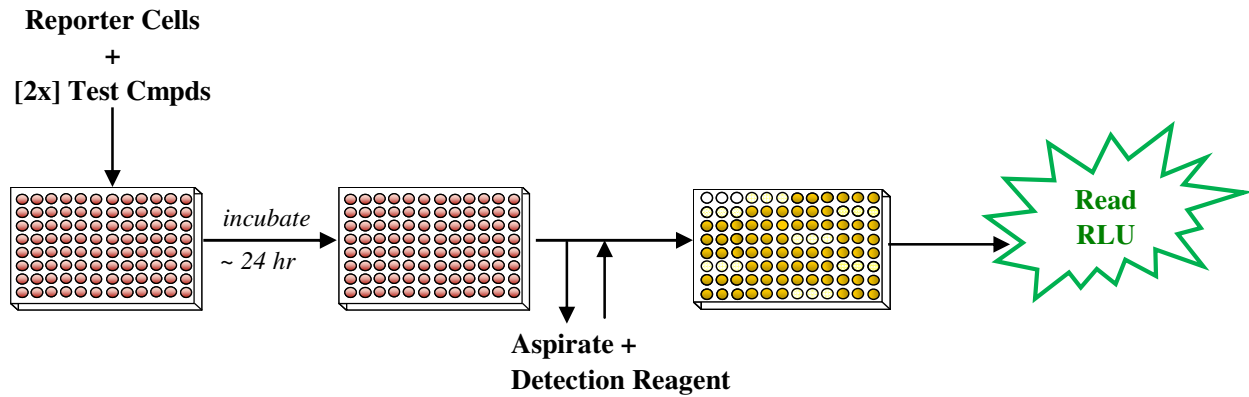
- *Reporter Cells.* This study will utilize proprietary nuclear receptor expression vectors encoding either the full-length nuclear receptor sequence or a hybrid receptor comprising the N-terminal Gal4 DNA binding domain fused to the ligand binding domain of the specific human nuclear receptor. The reporter vectors to be used in these studies comprise the firefly luciferase gene functionally linked to either an upstream NR response element (NRE) or the Gal4 activation sequence (UAS).
- *Compound Handling.* If required, master stocks of Test Compounds will be further diluted in DMSO to gain 1,000x-concentrations.
- *Setup of NR Assays.* The NR Assays are performed as depicted in **Figure xx** (agonist or inverse-agonist) or **Figure yy** (antagonist mode assay including optional Live Cell Multiplex Assay). *In brief,*

*Step 1:* A suspension of Reporter Cells is prepared in Cell Recovery Medium (CRM; containing 10% charcoal-stripped FBS). For antagonist assays, cells are co-mixed with 2x-concentration of EC80 of the respective challenge agonist, then 100 µl of treated cell suspension is dispensed into the assay plates.

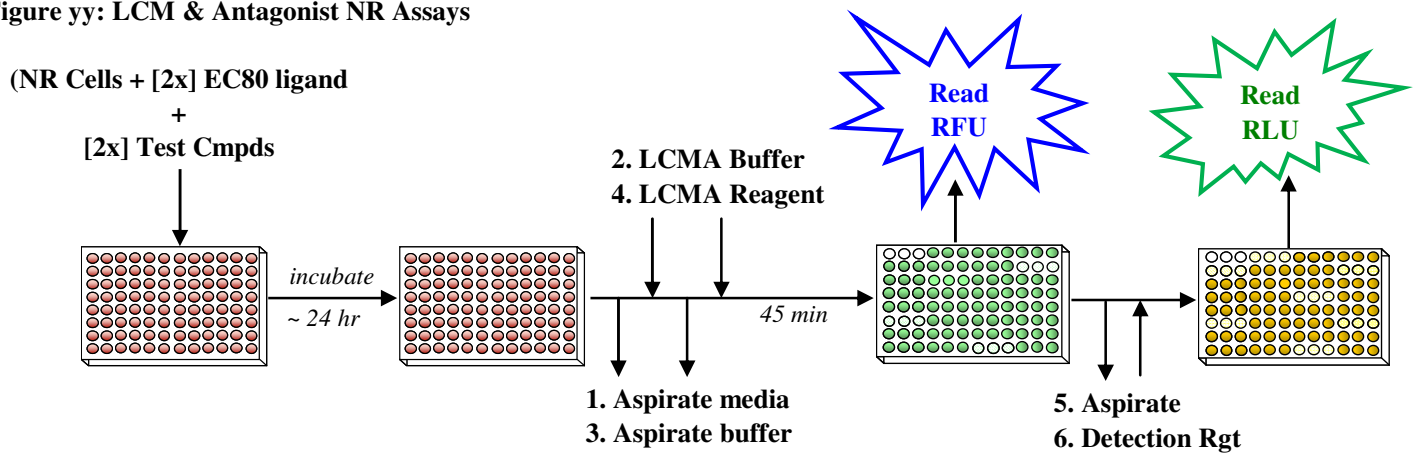
*Step 2:* Immediately prior to assay setup, test compounds are diluted using compound screening medium (CSM; containing charcoal stripped FBS) to generate '2x-concentration' treatment media. 100 µl of each treatment medium is dispensed into triplicate assay wells pre-dispensed with Reporter Cells. Assay plates are incubated at 37°C for 24 hr.

*Step 3:* Following the 24 hr incubation period, treatment media are discarded. For inverse-agonist and antagonist assays, wells are rinsed once with LCM Buffer, then LCM substrate is added. Following incubation at 37°C for 45 min, fluorescence is measured to determine relative numbers of live cells per assay well. LCM substrate is then discarded and 100 µl/well of Luciferase Detection Reagent is added. RLU's are quantified from each assay well to determine NR activity.

**Figure xx: Agonist Assays**



**Figure yy: LCM & Antagonist NR Assays**



## Data Reduction

Microsoft Excel is used to manage and archive assay data, as well as to calculate averaged RLU values +/- Standard Deviation (SD), Fold-Activation (*aka*, Signal-to-Background; S/B), Percent Coefficients of Variation (%CV), Fold-Inhibition, Percent-Inhibition, and Z' values. GraphPad Prizm software will be used to generate appropriate graphical representations of data.

## Retention of Client's Test Samples

Test Samples are the sole possession of the Client. Unless otherwise requested, INDIGO Biosciences will retain test samples in -20°C storage for a period of 3 months, after which time they will be disposed of. Clients may request that, upon completion of the study, unused portions of their test compounds are disposed of immediately, or returned at the clients expense.

## Retention of Client's Records

Unless otherwise requested, INDIGO Biosciences will permanently archive electronic versions of all quotes, reports to, and communications with, the Study Sponsor. All client information & study data is confidential and will at no time be released to a third party without prior written consent from the client.

## Graphical & Tabular Data Presentation

Unless otherwise requested, agonist data will be calculated as "**Fold-Activation**" and graphically presented in **dot-plot** form (5 or fewer treatment doses per test compd) or plotted *via non-linear regression* (6 or more treatment doses per test compd).

Antagonist data will be calculated as "Percent-Inhibition" and "Fold-Inhibition" and, unless otherwise directed, will be graphically presented as **% Inhibition** in **dot-plot or non-linear regression** form, depending on the number of doses tested per test compound (see above).

All agonist and antagonist *reference compound* dose-response data will be plotted using variable slope, robust fit, non-linear regression.

## Human PPAR delta (NR1C2) Assays

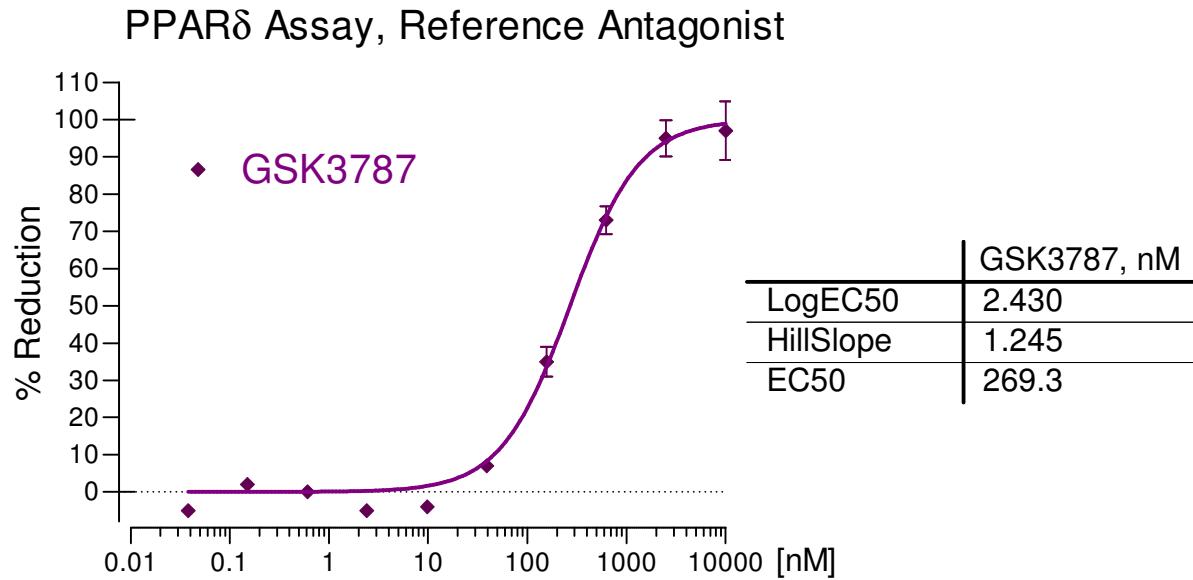
(Example 3: The following is Sample Data that will be provided in an accompanying “Data Compilation” file, Excel format)

Compound	Conc. (nM)	Human PPAR $\delta$ Antagonist Assays							
		Luc1	Luc2	Luc3	AVG	SD	% CV	Fold-Reduction	% Reduction
<b>DMSO</b>	0.10%	520,362	463,311	457,291	<b>480,321</b>	34,806	7.2	<b>1.0</b>	<b>0.0</b>
<b>TC1</b>	555	435,547	514,445	515,760	<b>488,584</b>	45,936	9.4	0.98	-1.7
	1667	491,486	515,977	505,481	<b>504,315</b>	12,287	2.4	0.95	-5.0
	5000	475,382	490,895	493,488	<b>486,588</b>	9,791	2.0	0.99	-1.3
<b>TC2</b>	555	478,811	442,159	437,255	<b>452,741</b>	22,709	5.0	1.06	<b>5.7</b>
	1667	384,533	372,010	388,022	<b>381,522</b>	8,420	2.2	1.26	<b>21</b>
	5000	316,854	290,853	324,339	<b>310,682</b>	17,575	5.7	1.55	<b>35</b>
<b>TC3</b>	555	478,811	442,159	437,255	<b>452,741</b>	22,709	5.0	1.06	<b>5.7</b>
	1667	222,918	215,658	224,940	<b>221,172</b>	4,881	2.2	2.17	<b>54</b>
	5000	176,336	161,866	180,502	<b>172,901</b>	9,781	5.7	2.78	<b>64</b>
<b>TC4</b>	555	494,759	467,800	411,150	<b>457,903</b>	42,674	9.3	1.05	<b>4.7</b>
	1667	450,967	503,161	504,826	<b>486,318</b>	30,626	6.3	0.99	-1.2
	5000	503,531	486,548	466,122	<b>485,400</b>	18,731	3.9	0.99	-1.1
<b>TC5</b>	555	524,593	429,826	451,315	<b>468,578</b>	49,686	10.6	1.03	<b>2.4</b>
	1667	521,317	552,656	481,707	<b>518,560</b>	35,555	6.9	0.93	-8.0
	5000	494,880	556,004	568,122	<b>539,669</b>	39,259	7.3	0.89	-12
<b>TC6</b>	555	460,794	485,254	414,629	<b>453,559</b>	35,864	7.9	1.06	<b>5.6</b>
	1667	483,682	475,865	475,833	<b>478,460</b>	4,522	0.9	1.00	<b>0.4</b>
	5000	387,606	403,508	440,185	<b>410,433</b>	26,965	6.6	1.17	<b>15</b>
<b>TC7</b>	555	494,759	467,800	411,150	<b>457,903</b>	42,674	9.3	1.05	<b>4.7</b>
	1667	432,177	482,196	483,792	<b>466,055</b>	29,350	6.3	1.03	<b>3.0</b>
	5000	482,551	466,275	446,701	<b>465,175</b>	17,950	3.9	1.03	<b>3.2</b>
<b>TC8</b>	555	542,975	472,609	413,663	<b>476,416</b>	64,740	13.6	1.01	<b>0.8</b>
	1667	572,398	509,642	503,021	<b>528,353</b>	38,287	7.2	0.91	-10
	5000	520,362	463,311	457,291	<b>480,321</b>	34,806	7.2	1.00	<b>0.0</b>
<b>TC9</b>	555	390,729	413,380	346,666	<b>383,592</b>	33,925	8.8	1.25	<b>20</b>
	1667	165,078	164,482	174,833	<b>168,131</b>	5,811	3.5	2.86	<b>65</b>
	5000	70,805	65,908	66,516	<b>67,743</b>	2,669	3.9	7.09	<b>86</b>
<b>TC10</b>	555	524,697	471,187	408,744	<b>468,210</b>	58,034	12.4	1.03	<b>2.5</b>
	1667	497,062	467,832	456,683	<b>473,859</b>	20,853	4.4	1.01	<b>1.3</b>
	5000	409,243	531,546	518,877	<b>486,555</b>	67,253	13.8	0.99	-1.3
Reference Antagonist: <b>GSK3787</b>	0.038	549,350	492,583	485,437	<b>509,123</b>	35,020	6.9	0.94	-6.0
	0.15	487,732	437,940	484,921	<b>470,198</b>	27,971	5.9	1.02	<b>2.1</b>
	0.61	505,493	451,291	485,051	<b>480,612</b>	27,372	5.7	1.00	-0.1
	2.4	517,721	545,878	543,161	<b>535,587</b>	15,531	2.9	0.90	-8.0
	9.8	517,721	545,878	543,161	<b>535,587</b>	15,531	2.9	0.90	-4.0
	39	443,734	441,804	451,402	<b>445,647</b>	5,077	1.1	1.08	<b>7.2</b>
	156	327,404	348,817	267,534	<b>314,585</b>	42,130	13.4	1.53	<b>35</b>
	625	123,876	137,076	130,867	<b>130,606</b>	6,604	5.1	3.68	<b>73</b>
	2500	26,941	25,540	24,310	<b>25,597</b>	1,316	5.1	18.8	<b>95</b>
	10000	5,710	4,817	4,941	<b>5,323</b>	484	9.1	90.2	<b>97</b>

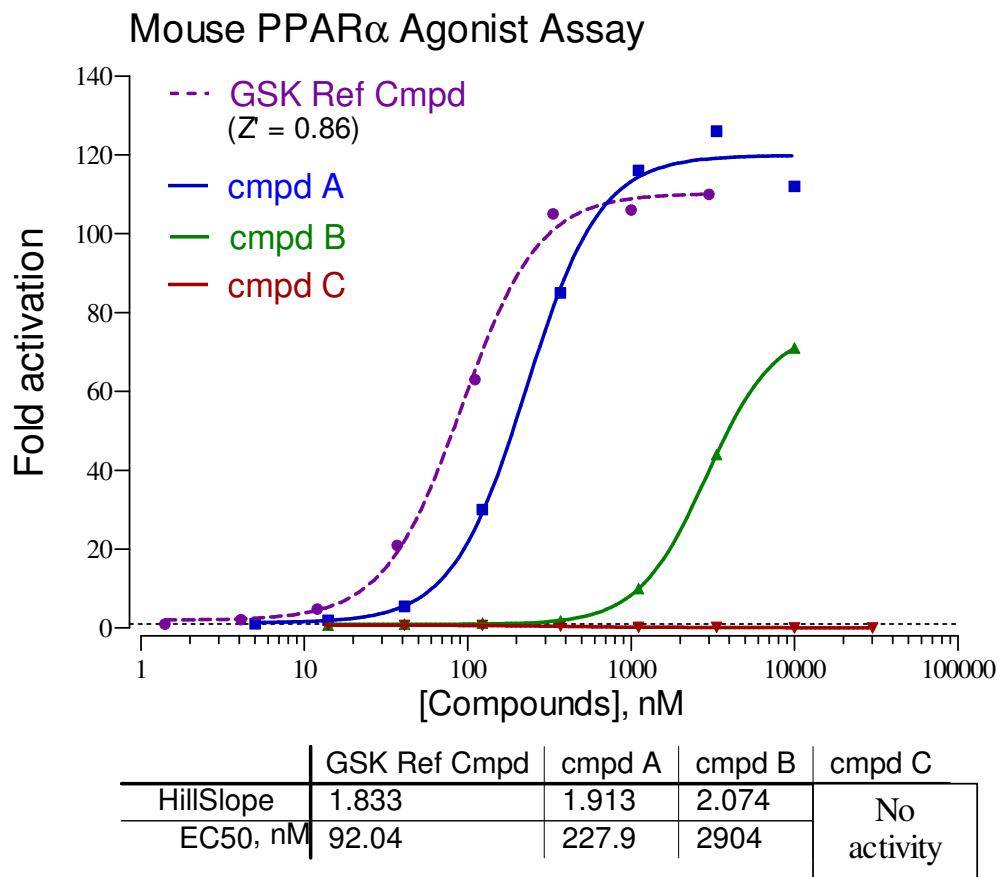
Values highlighted in Green represent  $\geq 50\%$  reduction (i.e.,  $\geq 2$ -fold reduction) in receptor activity.



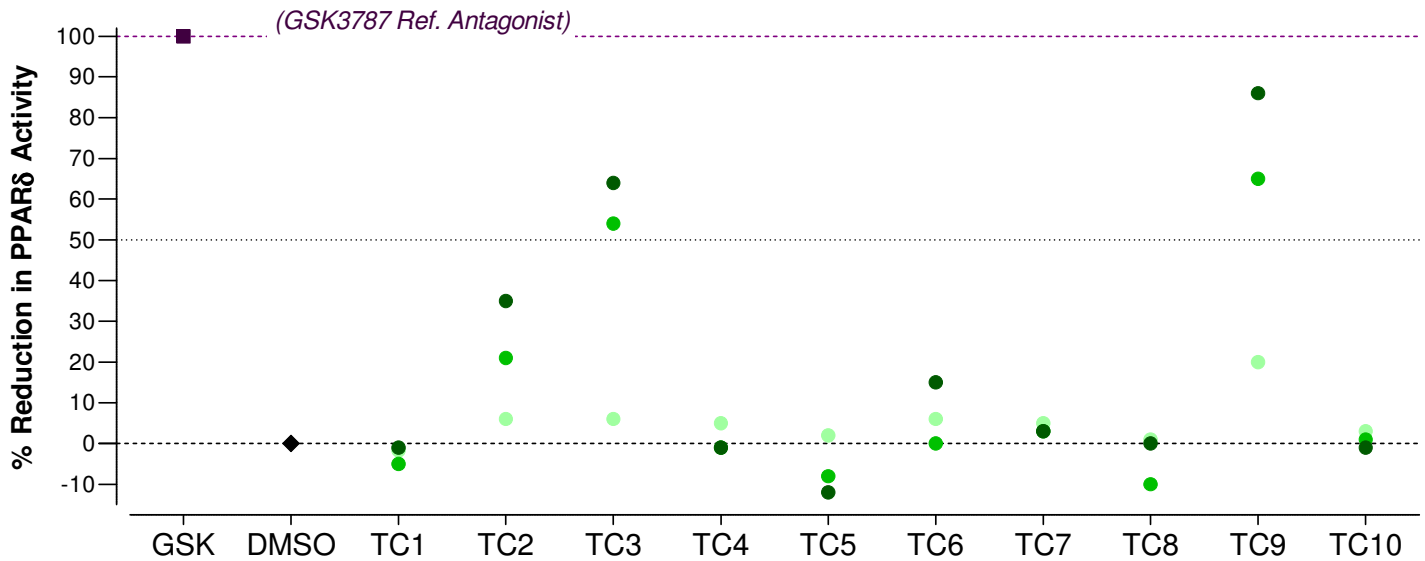
**(Example 4:** The following are miscellaneous sample graphical presentation styles for data generated from reference and test compound samples



Example of curve-fit data presentation: A minimum of 6 test concentrations are required to establish EC50 or IC50 values of test compounds relative to a reference compound.



Example of Dot-plot format data presentation: analyses of 1 – 3 concentrations of test compounds.



**Human PPARδ antagonist assay.**

Test compounds ● 5 μM, ● 1.67 μM, and ● 0.55 μM.

◆ 0.1% DMSO = 0% inhibition. ■ 40 μM GSK3787 (reference antagonist).

PPARδ Reporter cells are co-treated with ~EC<sub>80</sub> of GW0742 as the challenge agonist.

Percent Inhibition is:  $[100 * (1 - (RLU^{Test\ Cmpd} / RLU^{Vehicle\ control}))]$ .



*(~ End of Report ~)*