FINAL STUDY REPORT
(Example)

Company X

STUDY SPONSOR

...

AUTHORS

Study Manager
INDIGO Biosciences

Study Director
INDIGO Biosciences

...

DATA FILES ACCOMPNAYING THIS STUDY REPORT

1.) CompanyX_Data Compilation.xls (Microsoft Excel file)
2.) CompanyX_Test Cmpd Data.pzf (GraphPad Prism file)
3.) CompanyX_Ref Cmpd Data.pzf (GraphPad Prism file)

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INDIGO PROJECT IDENTIFICATION

INDIGO Contract **#xxxxxxx**

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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 the Study Findings *(Example)*

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*
PPAR†	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	
ROR†	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 *(Example)*

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

▪ *Plasmids.* 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). *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 10% 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. RLUs are quantified from each assay well to determine NR activity.

Figure xx: Agonist Assays

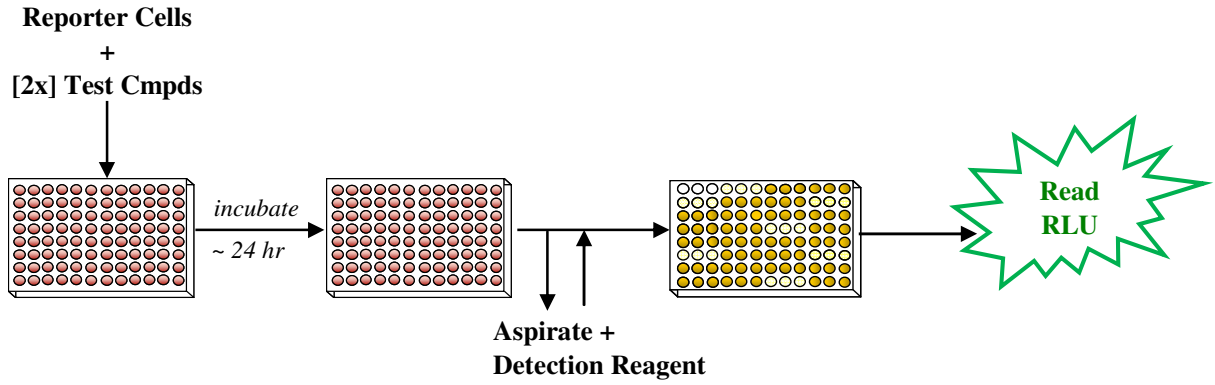
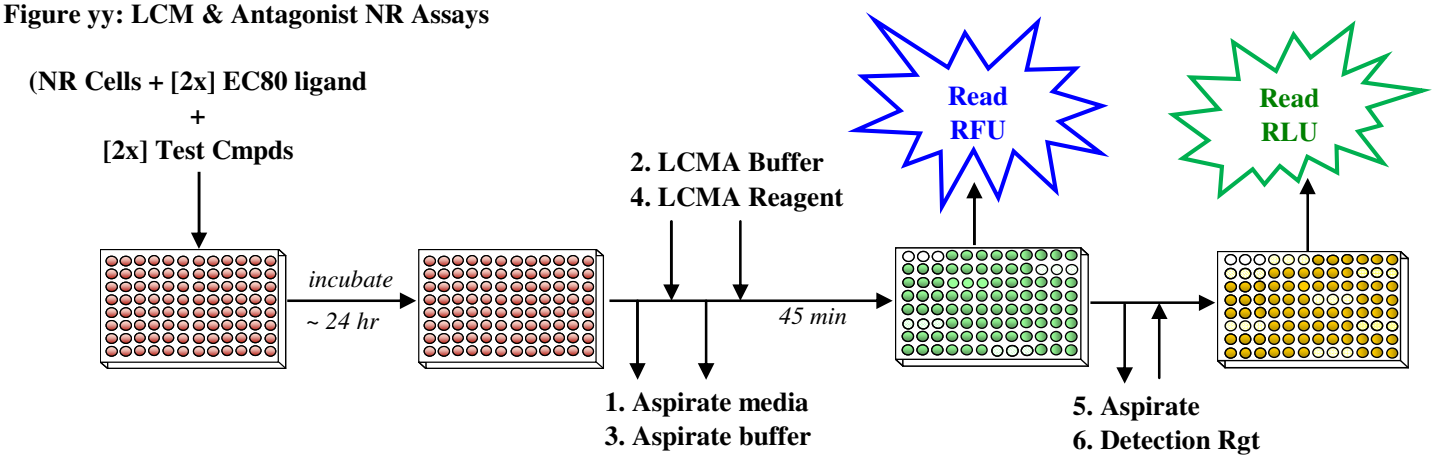


Figure yy: LCM & Antagonist NR Assays



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.

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

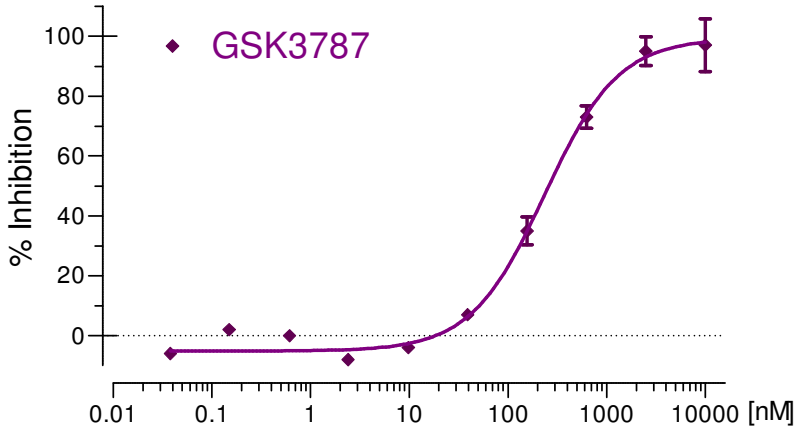
Unless otherwise requested, agonist data will be calculated as "**Fold-Activation**" and graphically presented in dot-plot form (5 or fewer treatment doses per test cmpd) or plotted *via* non-linear regression (6 or more treatment doses per test cmpd).

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 non-linear regression.

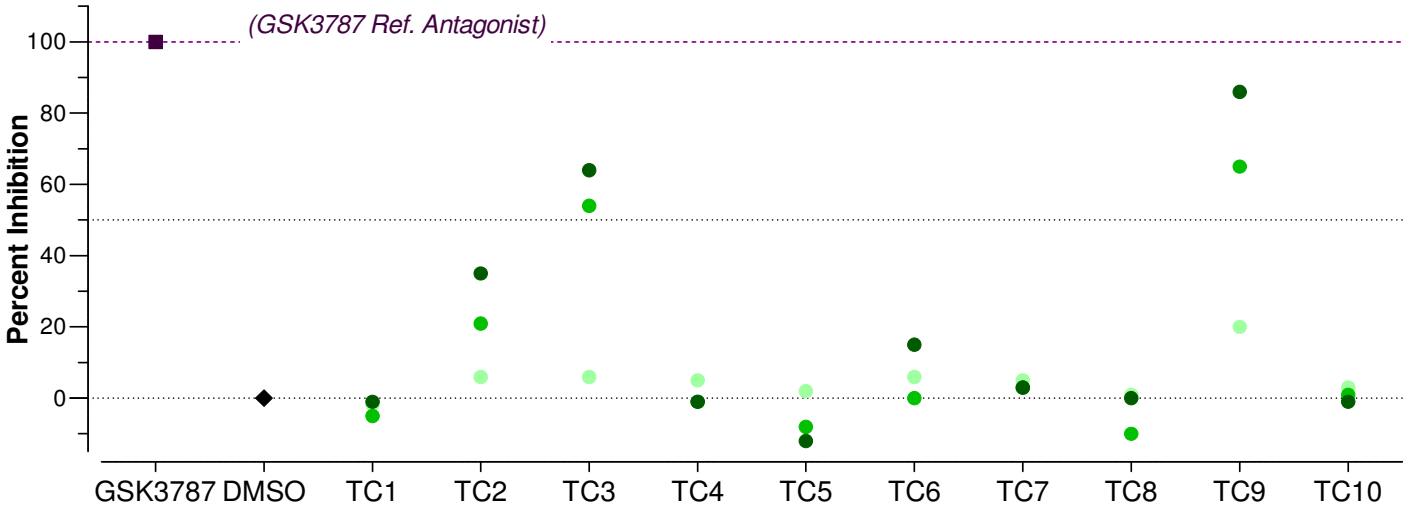
Human PPAR delta (NR1C2) Assays *(Sample Data)*

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



PPARδ Antagonist Assay

Bottom	-5.173
Top	99.35
LogEC50	2.372
HillSlope	1.161
EC50	235.7
Span	104.5



Human PPARd *antagonist* assay. Test componds • 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_{80} of GW0742 as the challenge agonist. Percent Inhibition is [100*(1-($RLU^{Test\ Cmpd}$ / $RLU^{Vehicle\ control}$))].

(~ End of Report ~)