# Human Peroxisome Proliferator-Activated Receptor Assays

## PANEL

**PPARα, PPARδ, PPARγ**

**32 Assays each in 96-well Format**
Product #IB00131-32P

▪

**Technical Manual**
*(version 7.2)*

**www.indigobiosciences.com**

3006 Research Drive, Suite A1, State College, PA 16801, USA

Customer Service:
814-234-1919; FAX 814-272-0152
customerserv@indigobiosciences.com

Technical Service:
814-234-1919
techserv@indigobiosciences.com

# Human PPAR Assays PANEL
# PPARα, PPARδ, PPARγ
## 32 Assays each in 96-well Format

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## I. Description

**▪ The Assay System ▪**

INDIGO's **PANEL** of **PPAR Reporter Assays** utilizes non-human mammalian cells engineered to express **Human Peroxisome Proliferator-Activated Receptors**: **PPARα** (NR1C1), **PPARδ** (NR1C2), or **PPARγ** (NR1C3), all ligand-dependent transcription factors that are commonly referred to as **PPARα**, **PPARδ** and **PPARγ**.

INDIGO's PPAR Reporter Cells include the luciferase reporter gene functionally linked to a responsive promoter. Thus, quantifying changes in luciferase expression in the treated reporter cells provides a sensitive surrogate measure of the changes in PPARα, PPARδ, or PPARγ activity. The principal application of this assay panel is in the screening of test samples to quantify any functional activity, either agonist or antagonist, that they may exert against the three human PPAR's.

PPAR Reporter Cells are prepared using INDIGO's proprietary **CryoMite™** process. This cryo-preservation method yields exceptional cell viability post-thaw, and provides the convenience of immediately dispensing healthy, division-competent reporter cells into assay plates. There is no need for cumbersome intermediate treatment steps such as spin-and-rinse of cells, viability determinations or cell titer adjustments prior to assay setup.

INDIGO's nuclear receptor Assays are all-inclusive cell-based assay systems. In addition to PPAR Reporter Cells, this kit provides two optimized media for use during cell culture and in diluting the user's test samples, a reference agonist, Luciferase Detection Reagent, and a cell culture-ready assay plate.

**▪ The Assay Chemistry ▪**

INDIGO's nuclear receptor assay kits capitalize on the extremely low background, high-sensitivity, and broad linear dynamic range of bio-luminescence reporter gene technology.

Reporter Cells incorporate the cDNA encoding beetle luciferase, a 62 kD protein originating from the North American firefly (*Photinus pyralis*). Luciferase catalyzes the mono-oxidation of D-luciferin in a $Mg^{+2}$-dependent reaction that consumes $O_2$ and ATP as co-substrates, and yields as products oxyluciferin, AMP, $PP_i$, $CO_2$, and photon emission. Luminescence intensity of the reaction is quantified using a luminometer, and is reported in terms of Relative Light Units (RLU's).

Assay kits feature a luciferase detection reagent specially formulated to provide stable light emission between 5 and 90+ minutes after initiating the luciferase reaction. Incorporating a 5 minute reaction-rest period ensures that light emission profiles attain maximal stability, thereby allowing assay plates to be processed in batch. By doing so, the signal output from all sample wells, from one plate to the next, may be directly compared within an experimental set.

**▪ Preparation of Test Compounds ▪**

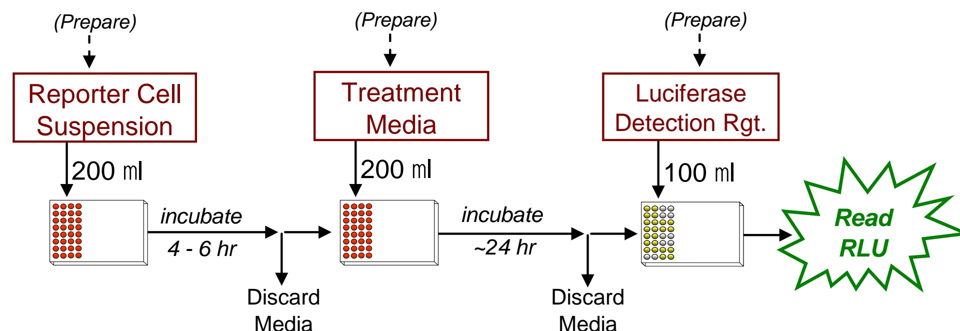
Test compounds are typically solvated at high-concentration in DMSO and stored frozen as master stocks. Immediately prior to setting up an assay, the master stocks are serially diluted using **Compound Screening Medium (CSM)** to achieve the desired assay concentrations. Do not use DMSO to further dilute test compound solutions. This method of dilution avoids the significant adverse effects of introducing high concentrations of DMSO into the assay. The final concentration of total DMSO carried over into assay reactions should *never* exceed 0.4%.

> *NOTE:* CSM is formulated to help stabilize hydrophobic test compounds in the aqueous environment of the assay mixture. Nonetheless, high concentrations of extremely hydrophobic test compounds diluted in CSM may lack long-term stability and/or solubility, especially if further stored at low temperatures. Hence, it is recommended that test compound dilutions are prepared in CSM immediately prior to assay setup, and are considered to be 'single-use' reagents.

## ▪ Assay Scheme ▪

NOTE: These PPAR assay protocols includes **Day 1** steps and dispensed volumes that are different from the conventional 96-well and 3x32-well format assay protocols that users may be accustomed to.

*In brief*, 200 µl of respective PPAR Reporter Cell suspensions are dispensed into wells of the assay plate and pre-incubated for 4-6 hours. Following the pre-incubation period, culture media are discarded and 200 µl/well of the prepared 1x-concentration treatment media are added. Following an additional 22-24 hr incubation, treatment media are discarded and Luciferase Detection Reagent is added. Following a 5 min. rest period, the intensity of light emission (in units of 'Relative Light Units'; RLU) from each assay well is quantified using a plate-reading luminometer.




## ▪ Assay Performance ▪

**Figures 2**, **3**, and **4** present performance data for the PPARα, PPARδ, and PPARγ assays. To assess the level of background signal contributed by non-specific factors that may cause activation of the luciferase reporter gene, "mock" reporter cells, which contain only the luciferase vector, were treated with agonist, as noted in respective figures (mock reporter cells are not provided with assay kits). For each assay, luminescence was quantified using a GloMax-Multi+ luminometer (Promega). Average relative light units (RLU) and corresponding standard deviation (SD) values were determined for each treatment concentration ($n \geq 6$). Signal-to-background (S/B) and Z' values were calculated as described by Zhang, *et al*. (1999)[1]. Non-linear regression and $EC_{50}$ analyses were performed using GraphPad Prism software.

[1] Zhang JH, Chung TD, Oldenburg KR. (1999) A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. J Biomol Screen.:**4**(2), 67-73.

$$Z' = 1 - [3*(SD^{Control} + SD^{Background}) / (RLU^{Control} - RLU^{Background})]$$

Human PPARα  Agonist Assays

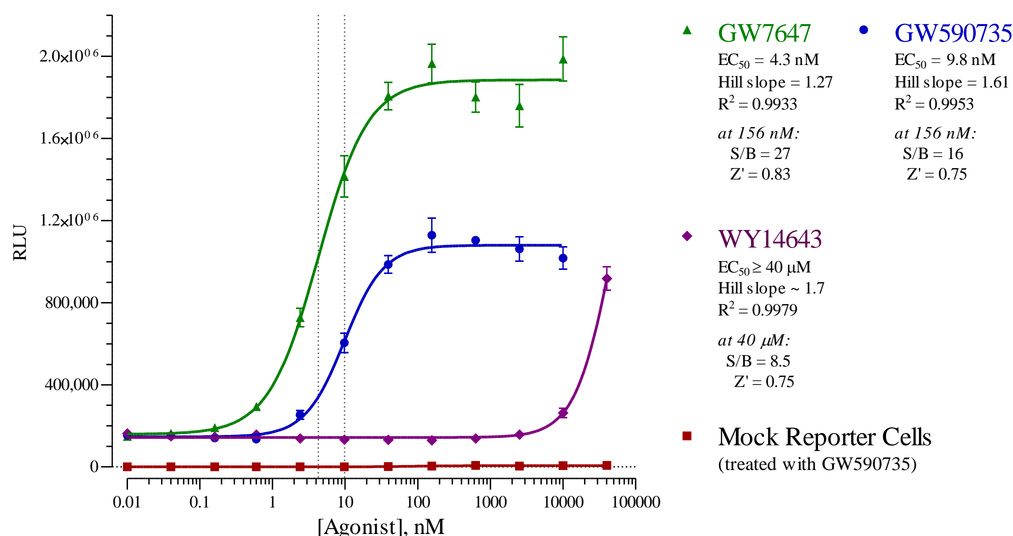



**Figure 2.** ***Agonist*** **dose-response analyses of Human PPARα.**
Analyses of PPARα Reporter Cells using GW590735 (provided), GW7647 and WY14643 (Tocris). Final assay concentrations varied between 40 μM and 0.010 nM and included a 'no-treatment' control (n ≥ 6; highest [DMSO] ≤ 0.1% *f.c.* **Appendix 1** describes an abbreviated 8-point dilution scheme for GW590735). Mock reporter cells demonstrate no significant background luminescence (≤ 0.1% that of the reporter cells at $EC_{Max}$). Thus, luminescence results strictly through ligand-activation of PPARα expressed in these reporter cells. Z' scores confirm the robust performance of this PPARα Assay.

Human PPARδ Assays: Agonist dose-responses

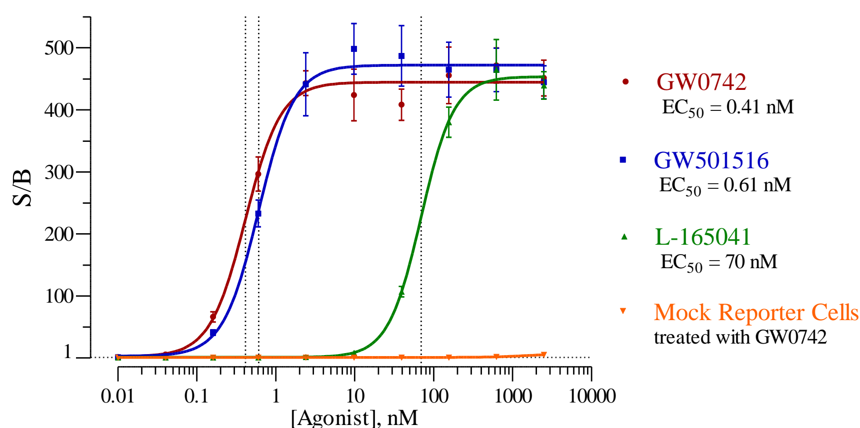



**Figure 3a.** ***Agonist*** **dose-response analyses of Human PPARδ.**
Validation of the PPARδ Assay was performed using manual dispensing of the reference agonists GW0742 (provided), GW501516 and L-165041 (Tocris). Final assay concentrations of agonist treatment media ranged between 40 μM and 10 pM, and included a 'no-treatment' control (n ≥ 6 / treatment; highest [DMSO] ≤ 0.1% *f.c.* **Appendix 2** describes an abbreviated 8-point dilution scheme that we find suitable for GW0742.) Mock Reporter Cells were identically treated with GW0742.

PPARδ reporter cells treated with 625 nM GW0742 yielded an average RLU value with CV = 6.0%, S/B ~ 430, and a corresponding Z'= 0.82. Mock reporter cells treated with GW0742 demonstrate no significant background luminescence (≤ 1% that of the reporter cells at $EC_{Max}$). Thus, luminescence results through ligand-dependent activation of human PPARδ expressed in these reporter cells.

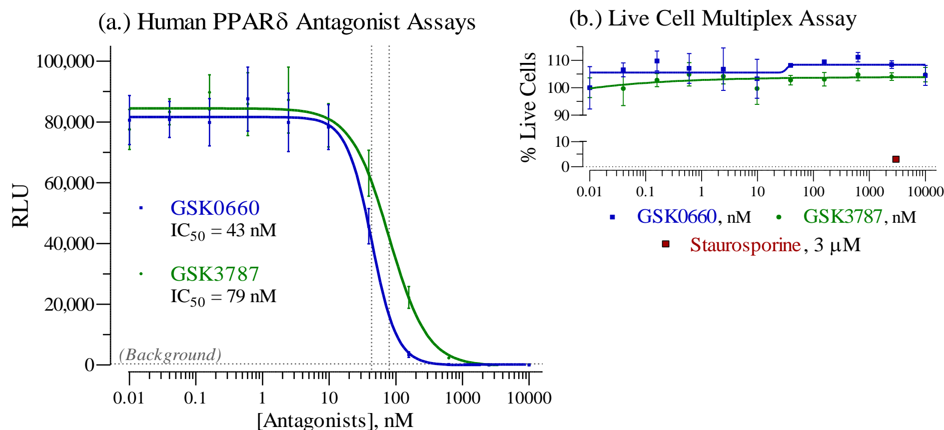



**Figure 3b.** ***Antagonist*** **dose-response analyses of Human PPARδ performed in combination with the INDIGO Live Cell Multiplex Assay.**

**(a.)** PPARδ antagonist assays were performed using GSK0660 and GSK37887 (Tocris).
**(b.)** To confirm that the observed drop in RLU values resulted from receptor inhibition, and *not* induced cell death, the relative numbers of live cells in each assay well were determined at the end of the treatment period using INDIGO's Live Cell Multiplex (LCM) Assay (#LCM-01).

Final assay concentration of the agonist GW0742 was 1 nM (approximating $EC_{75}$), and concentrations of the respective antagonists ranged between 10 µM and 10 pM ($n \geq 6$ per treatment; highest [DMSO] ≤ 0.15% *f.c.*). Included were cells treated with 3.0 µM Staurosporine as a positive control for cytotoxic response. Assay plates were incubated for 23 hrs, then processed according to the LCM Assay protocol to quantify relative numbers of live cells per treatment condition. Plates were then further processed to quantify PPARδ activity for each treatment condition. Averaged RFU values from each antagonist treatment group were normalized to the average RFU value of "no antagonist treatment" assay wells, which corresponds to 100% Live Cells in the LCM assay.

GSK0660 and GSK3787 treatments both caused dose-dependent reductions in RLU values, down to "background" levels. However, the LCM Assay reveals no decrease in the numbers of live cells per assay well, up to the maximum treatment concentrations of 10 µM. Hence, the observed reductions in RLU values can be attributed to the inhibition of PPARδ activity by the treatment compounds, and *not* to induced cell death.

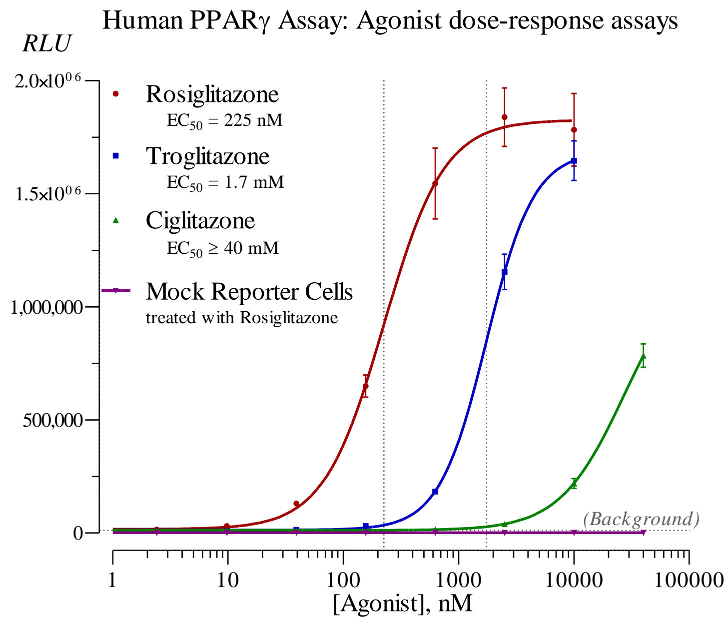



**Figure 4a. Agonist dose-response analyses of Human PPARγ.**

Validation of the PPARγ Assay was performed using manual dispensing and following the protocol described in this Technical Manual, using the reference agonists Rosiglitazone (provided), Troglitazone (Tocris) and Ciglitazone (Tocris). PPARγ Reporter Cells and Mock reporter cells were identically treated with Rosiglitazone, as described in **Appendix 3**.

PPARγ reporter cells treated with 2,500 nM Rosiglitazone yielded an average RLU value with CV=7%, S/B = 162 and a corresponding Z'= 0.78. Similarly treated mock reporter cells demonstrate no significant background luminescence (≤ 0.05% that of $EC_{Max}$). Thus, luminescence results strictly through ligand-activation of the PPARγ expressed in these reporter cells.

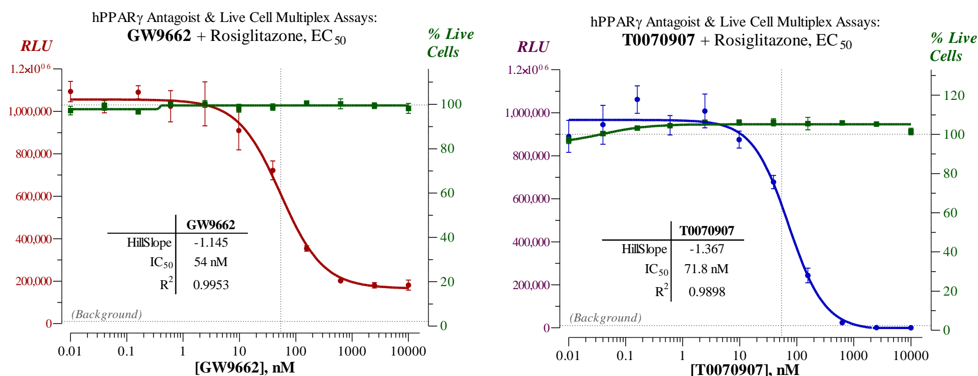



**Figure 4b.** ***Antagonist*** **dose-response analyses of Human PPARγ performed in combination with the INDIGO Live Cell Multiplex Assay.**

Antagonist assays were performed using T0070907 (Tocris), and GW9662 (Tocris). To confirm that the observed drop in RLU values resulted from receptor inhibition, as opposed to induced cell death, the relative numbers of live cells in each assay well were determined using INDIGO's Live Cell Multiplex (LCM) Assay (#LCM-01). Final assay concentrations of the respective antagonists ranged between 10 μM and 10 pM, including a 'no antagonist' control (n ≥ 6 per treatment; highest [DMSO] ≤ 0.15% *f.c.*). Each treatment also contained 220 nM (approximating $EC_{50}$) Rosiglitazone as challenge agonist. Assay plates were incubated for 22 hrs, then processed according to the LCM Assay protocol to quantify relative numbers of live cells per treatment condition. Plates were then further processed to quantify PPARγ activity for each treatment condition. Averaged RFU values from each antagonist treatment group were normalized to the average RFU value of "no antagonist treatment" assay wells, which corresponds to 100% Live Cells in the LCM assay.

T0070907 and GW9662 both caused dose-dependent reduction in RLU values. The LCM Assay reveals no significant variance in the numbers of live cells per assay well, up to the maximum treatment concentration of 10 μM. Hence, the observed reduction in RLU values can be attributed to dose-dependent inhibition of PPARγ activity, and *not* to cell death.

## *II. Product Components & Storage Conditions*

This Human PPAR Assays PANEL contains materials to perform 32 PPARα assays, 32 PPARδ assays, and 32 PPARγ assays, all in a single 96-well plate format. All reagents are supplied with sufficient extra volume to accommodate the needs of performing 3 individual groups of assays.

The individual aliquots of PPAR Reporter Cells and Detection Substrate and Detection Buffer are provided as single-use reagents. Once thawed, reporter cells can NOT be refrozen with any hope of retaining downstream assay performance. Therefore, extra volumes of these reagents should be discarded after assay setup.

Assay kits are shipped on dry ice. Upon receipt, individual kit components may be stored at the temperatures indicated on their respective labels. Alternatively, the entire kit may be further stored at -80°C.

To ensure maximal viability, "Reporter Cells" must be maintained at -80°C until immediately prior to use.

The date of product expiration is printed on the Product Qualification Insert (PQI) enclosed with each kit.

| *Kit Components* | *Amount* | *Storage Temp.* |
|---|---|---|
| ▪ PPARα Reporter Cells | 1 x 0.60 mL | **-80°C** |
| ▪ PPARδ Reporter Cells | 1 x 0.60 mL | **-80°C** |
| ▪ PPARγ Reporter Cells | 1 x 0.60 mL | **-80°C** |
| ▪ Cell Recovery Media (CRM) | 2 x 10.5 mL | -20°C |
| ▪ Compound Screening Media (CSM) | 1 x 45 mL | -20°C |
| ▪ *PPARα reference agonist:* GW590735, 10 mM (in DMSO) | 1 x 30 µL | -20°C |
| ▪ *PPARδ reference agonist:* GW0742, 1.0 mM (in DMSO) | 1 x 30 µL | -20°C |
| ▪ *PPARγ reference agonist:* Rosiglitazone, 10 mM (in DMSO) | 1 x 30 µL | -20°C |
| ▪ Detection Substrate | 3 x 2.0 mL | **-80°C** |
| ▪ Detection Buffer | 3 x 2.0 mL | -20°C |
| ▪ 96-well format plate frame | 1 | ambient |
| ▪ snap-in, 8-well strips (white, sterile, cell culture treated) | 12 | ambient |

## *III. Materials to be Supplied by the User*

The following materials must be provided by the user, and should be made ready prior to initiating the assay procedure:

***DAY 1***

▪ cell culture-rated laminar flow hood.

▪ 37°C, humidified 5% $CO_2$ incubator for mammalian cell culture.

▪ 37°C water bath.

▪ 70% alcohol wipes

▪ 8- *or* 12-channel electronic, repeat-dispensing pipettes & sterile tips

▪ disposable media basins, sterile.

▪ sterile multi-channel media basins *or* deep-well plates, *or* appropriate similar vessel for generating serial dilutions of test & reference compound(s).

▪ antagonist reference compounds (optional).

***DAY 2*** plate-reading luminometer.

## *IV. Assay Protocol*

Review the entire Assay Protocol before starting. Completing the assay requires an overnight incubation. *Steps 1-12* are performed on **Day 1**, requiring less than 2 hours to complete. *Steps 13-18* are performed on **Day 2**, and require less than 1 hour to complete.

**▪ A word about Antagonist-mode assay setup ▪**

Receptor inhibition assays expose the Reporter Cells to a constant, sub-maximal concentration (typically $EC_{50}$ – $EC_{85}$) of a known *agonist* AND the test compound(s) to be evaluated for antagonist activity. Adding the reference agonist to the bulk suspension of Reporter Cells (*i.e.,* prior to dispensing into assay wells) is the most efficient and precise method of setting up antagonist assays, and it is the method presented in the following protocol.

This PPAR Assay Panel kit provides a commonly used reference agonist for each PPAR assay; they may be used effectively to setup respective receptor inhibition assays.

▪ **PPARα**: GW590735 is provided as a 10 mM stock in DMSO; it may be used as an agonist of PPARα (Figure 2A) to set up antagonist screens. 33.3 nM GW590735 typically approximates $EC_{80}$ in this assay.

▪ **PPARγ**: GW0742 is provided as a 1.0 mM stock in DMSO; it may be used as an agonist of PPARδ (Figure 3A) to set up antagonist screens. 3.3 nM GW0742 typically approximates $EC_{75}$ in this assay.

▪ **PPARγ**: Rosiglitazone is provided as a 10 mM stock in DMSO; it may be used as an agonist of PPARγ (Figure 4A) to set up antagonist screens. 300 nM Rosiglitazone typically approximates $EC_{70}$ in this assay.

**DAY 1 Assay Protocol:** All steps must be performed using aseptic technique.

**1.)** Remove the **2 tubes** of **Cell Recovery Medium (CRM)** from freezer storage, thaw and equilibrate to 37°C using a water bath.

**2.) Rapid Thaw of the Reporter Cells:** *First*, retrieve the two tubes of **CRM** from the 37°C water bath and sanitize their outside surfaces with a 70% ethanol swab.

*Second*, retrieve **PPAR Reporter Cells** from -80°C storage: each PPAR will utilize 32 assay wells. W*ithout delay*, perform a rapid thaw of the frozen cells by transferring **6.4 ml** of pre-warmed CRM into each tube of frozen cells. Recap the tube of Reporter Cells and immediately place it in a 37°C water bath for 5 - 10 minutes. The resulting volume of respective PPAR cell suspensions will be **7.0 ml** per tube.

**3.)** Work in the cell culture hood to *carefully* mount four sterile 8-well strips (for *each* PPAR assay to be performed) into the blank assay plate frame. Strip-wells are fragile. Note that they have keyed ends (square and round), hence, they will fit into the plate frame in only one orientation.

**4.)** Retrieve the tube of Cell Suspensions from the water bath and sanitize the outside surface with a 70% alcohol swab.

**5.)** Dispense **200 µl / well** of a specific PPAR cell suspensions into 32-wells of the assay plate.

> *NOTE 5.1:* Increased well-to-well variation (= increased standard deviation!) will occur if care is not taken to prevent cells from settling during the dispensing period. Likewise, take care to dispense uniform volumes across the assay plate.
>
> *NOTE 5.2:* Users sometimes wish to examine the reporter cells using a microscope. If so, the extra volume of cell suspension provided with each kit may be dispensed into a clear 96-well cell culture treated assay plate. Continue to process the assay plate in identical manner to the white assay plate.

**6.) Pre-incubate reporter cells:** Place the assay plate into a 37°C, ≥ 85% humidity, 5% $CO_2$ incubator for 4 - 6 hours.

**7.)** ***Near the end of the 4-6 hour pre-incubation period:*** Remove **Compound Screening Medium (CSM)** from freezer storage and thaw in a 37°C water bath.

**8.) Prepare the Test Compound and Reference Compound treatment media at the desired final assay concentrations:** Use **CSM** to prepare an appropriate dilution series of the reference and test compound stocks. Prepare all treatment media at the desired final assay concentrations. In *Step 10*, the prepared treatment media will be dispensed at 200 µl / well into the assay plate. Manage dilution volumes carefully; this assay kit provides **45 ml** of CSM.

> NOTE: Total DMSO carried over into assay reactions should never exceed 0.4%.

***a. Agonist*-mode assays**. This PPAR Assay Panel kit provides a commonly used reference agonist for each PPAR assay.

▪ **PPARα.** Agonist GW590735 is provided as a 10 mM stock in DMSO. The PPARα assay typically exhibits a complete dose-response to GW590735 using an *assay* concentration range of: 300, 100, 33.3, 11.1, 3.70, 1.23, 0.412 and 0 nanoMolar (nM), as depicted in Figure 2A. **APPENDIX 1** provides an example for generating such a dilution series.

▪ **PPARδ**. Agonist GW0742 is provided as a 1.0 mM stock in DMSO. The PPARδ assay typically exhibits a complete dose-response to GW0742 using an *assay* concentration range of: 90, 30, 1.0, 3.33, 1.11, 0.370, 0.123, 0.412 and 0 nM (Figure 3A). **APPENDIX 2** provides an example for generating such a dilution series.

▪ **PPARγ**. Agonist Rosiglitazone is provided as a 10 mM stock in DMSO. The PPARγassay typically exhibits a complete dose-response to Rosiglitazone using an *assay* concentration range of: 2000, 1000, 500, 250, 125, 62.5, 31.3, 15.6 and 0 nM (Figure 4A). **APPENDIX 3** provides an example for generating such a dilution series.

*~ or ~*

***b. Antagonist*-mode assays**. When setting antagonist assays, first supplement a bulk volume of CSM with the appropriate challenge agonist to achieve the desired final assay-concentration (refer to "*A word about antagonist-mode assay setup*", pg. 10). The agonist-supplemented CSM is then used to generate dilutions of test compound samples to achieve their final assay concentrations.

**9.)** ***At the end of the cell pre-incubation period:*** **Discard the culture media**. Because the assay plate is composed of a frame with snap-in strip-wells, the practice of physically ejecting media is NOT advised. Complete removal of the media is efficiently performed by tilting the plate on edge and aspirating media using an 8-pin manifold (*e.g.,* Wheaton Science Microtest Syringe Manifold, # 851381) affixed to a vacuum-trap apparatus. Do *not* touch the well bottom, or run the tip of the aspiration device around the bottom circumference of the assay well. Such practices will result in destruction of the reporter cells and greatly increased well-to-well variability.

**10.)** Dispense **200 µl** of each prepared control media and treatment media into appropriate wells of the assay plate.

**11.)** Move the assay plate into a 37°C, humidified 5% $CO_2$ incubator for an additional 22 - 24 hours.

> *NOTE:* Ensure a high-humidity (≥ 85%) environment within the cell culture incubator. This is critical to prevent the onset of deleterious "edge-effects" in the assay plate.

**12.)** For greater convenience on Day 2, retrieve the appropriate number of vials of **Detection Substrate** *and* **Detection Buffer** from freezer storage and place them in a dark refrigerator (4°C) to thaw overnight.

**DAY 2 Assay Protocol:** Subsequent manipulations do *not* require special regard for aseptic technique, and may be performed on an open bench top.

**13.)** 30 minutes before intending to quantify receptor activity, remove **Detection Substrate** and **Detection Buffer** from the refrigerator and place them in a low-light area so that they may equilibrate to room temperature. Once at room temperature, gently invert each tube several times to ensure homogenous solutions.

> *NOTE:* Do NOT actively warm Detection Substrate above room temperature. If these solutions were not allowed to thaw overnight at 4°C, a room temperature water bath may be used to expedite thawing.

**14.)** Set the plate-reader to "luminescence" mode. Set the instrument to perform a single 5 second "plate shake" prior to reading the first assay well. Read time may be set to 0.5 second (500 mSec) per well, *or less*.

**15.)** *Immediately before proceeding to Step 16*: To read **32 assay wells**, transfer the entire volume of 1 vial of Detection Buffer into 1 vial of Detection Substrate, thereby generating a 4 ml volume of **Luciferase Detection Reagent (LDR)**. Mix gently to avoid foaming.

**16.)** Following 22 - 24 hours incubation in treatment media, remove media contents from each well of the assay plate (as before in *Step 9*).

**17.)** Add 100 µl of **LDR** to each well of the assay plate. Allow the assay plate to rest at room temperature for at least 5 minutes following the addition of LDR. Do not shake the assay plate during this period.

**18.)** Quantify luminescence.

## *V. Related Products*

| **PPARα Assay Products** | |
|---|---|
| ***Product No.*** | ***Product Descriptions*** |
| IB00111-32 | Human PPARα Reporter Assay System<br>3x 32 assays in 96-well format |
| IB00111 | Human PPARα Reporter Assay System<br>1x 96-well format assay |
| IB00112 | Human PPARα Reporter Assay System<br>1x 384-well format assays |
| **PPARδ Assay Products** | |
| IB00121-32 | Human PPARδ Reporter Assay System<br>3x 32 assays in 96-well format |
| IB00121 | Human PPARδ Reporter Assay System<br>1x 96-well format assay |
| IB00122 | Human PPARδ Reporter Assay System<br>1x 384-well format assays |
| **PPARγ Assay Products** | |
| IB00101-32 | Human PPARγ Reporter Assay System<br>3x 32 assays in 96-well format |
| IB00101 | Human PPARγ Reporter Assay System<br>1x 96-well format assays |
| IB00102 | Human PPARγ Reporter Assay System<br>1x 384-well format assays |
| Bulk volumes of assay reagents may be custom manufactured to accommodate any scale of HTS. Please Inquire. | |

| **Panel of PPAR Assays** | |
|---|---|
| ***Product No.*** | ***Product Description*** |
| IB00131-32P | Human PPARγ, PPARα and PPARδ Reporter Assay PANEL<br>32 assays each in 1x 96-well plate |

| Mouse/Rat PPARγ Assay Products | |
|---|---|
| ***Product No.*** | ***Product Descriptions*** |
| MR00101-32 | Mouse/Rat PPARγ Reporter Assay System<br>3x 32 assays in 96-well format |
| MR00101 | Mouse/Rat PPARγ Reporter Assay System<br>1x 96-well format assay |
| MR00102 | Mouse/Rat PPARγ Reporter Assay System<br>1x 384-well format assays |

| Panel of Mouse PPAR Assay Products | |
|---|---|
| ***Product No.*** | ***Product Description*** |
| MR00131-32P | mrPPARγ, mPPARα and mPPARδ Reporter Assay PANEL<br>32 assays each in 1x 96-well plate |

| LIVE Cell Multiplex (LCM) Assay | |
|---|---|
| ***Product No.*** | ***Product Descriptions*** |
| LCM-01 | Reagent volumes sufficient to perform **96** Live Cell Assays in 1x96-well, or 2x48-well, or 3x32-well assay plate formats |
| LCM-05 | Reagent in 5x-bulk volume to perform **480** Live Cell Assays in any combination of 1x96-, 2x48-, or 3x32-well assay plate formats |
| LCM-10 | Reagent in 10x-bulk volume to perform **960** Live Cell Assays in any combination of 1x96-, 2x48-, or 3x32-well assay plate formats |

Please refer to INDIGO Biosciences website for updated product offerings.

**www.indigobiosciences.com**

## *VI. Limited Use Disclosures*

Products commercialized by INDIGO Biosciences, Inc. are for RESEARCH PURPOSES ONLY – not for therapeutic or diagnostic use in humans.

“CryoMite” is a Trademark ™ of INDIGO Biosciences, Inc. (State College, PA, USA)

Product prices, availability, specifications and claims are subject to change without prior notice.

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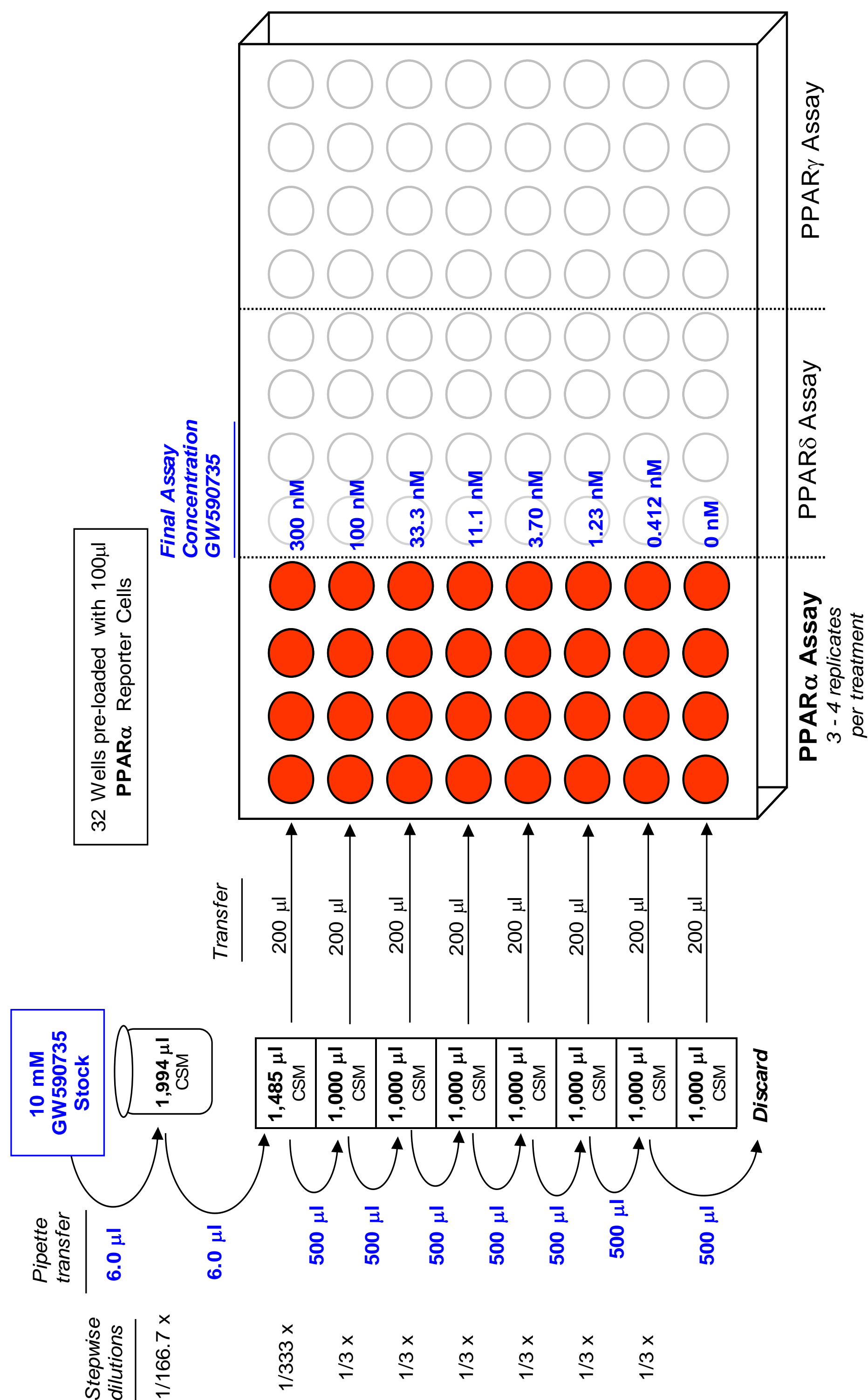
**APPENDIX 1**

Example scheme for the serial dilution of GW590735 reference agonist, and the setup of a PPARα dose-response assay.



**APPENDIX 2**

Example scheme for the serial dilution of GW0742 reference agonist, and the setup of a PPARδ dose-response assay.

32 Wells pre-loaded with 100µl **PPARδ** Reporter Cells

100 ml CSM **"0 nM"**

PPARγ Assay

**PPARδ Assay**
*3 - 4 replicates per treatment*

PPARα Assay

*Final Assay Concentration GW0742*

60.0 nM
20.0 nM
6.67 nM
2.22 nM
0.741 nM
0.247 nM
0.0823 nM
0.0274 nM

*Transfer*

200 µl
200 µl
200 µl
200 µl
200 µl
200 µl
200 µl
200 µl

1.0 mM GW0742 Stock

904 µl CSM

1,485 µl CSM
1,000 µl CSM
1,000 µl CSM
1,000 µl CSM
1,000 µl CSM
1,000 µl CSM
1,000 µl CSM
1,000 µl CSM

*Discard*

*Pipette transfer*

6.0 µl
15 µl
500 µl
500 µl
500 µl
500 µl
500 µl
500 µl
500 µl
500 µl

*Stepwise dilutions*

1/167 x
1/100 x
1/3 x
1/3 x
1/3 x
1/3 x
1/3 x
1/3 x
1/3 x

**APPENDIX 3**

Example scheme for the serial dilution of Rosiglitazone reference agonist, and the setup of a PPARγ dose-response assay.

**10 mM Rosiglitazone Stock**

| Stepwise dilutions | Pipette transfer | Tube | Transfer | Final Assay Concentration Rosiglitazone |
|---|---|---|---|---|
| 1/50 x | 10 µl | 490 µl CSM | | |
| 1/100 x | 20 µl | 1,980 µl CSM | 200 µl | 2,000 nM |
| 1/2 x | 1,000 µl | 1,000 µl CSM | 200 µl | 1,000 nM |
| 1/2 x | 1,000 µl | 1,000 µl CSM | 200 µl | 500 nM |
| 1/2 x | 1,000 µl | 1,000 µl CSM | 200 µl | 250 nM |
| 1/2 x | 1,000 µl | 1,000 µl CSM | 200 µl | 125 nM |
| 1/2 x | 1,000 µl | 1,000 µl CSM | 200 µl | 62.5 nM |
| 1/2 x | 1,000 µl | 1,000 µl CSM | 200 µl | 31.3 nM |
| 1/2 x | 1,000 µl | 1,000 µl CSM | 200 µl | 15.6 nM |
| | 1,000 µl | *Discard* | | |

32 Wells pre-loaded with 100µl **PPARγ** Reporter Cells

100 ml CSM **"0 nM" Control**

PPARα Assay

PPARδ Assay

**PPARγ Assay** *3 - 4 replicates per treatment*