



**Human Adrenoceptor Beta 3  
(ADRB3)  
Reporter Assay System**

**384-well Format Assays**  
Product # IB32202

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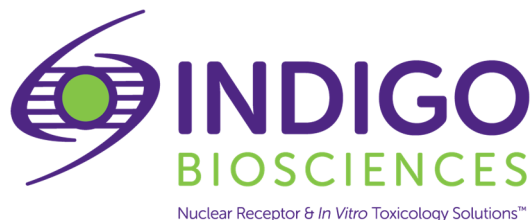
**Technical Manual**  
*(version 8.0)*

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**Human ADRB3 Reporter Assay  
 384-well Format Assays**

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## I. Description

### ▪ Background ▪

The adrenoceptors (*a.k.a.* adrenergic receptors) mediate the action of the sympathetic nervous system and are activated in response to “fight-or-flight” signals. They are divided into three types, adrenoceptor  $\alpha$ 1-,  $\alpha$ 2-, and  $\beta$ . Each type is further composed of three subtypes resulting in 9 different types ( $\alpha$ 1A,  $\alpha$ 1B,  $\alpha$ 1D,  $\alpha$ 2A,  $\alpha$ 2B,  $\alpha$ 2C,  $\beta$ 1,  $\beta$ 2, and  $\beta$ 3)<sup>1</sup>.

Adrenoceptors belong to the G-Protein-coupled receptor (GPCR) family. They all display the characteristic seven transmembrane helices, the extracellular loops which contribute to ligand binding, and the intracellular carboxy tail that associates with trimeric G proteins. All nine types of adrenoceptors are activated by the same endogenous catecholamines (epinephrine and norepinephrine); however, the specificity of their responses depends on the G-proteins and effector systems they associate with in a tissue and time specific manner<sup>1</sup>.

**Adrenoceptor Beta 3 (ADRB3)** was the last discovered member of the  $\beta$ -adrenoceptor sub-family and is the least studied. However, its utility as a therapeutic target has been validated by the approval of the ADRB3 agonists Mirabegron and Vibegron, which are used to treat overactive bladder syndrome. An additional feature that makes it a potential target for chronic conditions is that it lacks sites for phosphorylation by protein kinase A and  $\beta$ -adrenoceptor kinase, which are present on the  $\beta$ 1 and  $\beta$ 2-adrenoceptors, making it potentially resistant to desensitization<sup>2</sup>. ADRB3 is of additional interest as a target for the treatment of metabolic disorders, due to its role in lipolysis and thermogenesis and its expression in white and beige human adipocytes<sup>3</sup>. Further, targeting ADRB3 may be useful for the treatment of cancer, since it has been demonstrated that ADRB3 is over-expressed in the tumor microenvironment, and has also been implicated in other processes including angiogenesis and cancer progression<sup>4</sup>. Its involvement and expression in a variety of physiological processes and cell types, respectively, makes it a promising target in the treatment of multiple diseases.

### ▪ The Assay System ▪

This assay utilizes proprietary human cells engineered to provide constitutive, high-level expression of the **Human Adrenoceptor Beta 3 (ADRB3)**.

INDIGO's Reporter Cells contain an engineered luciferase reporter gene functionally linked to tandem cyclic AMP Response Elements (CRE) and a minimal promoter. Activated adenylyl cyclase results in the production of cAMP, which binds the transcription factor CREB (cAMP Response Element-Binding Protein). Activated CREB binds to CRE sequences, seeding the formation of a complete transcription complex that drives luciferase gene expression. Quantifying relative changes in luciferase enzyme activity in the treated reporter cells relative to the untreated reporter cells provides a sensitive surrogate measure of drug-induced changes in ADRB3 activity. The principal application of this reporter assay is in the screening of test compounds to quantify any functional activities, either activating or inhibitory, that they may exert against ADRB3.

The Reporter Cells in this kit are transiently transfected and prepared as frozen stocks using INDIGO's proprietary **CryoMite™** process. This cryo-preservation method allows for the immediate dispensing of healthy, division-competent reporter cells into assay plates. There is no need for intermediate treatment steps such as spin-and-rinse of cells, viability determinations or cell titer adjustments prior to assay setup.

INDIGO's assay kits provide the convenience of an all-inclusive cell-based assay system. In addition to ADRB3 Reporter Cells, provided are two optimized media for use in recovering the cryopreserved cells and for diluting test samples, the reference activator Isoproterenol, Luciferase Detection Reagents, and a cell culture-ready assay plate, and a detailed assay protocol.

### ▪ The Assay Chemistry ▪

INDIGO's receptor reporter assays 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<sup>+2</sup>-dependent reaction that consumes O<sub>2</sub> and ATP as co-substrates, and yields as products, oxyluciferin, AMP, PP<sub>i</sub>, CO<sub>2</sub>, and photon emission. Luminescence intensity of the reaction is quantified using a luminometer and is reported in terms of Relative Light Units (RLU's).

INDIGO's Receptor Assays feature a luciferase detection reagent specially formulated to provide stable light emission between 30 and 100+ minutes after initiating the luciferase reaction. Incorporating a 30-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.

### ▪ Considerations for the Preparation and Automated Dispensing of Test compounds ▪

Small molecule compounds are typically solvated at high concentration (ideally 1,000x-concentrated) in DMSO and stored frozen as master stocks. For **384-well format assays** these master stocks will be diluted by one of two alternative methods, the selection of which will be dictated by the type of dispensing instrument that is to be used. This Technical Manual provides detailed protocols for each of these two alternative methods:

- a.) Assay setups in which a conventional **tip-based** instrument is used to dispense test compounds into assay wells (in black text). Use **Compound Screening Medium (CSM)** to generate a series of **2x-concentration** test compound treatment media, as described in *Step 2a* of the **Assay Protocol**. The final concentration of DMSO carried over into assay reactions should not exceed 0.4%; strive to use 1,000x-concentrated stocks when they are prepared in any organic solvent.

*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.

*or,*

- b.) Assay setups in which an **acoustic transfer** device is used to dispense test compounds into assay wells (in blue text). Use DMSO to make a series of **1,000x-concentrated** test compound stocks that correspond to each desired final assay concentrations, as described in *Step 2b* of the **Assay Protocol**.

▪ **Considerations for Automated Dispensing of Other Assay Reagents** ▪

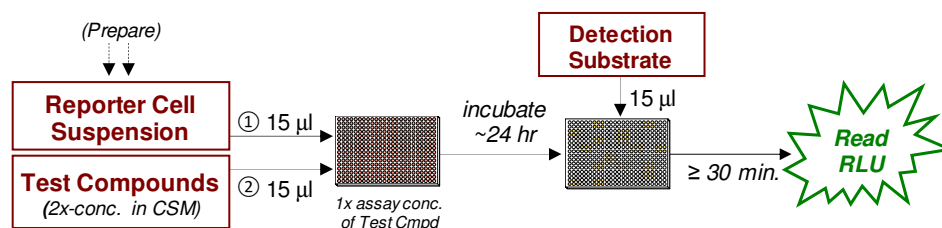
When dispensing into a small number of assay plates, first carefully consider the dead volume requirement of your tip-based dispensing instrument before committing assay reagents to its setup. In essence, "dead volume" is the volume of reagent that is dedicated to the instrument; it will *not* be available for final dispensing into assay wells. The following Table provides information on reagent volume requirements, and available excesses on a *per kit* basis. Always pool the individual reporter cell suspensions and all other respective assay kit reagents before processing multiple 384-well assay plates.

Stock Reagent & Volume provided	Volume to be Dispensed (384-well plate)	Excess rgt. volume available for instrument dead volume
when using tip dispensing of test cmpds <b>Reporter Cell Suspension</b> 7.5 ml	15 µl / well 5.8 ml / plate	~ 1.7 ml
when using acoustic dispensing of test cmpds <b>Reporter Cell Suspension</b> 15 ml	30 µl / well 11.5 ml / plate	~ 3.4 ml
<b>Detection Substrate</b> 7.8 ml	15 µl / well 5.8 ml / plate	~ 2 ml

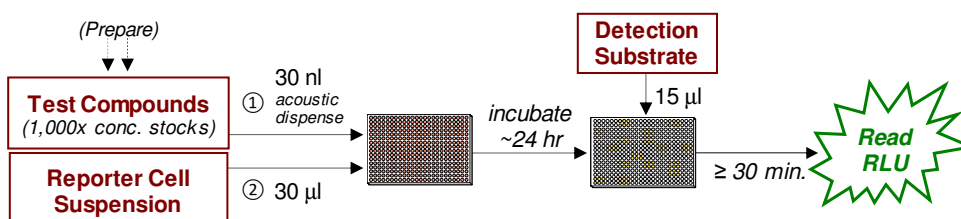
▪ **Assay Scheme** ▪

The *Day 1* preparation, volumes, and chronology of dispensed cells and test compounds are different between assay setups using a *tip-based dispenser (1a)* and those using an *acoustic transfer device (1b)*. Following 22 -24 hours incubation Detection Substrate is added. Light emission from each assay well is quantified using a plate-reading luminometer.

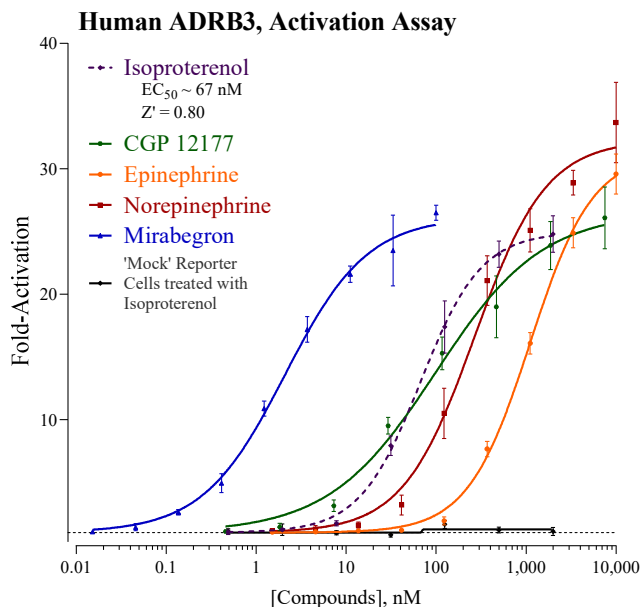
**Figure 1a.** Assay workflow if using conventional **tip-based** dispensing of test compounds.



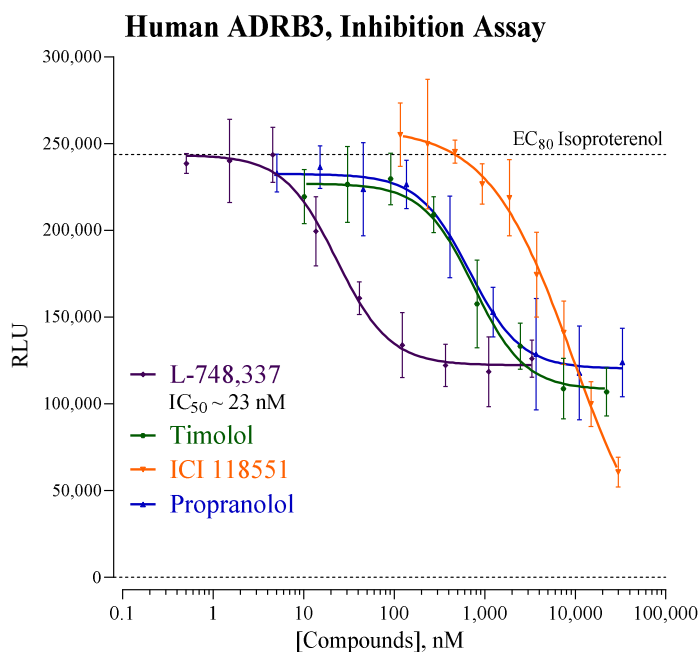
**Figure 1b.** Assay workflow if using **acoustic** dispensing of test compounds.



▪ Assay Performance ▪▪



**Figure 2. Activation of ADRB3.** Reporter cells were treated with the reference activators Isoproterenol (provided), CGP 12177, Epinephrine, Norepinephrine, and Mirabegron. The absence of signal in Isoproterenol treated ‘Mock’ cells (which contain the CRE-Luc reporter vector, but do *not* express ADRB3) confirms that the observed ligand-dependent response is specific to ADRB3 activation.



**Figure 3. Inhibition of ADRB3.** Reporter cells were co-treated with an EC<sub>80</sub> concentration of the reference activator Isoproterenol and varying concentrations of the  $\beta_3$  adrenoceptors selective inhibitor L-748,337, and the non-selective  $\beta$ -adrenoceptor inhibitors Timolol and Propranolol, and the selective  $\beta_2$ -adrenoceptor inhibitor ICI 118551. INDIGO’s Live Cell Multiplex (LCM) Assay confirmed that no treatment concentrations were cytotoxic (data not shown).

For both the activation assay (Figure 2) and inhibition assay (Figure 3), luminescence was quantified and values of average ( $n = 4$ ) RLU, standard deviation (SD), Fold-Activation, and  $Z'^5$  values were calculated. The least-squares method of non-linear regression was used to plot activity changes vs.  $\text{Log}_{10}$  [Compound, nM], and EC<sub>50</sub> /IC<sub>50</sub> values were determined, using GraphPad Prism software. All chemicals were procured from Cayman Chemical (Ann Arbor, MI, USA) except for CGP 12177, which was procured from Tocris (Bristol, UK).

## II. Product Components & Storage Conditions

This assay kit contains materials to perform assays in a single 384-well assay plate.

**Cryopreserved mammalian cells are temperature sensitive! To ensure maximal viability the tube of Reporter Cells must be maintained at -80°C until immediately prior to the rapid-thaw procedure described in this protocol.**

Assay kits are shipped on dry ice. Upon receipt of the kit transfer it to -80°C storage. If you wish to first inventory the individual kit components be sure to first transfer and submerge the tube of cells in dry ice.

The aliquot of Reporter Cells is provided as a single-use reagent. Once thawed, the cells can NOT be refrozen. Nor can they be maintained in extended culture with any hope of retaining downstream assay performance. Therefore, extra volumes of these reagents should be discarded after assay setup.

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

<u>Kit Components</u>	<u>Amount</u>	<u>minimum Storage Temp.</u>
▪ ADRB3 Reporter Cells	1 x 1.0 mL	<b>-80°C</b>
▪ Cell Recovery Medium (CRM)	1 x 7 mL	-20°C
▪ Compound Screening Medium (CSM)	1 x 45 mL	-20°C
▪ Isoproterenol, 2.0 mM (in DMSO)	1 x 80 µL	-20°C
▪ Detection Substrate (Note: contains DTT)	1 x 7.8 mL	<b>-80°C</b>
▪ 384-well assay plate (white, sterile, cell-culture ready)	1	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

- dry ice container
- cell culture-rated laminar flow hood.
- 37°C, humidified 5% CO<sub>2</sub> incubator for mammalian cell culture.
- 37°C water bath.
- 70% alcohol wipes
- 8-channel electronic, repeat-dispensing pipettes & tips suitable for dispensing 15 µL.
- disposable media basins, sterile.
- sterile multi-channel media basins *or* deep-well plates, *or* appropriate similar vessel for generating dilution series of reference compound(s) and test compound(s).
- *Optional*: antagonist reference compound (*e.g.*, Fig. 3.)
- *Optional*: clear 384-well assay plate for viewing cells on *Day 2*.

**DAY 2** plate-reading luminometer.

## IV. Assay Protocol

Review the entire Assay Protocol before starting. Completing the assay requires an overnight incubation. *Steps 1-8* are performed on **Day 1**, requiring less than 2 hours to complete. *Steps 9-13* are performed on **Day 2** and require less than 1 hour to complete.

### ▪ A word about antagonist-mode assay setups ▪

When setting up receptor inhibition assays the Reporter Cells are co-treated with a fixed sub-maximal concentration (typically between EC<sub>50</sub> – EC<sub>85</sub>) of the reference agonist AND varying concentrations of the test compound(s). This ADRB3 Assay kit includes a 2.0 mM stock solution of **Isoproterenol** that may be used to setup inhibition-mode assays. 200 nM of Isoproterenol approximates EC<sub>80</sub> in this assay. Hence, it is a suitable concentration of challenge agonist to use when screening test materials for inhibitory activities.

Add Isoproterenol to a bulk volume of **CSM**, as described above. This agonist-supplemented medium is then used to prepare serial dilutions of test material stocks to achieve the desired respective assay concentrations. This is an efficient and precise method of setting up inhibition assays, and it is the method presented in *Step 5b* of this protocol, and *Step 6b* of the protocol when using an acoustic transfer device to dispense test compounds.

Note that when using a *tip-based instrument* for the dispensing of 2x-concentrated test compounds the cell suspension must also be supplemented with a **2x**-concentration (400 nM) of the challenge agonist Isoproterenol.

When using an *acoustic transfer* device for the dispensing of 1,000x-concentrated test compounds the cell suspension should be supplemented with a **1x**-concentration (200 nM) of the challenge agonist Isoproterenol.

### DAY 1 Assay Protocol:

All steps should be performed using proper aseptic technique.

**1.) Remove Cell Recovery Medium (CRM) and Compound Screening Medium (CSM)** from freezer storage and thaw in a 37°C water bath.

**2.) Prepare dilutions of treatment compounds:** Prepare Test Compound treatment media for *Agonist-* or *Antagonist-mode* screens. NOTE that test and reference compounds will be prepared differently when using tip-dispensing vs. **acoustic dispensing**. Regardless of the method, residual DMSO carried over into assay reactions should not exceed 0.4%.

- a. *Tip dispensing method:* In *Step 6*, 15 µl / well of the prepared treatment media is added to the assay that has been pre-dispensed with 15 µl /well of Reporter Cells. Hence, to achieve the desired *final* assay concentrations one must prepare treatment media with a **2x**-concentration of the test and reference material(s). Use **CSM** to prepare the appropriate dilution series. Plan dilution volumes carefully; this assay kit provides 45 ml of CSM.
- b. *Acoustic dispensing method:* In *Step 6*, 30 nl / well of **1,000x**-concentrated test compound solutions are added to the assay plate using an acoustic transfer device.

**Preparing the positive control:** This assay kit includes a 2.0 mM stock solution of Isoproterenol, an agonist of ADRB3. The following 7-point treatment series, with concentrations presented in **4-fold** decrements, provides a complete dose-response: 2,000, 500, 125, 31.3, 7.81, 1.95, and 0.488 nM. Always include 'no treatment' (or 'vehicle') control wells.

**APPENDIX 1a** provides an example for generating such a dilution series to be used when *tip-dispensing* compound solutions prepared in CSM (15 µl / well).

**APPENDIX 1b** provides an example for generating such a series of 1,000x-concentrated solutions of compounds prepared in DMSO to be used when performing *acoustic dispensing* (transfer 30 nl / well).

(continued)



**When using *tip-based* instrumentation for dispensing test compounds ...**

**3.)** *First*, retrieve the tube of **CRM** from the 37°C water bath, sanitize the outside with a 70% ethanol swab.

*Second*, retrieve **Reporter Cells** from -80°C storage and immerse in dry ice to transport the tube to a laminar flow hood. Perform a *rapid thaw* of the frozen cells by transferring a 6.5 ml volume of 37°C CRM into the tube of frozen cells. Recap the tube of Reporter Cells and place it in a 37°C water bath for 5 - 10 minutes. The resulting volume of cell suspension will be 7.5 ml.

**4.)** Retrieve the tube of Reporter Cell Suspension from the water bath. Sanitize the outside surface of the tube with a 70% alcohol swab, then transfer it into the cell culture hood.

**5.)** *Gently* invert the tube of cells several times to gain a homogenous suspension.

**a. for Agonist-mode assays:** Dispense **15 µl / well** of cell suspension into the assay plate.

~ or ~

**b. for Antagonist-mode assays:** First supplement the bulk volume of Reporter Cell suspension with a 2x-concentration of the challenge agonist Isoproterenol (refer to "A word about antagonist-mode assay setup", pg. 8). Dispense **15 µl / well** of cell suspension into the assay plate.

**6.)** Dispense **15 µl / well** of 2x-concentrated treatment media (from *Step 2a*) into the assay plate.

**When using an *acoustic transfer* device for dispensing test compounds ...**

**3.)** Dispense **30 nl / well** of the 1,000x-concentrated compounds (in DMSO solutions, from *Step 2b*) into the assay plate.

**4.)** *First*, retrieve the tube of **CRM** from the 37°C water bath, sanitize the outside with a 70% ethanol swab.

*Second*, retrieve **Reporter Cells** from -80°C storage and immerse in dry ice to transport the tube to a laminar flow hood. Perform a *rapid thaw* of the frozen cells by transferring a **6.5 ml** volume of 37°C CRM into the tube of frozen cells. Recap the tube of cells and place it in a 37°C water bath for 5 - 10 minutes. The resulting volume of cell suspension will be 7.5 ml.

**5.)** Retrieve the tube of cell suspension from the water bath. Sanitize the outside surface of the tube with a 70% alcohol swab. Add an additional **7.5 ml** of **CSM** to the tube. The resulting volume of cell suspension will be 15 ml.

**6.)** *Gently* invert the tube of cells several times to gain a homogenous cell suspension.

**a. for Agonist-mode assays:** Dispense **30 µl / well** of cell suspension into the assay plate that has been pre-dispensed with test compounds.

~ or ~

**b. for Antagonist-mode assays:** First supplement the bulk volume of Reporter Cell suspension with the challenge agonist Isoproterenol to achieve an EC<sub>50</sub> – EC<sub>80</sub> concentration (refer to "A word about antagonist-mode assay setup", pg. 8). Then dispense **30 µl / well** of the supplemented cell suspension into the assay plate that has been pre-dispensed with test compounds.

*NOTE:* Take special care to prevent cells from settling during the dispensing period. Allowing cells to settle during the transfer process, and/or lack of precision in dispensing uniform volumes across the assay plate *will* cause well-to-well variation (= increased Standard Deviation) in the assay.

(continued)

*NOTE:* Following the dispensing of Reporter Cells and test compounds INDIGO recommends performing a *low-speed* spin of the assay plate (with lid) for  $\leq 1$  minute using a room temperature centrifuge fitted with counter-balanced plate carriers.

- 7.) Transfer the assay plate into a 37°C, humidified, 5% CO<sub>2</sub> incubator for 22 - 24 hours.

*NOTE:* Ensure a high-humidity ( $\geq 70\%$ ) environment within the cell culture incubator. This is critical to prevent the onset of deleterious "edge-effects" in the assay plate.

- 8.) For greater convenience on *Day 2*, retrieve **Detection Substrate** from freezer storage and place 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 a bench top or in a **fume hood**.

- 9.) Approximately 30 minutes before intending to quantify receptor activity remove **Detection Substrate** from the refrigerator and place it in a low-light area so that it may equilibrate to room temperature. Gently invert the tube several times to ensure a homogenous solution.

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

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

- 11.) Following 22 - 24 hours of incubation dispense **15  $\mu$ l / well** of **Detection Substrate** into all wells of the assay plate.

*NOTE:* 'Detection Substrate' contains a high concentration of DTT, which produces a strong odor that some users may find objectionable. It is advised to work in a **fume hood** when dispensing Detection Substrate into the assay plate and during the following 'plate rest' period.

*NOTE:* Perform this reagent transfer carefully to avoid bubble formation! Scattered micro-bubbles will not pose a problem. However, bubbles covering the surface of the reaction mix, or large bubbles clinging to the side walls of the well, will cause lens-effects that will degrade the accuracy and precision of the assay data. INDIGO recommends performing a final *low-speed* spin of the assay plate (with lid) for  $\leq 1$  minute using a room temperature centrifuge fitted with counter-balanced plate carriers.

- 12.) Allow the plate(s) to rest at room temperature for 30 minutes. Do not shake the assay plate(s) during this period.

*NOTE:* the luminescent signal is unstable during the first 30 minutes of the luciferase reaction, however, after the initial 30-minute reaction period the luminescence signal achieves a stable emission output.

- 13.) Quantify luminescence.

- 14.) Data analyses.

## ***V. Related Products***

<b><i>Product No.</i></b>	<b><i>Product Descriptions</i></b>
<b>Human ADRB3 Assays</b>	
IB32201	Human ADRB3 Reporter Assay System 1x 96-well format assay
IB32202	Human ADRB3 Reporter Assay System 1x 384-well format assay
<b>Human ADRA1A Assays</b>	
IB31001	Human ADRA1A Reporter Assay System 1x 96-well format assay
IB31002	Human ADRA1A Reporter Assay System 1x 384-well format assay
<b>Human ADRA1B Assays</b>	
IB31101	Human ADRA1B Reporter Assay System 1x 96-well format assay
IB31102	Human ADRA1B Reporter Assay System 1x 384-well format assay
<b>Human ADRA1D Assays</b>	
IB31201	Human ADRA1D Reporter Assay System 1x 96-well format assay
IB31202	Human ADRA1D Reporter Assay System 1x 384-well format assay
<b>Human ADRA2A Assays</b>	
IB34001	Human ADRA2A Reporter Assay System 1x 96-well format assay
IB34002	Human ADRA2A Reporter Assay System 1x 384-well format assays
<b>Human ADRB1 Assays</b>	
IB32001	Human ADRB1 Reporter Assay System 1x 96-well format assay
IB32002	Human ADRB1 Reporter Assay System 1x 384-well format assay

<b>Human ADRB2 Assays</b>	
IB32101	Human ADRB2 Reporter Assay System 1x 96-well format assay
IB32102	Human ADRB2 Reporter Assay System 1x 384-well format assay
<b>NFAT Assays</b> (recommended for receptor specificity screening)	
IB18001	NFAT Reporter Assay System 1x 96-well format assay
Bulk volumes of Assay Reagents may be custom manufactured to accommodate any scale of HTS. Please Inquire.	

<b>LIVE Cell Multiplex (LCM) Assay</b>	
LCM-01	Reagent volumes sufficient to perform <b>96</b> Live Cell Assays
LCM-05	Reagent in <b>5x bulk volume</b> to perform <b>480</b> Live Cell Assays contained in 5 x 96-well assay plates
LCM-10	Reagent in <b>10x bulk volume</b> to perform <b>960</b> Live Cell Assays contained in 10 x 96-well assay plates

<b>INDIGlo Luciferase Detection Reagent</b>	
LDR-10, -25, -50, -500	INDIGlo Luciferase Detection Reagents in 10 mL, 25 mL, 50 mL, and 500 mL volumes

Please refer to INDIGO Biosciences website for updated product offerings.

**[www.indigobiosciences.com](http://www.indigobiosciences.com)**

## VI. Literature Citations

- <sup>1</sup> Perez DM (2020)  $\alpha$ 1-Adrenergic Receptors in Neurotransmission, Synaptic Plasticity, and Cognition. *Front. Pharmacol.* 11:581098. doi: 10.3389/fphar.2020.581098.
- <sup>2</sup> Schena G (2019) Everything You Always Wanted to Know about  $\beta_3$ -AR\* (\*But Were Afraid to Ask). *Cells.* 8, 357; doi:10.3390/cells8040357.
- <sup>3</sup> Michel LYM. (2020). The Beta3 Adrenergic Receptor in Healthy and Pathological Cardiovascular Tissues. *Cells.* 9, 2584; doi:10.3390/cells9122584.
- <sup>4</sup> Pasha A (2024). Inside the Biology of the  $\beta_3$ -Adrenoceptor. *Biomolecules.* 14, 159. doi: 10.3390/biom14020159.
- <sup>5</sup> Zhang JH, *et al.* (1999) A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screen.*:4(2), 67-73.

$$Z' = 1 - [3*(SD^{Ref EC100} + SD^{Untreated}) / (RLU^{Ref EC100} - RLU^{Untreated})]$$

## VII. Limited Use Disclosures

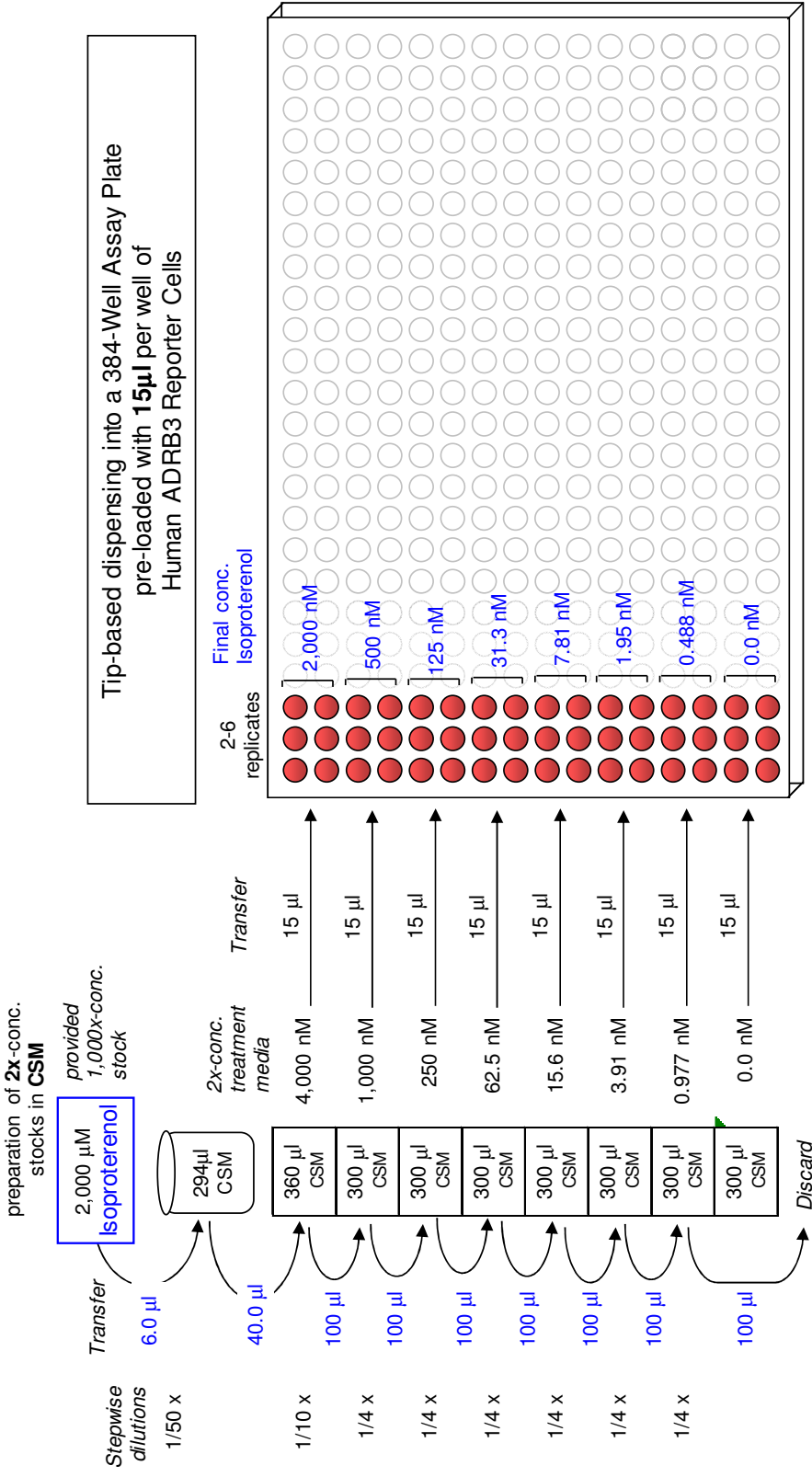
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Product prices, availability, specifications, claims and technical protocols are subject to change without prior notice. The printed Technical Manual provided in the kit box will always be the most recently updated version available.

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**APPENDIX 1a for tip-based dispensing.** Example scheme for the serial dilution of Isoproterenol using CSM to generate **2x-concentrated** treatment media. A *tip-based* instrument is used to dispense 15  $\mu\text{l}$  / well into an assay plate that has been *pre-dispensed* with 15  $\mu\text{l}$  / well of ADRB3 Reporter Cells suspension.



**APPENDIX 1b for acoustic dispensing.** Example scheme for the serial dilution of Isoproterenol using **DMSO** to generate **1,000x-concentrated** stocks. 30 nl / well of these prepared stocks are first dispensed into *empty* wells of the assay plate using an acoustic transfer device, followed by the dispensing of 30  $\mu$ l / well of ADRB3 reporter cells.

