PathHunter® PTH Bioassay Kit

For chemiluminescent detection of parathyroid hormone (PTH) activity

Simple Solutions for Complex Biology

DiscoveRx

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Read the entire product insert before beginning the assay
For additional information or Technical Support, contact SupportUS@discoverx.com or visit www.discoverx.com.

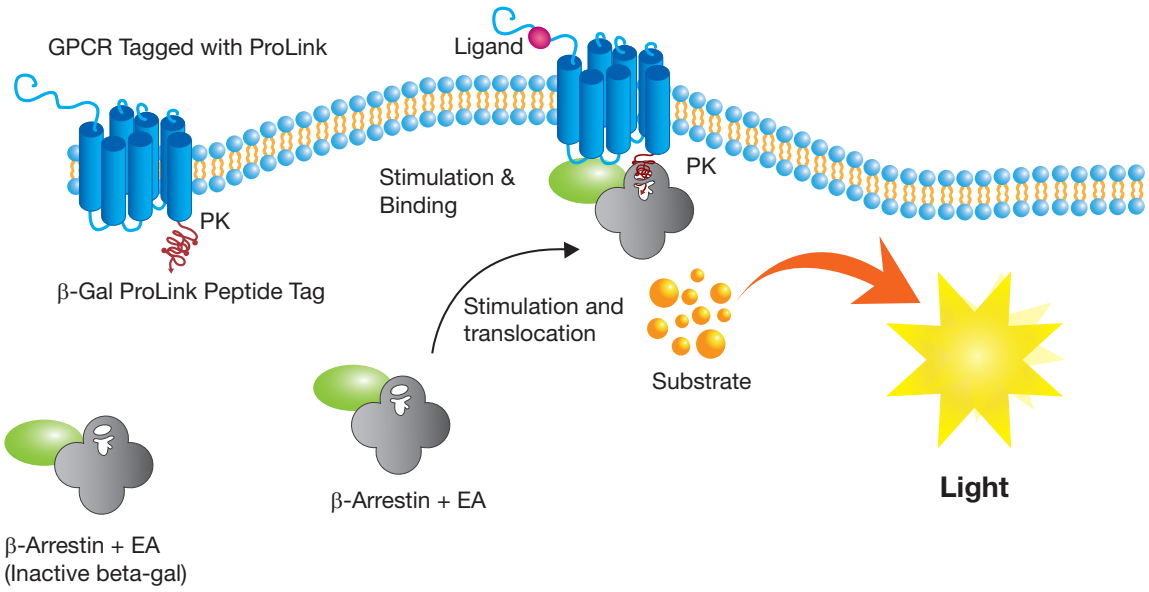
Overview

Intended Use

PathHunter® Parathyroid Hormone (PTH) Bioassay kits are ready to use kits that contain everything necessary to perform a functional assay with live cells, without any continuous cell culture. This kit has been designed to rapidly detect the potency of your biologic or the presence of neutralizing antibodies against the biologic. The Bioassay kits include single use vials of frozen cells expressing the PTH receptor, chemiluminescent detection reagents, control agonist, optimized cell plating reagent, protein dilution buffer and plates. Simply thaw and plate the pre-validated cells and treat with test reagents within 24 to 48 hours. Assays have been designed for 96-well formats.

Technology Principle: PathHunter® PTH Bioassay

The PathHunter PTH Bioassay monitors GPCR activity by detecting the interaction of β-Arrestin with the activated PTH GPCR using β-galactosidase (β-gal) enzyme fragment complementation. In this system, the GPCR of interest is fused in frame with the small, 42 amino acid fragment of β-gal called ProLink™ and co-expressed in cells stably expressing a fusion protein of β-Arrestin and the larger, N-terminal deletion mutant of β-gal (called enzyme acceptor or EA). Activation of the GPCR stimulates binding of β-Arrestin to the ProLink-tagged GPCR and forces complementation of the two enzyme fragments of β-galactosidase, resulting in the formation of an active β-gal enzyme. This action leads to an increase in enzyme activity that can be measured using chemiluminescent PathHunter Bioassay Detection Reagents. Because arrestin recruitment occurs independent of G-protein coupling, these assays provide a direct, universal platform for measuring receptor activation, thus providing an ideal tool to characterize functional biologics.



Materials Provided

Description	Contents (93-0315Y2-00047)	Contents (93-0315Y2-00048)
PathHunter PTH Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit	200 dp	1,000 dp
• Detection Reagent 1	2 mL	10 mL
• Detection Reagent 2	8 mL	40 mL
AssayComplete Cell Plating 0 Reagent	1 x 100 mL	3 x 100 mL
Protein Dilution Buffer	1 x 50 mL	2 x 50 mL
Control Ligand (PTH (1-34))	1 vial	1 vial
96-well Tissue Culture Treated Plates	2 plates	10 plates

Storage Conditions

PathHunter PTH Bioassay Cells

Cells are shipped on dry ice and should arrive in a frozen state. To ensure maximum cell viability, thaw the vials as soon as possible upon receipt.

If continued storage of the frozen vials is necessary, store as follows:

- Short term (2 weeks or less): Store vials at -80°C immediately upon arrival. (DO NOT store at –80°C for more than 2 weeks).
- Long term (greater than 2 weeks): Vials should ONLY be stored in the vapor phase of liquid nitrogen (LN_2).

Safety Warning: A face shield, gloves and lab coat should be worn at all times when handling frozen vials. The manufacturer of the cryovial recommends storing the vials in the vapor phase above the liquid N_2. Upon thawing, if LN_2 is present in the cryovial, it rapidly converts back to its gas phase which can result in the explosion of the vial upon its removal from the liquid nitrogen tank.

PathHunter Bioassay Detection Kit

Store at -20°C. Once thawed, the detection reagents can be kept at 4°C for up to 4 days. For longer storage (up to the expiration date listed in the kit certificate of analysis), the reagent should be aliquoted and stored at -20°C until needed. Avoid multiple freeze-thaw cycles.

To make aliquots suitable for testing one assay plate each, 1 mL of Detection Reagent 1 per aliquot should be dispensed and frozen down. 4 mL of Detection Reagent 2 per aliquot should be dispensed and frozen down separately. Do not mix the two reagents during aliquoting.

AssayComplete Cell Plating 0 Reagent

Once thawed, the Cell Plating Reagent can be stored at 4°C for up to 4 weeks. For longer storage (up to the expiration date listed in the kit certificate of analysis), the reagent should be aliquoted and stored at -20°C until needed. Avoid multiple freeze-thaw cycles. To make aliquots suitable for testing one assay plate each, 30 mL of reagent per aliquot should be dispensed and frozen down.

Protein Dilution Buffer

Once thawed, the Protein Dilution Buffer can be stored at 4°C for up to 4 weeks. For longer storage (up to the expiration date listed in the kit certificate of analysis), the reagent should be aliquoted and stored at -20°C until needed. Avoid multiple freeze-thaw cycles. To make aliquots suitable for testing one assay plate each, 10 mL of reagent per aliquot should be dispensed and frozen down. This amount may vary depending on your stock sample concentrations and should be adjusted accordingly.

96-well Tissue Culture Treated Plates

Store at room temperature.

Additional Materials Required

The following equipment and additional materials are required to perform these assays:

Equipment
Single and multichannel micro-pipettors and pipette tips
Multimode or luminescence plate reader
V-Bottom 96-well compound dilution plates (DiscoveRx, Cat. #92-0011 or similar)
Disposable Reagent Reservoir (Thermo Scientific, Cat. #8094 or similar)

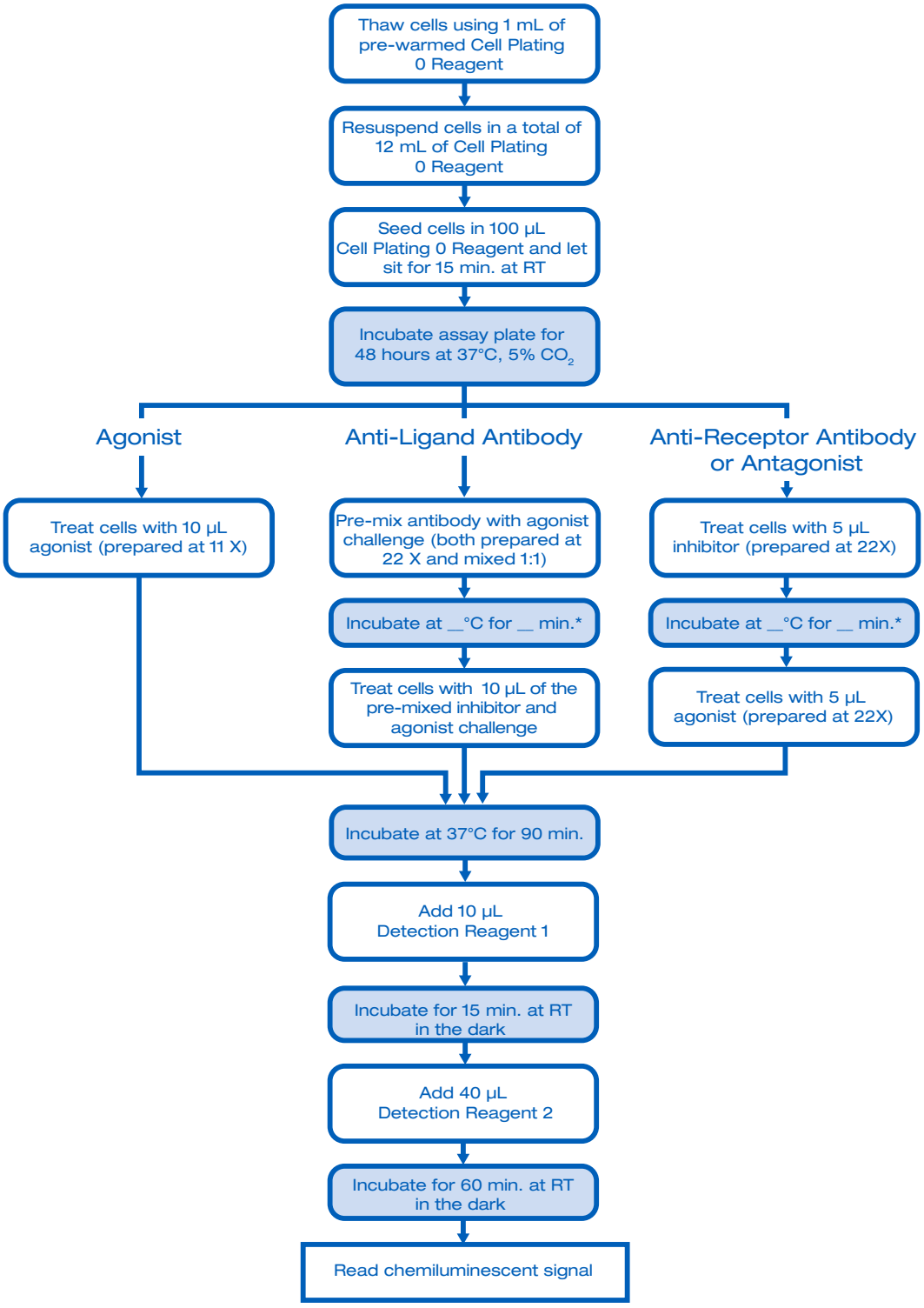
Protocol Schematic

Tip: *Use this sheet to note your assay specific conditions. Post on your bench to use as a quick reference guide.*

Assay Name:_____

Product Number: _____

Quick-Start Procedure: In a 96-well tissue culture treated plate perform the following:



* Incubation times and temperatures.

Detailed Protocols

PathHunter PTH Bioassay Cell Preparation:

The following protocol is for thawing and plating frozen PathHunter PTH Bioassay cells from cryovials. These assays are optimized for 96-well plates. For volumes associated with 384-well format, please refer to page 12.

1. Before thawing the cells, ensure that all the necessary materials required are set up in the tissue culture hood. This includes:

    b. One 25 mL reagent reservoir.

    c. One 15 mL conical tube.

    d. A micropipettor (P1000) set to dispense 1 mL.

    e. A multichannel pipette and tips set to dispense 100 µL.

    f. A bottle of Cell Plating 0 Reagent (pre-warmed in a 37˚C water bath for 15 minutes). Refer to the target-specific datasheet for the recommended Cell Plating Reagent.

    g. A white-walled 96-well assay plate.

2. Dispense 12 mL of Cell Plating Reagent into the 15 mL conical tube.

3. Remove the cryovial from liquid nitrogen and immediately place in dry ice.

DO NOT use heated water bath to thaw the vial. Wait at least one minute for any liquid N_2 inside the vial to evaporate, then remove the cryovial from dry ice, wipe down quickly with 70% EtOH, and bring it into the tissue culture hood right away. **DO NOT** touch the sides or bottom of the vial to avoid thawing of the cell pellet through body heat.

4. Immediately add 1 mL (using P1000) of pre-warmed Cell Plating Reagent from the 15 mL conical tube to the cryovial, thawing the cell pellet. The medium should be added slowly along the side of the wall of cryovial tube. Mix the cells gently by pipetting up and down several times to break up any clumps. Transfer the cell suspension to the conical tube containing the remaining 11 mL of Cell Plating Reagent. Remove any medium/suspension left in the tube to ensure complete recovery of all the cells from the vial.

5. Mix the tube by inversion to ensure the cells are properly mixed in the medium without creating any froth in the suspension and immediately pour the suspension into the reagent reservoir.

6. Add 100 µL of cells to each well of the 96-well assay plate using the multichannel pipette.

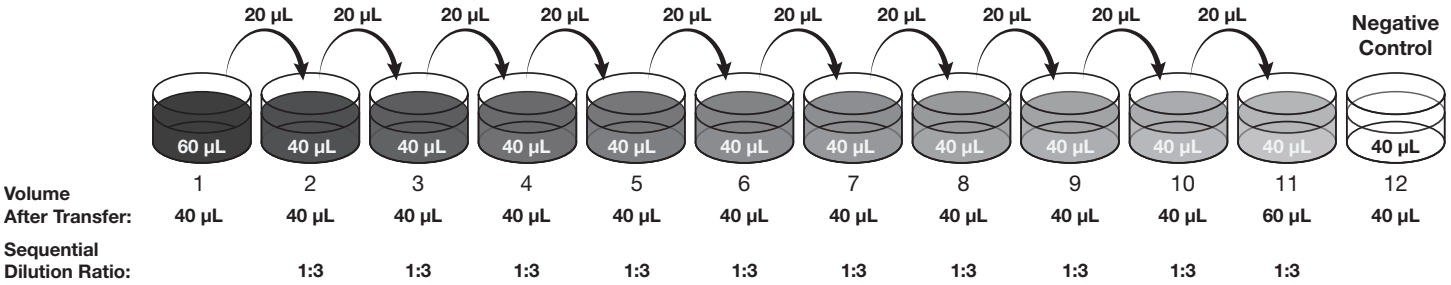
7. Let the assay plate sit for 15 minutes at room temperature, then gently place the assay plate in a tissue culture incubator set to 37˚C, 5% CO_2 for 48 hours.

Ligand Preparation

The following protocol is designed for testing purified biologics. The PathHunter assays can also be run in the presence of high levels of serum or plasma without significantly impacting assay performance. Therefore, standard curves of control can typically be prepared in neat serum or plasma and added directly to cells without further dilution. For the best results, the optimized minimum required dilution (MRD) of crude samples should be empirically determined.

Method 1

A 1:3 serial dilution. This method is simple to run and covers a broad concentration range, making it ideal for initial assay optimization. The volumes listed below are designed for running samples from one dose response curve in duplicate on the assay plate (as pictured in the assay plate map on page 11).



	1	2	3	4	5	6	7	8	9	10	11	12
Volume After Transfer:	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	60 µL	40 µL
Sequential Dilution Ratio:		1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	1:3	

1. Sample Preparation - Samples are prepared differently based on their primary mode of action: agonists that activate the pathway, anti-receptor antibodies or antagonists that bind to the receptor and block agonist activity, or anti-ligand antibodies that bind to and prevent agonist ligands from binding to the receptor.

    a. Agonists - Prepare the reference agonist (PTH) or sample agonist dose response curve. Agonists are prepared at 11X the desired final concentration. For reference agonist:

        i. Prepare a 500 µM stock concentration of the reference agonist (PTH (1-34)) by adding 243 µL of reconstitution buffer to the lyophilized powder.

        ii. In a separate tube, dilute the stock concentration of PTH (1-34) to make a 11 µM PTH (1-34) concentration (11X the final concentration of 1 µM). Do this by adding 4.4 µL of the stock concentration of PTH (1-34) to 195.6 µL of Protein Dilution Buffer (PDB).

        iii. Add 60 µL of the 11 µM PTH (1-34) to well column 1 of the dilution plate (e.g. a V-bottom polypropylene 96-well dilution plate, DiscoveRx Cat. #92-0011 or similar).

        iv. Add 40 µL of Protein Dilution Buffer (PDB) to wells column 2 through column 12 of a master dilution plate.

        v. Using a clean tip, transfer 20 µL from column 1 into column 2 and mix by pipetting up and down several times. Replace the pipette tip, and transfer 20 µL from column 2 into column 3. Mix by pipetting up and down several times. Repeat this process until column 11 is reached, resulting in an eleven point, 1:3 dilution series. No ligand is transferred to column 12 as this will serve as a negative control.

    b. Anti-receptor antibodies or antagonists - These samples are prepared at 22X the desired final concentration, and are typically pre-incubated with the cells prior to adding a challenge dose of the reference agonist.

        i. Add 60 µL of 22X sample to column 1 on a new row of the master dilution plate.

ii. Add 40 µL of Protein Dilution Buffer (PDB) to columns 2 through 12 of this row of the master dilution plate.

iii. Using a clean tip, transfer 20 µL from column 1 into column 2 and mix by pipetting up and down several times. Replace the pipette tip, and transfer 20 µL from column 2 into column 3. Mix by pipetting up and down several times. Repeat this process until column 11 is reached, resulting in an eleven point, 1:3 dilution series. No ligand is transferred to column 12 as this will serve as a negative control.

iv. Prepare the agonist challenge. Determine the EC_{80} of the reference agonist and prepare the agonist challenge at 22x this desired final concentration.

v. Remove the assay plate from the 37°C, 5% CO_2 incubator.

vi. Add 5 µL of 22x inhibitor to the appropriate wells of the assay plate. Pre-incubating the inhibitor on the cells prior to the addition of the agonist may decrease background signal and improve the assay window. The optimal pre-incubation time can be determined empirically. A typical incubation time is 15-30 min.

vii. Once the pre-incubation is complete, add 5 µL of 22x agonist challenge to the appropriate wells of the assay plate.

c. Anti-ligand antibody - These samples are prepared at 22X the desired final concentration, and are typically pre-incubated with their target antigen prior to adding to the assay plate.

i. Add 60 µL of 22X sample to column 1 on a new row of the master dilution plate.

ii. Add 40 µL of Protein Dilution Buffer (PDB) to columns 2 through 12 of this row of the master dilution plate.

iii. Using a clean tip, transfer 20 µL from column 1 into column 2 and mix by pipetting up and down several times. Replace the pipette tip, and transfer 20 µL from column 2 into column 3. Mix by pipetting up and down several times. Repeat this process until column 11 is reached, resulting in an eleven point, 1:3 dilution series. No ligand is transferred to column 12 as this will serve as a negative control.

iv. Prepare the agonist challenge. Determine the EC_{80} of the reference agonist and prepare the agonist challenge at 22x this desired final concentration.

v. Add 20 µL of 22x agonist challenge and 20 µL of the 22x inhibitor dilution series to columns 1-11 of a pre-mixing plate (e.g. a V-bottom polypropylene 96-well dilution plate, DiscoveRx Cat. #92-0011 or similar). 40 µL of PDB can be added to the negative control well of column 12 to maintain equal volumes in all wells. Mix the contents of the pre-mixing plate wells thouroughly by pipetting up and down. Pre-incubating the antibody with the agonist challenge before continuing may decrease background signal and improve the assay window. The optimal pre-incubation time can be determined empirically. A typical incubation time is 15-30 minutes.

vi. After the pre-incubation, add 10 µL from each well of the pre-incubation plate to the appropriate wells of the assay plate.

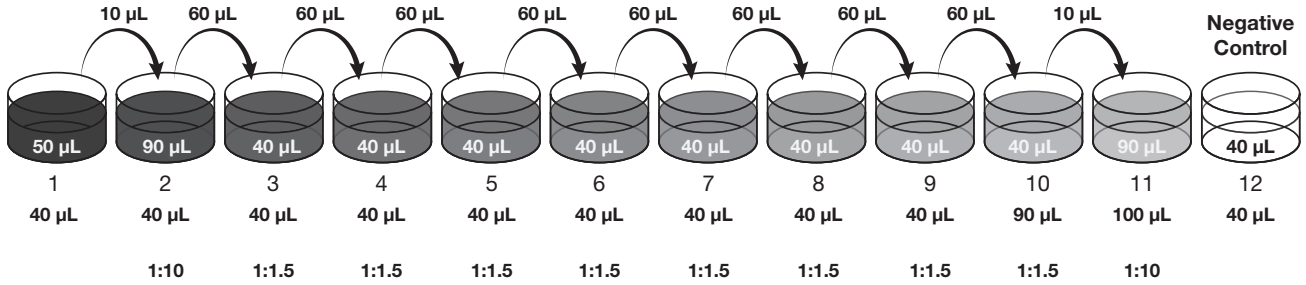
2. Remove the assay plate from the 37°C, 5% CO2 incubator. Allow the plate to equilibrate to room temperature for 15 minutes.

3. Add 10 µL from the agonist reference curve on the master dilution plate to the appropriate wells of the assay plate.

4. Return the assay plate to the 37°C, 5% CO_2 incubator and incubate 90 minutes.

Method 2

This method covers a smaller concentration range in order to generate more data points within the linear range of the dose response curve, and is suitable for testing ligands with known pharmacology in this assay platform. The volumes listed below are designed for running samples from one dose response curve in triplicate on the assay plate (as pictured in the example assay plate map on page 11).



	1	2	3	4	5	6	7	8	9	10	11	12
Volume After Transfer:	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	40 µL	90 µL	100 µL	40 µL
Sequential Dilution Ratio:		1:10	1:1.5	1:1.5	1:1.5	1:1.5	1:1.5	1:1.5	1:1.5	1:1.5	1:10	

1. Sample Preparation - Samples are prepared differently based on their primary mode of action: agonists that activate the pathway, anti-receptor antibodies or antagonists that bind to the receptor and block agonist activity, or anti-ligand antibodies that bind to and prevent agonist ligands from binding to the receptor.

    a. Agonists - Prepare the reference agonist (PTH) or sample agonist dose response curve. Agonists are prepared at 11X the desired final concentration. For reference agonist:

        i. Prepare a 500 µM stock concentration of the reference agonist (PTH (1-34)) by adding 243 µL of reconstitution buffer to the lyophilized powder.

        ii. In a separate tube, dilute the stock concentration of PTH (1-34) to make a 11 µM PTH (1-34) concentration (11X the final concentration of 1 µM). Do this by adding 4.4 µL of the stock concentration of PTH (1-34) to 195.6 µL of Protein Dilution Buffer (PDB).

        iii. Add 50 µL of the 11 µM PTH (1-34) to column 1 of the dilution plate (e.g. a V-bottom polypropylene 96-well dilution plate, DiscoveRx Cat. #92-0011 or similar).

        iv. Add 90 µL of Protein Dilution Buffer (PDB) to wells column 2 and column 11 of a master dilution plate. Then add 40 µL of PDB to the remaining nine empty wells in the same row.

        v. Using a clean tip, transfer 10 µL from well column 1 into well column 2 and mix by pipetting up and down several times with a pipettor set to 60 µL.

        vi. Replace the pipette tip, and transfer 60 µL from well column 2 into well column 3. Mix by pipetting up and down several times. Repeat this process until column 10 is reached.

        vii. Using a clean tip, transfer 10 µL from well column 10 into well column 11 and mix by pipetting up and down several times with a pipettor set to 60 µL. No ligand is transferred to well column 12 as this will serve as a negative control.

    b. Anti-receptor antibodies or antagonists - These samples are prepared at 22X the desired final concentration, and are typically pre-incubated with the cells prior to adding a challenge dose of the reference agonist.

        i. Add 50 µL of 22X sample to column 1 on a new row of the master dilution plate.

        ii. Add 90 µL of PDB to columns 2 and 11. Then add 40 µL of PDB to the remaining nine empty wells in the same row.

iii. Using a clean tip, transfer 10 µL from column 1 into column 2 and mix by pipetting up and down several times with a pipettor set to 60 µL.

iv. Replace the pipette tip, and transfer 60 µL from column 2 into column 3. Mix by pipetting up and down several times. Repeat this process until column 10 is reached.

v. Using a clean tip, transfer 10 µL from column 10 into column 11 and mix by pipetting up and down several times with a pipettor set to 60 µL.

vi. Prepare the agonist challenge. Determine the EC_{80} of the reference agonist and prepare the agonist challenge at 22x this desired final concentration.

vii. Remove the assay plate from the 37°C, 5% CO_2 incubator.

viii. Add 5 µL of 22x inhibitor to the appropriate wells of the assay plate. Pre-incubating the inhibitor on the cells prior to the addition of the agonist may decrease background signal and improve the assay window. The optimal pre-incubation time can be determined empirically. A typical incubation time is 15-30 min.

ix. Once the pre-incubation is complete, add 5 µL of 22x agonist challenge to the appropriate wells of the assay plate.

c. Anti-ligand antibody - These samples are prepared at 22X the desired final concentration, and are typically pre-incubated with their target antigen prior to adding to the assay plate.

i. Add 50 µL of 22X sample to column 1 on a new row of the master dilution plate.

ii. Add 90 µL of PDB to columns 2 and 11. Then add 40 µL of PDB to the remaining nine empty wells in the same row.

iii. Using a clean tip, transfer 10 µL from column 1 into column 2 and mix by pipetting up and down several times with a pipettor set to 60 µL.

iv. Replace the pipette tip, and transfer 60 µL from column 2 into column 3. Mix by pipetting up and down several times. Repeat this process until column 10 is reached.

v. Using a clean tip, transfer 10 µL from column 10 into column 11 and mix by pipetting up and down several times with a pipettor set to 60 µL.

vi. Prepare the agonist challenge. Determine the EC_{80} of the reference agonist and prepare the agonist challenge at 22x this desired final concentration.

vii. Add 20 µL of the 22x inhibitor dilution series and 20 µL of the 22x agonist challenge to columns 1-11 of a pre-mixing plate (e.g. a V-bottom polypropylene 96-well dilution plate, DiscoveRx Cat. #92-0011 or similar). 40 µL of PDB can be added to the negative control well of column 12 to maintain equal volumes in all wells. Mix the contents of the pre-mixing plate wells thouroughly by pipetting up and down. Pre-incubating the antibody with the agonist challenge before continuing may decrease background signal and improve the assay window. The optimal pre-incubation time can be determined empirically. A typical incubation time is 15-30 minutes.

viii. After the pre-incubation, add 10 µL from each well of the pre-incubation plate to the appropriate wells of the assay plate.

2. Remove the assay plate from the 37°C, 5% CO_2 incubator. Allow the plate to equilibrate to room temperature for 15 minutes.
3. Add 10 µL from the agonist reference curve and any agonist samples on the master dilution plate to the appropriate wells of the assay plate.
4. Return the assay plate to the 37°C, 5% CO_2 incubator and incubate for 90 minutes.

Detection

1. Using a multichannel pipette, add 10 µL of Detection Reagent 1 to each well of the assay plate.
2. Incubate the plate at room temperature for 15 minutes in the dark.
3. Using a multichannel pipette add 40 µL of Detection Reagent 2 to each well of the assay plate.
4. Incubate the plate at the temperature and time indicated on the product insert in the dark.
5. Read sample on a standard luminescence plate reader at 0.1 to 1 second/well for PMT readers of 5-10 seconds for imager.

Note: For crude biologic samples, gently removing the liquid from all wells and replacing with 100 µL of Cell Plating Reagent before the addition of the detection reagents can result in higher signal. Additional Cell Plating Reagent is necessary for this method.

Representative Plate Maps for Agonist/Inhibitor Curves

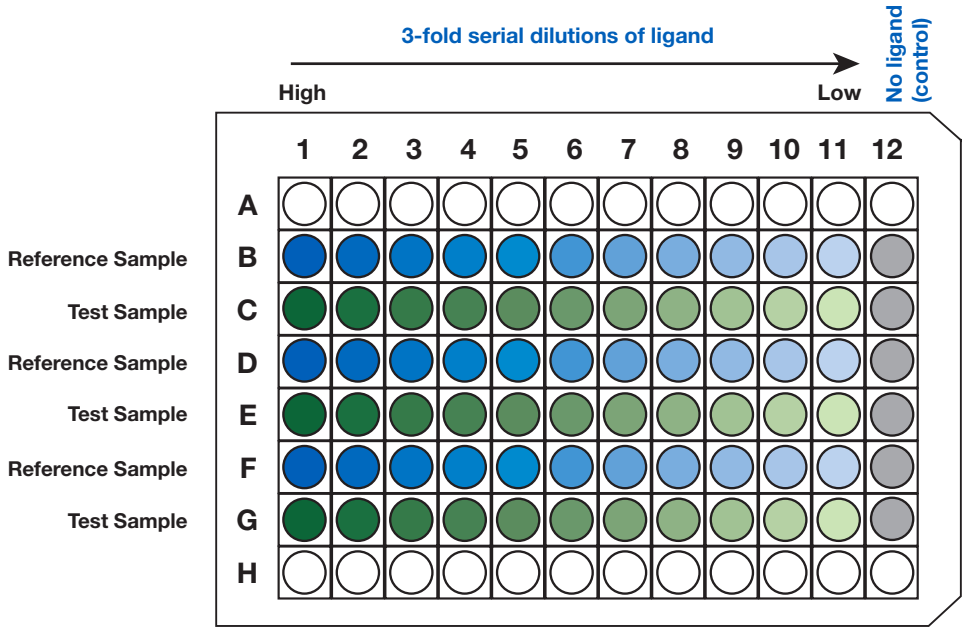


Figure 1. Serial dilution method 1. This plate map shows 11-point dose curves with three data points at each concentration for one reference and one test sample per plate.

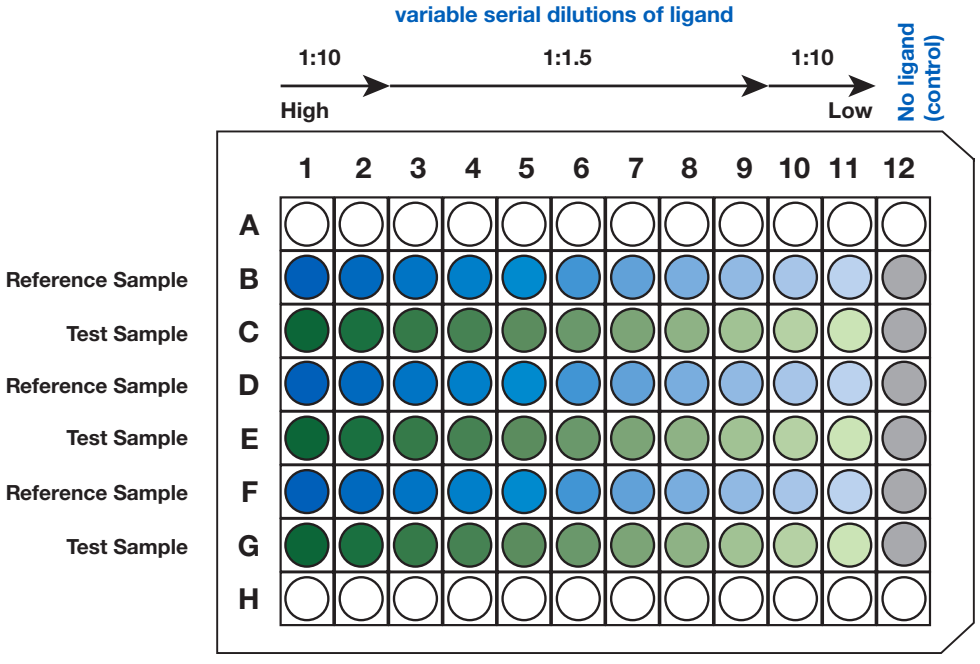


Figure 2. Serial dilution method 2. This plate map shows a 11 point dose curve with 3 data points at each concentration for one reference and one test sample per plate, with a variable serial dilution scheme.

Assay Formats Table

PathHunter Assay Format		
Plate Format	96-well	384-well
Total Volume	160 µL	37.5 µL
Cell Numbers	10,000	5,000
AssayComplete Cell Plating Reagents*	100 µL	20 µL
Test Sample	10 µL	5 µL
Bioassay Detection Reagents	50 µL	12.5 µL

*AssayComplete Cell Plating Reagent volume used to resuspend cells for assay plates

Related Products	
Description	**Ordering Information**
PathHunter Bioassay Detection Reagents	www.discoverx.com/detectionreagents
Cell Culture Kits, Reagents & Consumables	www.discoverx.com/cell-culture-kits-reagents-consumables
AssayComplete Cell Plating Reagents	www.discoverx.com/cellplatingreagents
Control Ligands	www.discoverx.com/controlligands

Instrument Compatibility Chart		
Assay	**Instrument**	**Read-Out**
All PathHunter® assays HitHunter® cAMP HitHunter® cGMP	**COMPATIBLE WITH ANY LUMINOMETER** **BMG:** PheraStar, Cytostar, LumiStar **Perkin Elmer:** TopCount, Victor 2 or V, Fusion, LumiCount, Envision, Micro-beta (Trilux), Viewlux, Northstar **GE:** LEAD seeker, Farcyte **Molecular Devices:** FLIPR, SpectraMax M3/ M4/M5/M5e, FlexStation 3, SpectraMax L **Tecan:** Ultra, Evolution **Turner BioSystems:** Modulus Microplate **Caliper** LabChip 3000 & EZ Reader **Berthold Technologies:** Mithras LB940, CentroLIApc **Hamamatzu** FDS6000, FDSS/RayCatcher **Thermo Scientific:** Luminoskan Ascent **Biotek:** Synergy 2	Luminescence

*For other instruments not listed here, please contact technical support at SupportUS@discoverx.com or SupportEU@discoverx.com to ensure compatibility.

Troubleshooting Guide

Problem	Cause	Solution
No Response	Improper thawing procedure	Refer to thawing instructions on page 5 of this user manual.
	Improper ligand used or improper ligand incubation time	See datasheet for recommended ligand and assay conditions
	Improper preparation of ligand (agonist or antagonist)	Refer to vendor specific datasheet to ensure proper handling, dilution and storage of ligand
	Improper time course for induction	Optimize time course of induction with agonist and antagonist
Decreased Response	Cells are not adherent and exhibit incorrect morphology	Confirm adherence of cells using microscope
Low or No Signal	Improper preparation of detection reagents	Detection reagents should ideally be prepared just prior to use and are sensitive to light
	Problem with microplate reader	Microplate reader should be in luminescence mode. Read at 0.1-1 sec/well.
Experimental S:B does not match datasheet value	Incorrect incubation temperature	Confirm assay conditions
		Check and repeat assay at correct incubation temperature as indicated on the assay datasheet
	Improper preparation of ligand (agonist or antagonist)	Some ligands are difficult to handle. Confirm the final concentration of ligands
EC_{50} is right-shifted	Improper ligand handling or storage	Check ligand handling requirements
	Difference in agonist binding affinity	Confirm that the ligand used is comparable to the ligand in the Product Insert
	Problems with plate type and compound stability	Hydrophobic compounds should be tested for solubility and may be diluted in Protein Dilution Buffer

For additional information or technical support, please contact technical support at SupportUS@discoverx.com or SupportEU@discoverx.com.

Limited Use License Agreement

A. This product and/or its use is covered by U.S. patents #6,342,345 B1, #7,135,325, B2, #8,101,373 B2 and/or related foreign patents and pending applications and trade secrets that are either owned by or licensed to DiscoveRx Corporation ("DiscoveRx"). The cells and detection reagents (collectively "Materials") purchased from DiscoveRx are expressly restricted in their use. DiscoveRx has developed a Cell-Based assay ("Assay") that employs genetically modified cells and vectors (collectively, the “Cells”), and related detection reagents (the “Reagents”) (collectively referred to as “Materials”). By purchasing and using the Materials, the Purchaser agrees to comply with the following terms and conditions of this label license and recognizes and agrees to such restrictions:

1. Purchaser will not further propagate the Cells included in the Assay kit.
2. Purchaser is permitted to use the Cells only for use in the Assay and in connection with Reagents purchased from DiscoveRx or its authorized distributor.
3. The Materials are not transferable and will be used only at the site for which they were purchased. Transfer to another site owned by Purchaser will be permitted only upon written request by Purchaser followed by subsequent written approval by DiscoveRx.
4. The Reagents contain or are based upon the proprietary and valuable know-how developed by DiscoveRx, and the Reagents have been optimized by DiscoveRx to function more effectively with the Cells in performing the Assay. Purchaser will not analyze or reverse engineer the Materials nor have them analyzed on Purchaser’s behalf.
5. In performing the Assay, Purchaser will use only Reagents supplied by DiscoveRx or an authorized DiscoveRx distributor for the Materials.
6. Purchaser will not use the Cells with any other reagents or substrates, other than the Reagents that are provided by or purchased from DiscoveRx or an authorized DiscoveRx distributor, in connection with the Materials.
7. The number of Assays performed will not exceed the authorized number for which Materials were purchased.

B. The use of this cell line may require third party licenses. Purchaser shall have the sole responsibility for obtaining any such licenses prior to use of the cell lines.

If the purchaser has any further questions regarding the rights conferred with purchase of the Materials, please contact:

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Fremont, CA 94538
tel | 510.771.3527
Agreements@discoverx.com