User Manual

PathHunter® IL-15 Bioassay Kit (Reporter)

For Detection of IL-15 Pathway Signaling through Activation of Endogenous Receptors

For Bioassay Kits with control

93-1178Y055-00185: 2-Plate Kit

93-1178Y055-00186: 10-Plate Kit

For Bioassay Kit without control

93-1178Y055-00196: 10-Plate Kit

Document Number 70-446 Rev 0

Table of Contents

Table of Figures

1. Overview

The intended use of the PathHunter IL-15 Bioassay Kit (Reporter) is to determine the relative potency of IL-15 therapeutics. The PathHunter IL-15 Bioassay Kit (Reporter) with control contains all the materials needed to perform a complete assay, including cryopreserved, single-use cells, detection reagents, cell plating reagent, agonist for stimulating the cells, and assay plates. A 10-Plate PathHunter IL-15 Bioassay Kit (Reporter) is also offered without control agonist but contains all other components listed above to run the assay. This bioassay has been optimized for a 96-well plate format.

2. Assay Principle

This bioassay utilizes Enzyme Fragment Complementation (EFC) technology, where the β-galactosidase (β-gal) enzyme is split into two fragments, the ProLink™ (PK), and the Enzyme Acceptor (EA). Independently, these fragments have no β-gal enzymatic activity; however, when forced to complement through protein-protein interactions, they form an active β-gal enzyme.

PathHunter Signaling Reporter assays detect target pathway signaling through the activation of endogenous receptors or receptors introduced into cells with a reporter gene construct. Ligand-mediated stimulation of these receptors initiates pathway signaling and subsequent activation of transcription factors, which bind to a regulatory transcriptional element controlling reporter gene expression. In the PathHunter IL-15 Bioassay (Reporter) kit, binding of IL-15 to the IL-15 receptor drives the expression of a reporter protein tagged with the small enhanced ProLabel® (ePL) β-galactosidase (β-gal) enzyme donor fragment. Reporter activity is measured by lysing the IL-15 Bioassay (Reporter) cells with a detection reagent containing the complementary β-gal enzyme acceptor (EA) fragment and luminescent enzyme substrate.

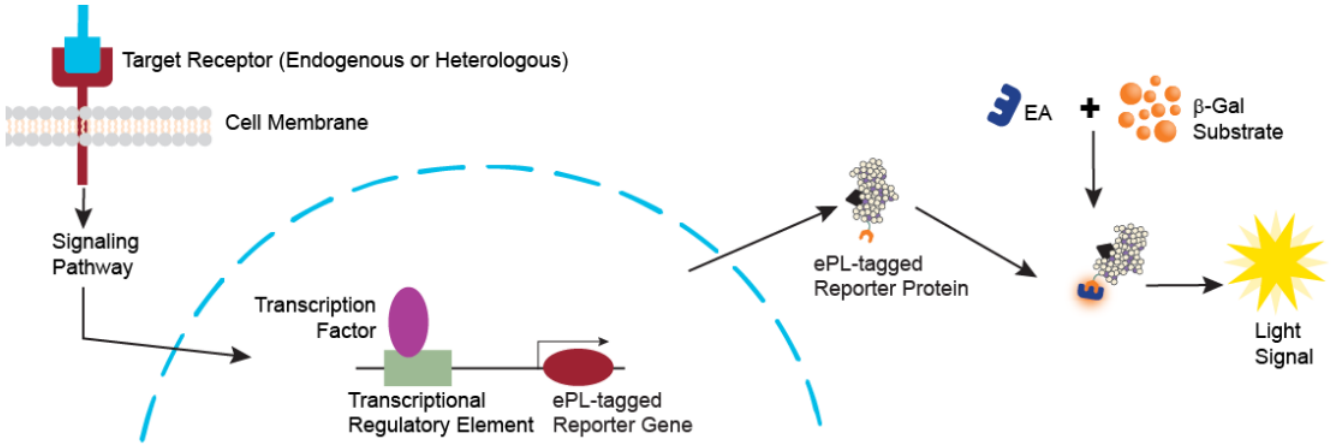


Figure 1. Schematic of the assay principle for the PathHunter IL-15 Bioassay (Reporter).

3. Materials Provided in the PathHunter IL-15 Bioassay Kit (Reporter)

Table 1: Materials Provided

List of Components	93- 1178Y055-00185 (2-Plate Kit)	93-1178Y055-00186 (10-Plate Kit)	93- 1178Y055-00196 (10-Plate Kit without control)
PathHunter REH IL-15 Signaling Reporter Bioassay Cells (1.2×10^6 cells in 0.1 mL per vial) ¥	2 Vials	10 Vials	10 Vials
AssayComplete™ Cell Plating 0 Reagent (100 mL per bottle)	1 x 100 mL	2 x 100 mL	2 x 100 mL
AssayComplete™ Protein Dilution Buffer (50 mL per bottle)	1 x 50 mL	2 x 50 mL	2 x 50 mL
Control Agonist (Recombinant Human IL-15) (10 µg per vial)	1	1	N/A*
PathHunter ProLabel/ProLink Detection Kit (mL per bottle) EA Reagent (Bottle) Lysis Buffer (Bottle) Substrate Reagent (Bottle) Positive Control (Vial)	 1 x 20 mL 1 x 20 mL 1 x 80 mL 1 x 200 µL	 1 x 20 mL 1 x 20 mL 1 x 80 mL 1 x 200 µL	 1 x 20 mL 1 x 20 mL 1 x 80 mL 1 x 200 µL
96-Well White, Clear Flat-Bottom, TC-Treated, Sterile Plates with Lid	2 Plates	10 Plates	10 Plates

***Note**: For 93-1178Y055-00196 control is not provided in the kit, and would need to be obtained separately if needed

¥ **Note**: Excess cells per vial are provided and may be discarded if not needed.

4. Storage Conditions

PathHunter REH IL-15 Signaling Reporter Bioassay Cells

Cells are shipped on dry ice and should arrive in a frozen state. To ensure maximum cell viability, store the vials of bioassay cells in the vapor phase of liquid nitrogen as soon as possible upon receipt. Please contact technical support immediately, if the cells received were already thawed.

- Short-term (24 hours or less): Store vials at -80°C immediately upon arrival. (DO NOT store at -80°C for longer than 24 hours).
- Long-term (greater than 24 hours): Vials should ONLY be stored in the vapor phase of liquid nitrogen.

Safety Warning: A face shield, gloves and lab coat should be worn at all times when handling frozen vials. Use tongs to remove cryovials from liquid nitrogen storage and place the vials immediately on dry ice in a covered container. Wait at least 1 minute for any liquid nitrogen that may be present inside the vial to evaporate. Do not touch the bottom of the vials at any time to avoid inadvertent thawing of the cells.

PathHunter PL/PK Detection Kit

Upon receipt, store reagents at -20°C. The detection kit is stable until the expiration date listed on the outer label of the kit box. Thaw reagents at room temperature before use. Once thawed, the reagents can be stored for up to 1 month at 2-8°C. For long-term storage (up to the expiration date listed in the kit's Certificate of Analysis), the reagent should be re-frozen in opaque containers at -20°C until needed. Avoid multiple freeze-thaw cycles.

For the ten-plate kit: If all the plates will not be used at the same time, we recommend making five aliquots for each reagent. Each aliquot will be adequate for two assay plates. Make five aliquots of 4 mL each for EA Reagent, and

Lysis Buffer. For Substrate Reagent, prepare five aliquots of 16 mL each. Sufficient reagent volumes are provided in the kit to make these aliquots.

AssayComplete Cell Plating 0 Reagent (CP0)

Upon receipt, store at -20°C. 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's 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 can be dispensed and frozen down.

AssayComplete Protein Dilution Buffer (PDB)

Upon receipt, store at -20°C. 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's 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, 5 mL of reagent per aliquot can be dispensed and frozen down.

Recombinant Human IL-15 control agonist (If supplied in the kit)

Upon receipt, store at -20°C, until ready to use (up to the date listed in the kit’s Certificate of Analysis). Centrifuge the vial before opening to maximize recovery. Reconstitute to a concentration of 100 µg/mL by adding 0.1 mL of supplied reconstitution buffer to the 10 µg vial. Once prepared, the stock solution should be stored as suitable aliquots (e.g. 30 µL) at -20°C until needed. Do not freeze/thaw more than twice. Reconstituted control is stable for 12 months at -20°C to -80°C, or 1 week at 2-8°C.

96-Well Tissue Culture-Treated Plates

Upon receipt, store at room temperature.

5. Additional Materials and Equipment Recommended for Assay

The below equipment and additional materials are required to perform these assays. Equivalent reagents may be used. All plastic materials should be stored at temperatures specified by suppliers.

Table 2: Additional Materials and Equipment Recommended

Product Description	Vendor (Catalog Number)
Recombinant Human IL-15	DiscoverX (92-1265), or similar
96-well Green, V-bottom, Untreated, Non-Sterile Dilution Plates (Master Dilution Plate; for preparing serial dilutions of samples)	DiscoverX (92-0011), or similar
Disposable polystyrene reagent reservoirs (25mL), sterile	Thermo Fisher Scientific (Cat.#. 8094), or similar
15 mL LightSafe polypropylene tubes, sterile	Millipore Sigma (Cat # Z688320), or similar
Multimode or luminescence plate reader	Refer to the Instrument Compatibility Chart at discoverx.com/instrument-compatibility
50 mL and 15 mL Polypropylene tubes, sterile	
1.5 mL polypropylene microcentrifuge tubes, sterile	
Tissue culture disposable pipettes (1 mL - 25 mL), sterile	
Disposable pipet tips for P20, P100, P1000 pipetmans	
Sterile biosafety cabinet	
Automated cell counter or hemocytometer	
Humidified tissue culture incubator	
Single and multichannel pipettors (e.g. P20, P100, P1000)	

6. Unpacking Cell Cryovials

Cryovials are shipped on dry ice and contain cells in 0.1 mL of AssayComplete™ Freezing Reagent (refer to the CoA for cell number provided in the vial). Upon receipt, the vials should be transferred to the vapor phase of liquid nitrogen for storage beyond 24 hours. The following procedures are for the safe storage and removal of cryovials from liquid nitrogen storage.

Appropriate personal protective equipment (PPE), including a face shield, gloves, and a lab coat should be worn during these procedures.

1. PathHunter REH IL-15 Signaling Reporter Bioassay Cells must arrive in a frozen state on dry ice.

Contact technical support immediately, if cells received were already thawed.

2. Frozen cells must be transferred to either liquid nitrogen storage or a -80°C freezer immediately upon arrival. If cells will be thawed and used within 24 hours, they can be stored temporarily at -80°C. For long-term storage, place vials in the vapor phase of liquid nitrogen storage.

Cryovials should be stored in the vapor phase of liquid nitrogen ONLY. These vials are not rated for storage in the liquid phase.

3. Using tongs, remove the cryovials from the liquid nitrogen storage, and place them immediately in dry ice, in a covered container. Wait for at least one minute for any liquid nitrogen inside the vial to evaporate.

4. Proceed with the thawing protocol in the detailed assay protocol section. Refer to the CoA for the appropriate AssayComplete products mentioned in the protocol below.

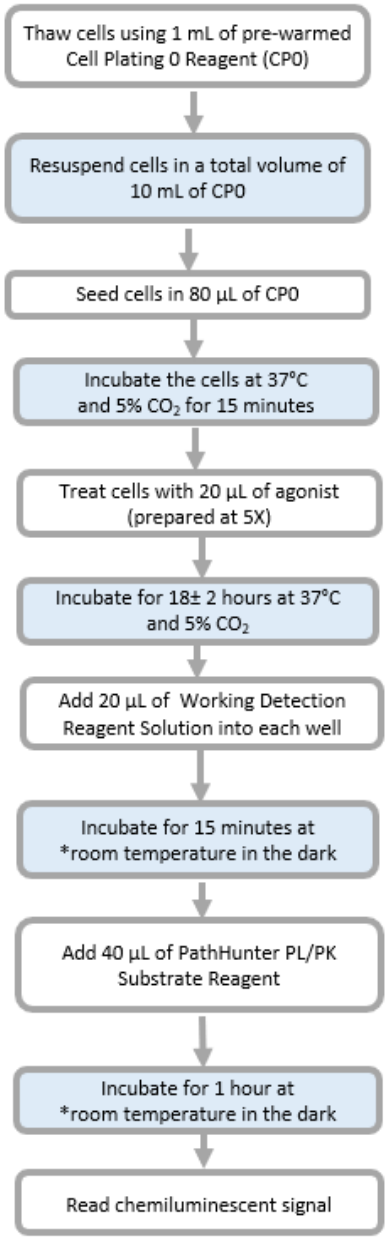
7. Protocol Schematic

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

Assay Name: _____Date: _____

Product Details: _____

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



*Room temperature refers to a range of 23-25°C

8. Detailed Assay Protocol

This assay is performed under aseptic conditions. Preparation of cells, Reference Standards/Test Samples are performed in a Biological Safety Cabinet following good aseptic technique. All appropriate materials are either certified sterile or prepared aseptically.

Prepared volumes may be scaled up or down if required.

If purchasing the bioassay kit without control, control agonist can be sourced per the details in the Additional Materials and Equipment Recommended for Assay table.

8.1 Bioassay Cell Preparation (Day 1)

The following protocol is for thawing and plating the cryopreserved REH IL-15 Signaling Reporter Bioassay Cells from cryovials.

1. Prior to thawing the cells, ensure that all the required materials are set up in the tissue culture hood. These include:
    a. One sterile 25 mL reagent reservoir
    b. One sterile 50 mL conical tube
    c. A micro pipettor (P1000 & P100) set to dispense 1 mL and 30 µL.
    d. A multichannel pipette and tips set to dispense 80 µL.
    e. A bottle of AssayComplete™ Cell Plating 0 Reagent (CP0), **pre-warmed in a 37˚C water bath for 15 minutes**
    f. A 96-Well White, Clear, Flat-bottom Tissue Culture-Treated Sterile Assay Plate (provided with the kit)
2. Dispense 10 mL of CP0 into the 50 mL conical tube.
3. Remove the cryovial from the liquid nitrogen vapor and immediately place it in dry ice.
4. Remove the cryovial from dry ice and ensure cap is tightened. Holding vial by the cap, immediately thaw vial in 37°C water bath for 30 seconds (+/- 5 seconds), gently agitating the vial to thaw cells.

    DO NOT LEAVE VIAL IN WATER BATH.

5. Visually inspect bottom of vial after 20 seconds. If pellet is thawed, remove vial from water bath, wipe down the outside surface of the cryovial quickly with 70% ethanol, and immediately bring it into the tissue culture hood. If ice is still visible, return vial to water bath for additional 10-15 seconds.
6. Add 1.0 mL of pre-warmed CP0 from the 50 mL conical tube, to the cryovial. The reagent should be added slowly along the wall of the cryovial tube. Slowly pipet up and down several times to uniformly suspend the cells.
7. Transfer the cell suspension to the conical tube containing the remaining 9.0 mL of CP0. Remove all the suspension from the cryovial tube to ensure maximum recovery of all the cells.
8. Replace the cap on the conical tube and mix by gentle inversion 2-3 times to ensure that the cells are properly resuspended in the reagent, without creating any froth in the suspension. Take out 30 µL of cell suspension for counting.
9. Using Hemocytometer (manual) or automatic cell counter (i.e. Nexcellom T4 cell counter), count cells and adjust cell density to 6.25 x 10^4 cell / mL (should use roughly 19.2 mL total volume of CP0 per vial).

10. Using a manual 12-channel multichannel pipet, transfer 80 µL of the cell suspension (5K cells/well) to each well of the 96-well assay plate, one row at a time, using reverse pipetting. Mix cells in trough by pipetting up/down 2-3 times before aspirating and pipetting cells into each subsequent row in the assay plate. Discard any leftover cells.
11. Replace the lid and leave the plate at room temperature in biosafety cabinet for 15 minutes (but no more than 30 minutes) to allow the cells to settle uniformly in the well, to minimize potential edge effects.

Note: If sample preparation will take longer than 30 minutes, transfer assay plate to humidified tissue culture incubator at 37°C, 5% CO_2 until sample prep is complete.

12. Proceed directly to preparation of IL-15 working stocks and serial dilutions.

8.2 Sample Preparation (IL-15)

1. Before beginning, ensure all required reagents are assembled in biosafety cabinet:
   a. Micropipettes (P20, P100, P1000)
   b. A multichannel pipette and tips set to dispense 20 µL
   c. An aliquot of AssayComplete™ Protein Dilution Buffer, thawed and equilibrated to room temperature (22°C - 25°C).
   d. A 96-Well Green, V-Bottom, Untreated, Non-Sterile Dilution Plate, labeled ‘Master dilution plate’ (MDP)
   e. Sterile microfuge tubes, for preparation of intermediate, working or nominal testing concentrations of IL-15.
2. Prepare IL-15 intermediate and working stock solutions in Protein Dilution Buffer (PDB) as shown in examples in Table 3:
   a. IL-15 used for assay qualification is supplied as a lyophilized powder
   b. Reconstitute lyophilized ligand to a concentration of 100 µg/mL in PDB.
      - e.g. for 10 µg size, resuspend in 100 µL of PDB.
   c. It is recommended to use a minimum aliquot size of 10 µL for preparation of all sample / stock dilutions.

Table 3. Example of Preparation of IL-15 Intermediate and Working Stocks

Intermediate and Working Stock Solutions	Final concentration µg/mL	Volume of IL-15 (Stock concentration)	Volume Protein Dilution Buffer, µL
IL-15 Reference Standard (RS), Intermediate Stock 1	10	10 µL (of 100 µg//mL stock)	90
IL-15 RS, Working Stock	1	10 µL (of 10 µg/mL stock)	90

3. On the day of assay, prepare serial dilutions of the IL-15 reference standard in Row A of a fresh 96-well master dilution plate (MDP) as 5X stocks in PDB as per Table 4 below. Sufficient volumes to run duplicate wells per dose (pseudoreplicates) in the assay plate are provided.
   a. Add appropriate volume of sample diluent PDB to Row A of the MDP, as indicated in column 6 of Table 4.
   b. Mix 20 µL of IL-15 RS working stock (prepared in Table 3) to Well 2 of Row A with ligand diluent present in well to prepare top dose in dose curve.
   c. Prepare serial dilutions by transferring the indicated volume of IL-15 in column 5 of Table 4 from the source well, indicated in column 4, to the destination well, indicated in column 1. Pipet up and down several times to mix in destination wells. Replace pipet tips between each serial dilution.

Note: No sample is added to column 1, to minimize potential for hook effects at top dose

Note: Vehicle is added to well 1 to serve as the negative control.

Table 4. Example of Preparation of IL-15 (Reference Standard) Serial Dilutions

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Well Location on Master Dilution Plate (MDP)	**Concentration (5X) of sample on MDP Row A), ng/mL**	**Dilution Ratio**	**Sample Source Location**	**Volume Sample, µL**	**Volume Ligand Diluent, µL**	**Final [Sample] in Assay (1X), ng/mL**
Row A, Well 1	0	--	--	--	75	0
Row A, Well 2	250	1:4	IL-15 RS Working Stock	20	60	50
Row A, Well 3	62.5	1:4	Row A, Well 2	20	60	12.5
Row A, Well 4	15.6	1:4	Row A, Well 3	30	90	3.13
Row A, Well 5	6.25	1:2.5	Row A, Well 4	50	75	1.25
Row A, Well 6	2.5	1:2.5	Row A, Well 5	50	75	0.5
Row A, Well 7	1.0	1:2.5	Row A, Well 6	50	75	0.2
Row A, Well 8	0.4	1:2.5	Row A, Well 7	50	75	0.08
Row A, Well 9	0.16	1:2.5	Row A, Well 8	50	75	0.032
Row A, Well 10	0.064	1:2.5	Row A, Well 9	50	75	0.0128
Row A, Well 11	0.016	1:4	Row A, Well 10	25	75	0.0032
Row A, Well 12	0.004	1:4	Row A, Well 11	25	75	0.0008

4. On the day of assay, prepare samples to be tested as a 100% nominal concentration (NC) as shown in example in Table 5. Volumes may be scaled up or down as needed. It is recommended to use a minimum aliquot size of 10 µL for preparation of all sample / stock dilutions These can be prepared in sterile 1.5 mL microcentrifuge tubes.

Note: Depending on sample concentration, intermediate dilutions of test sample(s) may need to be prepared (as demonstrated in Table 3 for the IL-15 Reference Standard) to generate the 1 µg/mL solution needed to prepare the Test Sample Working Stock in Table 5.

Table 5. Example of Preparation of Test Sample Working Stocks

Test Sample (NC%)	[Test], ng/mL	Volume of 1 µg/mL Test Sample, µL	Volume Dilution Buffer, µL
Test Sample 1 Working Stock (100%)	250	37.5	112.5
Test Sample 2 Working Stock (100%)	250	37.5	112.5

5. On the day of assay, prepare serial dilutions of the first test sample (Test Sample 1) in Row B of the 96-well master dilution plate (MDP) as 5X stocks in PDB as per Table 6 below. Sufficient volumes to run duplicate wells per dose (pseudoreplicates) in the assay plate are provided.
   a. Add appropriate volume of sample diluent (PDB) to Row B of the MDP, as indicated in column 6 of Table 6.
   b. Add 120 µL of Test Sample 1 Working Stock (prepared in Table 5) to Well 2 of Row B in the MDP.
   c. Prepare serial dilutions by transferring the indicated volume of Test Sample 1 in column 5 from the source well indicated in column 4 to the destination well, indicated in column 1. Pipet up and down several times to mix in destination wells. Replace pipet tips between each serial dilution.

Note: No sample is added to column 1, to minimize potential for hook effects at top dose

Note: Vehicle is added to well 1 to serve as the negative control.

Table 6. Example of Preparation of Test Sample Serial Dilutions (e.g. Test Sample 1; 100% NC)

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Well Location on Master Dilution Plate (MDP)	Concentration (5X) of sample on MDP, ng/mL	Dilution Ratio	Sample Source Location	Volume Sample, µL	Volume Ligand Diluent, µL	Final [Sample] in Assay (1X), ng/mL
Row B, Well 1	0	--	--	--	75	0
Row B, Well 2	250		Test Sample 1 Working Stock (Table 5)	120	--	50
Row B, Well 3	62.5	1:4	Row B, Well 2	30	90	12.5
Row B, Well 4	15.6	1: 4	Row B, Well 3	30	90	3.125
Row B, Well 5	6.25	1: 2.5	Row B, Well 4	50	75	1.25
Row B, Well 6	2.5	1: 2.5	Row B, Well 5	50	75	0.5
Row B, Well 7	1.0	1: 2.5	Row B, Well 6	50	75	0.2
Row B, Well 8	0.4	1: 2.5	Row B, Well 7	50	75	0.08
Row B, Well 9	0.16	1: 2.5	Row B, Well 8	50	75	0.032
Row B, Well 10	0.064	1: 2.5	Row B, Well 9	50	75	0.0128
Row B, Well 11	0.016	1:4	Row B, Well 10	25	75	0.0032
Row B, Well 12	0.004	1:4	Row B, Well 11	25	75	0.0008

6. Prepare serial dilutions for second test sample (Test Sample 2), using row C in the master dilution plate, using the same dilution series as detailed in Table 6.
7. Retrieve assay plate containing cells from biosafety cabinet or incubator.
8. Pipette serial dilutions for each sample (as pseudoreplicates) into the assay plate as shown in the Representative Plate Map, and as follows:
    a. Transfer 20 µL of prepared serial dilutions from Row A of the MDP into Rows B and E of the assay plate containing cells.
    b. Transfer 20 µL of prepared serial dilutions from Row B of the MDP into Rows C and F of the assay plate containing cells.
    c. Transfer 20 µL of prepared serial dilutions from Row C of the MDP into Rows D and G of the assay plate containing cells
9. Replace assay plate lid and gently mix the contents by slowly moving the plate back and forth 3-4 times on the surface of the tissue culture hood, in a criss-cross pattern.
10. Incubate assay plate in a humidified tissue culture incubator at 37˚C and 5% CO_2 for 18 ± 2 hours after drug/ligand addition.

Representative Plate Map

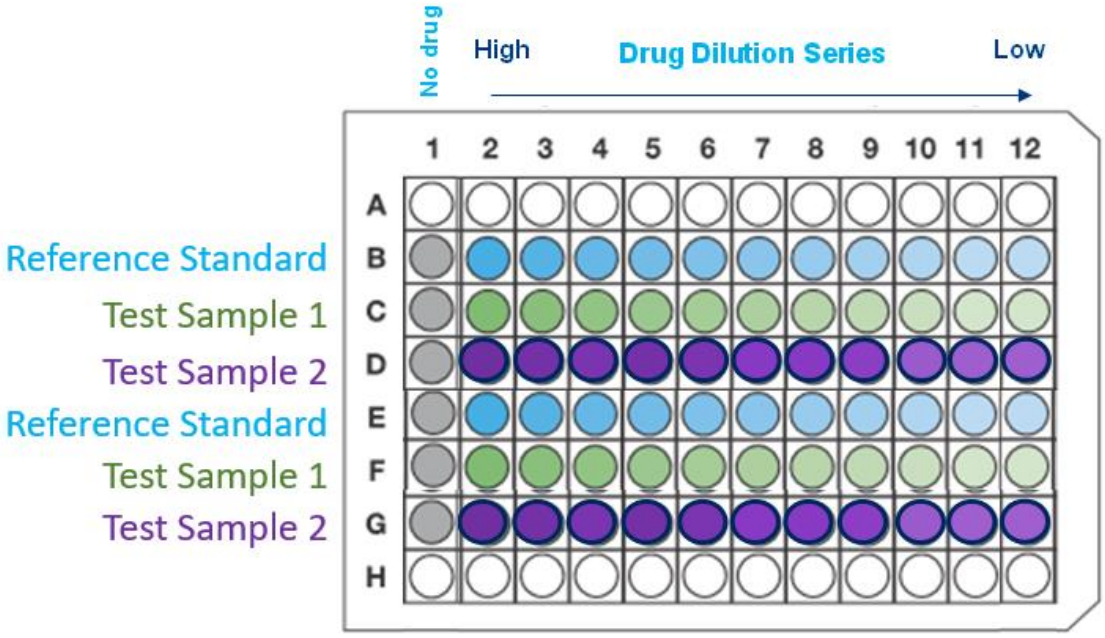


Figure 2. Representative Assay Plate Map: This plate map shows 3 interdigitated 11-point dose curves, with two replicates per dose point, for two test samples and one reference sample tested using the same dilution scheme.

Note: Column 1 contains no drug (vehicle only). Rows A and H contain cells with assay medium only.

8.3 Addition of Detection Reagent

Signal Detection (Day 2)

1. Thaw one aliquot each of the PathHunter PL/PK Substrate Reagent, Lysis Buffer and EA reagent and equilibrate to room temperature.
2. Prepare a working detection solution by mixing the Lysis buffer and EA reagent in a 15 mL conical tube. Refer to the table below for the volume ratio.

Table 7. Working Detection solution preparation

Working Detection Solution		
Component	**Ratio**	**Volume per Plate (mL)**
Lysis buffer	1	1.5
EA reagent	1	1.5

3. Replace the cap of the tube and mix by gentle inversion. Pour detection mixture into a 25 mL reagent reservoir.
4. Remove assay plate from incubator and remove lid. Using a multichannel pipet, add 20 µL of the working Detection Reagent solution from the reservoir into each well of the assay plate.
5. Incubate the assay plate for 15 minutes (+/- 5 minutes) at room temperature (22°C - 25°C) in the dark (e.g. in a drawer or covered location on the bench top).

Detection reagents are light sensitive, thus incubation in the dark is necessary.

6. Retrieve assay plate and remove lid. Using a multichannel pipet, add 40 µL of the PathHunter PL/PK Substrate Reagent from the reservoir into each well of the assay plate.
7. Replace lid on assay plate and gently mix the contents by slowly moving the plate back and forth 3-4 times on the surface of the tissue culture hood, in a criss-cross pattern.
8. Incubate the assay plate for 1 hour (+/- 15 minutes) at room temperature (22°C - 25°C) in the dark (e.g. in a drawer or covered location on the bench top).
9. Read samples on a standard luminescence plate reader at 0.1 to 1 second/well for photomultiplier tube readers or 5 to 10 seconds for imager.
    - A luminescence reader usually collects signal from all wavelengths. Some instrument manufacturers may include a cutoff filter at high wavelengths, but usually no wavelength setting is needed for luminescence readout.
10. Data analysis and plotting graph can be performed using your choice of statistical analysis software (e.g. GraphPad Prism, Molecular Devices Softmax Pro, BioTek Gen5, Microsoft Excel, etc.)

Data Analysis

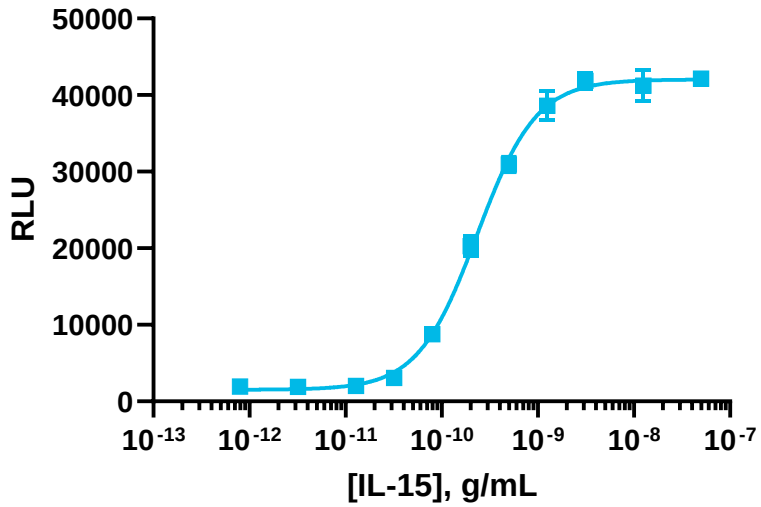
1. Import raw RLU (Relative Light Unit) data into appropriate version of SoftMax Pro or PLA 3.0 or similar software to calculate relative potency of test sample relative to the reference standard.
2. Reference standard data should be analyzed with a 4PL curve fit, with log of standard concentrations on the x-axis and average RLU values on the y-axis.
3. Test sample data should be analyzed with a 4PL curve fit, with log of standard concentrations on the x-axis and average RLU values on the y-axis.
4. The appropriate tests for parallelism should be performed within the chosen software system to assess validity of the potency of each test sample.

9. Typical Results

The following graph is an example of a typical dose-response curve for the PathHunter IL-15 Bioassay Kit (Reporter) generated using the protocol outlined in this user manual. The data shows potent, dose-dependent binding of drug to the IL-15 receptor that drives the expression of a reporter protein tagged with (β-gal) enzyme donor fragment. Lysis of IL-15 Bioassay (Reporter) cells with a detection reagent leads to formation of (β-gal) enzyme and upon interaction with substrate causes chemiluminescence that indicates reporter activity.

The plate was read on an EnVision® Multimode Plate Reader using a 0.2 sec /well integration time and data analysis, plotted below, was conducted using (A) GraphPad Prism and (C) PLA 3.0 (Stegmann Systems).

A



B

S/B	EC_{50}, pg/mL
21.7	233.6

C

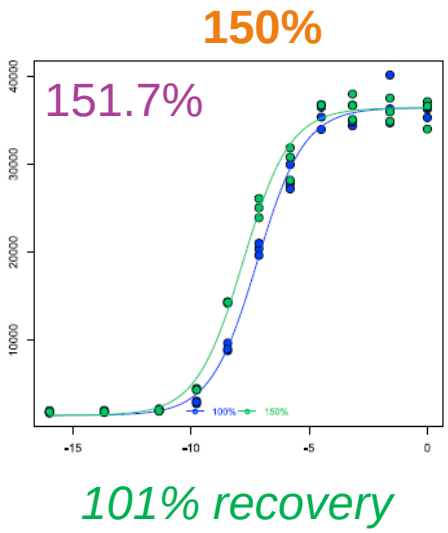


Figure 3. A Representative dose curve for IL-15 in the PathHunter IL-15 Bioassay Kit (Reporter) plotted in GraphPad Prism. B Signal: background (S/B) and EC50 for data plotted in (A). C Example relative potency (RP) data from PLA 3.0 for a 150% nominal concentration of IL-15. Data shown is the restricted model (common slope and asymptotes) based on a 95% confidence interval around the 4PL fit model, where the dark blue curve represents the IL-15 RS, while light green corresponds to the 150% test sample. Relative Potency (RP; represented as %) calculated by PLA 3.0 is shown in pink font in the upper left-hand corner of the graph. Percent recovery is calculated as observed potency value / expected potency value x 100.

Document Revision History

Revision Number	Date Released	Revision Details
0	February 2024	New Document

Limited Use License Agreement

These products may be covered by issued US and/or foreign patents, patent application and subject to Limited Use Label License.

Please visit discoverx.com/license-policies/ for limited use label license and trademark information for this product.

Contact Information

Eurofins DiscoverX

42501 Albrae Street, Fremont, CA 94538

Web: discoverx.com/bioassays

Technical Support: discoverx.com/support/

PathHunter® IL- 15 Bioassay Kit (Reporter) User Manual 70-446 Revision 0