User Manual

PathHunter® PD-1 Bioassay Kit (SHP1 Signaling)

For Detection of PD-L1 Induced PD-1 Signaling

For Bioassay Kits with control:

93-1104Y19-00117: 2-Plate Kit

93-1104Y19-00118: 10-Plate Kit

For Bioassay Kits without control:

93-1104Y19-00193: 10-Plate Kit

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Document Number 70-399 Revision 2

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Overview

The PathHunter PD-1 Bioassay Kit (SHP1 Signaling) provides a robust, highly sensitive, and easy-to-use functional co-culture cell-based assay to determine drug potency and detect neutralizing antibodies. The bioassay kit contains all the materials needed for a complete assay, including cryopreserved cells, detection reagents, cell plating reagent, positive control agonist, and assay plates. The ready-to-use cryopreserved cells have been manufactured to ensure assay reproducibility and faster implementation from characterization to lot release. This bioassay has been optimized for a 96-well plate format. The protocol can be optimized further for running the assay in a high-throughput 384-well plate format.

Assay Principle

The PathHunter PD-1 Bioassay (SHP1 Signaling) utilizes the Enzyme Fragment Complementation (EFC) technology to interrogate receptor activity. EFC consists of two β-galactosidase (β-gal) enzyme fragments, the Enzyme Donor (ED) and Enzyme Acceptor (EA) fragments, which independently have no β-gal activity. However, when forced to complement, they form an active β-gal enzyme that hydrolyzes a substrate to produce a chemiluminescent signal.

The PathHunter PD-1 Bioassay (SHP1 Signaling) consists of human cells engineered to stably express an ED-tagged PD-1 receptor, while EA is fused to the phosphotyrosine-binding SH2 domain of the intracellular signaling protein, SHP1. Antibody-induced activation of the receptor results in phosphorylation of the receptor's cytosolic tail. The SH2-domain fused to EA binds the phosphorylated receptor, forcing complementation of ED and EA, resulting in formation of an active β-gal enzyme, which hydrolyzes the substrate to produce a chemiluminescent signal.

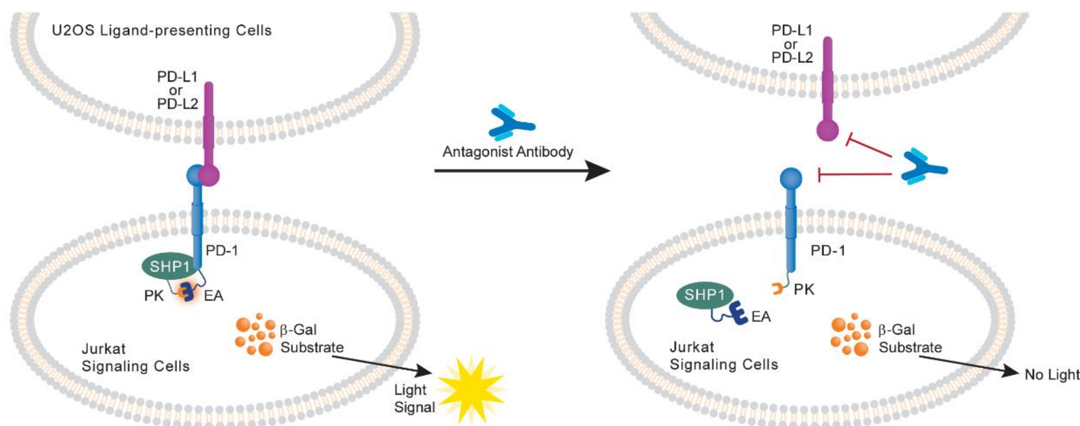


Figure 1. Assay Principle Full-length PD-1 receptor was engineered with the ED fragment fused to its C-terminus, and the SH2-domain of SHP1 was engineered with the complementary EA fragment. These constructs were stably expressed in Jurkat cells, while untagged full-length PD-L1 or PD-L2 was stably expressed in U2OS cells (ligand-presenting cells). Ligand engagement, through co-culture with ligand-presenting cells, results in phosphorylation of PD-1, leading to the recruitment of SHP1-EA. This forces complementation of the EFC components to create an active β-gal enzyme. This active enzyme hydrolyzes substrate to create chemiluminescence as a measure of receptor activity. Addition of an antagonist (e.g. antibody to PD-L1, PD-L2 or PD-1) blocks PD-1 signaling, and will prevent complementation, resulting in a loss of signal.

Materials Provided

List of Components	**93-1104Y19-00117 (2-Plate Kit)**	**93-1104Y19-00118 (10-Plate Kit)**	**93-1104Y19-00193 (10-Plate Kit without control)**
PathHunter Jurkat PD-1 Bioassay Cells (3×10^6 cells in 0.1 mL per vial)	2 Vials	10 Vials	10 Vials
PathHunter U2OS PD-L1 Bioassay Cells (4.8×10^6 cells in 0.2 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
Anti-PD-1 Antibody (100 µg per vial)	1 x 100 µg	1 x 100 µg	N/A**
PathHunter Bioassay Detection Kit Detection Reagent 1 (Bottle) Detection Reagent 2 (Bottle)	1 x 3 mL 1 x 12 mL	1 x 15 mL 1 x 60 mL	1 x 15 mL 1x 60 mL
96-Well White, Flat-Bottom, TC-Treated, Sterile Plates with Lid	2 Plates	10 Plates	10 Plates

*Cell Plating 0 Reagent (CP0) is also used for diluting control agonist and antagonists in the bioassay.

****Note:** For 93-1104Y19-00193 control ligand is not included in the kit and would need to be ordered separately.

Storage Conditions

PathHunter Jurkat PD-1 Bioassay Cells and PathHunter U2OS PD-L1 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 always worn 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.

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 on 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, 20 mL of reagent per aliquot can be dispensed and frozen down.

Anti-PD-1 Antibody

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. Once prepared, the excess stock solution can be stored as suitable aliquots (e.g. 30 µL) at -20°C until needed. Do not freeze/thaw more than twice.

PathHunter Bioassay Detection Kit

Upon receipt, store at -20°C. Once thawed, the detection reagents can be kept at 4°C for up to 4 days. For long-term storage (up to the expiration date listed on the kit's Certificate of Analysis), the reagent should be aliquoted and stored at -20°C until needed. Avoid multiple freeze-thaw cycles.

For the ten-plate kit: If not using all the plates at the same time, we recommend making five aliquots for each of the two detection reagents. Each aliquot will be adequate for two assay plates. Make five aliquots of 3 mL each for Detection Reagent 1, and five aliquots of 12 mL each for Detection Reagent 2. Sufficient volumes for each reagent component have been provided in the kit to make these aliquots.

96-Well White, Flat-Bottom, TC-Treated, Sterile Plates with Lid

Upon receipt, store at room temperature.

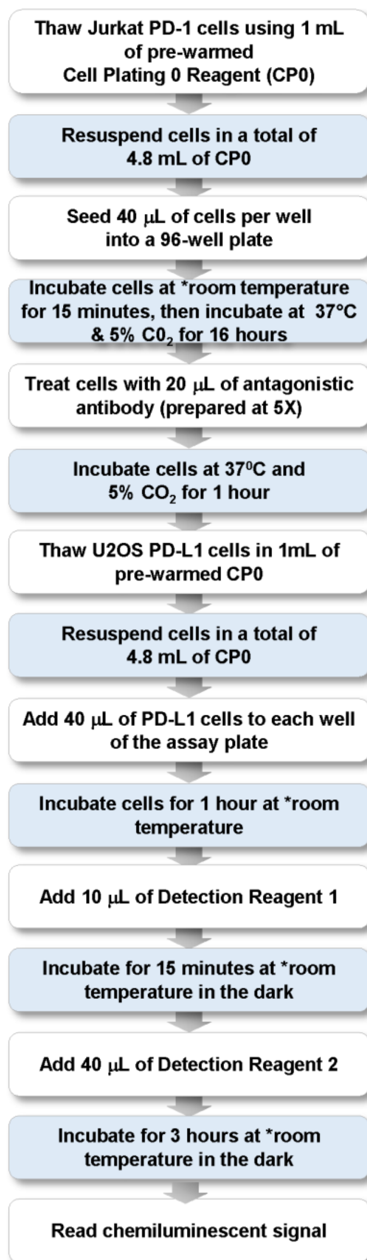
Additional Materials Required

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

Material	Ordering Information
Anti-PD-1 Antibody [NAT 105]	Eurofins DiscoverX (Part # 92-1290), or similar
96-Well Green, V-Bottom, Untreated, Non-Sterile Dilution Plates	Eurofins DiscoverX (Part # 92-0011)
Multimode or luminescence plate reader	Refer to the Instrument Compatibility Chart at discoverx.com/instrument-compatibility
Sterile disposable reagent reservoir	Thermo Fisher Scientific, Cat. No. 8094 or similar
Single and multichannel micropipettes and pipette tips (10 µL-1,000 µL)	
Polypropylene tubes (50 mL and 15 mL)	
Microcentrifuge tubes (1.5 mL)	
Tissue culture disposable pipettes (1 mL-25 mL) and tissue culture flasks (T25 and T75 flasks, etc.)	

Protocol Schematic: Anti-Receptor Antibody

Quick-start Procedure: In a 96-well plate, perform the following steps.



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

Detailed Protocol: Anti-Receptor Antibody

This user manual provides a protocol for determining potency in a 96-well format.

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

Day 1: Bioassay Cell Preparation

The following protocol is for thawing and plating cryopreserved PathHunter Jurkat PD-1 Bioassay Cells from the cryovials.

1. Before thawing the cells, ensure that all the materials required are set up in the tissue culture hood. These include:
   a. One sterile 25 mL reagent reservoir
   b. One sterile 15 mL conical tube
   c. A micropipette (P1000) set to dispense 1 mL
   d. A multichannel pipette and tips set to dispense 40 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, Flat-Bottom, Tissue Culture-Treated Sterile Assay Plate (provided with the kit)
   g. A 96-Well Green, V-Bottom, Untreated, Non-Sterile Dilution Plate, to be used as the master dilution plate.
2. Dispense 4.8 mL of CP0 into the 15 mL conical tube.
3. Remove the cryovial from the liquid nitrogen vapor and immediately place it in dry ice.

DO NOT use heated water bath to thaw the vial. Wipe down the cryovial quickly with 70% ethanol 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. Add 1 mL of pre-warmed CP0 from the 15 mL conical tube into the cryovial, to thaw the cell pellet. The reagent should be added slowly along the wall of the cryovial tube. Slowly pipet the cell suspension up and down, several times, to uniformly resuspend the cells.
5. Transfer the cell suspension to the conical tube containing the remaining 3.8 mL of CP0. Remove any remaining suspension from the cryovial to ensure maximum recovery of all the cells.
6. Replace the cap on the conical tube and gently invert it several times to ensure that the cells are uniformly resuspended in CP0, without creating any froth in the suspension. Immediately pour the suspension into the sterile 25 mL reagent reservoir.
7. Transfer 40 µL of the cell suspension to each well of the 96-well assay plate using a multichannel pipette. Replace the lid and leave the plate at room temperature for 15 minutes, to allow the cells to settle uniformly in the well and minimize potential edge effects.
8. Incubate the assay plate at 37°C and 5% CO_2 for 16 hours before proceeding with the assay.

Day 2: Sample Preparation

The following protocol is an example for preparing serial dilutions of control antibody and agonist test samples.

When optimizing the assay conditions, it is recommended to include a standard curve of the control antibody (anti-PD-1 antibody [NAT105], included in the kit) to verify that the assay is working properly.

A 1:3 serial dilution for the control antibody (Anti-PD-1 Antibody), has been used in this protocol, as shown in Figure 2. Antibody Serial Dilutions. The volumes listed below are designed for running samples from one dose-response curve in triplicate on the assay plate (Refer to Figure 3: Representative Assay Plate Map).

1. Add 200 µL of Cell Plating 0 Reagent (CP0) to Wells A2 to A12 of the master dilution plate.
2. Prepare the control antibody dose-response curve. The antibody is prepared at 5X the desired final concentration with a top dose of 10 µg/mL.
    - 2.1. Add 200 µL of the control antibody prepared at 5X the desired final concentration (50 µg/mL) to Well A1 of the master dilution plate.
    - 2.2. Using a clean pipette tip, transfer 100 µL from Well A1 to Well A2, and mix thoroughly by pipetting up and down several times.
    - 2.3. Replace the pipette tip and transfer 100 µL from Well A2 to A3. Repeat this process until Well A11 is reached, resulting in an 11-point, 1:3 dilution series. No sample is transferred to Well A12 as this is the negative control.
3. Prepare test samples in a similar manner in additional rows of the master dilution plate.
4. Remove the assay plate from the incubator and place it in the tissue culture hood.
5. Transfer 20 µL from each well of the antibody dilution series from the master dilution plate to the appropriate wells of the assay plate.
6. Incubate the assay plate in a humidified incubator at 37°C and 5% CO_2 for 1 hour.

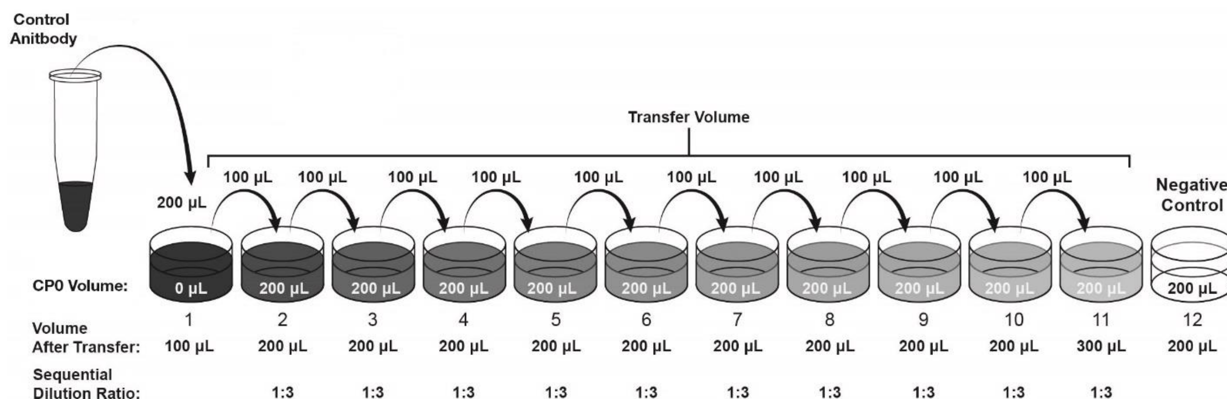


Figure 2. Anti-Receptor Antibody Serial Dilutions: Make eleven 3-fold serial dilutions of the antibody in a master dilution plate.

Day 2: Ligand-Presenting Cell Preparation and Addition

The following protocol is used for preparing the PathHunter U2OS PD-L1 Bioassay Cells from cryovials.

1. Thaw the ligand-presenting cells using the same protocol outlined for the PathHunter Jurkat PD-1 Bioassay Cells in Day 1: Bioassay Cell Preparation
2. Using a multichannel pipette, add 40 µL of cells to each well of the 96-well assay plate.
3. Incubate the assay plate for 1 hour at room temperature in the dark.

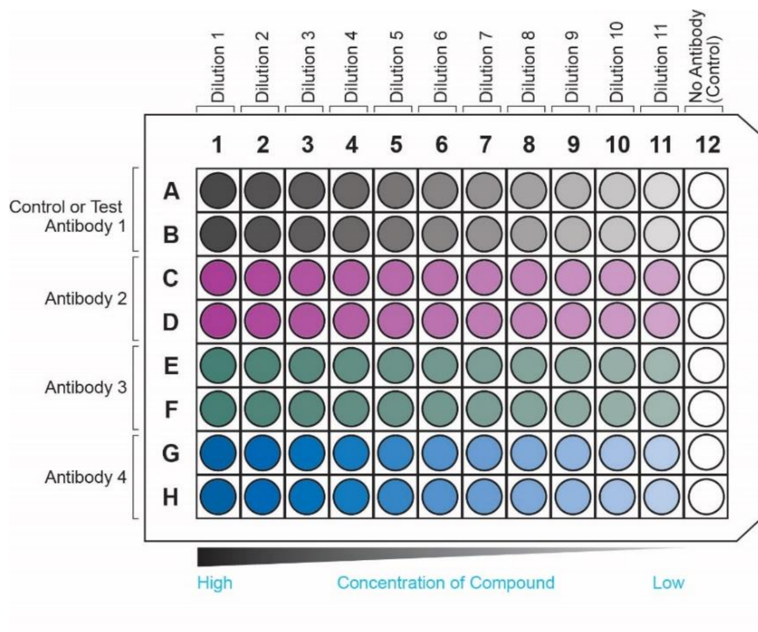


Figure 3. Representative Assay Plate Map: This plate map shows four 11-point dose curves, with two replicates per dose point, for three test samples and one reference sample tested using the same dilution scheme.

Day 2: Signal Detection

1. Thaw one aliquot each of Bioassay Detection Reagent 1 and Bioassay Detection Reagent 2 from the PathHunter Bioassay Detection kit and equilibrate to room temperature.
2. Using a serological pipet, transfer 2.3 mL of Bioassay Detection Reagent 1 into a sterile reservoir.
3. Remove assay plate from incubator and remove lid. Add 10 µL of the Bioassay Detection Reagent 1 from the reservoir into each row of the assay plate.
4. 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.
5. Incubate the assay plate for 15 minutes (+/- 5 minutes) at room temperature (23°C - 25°C) in the dark (e.g. in a drawer or covered location on the bench top).

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

Detection Reagents are light sensitive, hence incubation in the dark is necessary.

6. Using a 10 mL serological pipet, transfer 9.2 mL of Bioassay Detection Reagent 2 into a fresh sterile reservoir.

7. Remove assay plate from incubator and remove lid. Pipet 40 µL of the Bioassay Detection Reagent 2 from the reservoir into each row of the assay plate.

8. 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.

9. Incubate the assay plate for 3 hours at room temperature (23°C - 25°C) in the dark (e.g. in a drawer or covered location on the bench top).

10. Read the sample on a standard luminescence plate reader at 0.1 to 1 second/well for photomultiplier tube (PMT) readers or 5-10 seconds for an imager. To determine instrument compatibility, visit discoverx.com/instrument-compatibility.

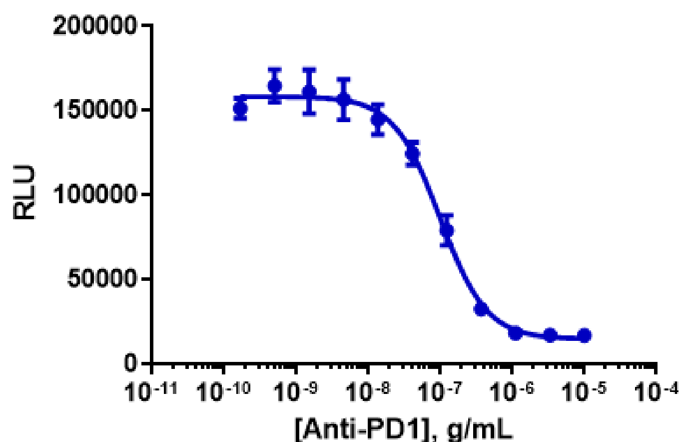
11. Data analysis can be performed using any statistical analysis software such as GraphPad Prism, SoftMax Pro, Gen5, or Microsoft Excel etc.

Typical Results

The following graph is an example of a typical dose-response curve for the PD-1 Signaling Bioassay generated using the anti-receptor antibody protocol outlined in this manual. The data shows potent, dose-dependent inhibition of PD-1 activation in Jurkat cells, when treated with an anti-PD-1 antibody.

The plate was read on the EnVision® Multimode Plate Reader and data analysis was conducted using GraphPad Prism.

A.



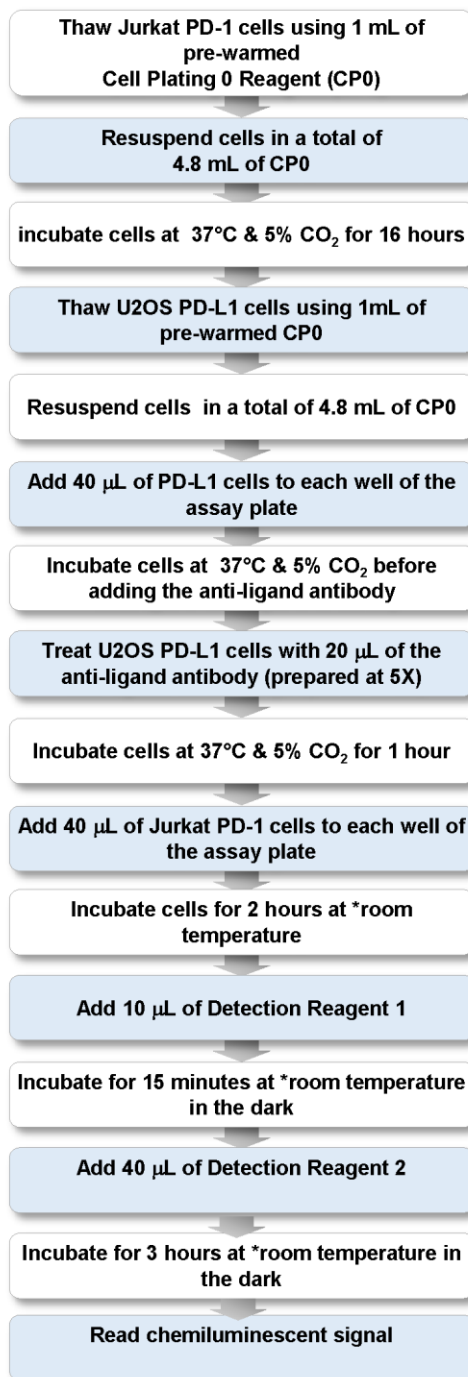
B.

IC_{50} (ng/mL)	99.6
S/B	9

Figure 4. Typical Results: Representative **A**, dose-response curve and **B**, the IC_{50} and assay window for the inhibition of PD-1 activation when an anti-PD-1 antibody is used.

Protocol Schematic: Anti-Ligand Antibody

The flowchart below summarizes the steps involved in the bioassay protocol.



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

Detailed Protocol: Anti-Ligand Antibody

The following sections describe the cell preparation and bioassay protocol for evaluating anti-ligand antibodies.

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

Day 1: Jurkat PD-1 Bioassay Cell Preparation

The following protocol is for thawing cryopreserved PathHunter Jurkat PD-1 Bioassay Cells from the cryovials (one cryovial per 96-well assay plate).

1. Before thawing the cells, ensure that all the materials required are set up in the tissue culture hood. These include:
    a. One sterile 25 mL reagent reservoir
    b. One sterile 15 mL conical tube
    c. One sterile T25 tissue culture flask (optional)
    d. A micropipette (P1000) set to dispense 1 mL
    e. A bottle of AssayComplete™ Cell Plating 0 Reagent (CP0), pre-warmed in a 37°C water bath for 15 minutes
2. Dispense 4.8 mL of CP0 into the 15 mL conical tube.
3. Remove the cryovial from the liquid nitrogen vapor and immediately place it in dry ice.

DO NOT use heated water bath to thaw the vial. Wipe down the cryovial quickly with 70% ethanol 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. Add 1 mL of pre-warmed CP0 from the 15 mL conical tube into the cryovial, to thaw the cell pellet. The reagent should be added slowly along the wall of the cryovial tube. Slowly pipet the cell suspension up and down, several times, to uniformly resuspend the cells.
5. Transfer the cell suspension to the conical tube containing the remaining 3.8 mL of CP0. Remove any remaining suspension from the cryovial to ensure maximum recovery of all the cells.
6. Slowly pipet the cell suspension up and down several times. Using the same pipet, transfer the cell suspension from the conical tube into a sterile T25 flask.
7. Incubate the flask at 37°C and 5% CO_2 for 16 hours before proceeding with the assay. Ensure that the cap of the flask is loosened slightly to allow for gas exchange.

Day 2: U2OS PD-L1 Bioassay Cell Preparation

The following protocol is for thawing and plating cryopreserved PathHunter U2OS PD-L1 Bioassay Cells from the cryovials (one cryovial per 96-well assay plate).

1. Before thawing the cells, ensure that all the materials required are set up in the tissue culture hood. These include:
    a. One sterile 25 mL reagent reservoir
    b. One sterile 15 mL conical tube
    c. A micropipette (P1000) set to dispense 1 mL

d. A multichannel pipette and tips set to dispense 40 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, Flat-Bottom, Tissue Culture-Treated Sterile Assay Plate (provided with the kit)
g. A 96-Well Green, V-Bottom, Untreated, Non-Sterile Dilution Plate, to be used as the master dilution plate

2. Dispense 4.8 mL of CP0 into the 15 mL conical tube.
3. Remove the cryovial from the liquid nitrogen vapor and immediately place it in dry ice.

DO NOT use heated water bath to thaw the vial. Wipe down the cryovial quickly with 70% ethanol 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. Add 1 mL of pre-warmed CP0 from the 15 mL conical tube into the cryovial, to thaw the cell pellet. The reagent should be added slowly along the wall of the cryovial tube. Slowly pipet the cell suspension up and down, several times, to uniformly resuspend the cells.
5. Transfer the cell suspension to the conical tube containing the remaining 3.8 mL of CP0. Remove any remaining suspension from the cryovial to ensure maximum recovery of all the cells.
6. Replace the cap on the conical tube and gently invert it several times to ensure that the cells are uniformly resuspended in CP0, without creating any froth in the suspension. Immediately pour the suspension into the sterile 25 mL reagent reservoir.
7. Transfer 40 µL of the cell suspension to each well of the 96-well assay plate using a multichannel pipette. Replace the lid and leave the plate at room temperature for 15 minutes, to allow the cells to settle uniformly in the well and minimize potential edge effects.
8. Incubate the assay plate at 37°C and 5% CO_2 while preparing the anti-ligand antibody dilutions.

Day 2: Anti-Ligand Antibody Preparation

The following protocol is an example for preparing serial dilutions of control antibody and anti-ligand test samples.

When optimizing the assay conditions, it is recommended to include a standard curve of a reference anti-PD-L1 antibody (e.g. Thermo Fisher [Monoclonal Antibody (MIH1), Functional Grade, eBioscience™]; Cat. No. 16-5983) to verify that the assay is working properly.

A 1:3 serial dilution for the control antibody (anti-PD-L1 antibody), has been used in this protocol, as shown in Figure 5. Antibody Serial Dilutions. The volumes listed below are designed for running samples from one dose-response curve in triplicate on the assay plate (Refer to Figure 6: Representative Assay Plate Map).

1. Add 200 µL of Cell Plating 0 Reagent (CP0) to Wells A2 to A12 of the master dilution plate.

Prepare the control antibody dose-response curve. The antibody is prepared at 5X the desired final concentration with a top dose of 10 µg/mL. Once prepared, the excess stock solution can be stored as suitable aliquots (e.g. 30 µL) at -20°C until needed. Do not freeze/thaw more than twice.

   1.1. Add 200 µL of the control antibody prepared at 5X the desired final concentration (50 µg/mL) to Well A1 of the master dilution plate.

   1.2. Using a clean pipette tip, transfer 100 µL from Well A1 to Well A2, and mix thoroughly by pipetting up and down several times.

   1.3. Replace the pipette tip and transfer 100 µL from Well A2 to A3. Repeat this process until Well A11 is reached, resulting in an 11-point, 1:3 dilution series. No sample is transferred to Well A12 as this is the negative control.

2. Prepare test samples in a similar manner in additional rows of the master dilution plate.
3. Remove the assay plate containing U2OS PD-L1 Bioassay Cells from the incubator and place it in the tissue culture hood.
4. For each sample, transfer 20 µL from each well of the antibody dilution series from the master dilution plate to the appropriate wells of the assay plate, as shown in Figure 6.
5. Incubate the assay plate in a humidified incubator at 37°C and 5% CO_2 for 1 hour.

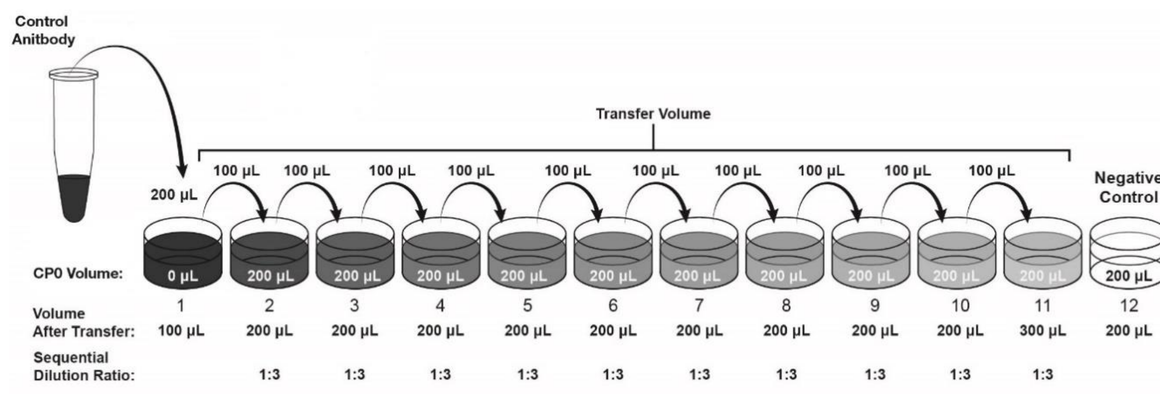


Figure 5. Anti-Ligand Antibody Serial Dilutions: Make eleven 3-fold serial dilutions of the antibody in a master dilution plate.

Day 2: Jurkat PD-1 Bioassay Cell Addition

1. Remove the T25 flask containing the resting Jurkat PD-1 Bioassay Cells (as prepared on Day 1; page 20) from the incubator.
2. Mix the cell suspension by slowly pipetting up and down several times without creating froth. Using the same pipet, transfer the cell suspension into a sterile 25 mL reagent reservoir.
3. Remove the assay plate containing U2OS PD-L1 Bioassay Cells from the incubator. These cells have been treated with the anti-PD-L1 antibody.
4. Add 40 µL of Jurkat PD-1 Bioassay Cells to each well of the assay plate using a multichannel pipette.
5. Cover the assay plate with its lid and mix the contents well by slowly tilting the plate back and forth 2-3 times.
6. Incubate the assay plate at room temperature for 2 hours.

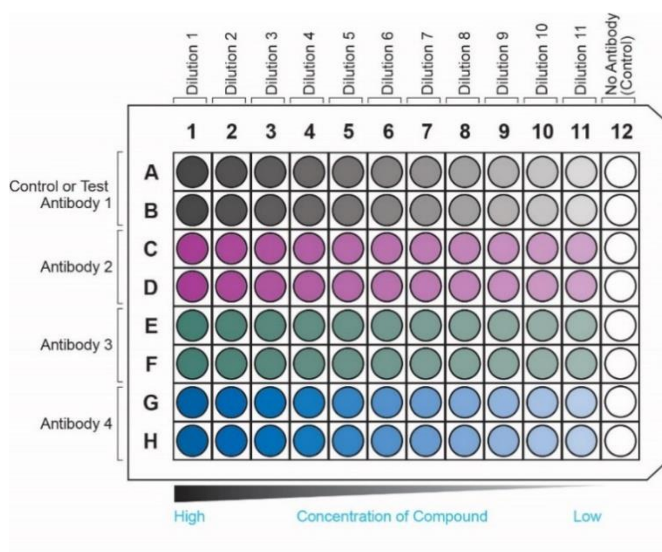


Figure 6. Representative Assay Plate Map: This plate map shows four 11-point dose curves, with two replicates per dose point, for three test samples and one reference sample tested using the same dilution scheme.

Day 2: Signal Detection

1. Thaw one aliquot each of Bioassay Detection Reagent 1 and Bioassay Detection Reagent 2 from the PathHunter Bioassay Detection kit and equilibrate to room temperature.
2. Using a serological pipet, transfer 2.3 mL of Bioassay Detection Reagent 1 into a sterile reservoir.
3. Remove assay plate from incubator and remove lid. Add 10 µL of the Bioassay Detection Reagent 1 from the reservoir into each row of the assay plate.
4. 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.
5. Incubate the assay plate for 15 minutes (+/- 5 minutes) at room temperature (23°C - 25°C) in the dark (e.g. in a drawer or covered location on the bench top).

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

   Detection Reagents are light sensitive, hence incubation in the dark is necessary.

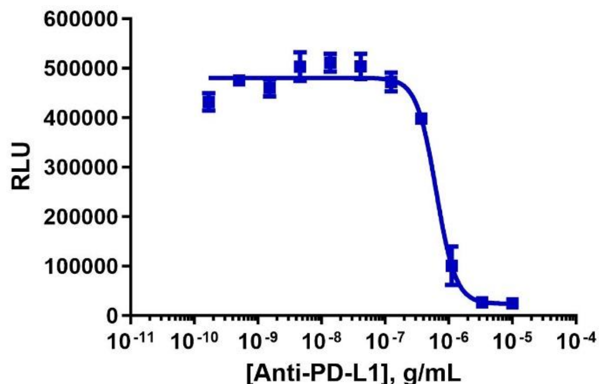
6. Using a 10 mL serological pipet, transfer 9.2 mL of Bioassay Detection Reagent 2 into a fresh sterile reservoir.
7. Remove assay plate from incubator and remove lid. Pipet 40 µL of the Bioassay Detection Reagent 2 from the reservoir into each row of the assay plate.
8. Replace lid on assay plate and 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.
9. Incubate the assay plate for 3 hours at room temperature (23°C - 25°C) in the dark (e.g. in a drawer or covered location on the bench top).
10. Read the sample on a standard luminescence plate reader at 0.1 to 1 second/well for photomultiplier tube (PMT) readers or 5-10 seconds for an imager. To determine instrument compatibility, visit discoverx.com/instrument-compatibility.
11. Data analysis can be performed using any statistical analysis software such as GraphPad Prism, SoftMax Pro, Gen5, or Microsoft Excel etc.

Typical Results

The following graph is an example of a typical dose-response curve for the PD-1 Signaling Bioassay generated using the anti-ligand antibody protocol outlined in this manual. The data shows potent, dose-dependent inhibition of PD-1 when U2OS PD-L1 cells are treated with an anti-PD-L1 antibody.

The plate was read on the EnVision® Multimode Plate Reader and data analysis was conducted using GraphPad Prism.

A.



B.

IC_{50} (ng/mL)	632
S/B	20.5

Figure 7. Typical Results: Representative **A**, dose-response curve and **B**, the IC_{50} and assay window for the inhibition of PD-1 activation when an anti-PD-L1 antibody is used.

Troubleshooting Guide

Problem	Potential Cause	Proposed Solution
No response	Incorrect thawing procedure	Refer to the thawing instructions in the Bioassay Cell Preparation section of this user manual.
	Incorrect ligand used or improper ligand incubation time	Refer to the Certificate of Analysis for recommended ligand and assay conditions.
	Incorrect preparation of the ligand (agonist or antagonist)	Refer to the vendor-specific datasheet to ensure proper handling, dilution, and storage of the ligand.
	Sub-optimal time course for induction	Optimize time course of induction with agonist and antagonist.
Decreased or no response	Cells are not adherent and exhibit incorrect morphology	Confirm adherence of cells using a microscope.
	Problem with microplate reader	The microplate reader should be in luminescence mode. Read at 0.1-1 seconds/well.
Low or no signal	Incorrect preparation of detection reagents	Detection reagents are sensitive to light and should ideally be prepared just prior to use.
	Problem with microplate reader	The microplate reader should be in luminescence mode. Read at 0.1-1 second/well.
Experimental S/B does not match the value noted in the Certificate of Analysis provided	Incorrect incubation temperature	Confirm assay conditions.
		Check and repeat the assay at the correct incubation temperature, as indicated on the assay datasheet.
	Incorrect 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	Ensure that the ligands are stored and incubated at the proper temperature.
	Difference in agonist binding affinity	Confirm that the ligand used is comparable to the ligand in the Certificate of Analysis.
	Problems with dynamic range or dilutions	Changing tips during dilution can help in avoiding carryover.
High variability between replicates	Instrument calibration	Ensure that the dispensing equipment is properly calibrated, and proper pipetting technique is used.

For questions on using this product, please contact Technical Support at discoverx.com/support/.

Document Revision History

Revision Number	Date Released	Revision Details
0	November 2017	• New document
1	December 2020	• New protocol for the evaluation of anti-ligand antibodies has been added on pages 10-16 • Branding updates
2	December 2023	• Branding updates

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