

Description

The Monkey PD1[Biotinylated]: PD-L1 Inhibitor Screening Assay Kit is a chemiluminescence assay designed for screening and profiling molecules that block the binding of PD-1 (programmed cell death protein 1) to PD-L1 (programmed cell death 1 ligand 1). This assay kit comes in a convenient 96-well format, with enough purified monkey biotin-labeled PD-1 (amino acids 25-167), monkey PD-L1 (amino acids 25-167), assay buffers and detection reagents for 100 enzyme reactions.

The key to this kit is the high affinity of biotin-labeled PD-1 for streptavidin-HRP. First, a 96-well plate is coated with PD-L1, followed by incubation with PD-1. Finally, the plate is treated with streptavidin-HRP followed by addition of HRP substrate. The chemiluminescence signal can be detected with a microplate reader capable of reading chemiluminescence. The signal generated is proportional to the binding between PD-1 and PD-L1.

Background

PD-L1 and PD-L2 binding to PD-1, a receptor expressed on T-cells, negatively regulates immune responses. PD-1 ligands PD-L1 and PD-L2 are found on the surface of many cancer cells, and their interaction with receptor PD-1 inhibits T cell activity and allows cancer cells to escape immune surveillance. This pathway is also involved in regulating autoimmune responses. Therefore, these proteins (termed immune checkpoints) are promising therapeutic targets for many types of cancer as well as multiple sclerosis, arthritis, lupus, and type I diabetes. Checkpoint inhibitors have remarkable efficacy in a wide range of cancer types and have revolutionized cancer treatment. PD-1 inhibitors nivolumab, pembrolizumab, cemiplimab and PD-L1 inhibitors atezolizumab, avelumab, and durvalumab are all FDA-approved drugs for immuno-therapy.

Applications

Screening small molecule inhibitors that block monkey PD-1 binding to monkey PD-L1 for drug discovery and high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
71153	PD-L1 (Monkey), Fc fusion (Human IgG1)*	10 µg	-80°C
71235	PD-1 (Monkey), Fc fusion (Human IgG1), Biotin-Labeled *	5 µg	-80°C
79311	3x Immuno Buffer 1	50 ml	-20°C
79728	Blocking Buffer 2	50 ml	+4°C
79742	Streptavidin-HRP	10 µl	+4°C
79670	ELISA ECL Substrate A (translucent bottle)	6 ml	Room Temp
	ELISA ECL Substrate B (brown bottle)	6 ml	Room Temp
79699	96-well white plate	1	Room Temp

*The concentration of the protein is lot-specific and will be indicated on the tube.

Materials Required but Not Supplied

- 1x PBS (Phosphate Buffer Saline) Buffer
- Microplate reader capable of reading chemiluminescence
- Adjustable micropipettor and sterile tips
- Orbital Shaker

Storage Conditions



This assay kit will perform optimally for up to **6 months** from date of receipt when the materials are stored as directed.

Safety



This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

This kit is compatible with up to 1% final DMSO concentration.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include “Blank”, “Positive Control”, “Ligand Control” and “Test inhibitor”.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to [Protein FAQs \(bpsbioscience.com\)](#).
- We recommend using Anti-PD-L1 Neutralizing Antibody (#71213) as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1 x, 1 x and 10 x the IC₅₀ value shown in the validation data below.
- For instructions on how to prepare reagent dilutions please refer to [Serial Dilution Protocol \(bpsbioscience.com\)](#).

Step 1: Plate coating with monkey PD-L1 protein

Coat the plate one day prior to running your samples.

1. Thaw **PD-L1** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **PD-L1** protein to 2 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **PD-L1** protein solution to each well, except “Ligand Control” wells.
4. Add 50 µl of PBS to “Ligand Control” wells.
5. Incubate at 4°C overnight.
6. Prepare **1x Immuno Buffer** by diluting 3-fold **3x Immuno Buffer 1** with distilled water.

Note: Reserve some 3x Immuno Buffer 1 for later steps.

7. Tap the plate onto clean paper towel to remove the liquid.
8. Wash the plate three times with 100 μ l of 1x Immuno Buffer 1 per well.
9. Tap the plate onto clean paper towel to remove the liquid.
10. Add 100 μ l of Blocking Buffer 2 to every well.
11. Incubate for 1 hour at Room Temperature (RT) with gentle agitation.
12. Tap the plate onto clean paper towel to remove the liquid.
13. Start your assay test immediately.

Step 2: Inhibition reaction

1. Prepare a Master Mix (25 μ l/well): N wells x (10 μ l of 3x Immuno Buffer 1 + 15 μ l of distilled water).
2. Add 25 μ l of Master Mix to every well.
3. Prepare the Test Inhibitor (5 μ l/well): for a titration prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 μ l.

3.1 If the Test Inhibitor is water-soluble: Prepare serial dilutions in 1x Immuno Buffer, 10-fold more concentrated than the desired final concentrations.

For the positive and negative controls, use 1x Immuno Buffer (Diluent Solution).

OR

3.2 If the Test inhibitor is soluble in DMSO: Prepare the test inhibitor at 100-fold the highest desired concentration in 100% DMSO, then dilute the inhibitor 10-fold in 1x Immuno Buffer, to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%.

Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 10% DMSO in 1x Immuno Buffer, to keep the concentration of DMSO constant.

For positive and negative controls, prepare 10% DMSO in 1x Immuno Buffer, (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

Note: The final concentration of DMSO should not exceed 1%.

4. Add 5 μ l of Test Inhibitor to each well labeled as "Test Inhibitor".
5. Add 5 μ l of Diluent Solution to the "Positive Control", "Ligand Control" and "Blank" wells.
6. Thaw biotin-labeled PD-1 on ice. Briefly spin the tube containing the enzyme to recover its full content.

7. Dilute PD-1 to 2 ng/μl with 1x Immuno Buffer (20 μl/well).
8. Initiate the reaction by adding 20 μl of diluted PD-1 to the wells designated “Positive Control”, Ligand Control” and “Test Inhibitor”.
9. Add 20 μl of 1x Immuno Buffer to the “Blank” wells.
10. Incubate at RT for 1 hour.

	Blank	Ligand Control	Positive Control	Test Inhibitor
Master Mix	25 μl	25 μl	25 μl	25 μl
Test Inhibitor	-	-	-	5 μl
Diluent Solution	5 μl	5 μl	5 μl	-
1x Immuno Buffer	20 μl	-	-	-
Diluted PD-1 (2 ng/μl)	-	20 μl	20 μl	20 μl
Total	50 μl	50 μl	50 μl	50 μl

11. Wash the plate three times with 100 μl of 1x Immuno Buffer per well.
12. Tap the plate onto clean paper towel to remove the liquid.
13. Add 100 μl of Blocking Buffer 2 to every well.
14. Incubate for 10 minutes at RT with gentle agitation.
15. Tap the plate onto clean paper towel to remove the liquid.

Step 3: Detection

1. Dilute Streptavidin-HRP 1000-fold with Blocking Buffer 2 (100 μl/well).
2. Add 100 μl of diluted Streptavidin-HRP to each well.
3. Incubate for 1 hour at RT with slow agitation.
4. Wash the plate three times with 200 μl of 1x Immuno Buffer per well.
5. Tap the plate onto clean paper towel to remove the liquid.
6. Add 100 μl of Blocking Buffer 2 to every well.
7. Incubate for 10 minutes at RT with gentle agitation.
8. Tap the plate onto clean paper towel to remove the liquid.

9. Just before use, mix 1 volume of ELISA ECL Substrate A and 1 volume of ELISA ECL Substrate B (100 μ l of mix/well).
10. Add 100 μ l of mix to every well.
11. Immediately read the plate in a luminometer or microtiter-plate reader capable of reading chemiluminescence.
12. The “Blank” value should be subtracted from all other values.

Reading Chemiluminescence

Chemiluminescence is the emission of light (luminescence) which results from a chemical reaction. The detection of chemiluminescence requires no wavelength selection because the method used is emission photometry and is not emission spectrophotometry.

To properly read chemiluminescence, make sure the plate reader is set for LUMINESCENCE mode. Typical integration time is 1 second, delay after plate movement is 100 msec. Do not use a filter when measuring light emission. Typical settings for the Synergy 2 BioTek plate reader are: use the “hole” position on the filter wheel; Optics position: Top; Read type: endpoint. Sensitivity may be adjusted based on the luminescence of a control assay without enzyme (typically we set this value as 100).

Example Results

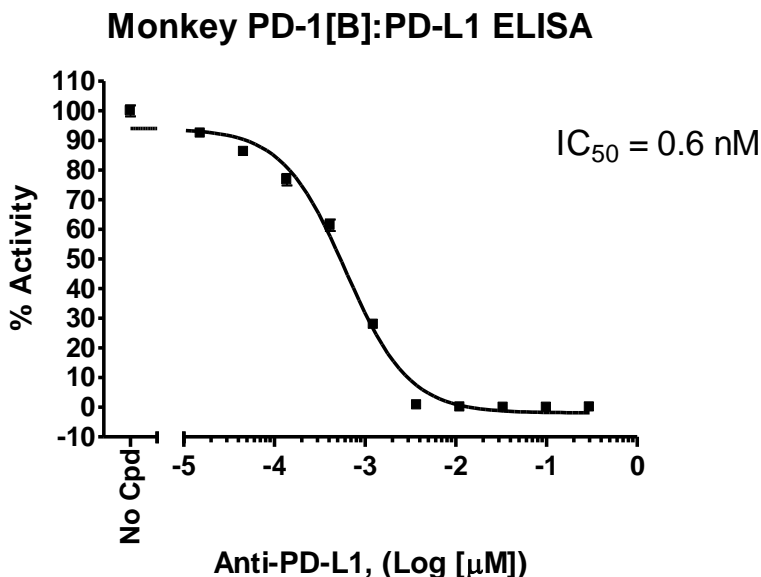


Figure 1: Inhibition of monkey PD-1 binding to monkey PD-L1 by Anti-PD-L1 Neutralizing Antibody. Binding of monkey PD-1 to monkey PD-L1 was measured in the presence of increasing concentrations of Anti-PD-L1 Neutralizing Antibody (#71213). Absorbance was measured using a Tecan UV/Vis spectrophotometric microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

Onlamoon N., *et al.*, 2008 *Immunol.* 124.1: 277-293

Wang C., *et al.*, 2014 *Cancer Immunol. Res.* 2.9: 846-856

Sasca D., *et al.* 2019 *Blood* 133: 2305-2319.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PD-1[Biotinylated]: PD-L2 Inhibitor Screening Colorimetric Assay	72019	96 reactions
PD1:PD-L2 Homogeneous Assay Kit	72015	384 reactions
PD-1[Biotinylated]: PD-L1 Homogeneous Assay Kit	72028	384 reactions
PD-1:PD-L1 TR-FRET Assay Kit	72032	96 reactions
PD-1:PD-L2 TR-FRET Assay Kit	72012	384 reactions
PD-1:PD-L2 Cell-Based Inhibitor Screening Assay Kit	79378	96 reactions

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