# PD-1: PD-L2[Biotinylated] Inhibitor Screening Colorimetric Assay Kit

#72017
96 reactions

**Description**

The PD1: PD-L2[Biotinylated] Inhibitor Screening Colorimetric Assay Kit is a colorimetric assay designed for screening and profiling molecules that block the binding of PD-1 (programmed cell death protein 1) and PD-L2 (programmed cell death 1 ligand 2). This assay kit comes in a convenient 96-well format, with enough purified PD-1 (amino acids 25-167), biotin-labeled PD-L2 (amino acids 20-219), assay buffers and detection reagents for 100 enzyme reactions.

The key to this kit is the high affinity of biotin-labeled PD-L2 for streptavidin-HRP. First, a 96-well plate is coated with PD-1, followed by incubation with PD-L2. Finally, the plate is treated with streptavidin-HRP followed by addition of a colorimetric HRP substrate to produce color, which can then be measured using a UV/Vis spectrophotometer microplate reader. The signal generated is proportional to the binding between PD-1 and PD-L2.

**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 PD-1 binding to PD-L2 for drug discovery and high throughput screening (HTS) applications.

**Supplied Materials**

| Catalog # | Name | Amount | Storage |
|---|---|---|---|
| 71106 | PD-1 (CD279), Fc fusion (Human)* | 10 µg | -80°C |
| 71108 | PD-L2 (CD273), Fc fusion, Biotin-labeled (Human)* | 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 |
| 79651 | Colorimetric HRP Substrate | 10 ml | +4°C |
| 79964 | 96-well transparent 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
- UV/Vis spectrophotometer microplate reader capable of reading absorbance at λ=450 nm*
- 1 N hydrochloric acid (aqueous)
- Adjustable micropipettor and sterile tips
- Orbital Shaker

**Alternately, a spectrophotometer reading at 650 nm may be used, but the sensitivity of the assay will be greatly reduced.*

**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-1 Neutralizing Antibody (#71120) 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 $IC_{50}$ 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 PD-1 protein**

Coat the plate one day prior to running your samples.

1. Thaw **PD-1** protein on ice. Briefly spin the tube to recover the full content.
2. Dilute **PD-1** protein to 2 µg/ml in PBS (50 µl/well).
3. Add 50 µl of diluted **PD-1** protein solution to each well.
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 µl of 1x Immuno Buffer 1 per well.

9. Tap the plate onto clean paper towel to remove the liquid.

10. Add 100 µl 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 µl/well): N wells x (10 µl of 3x Immuno Buffer 1 + 15 µl of distilled water).

2. Add 25 µl of Master Mix to every well.

5. Prepare the Test Inhibitor (5 µl/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 µl.

   5.1 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***

   5.2 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 in1x 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 in1x 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%.*

6. Add 5 µl of Test Inhibitor to each well labeled as “Test Inhibitor”.
7. Add 5 µl of Diluent Solution to the “Positive Control”, “Ligand Control” and “Blank” wells.
8. Thaw biotin-labeled PD-L2 on ice. Briefly spin the tube containing the enzyme to recover its full content.
9. Dilute PD-L2 to 1 ng/µl with 1x Immuno Buffer (20 µl/ well).
10. Initiate the reaction by adding 20 µl of diluted PD-L2 to the wells designated “Positive Control”, Ligand Control” and "Test Inhibitor".
11. Add 20 µl of 1x Immuno Buffer to the “Blank” wells.
12. Incubate at RT for 2 hours.

| | **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-L2 (1 ng/µl) | - | 20 µl | 20 µl | 20 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** | **50 µl** |

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

10. Incubate the plate at the RT until blue color is developed in the "Positive Control" wells.

    *Note: For this assay, it normally takes 1-2 minutes to fully develop the color. However, the optimal incubation time may vary, and should be determined empirically by the user.*

11. Add 100 µl of 1 N hydrochloric acid to each well.

12. Read the absorbance at 450 nm using a UV/Vis spectrophotometer microplate reader.

    *Note: The "Blank" absorbance value should be ~0.05 at 450 nm. Alternatively, the plate may be read at 650 nm without adding 1 N hydrochloric acid, but the Signal-to-Background ratio will be decreased.*

**Example Results**

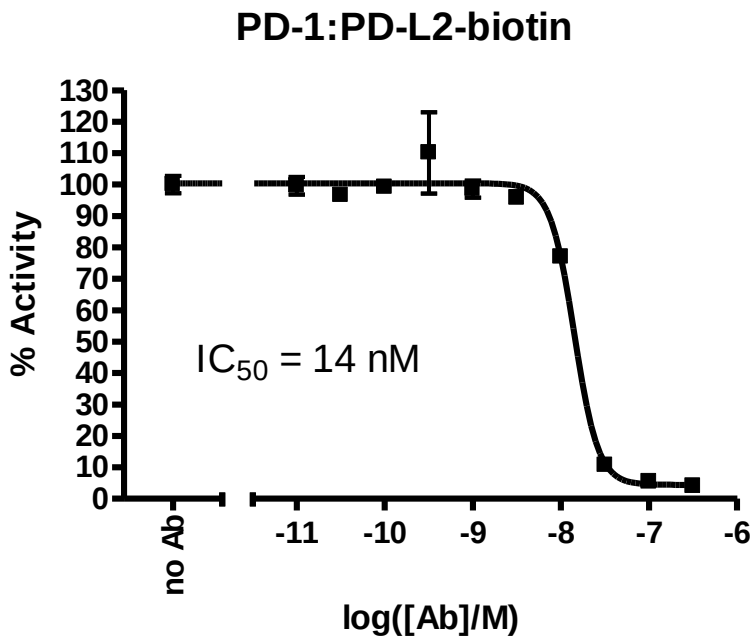


*Figure 1: Inhibition of PD-1 binding to PD-L2 by Anti-PD-1 Neutralizing Antibody.*
Binding of PD-1 to PD-L2 was measured in the presence of increasing concentrations of Anti-PD-1 Neutralizing Antibody (#71120). Absorbance was measured using a Tecan UV/Vis spectrophotometric microplate reader.

*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**

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

**Related Products**

| *Products* | *Catalog #* | *Size* |
|---|---|---|
| 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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