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Data Sheet

PD-1[Biotinylated]: PD-L1 Inhibitor Screening Assay Kit

Catalog # 79523
Size: 384 reactions

DESCRIPTION: Cell signaling through the PD-1 receptor upon binding the PD-L1 ligand attenuates immune responses and is exploited by both tumors and viruses. The *PD-1[Biotinylated]: PD-L1 Inhibitor Screening Assay Kit* is designed for screening and profiling inhibitors of this signaling. This kit comes in a convenient 384-well format with biotin-labeled PD-1, purified PD-L1, streptavidin-labeled HRP, and assay buffer for 400 binding reactions. The key to this kit is the high sensitivity of detection of biotin-labeled PD-1 by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, PD-L1 is coated on a 384-well plate. Next, PD-1 is incubated with PD-L1 on the plate. Finally, the plate is treated with streptavidin-HRP followed by addition of an HRP substrate to produce chemiluminescence, which can then be measured using a chemiluminescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71109	PD-1, Biotin-labeled	2 x 2 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71104	PD-L1	2 x 10 µg	-80°C	
79742	Streptavidin-HRP	2 x 10 µl	+4°C	
79311	3x Immuno Buffer 1	2 x 50 ml	-20°C	
79728	Blocking Buffer 2	2 x 50 ml	+4°C	
79670	ELISA ECL substrate A (transparent bottle)	2 x 6 ml	Room Temp	
	ELISA ECL substrate B (brown bottle)	2 x 6 ml	Room Temp	
78188	White 384-well microplate	1	+4°C	

MATERIALS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
Luminometer or fluorescent microplate reader capable of reading chemiluminescence
Rotating or rocker platform
Adjustable micropipettor and sterile tips

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APPLICATIONS: This kit is most useful for screening for inhibitors of PD-L1.

STABILITY: One year from date of receipt when stored as directed.

REFERENCE(S):

1. Lin, D., *et al. Proc Natl Acad Sci U.S.A.* 2008, **105:** 3011-3016.
2. Keir, M.E., *et al. Annu. Rev. Immunol*. 2008, **26:** 677-704.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Coating the plate with PD-L1:

1) Thaw **PD-L1** on ice. Upon first thaw, briefly spin tube containing **PD-L1** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **PD-L1** in aliquots at -80°C. *Note: PD-L1 is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **PD-L1** to 2 µg/ml in PBS.

3) Add 25 µl of diluted **PD-L1** solution to each well and incubate overnight at 4°C. Leave a couple of wells empty (uncoated), for use with the "Ligand Control" (see below).

4) Dilute **3x Immuno Buffer 1** to **1x Immuno Buffer 1** with water.

5) Decant to remove supernatant. Wash the plate 3 times with 75 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

6) Block wells by adding 50 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature. Decant to remove supernatant.

Step 1:

1) Prepare the master mixture: N wells × (5 µl **3x Immuno Buffer 1** + 7 µl H_2O).

2) Add 12 µl of master mixture to each well. Use uncoated wells for the "Ligand Control."

3) Add 3 µl of inhibitor solution to each well designated "Test Inhibitor." For the "Positive Control," "Ligand Control," and "Blank," add 3 µl of the same solution without inhibitor (inhibitor buffer).

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4) Thaw **PD-1, Biotin-labeled** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **PD-1, Biotin-labeled** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. *Note: **PD-1, Biotin-labeled** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer 1	5 µl	5 µl	5 µl	5 µl
H_2O	7 µl	7 µl	7 µl	7 µl
Test Inhibitor/Activator	–	–	–	3 µl
Inhibitor buffer (no inhibitor)	3 µl	3 µl	3 µl	–
1x Immuno Buffer 1	10 µl	–	–	–
PD-1-biotin (0.5 ng/µl)	–	10 µl	10 µl	10 µl
Total	**25 µl**	**25 µl**	**25 µl**	**25 µl**

5) Dilute **PD-1, Biotin-labeled** in **1x Immuno Buffer 1** at 0.5 µg/ml. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

6) Add 10 µl of **1x Immuno Buffer 1** to the well designated “Blank.”

7) Initiate reaction by adding 10 µl of diluted **PD-1, Biotin-labeled** (see Step 1-5) to wells labeled “Positive Control,” “Ligand Control,” and “Test Inhibitor,” Incubate at room temperature for two hours.

8) Decant to remove supernatant. Wash the plate 3 times with 75 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

9) Block wells by adding 50 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant.

Step 2:

1) Dilute **Streptavidin-HRP** 1000-fold with **Blocking Buffer 2**.

2) Add 50 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3) Wash plate 3 times with 75 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

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4) Block wells by adding 50 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

5) Just before use, mix on ice N wells × (25 µl **ELISA ECL substrate A** and 25 µl **ELISA ECL substrate B)**, then add 50 µl to each well. Discard any unused chemiluminescent reagent after use.

6) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. “Blank” value is subtracted from all readings.

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).

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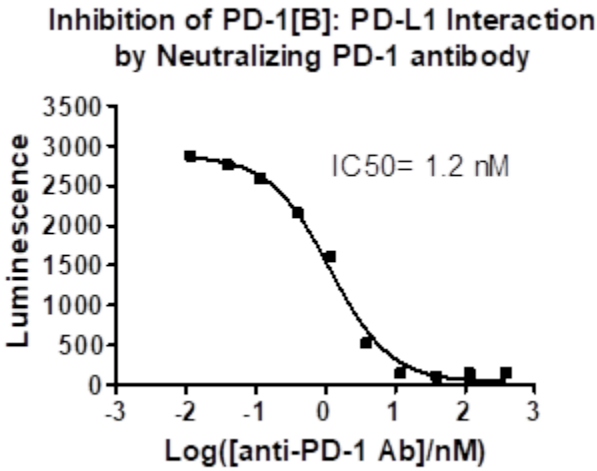
Example of Assay Results:



Inhibition of PD-1-PD-L1 binding by PD-1 Neutralizing Antibody (BPS Bioscience, #71120). Luminescence was measured using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS:

Product	Catalog #	Size
PD-1	71106	100 µg
PD-1, Biotin labeled	71109	50 µg
PD-L1	71104	100 µg
PD-L1, Biotin-labeled	71105	50 µg
PD-L2	71107	100 µg
PD-L2, Biotin-labeled	71108	50 µg
PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Assay Kit	72004	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Assay Kit	72006	96 rxns
PD-1 Neutralizing Antibody	71120	100 µg
PD-L1 Neutralizing Antibody	71213	100 µg
PD-1:PD-L1[Biotinylated] Inhibitor Screening Colorimetric Assay Kit	72016	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Colorimetric Assay Kit	72017	96 rxns
PD-1[Biotinylated]:PD-L1 Inhibitor Screening Colorimetric Assay Kit	72018	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Colorimetric Assay Kit	72019	96 rxns

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Luminescence signal of positive control reaction is weak	PD-1 or PD-L1 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh PD-1-biotin, (BPS Bioscience #71109) and fresh PD-L1 (BPS Bioscience #71104). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity of light detection.
	Chemiluminescent reagents mixed too soon	Chemiluminescent solution should be used within 15 minutes of mixing. Ensure both reagents are properly mixed.
Luminescent signal is erratic or varies widely among wells	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
Background (signal to noise ratio) is high	Insufficient washes	Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in PBST.
	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMSO level at <1% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of PD-1-biotin (BPS Bioscience #71109) to create a standard curve.

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