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Data Sheet

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

Catalog # 72027
Size: 96 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 for immune evasion. The Mouse *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 96-well format, with biotin-labeled mouse PD-1, purified mouse PD-L1, streptavidin-labeled HRP, and assay buffer for 100 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, mouse PD-L1 is coated on a 96-well plate. Next, mouse PD-1 is incubated with mouse 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	
71117	Mouse PD-L1, Fc fusion*	30 µg	-80°C	***Avoid freeze/ thaw cycles***
71118	Mouse PD-1, Fc fusion, Biotin-labeled*	10 µg	-80°C	
79742	Streptavidin-HRP	10 µl	+4°C	
	3x Mouse PD-1 Assay Buffer	50 ml	-20°C	
79728	Blocking Buffer 2	50 ml	+4°C	
79670	ELISA ECL substrate A (transparent bottle)	6 ml	Room Temp.	
	ELISA ECL substrate B (brown bottle)	6 ml	Room Temp.	
79699	White 96-well microplate	1	+4°C	

**The concentrations of Mouse PD-L1 and Mouse PD-1 are lot-specific and will be indicated on the tubes containing the protein.*

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline) pH 7.4
Luminometer or microplate reader capable of reading chemiluminescence
Rotating or rocker platform

APPLICATIONS: This kit is perfect for screening for inhibitors of mouse PD-L1 binding to mouse PD-1.

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

REFERENCES:

1. Lin, D., *et al. Proc. Natl. Acad. Sci. USA.* 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 **Mouse PD-L1** on ice. Upon first thaw, briefly spin tube containing **Mouse PD-L1** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **Mouse PD-L1** in aliquots at -80ºC. *Note: **Mouse PD-L1** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Dilute **Mouse PD-L1** to 5 µg/ml in PBS.

3) Add 50 µl of diluted **Mouse 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 Mouse PD-1 Assay buffer** to **1x Mouse PD-1 Assay buffer** with water. Prepare only enough **1x Mouse PD-1 Assay buffer** required for the assay.

5) Decant plate to remove supernatant. Wash the plate 3 times with 100 µl **1x PD-1 Assay Buffer**. Tap plate onto clean paper towels to remove liquid.

6) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 1 hour at room temperature. Decant to remove supernatant.

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Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Mouse PD-1 Assay Buffer** + 15 µl H_2O).

2) Add 25 µl of master mixture to each well. Include uncoated wells for the "Ligand Control".

3) Add 5 µl of inhibitor solution to each well designated "Test Inhibitor". For the "Positive Control", "Ligand Control" and "Blank", add 5 µl of the same solution without inhibitor (inhibitor buffer).

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

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Mouse PD-1 Assay Buffer	10 µl	10 µl	10 µl	10 µl
H_2O	15 µl	15 µl	15 µl	15 µl
Test Inhibitor/Activator	–	–	–	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	–
1x Mouse PD-1 Assay Buffer	20 µl	–	–	–
Mouse PD-1-biotin (5 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Dilute **Mouse PD-1-biotin** in **1x PD-1 Assay Buffer** at 5 ng/µl (100 ng/20 µl). Keep diluted protein on ice until use. Discard any unused diluted protein after use.

6) Add 20 µl of **1x Mouse PD-1 Assay Buffer** to the well designated "Blank".

7) Initiate reaction by adding 20 µl of diluted **Mouse PD-1-biotin** (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 100 µl **1x Mouse PD-1 Assay Buffer**. Tap plate onto clean paper towels to remove liquid.

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9) Block wells by adding 100 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 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

3) Wash plate three times with **1x Mouse PD-1 Assay Buffer**. Tap plate onto clean paper towels to remove liquid.

4) Block wells by adding 100 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 50 µl **ELISA ECL substrate A** and 50 µl **ELISA ECL substrate B**, then add 100 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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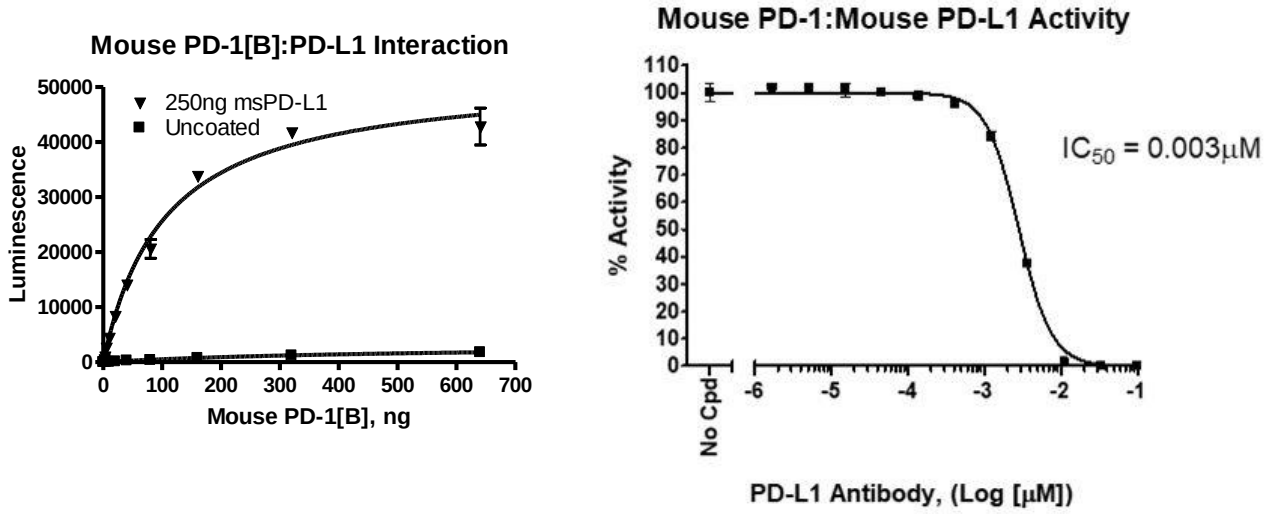
Example of Assay Results:



(Left) Mouse PD-1-PD-L1 binding activity, measured using the using the *Mouse PD-1[Biotinylated]:PD-L1 Inhibitor Screening Assay Kit*, (BPS Cat. #72027) and (Right) Inhibition of mouse PD-1:PD-L1 binding by Anti-PD-L1 Neutralizing Antibody (BPS Cat. #71213). 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.*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
Mouse PD-1, Fc fusion	71116	100 µg
Mouse PD-1, Fc fusion, Biotin labeled	71118	50 µg
Mouse PD-L1, Fc fusion	71117	100 µg
Mouse PD-L1, Fc fusion, Biotin labeled	71119	50 µg
Human PD-1, Fc fusion	71106	100 µg
Human PD-1, Fc fusion, Biotin labeled	71109	50 µg
Human PD-L1, Fc fusion	71104	100 µg
Human PD-L1, Fc fusion, Biotin-labeled	71105	50 µg
Human PD-L2, Fc fusion	71107	100 µg
Human PD-L2, Fc fusion, Biotin-labeled	71108	50 µg
PD-1 Neutralizing Antibody	71120	50 µg
PD-L1 Neutralizing Antibody	71213	50 µg
Human PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 rxns
Human PD-1:PD-L2[Biotinylated] Inhibitor Screening Assay Kit	72004	96 rxns
Human PD-1[Biotinylated]:PD-L2 Inhibitor Screening Assay Kit	72006	96 rxns
Human PD-1:PD-L1[B] Inhibitor Screening Colorimetric Assay Kit	72016	96 rxns
Human PD-1:PD-L2[B] Inhibitor Screening Colorimetric Assay Kit	72017	96 rxns
Human PD-1[B]:PD-L1 Inhibitor Screening Colorimetric Assay Kit	72018	96 rxns
Human PD-1[B]: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	Mouse PD-1 or Mouse PD-L1 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh Mouse PD-1-biotin, (BPS Bioscience #71118) and fresh Mouse PD-L1 (BPS Bioscience #71117). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	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.
	Inaccurate pipetting/technique	Run duplicates of all reactions. Use a multichannel pipettor. Use master mixes to minimize errors.
Luminescent signal is erratic or varies widely among wells	Bubbles in wells	Pipette slowly to avoid bubble formation. Tap plate lightly to disperse bubbles; be careful not to splash between wells.
	Insufficient washes	Increase number of washes. Increase wash volume.
Background (signal to noise ratio) is high	Sample solvent is inhibiting the enzyme	Run negative control assay including solvent. Maintain DMOUSE O level at <2% Increase time of enzyme incubation.
	Results are outside the linear range of the assay	Use different concentrations of Mouse PD-1-biotin (BPS Bioscience #71118) to create a standard curve.