6405 Mira Mesa Blvd Ste 100
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: support@bpsbioscience.com

Data sheet

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

Catalog #79863
Size: 96 reactions

DESCRIPTION: Cell signaling through the PD-1 receptor upon binding the PD-L1 ligand attenuates immune responses and this pathway is exploited by both tumors and viruses. The Monkey PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit is designed for screening and profiling inhibitors of this signaling pathway in the animal model cynomolgus monkey (*Macaca fascicularis*). This kit comes in a convenient 96-well format, with biotin-labeled monkey PD-L1, purified monkey PD-1, 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-L1 by streptavidin-HRP. Only a few simple steps on a microtiter plate are required for the assay. First, PD-1 is coated on a 96-well plate. Next, PD-L1 is incubated with PD-1 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	
71158	PD-1 (Monkey), Fc fusion (Human IgG1) HiP™	10 µg	-80°C	Avoid multiple freeze/ thaw cycles!
71154	PD-L1 (Monkey), Fc fusion (Human IgG1), Biotin-labeled HiP™	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	6 ml	Room Temp	
	ELISA ECL Substrate B	6 ml	Room Temp	
79699	96-well white microplate	1	+4°C	

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

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

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STABILITY: Up to 6 months from date of receipt, when stored as recommended.

REFERENCES:

Onlamoon, N. *et al. Immunol.* 2008, **124.1**: 277-293
Wang. C. *et al. Cancer Immunol. Res.* 2014, **2.9**: 846-856

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Coating the plate with monkey PD-1:

1) Thaw **monkey PD-1** on ice. Upon first thaw, briefly spin tube containing **monkey PD-1** to recover the full contents of the tube. Aliquot into single use aliquots. Immediately store remaining **monkey PD-1** in aliquots at -80°C. Note: **monkey PD-1** is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.
2) Dilute **monkey PD-1** to 2 µg/ml in PBS.
3) Add 50 µl of diluted **PD-1** 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. (Keep a portion of the **3x Immuno Buffer 1** undiluted, for use in Step 1 below).
5) Decant to remove supernatant. Wash the plate three times with 100 µl **1x Immuno Buffer 1**. 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 with slow shaking. Remove supernatant as described in step 5.

Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1** + 15 µl distilled water)
2) Add 25 µl of master mixture to each well. Use 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). Incubate at room temperature for one hour with slow shaking.

   Note: Inhibitor buffer should contain the same concentration of DMSO as the test inhibitor. For example, prepare 200x test compound in DMSO; dilute 1:20 in water to make a 5% DMSO solution. For the control, use 5% DMSO(aq) with no inhibitor. Final DMSO concentration in the assay will be 0.5%.

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4) Thaw **monkey PD-L1-biotin** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full contents of the tube. Aliquot **monkey PD-L1-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80°C. Note: **monkey PD-L1*-biotin*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

5) Dilute **monkey PD-L1-biotin** to 2 ng/µl (approximately 40 nM) in **1x Immuno Buffer 1**. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

6) Add 20 µl of **1x Immuno Buffer 1** to the well designated "Blank".

	Blank	**Ligand Control**	**Positive Control**	**Test Inhibitor**
3x Immuno Buffer 1	10 µl	10 µl	10 µl	10 µl
Distilled water	15 µl	15 µl	15 µl	15 µl
Test Inhibitor	-	-	-	5 µl
Inhibitor buffer (no inhibitor)	5 µl	5 µl	5 µl	-
1x Immuno Buffer 1	20 µl	-	-	-
Monkey PD-L1-biotin (2 ng/µl)	-	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

7) Initiate reaction by adding 20 µl of diluted **PD-L1-biotin** (see Step 1-5) to wells labeled "Positive Control", "Ligand Control" and "Test Inhibitor". Incubate at room temperature for one hour with slow shaking.

8) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

9) Block wells by adding 100 µl of **Blocking Buffer 2** to each well. Incubate for 10 minutes at room temperature. Remove supernatant as in Step 1-8.

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 Immuno Buffer 1**. Tap plate onto clean paper towel 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.

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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 milliseconds. 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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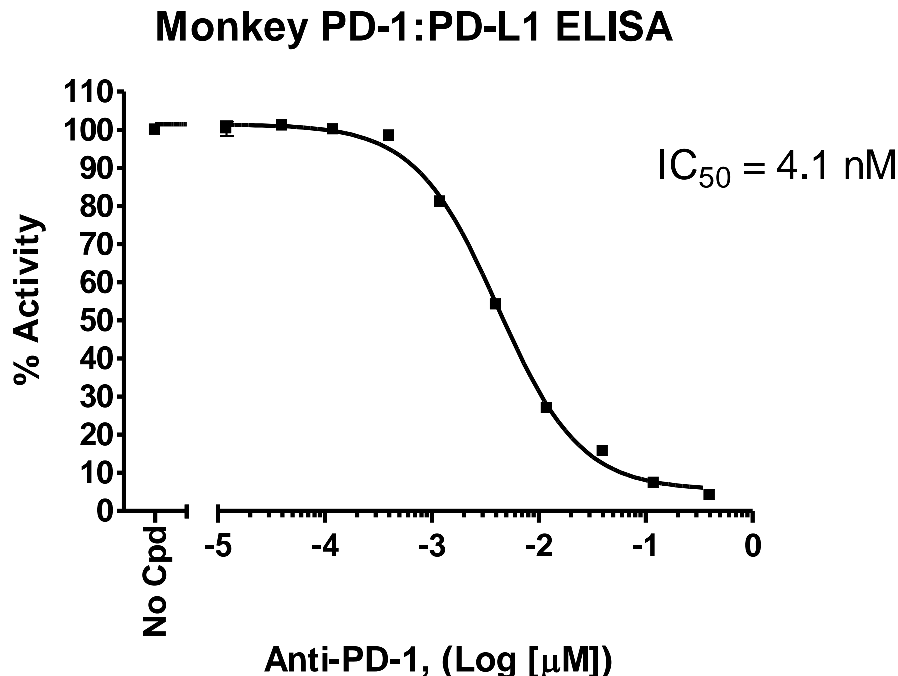
Example of assay results:



Inhibition of monkey PD-1:PD-L1 binding using the Anti-PD-1 Neutralizing Antibody (BPS Bioscience, #71120) in the *Monkey PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit*. 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 Name	Catalog#	Size
PD-1 (Monkey), Fc fusion (Human IgG1) HiP™	71158	100 µg
PD-1 (Monkey), Fc fusion (Human IgG1), Biotin-labeled HiP™	71235	50 µg
PD-L1 (Monkey), Fc fusion (Human IgG1) HiP™	71153	100 µg
PD-L1 (Monkey), Fc fusion (Human IgG1), Biotin-labeled HiP™	71154	50 µg
PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 reactions
PD-1 (CD279), Fc fusion (Human)	71106-1	100 µg
PD-L1 (CD274), Fc fusion, Biotin-labeled (Human) HiP™	71105-1	25 µg

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TROUBLESHOOTING GUIDE

Problem	Possible cause	Solution
Luminescence signal of positive control reaction is weak	Monkey PD-1 or monkey PD-L1-biotin has lost activity	Proteins lose activity upon repeated freeze/thaw cycles. Use fresh monkey PD-L1-biotin (BPS Bioscience #71154) and fresh monkey PD-1 (BPS Bioscience #71158).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.
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 monkey PD-L1-biotin (BPS Bioscience #71154) to create a standard curve

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