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Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Data Sheet

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

Catalog # 72017
Size: 96 reactions

DESCRIPTION: Cell signaling through the PD-1 receptor upon binding the PD-L2 ligand attenuates immune responses and is exploited by both tumors and viruses. The *PD-1:PD-L2[Biotinylated] Inhibitor Screening Colorimetric Assay Kit* is designed for screening inhibitors of PD-L2 ligand binding to PD-1 receptor. This kit comes in a convenient 96-well format, with biotin-labeled PD-L2, purified PD-1, streptavidin-labeled HRP, and assay buffer for 100 binding reactions. The key to this kit is the high affinity of biotin-labeled PD-L2 for 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-L2 is incubated with PD-1 on the plate. 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.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71108	PD-L2, Biotin-labeled	5 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71106	PD-1	10 µg	-80°C	
	Streptavidin-HRP	15 µl	+4°C	
79311	3x Immuno Buffer 1	50 ml	-20°C	
	Blocking Buffer	50 ml	+4°C	
	Colorimetric HRP substrate	10 ml	+4°C	
	Transparent 96-well microplate	1	+4°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
1N HCl (aqueous)
Rotating or rocker platform
UV/Vis spectrophotometer microplate reader capable of reading absorbance at 450 nm*

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

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APPLICATIONS: This kit is most useful for screening for inhibitors of PD1 binding to PD-L2.

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

REFERENCES:

1. Molnar, E., *et al. Proc Natl Acad Sci U.S.A*. 2008, **105:** 10483-10488.
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-1:

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

2) Dilute **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.

5) Decant to remove supernatant. Wash the plate 3 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** to each well. Incubate for 1 hour at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

Step 1:

1) Prepare the master mixture: N wells × (10 µl **3x Immuno Buffer 1**+ 15 µl H_2O)

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

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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 **PD-L2-biotin** on ice. Upon first thaw, briefly spin tube containing protein to recover full contents of the tube. Aliquot **PD-L2-biotin** into single use aliquots. Immediately store remaining undiluted protein in aliquots at -80°C. *Note: **PD-L2-biotin** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

	Blank	Ligand Control	Positive Control	Test Inhibitor
3x Immuno Buffer 1	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 Immuno Buffer 1	20 µl	–	–	–
PD-L2-biotin (1 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Dilute **PD-L2-biotin** in **1x Immuno Buffer 1**at 1 ng/µl (20 ng/20 µl). 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".

7) Initiate reaction by adding 20 µl of diluted **PD-L2-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 Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

9) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

Step 2:

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

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2) Add 100 µl to each well. Incubate for 1 hour at room temperature with slow shaking.

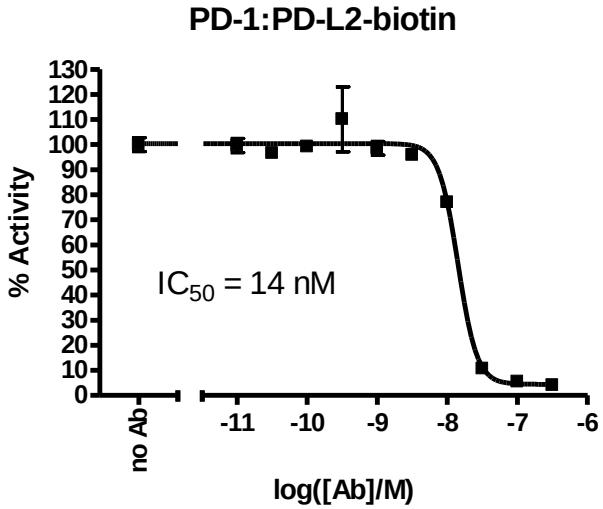
3) Wash plate three times with **1x Immuno Buffer 1**. Tap plate onto clean paper towels to remove liquid.

4) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

5) Add 100 µl of the **Colorimetric HRP substrate** to each well and incubate the plate at room temperature until blue color is developed in the positive control well. This usually takes 1-2 min to fully develop. However, the optimal incubation time may vary, and should be determined empirically by the user.

6) After the blue color is developed, add 100 µl of 1N HCl to each well. Read the absorbance at 450 nm using UV/Vis spectrophotometer microplate reader. The blank wells should exhibit an absorbance of ~ 0.05 at 450 nm. *Alternatively, the plate may be read at 650 nm without adding 1N HCl, but the Signal-to-Background ratio will be decreased.*

Example of Assay Results:



Inhibition of PD-1-PD-L2 binding by PD-1 Neutralizing antibody (BPS Cat. #71120) measured using the PD-1:PD-L2[Biotinylated] Inhibitor Screening Colorimetric Assay Kit (BPS Cat. #72017). *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
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 Colorimetric Kit	72016	96 rxns
PD-1[Biotinylated]:PD-L1 Inhibitor Screening Colorimetric Kit	72018	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Colorimetric Kit	72019	96 rxns
PD-1:PD-L1[Biotinylated] Inhibitor Screening Chemiluminescent Kit	72003	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Chemiluminescent Kit	72004	96 rxns
PD-1[Biotinylated]:PD-L1 Inhibitor Screening Chemiluminescent Kit	72005	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Chemiluminescent Kit	72006	96 rxns
PD-1 Neutralizing Antibody	71120	50 µg
PD-L1 Neutralizing Antibody	71213	50 µg

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Colorimetric signal of positive control reaction is weak	PD-1 or PD-L2 has lost activity	Protein loses activity upon repeated freeze/thaw cycles. Use fresh protein. Store protein in single-use aliquots. Increase time of protein incubation. Increase protein concentration.
	Incorrect settings on instruments	Refer to instrument instructions for settings to increase sensitivity.
	Colorimetric HRP substrate was not incubated long enough	Increase the amount of time that the colorimetric HRP substrate is incubated in the wells. Avoid azides.
Colorimetric 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.
	Signal is out of range of detection (too high)	Decrease the amount of time that the colorimetric HRP substrate is incubated in the wells
Background (signal to noise ratio) is high	Insufficient washes or blocking	Be sure to include blocking steps after wash steps. Increase number of washes. Increase wash volume. Increase Tween-20 concentration to 0.1% in TBST. Be sure to dilute Streptavidin-HRP in blocking buffer, not assay buffer.
	Sample solvent is inhibiting the protein	Run negative control assay including solvent. Maintain DMSO level at <1%. Increase time of protein incubation.
	Results are outside the linear range of the assay	Use different concentrations of protein to create a standard curve.