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Data Sheet

PD-1:PD-L2 Homogeneous Assay Kit

Catalog #72015

DESCRIPTION: The *PD-1:PD-L2 Homogeneous Assay Kit* is designed to measure the inhibition of PD-1 binding to PD-L2. The *PD-1:PD-L2 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format with purified biotinylated PD-L2, FLAG-tagged PD-1, and assay buffer to perform a total of 384 reactions. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing PD-1 and an inhibitor of choice is incubated with the biotinylated PD-L2 for 60 minutes. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
71198	PD-1-FLAG-Avi-His	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
71108	PD-L2-biotin	5 µg	-80°C	
79311	3x Immuno Buffer 1	4 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA FLAG acceptor beads, 5 mg/ml (PerkinElmer #AL112C)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate-384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for screening for inhibitors of PD-1 binding to PD-L2

CONTRAINDICATIONS: Only limited amounts of DMSO can be included, as it has been shown to disrupt PD-1:PD-L2 interaction. Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least 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.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Step 1:

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

2) Dilute one part **3x Immuno Buffer 1** with 2 parts of distilled water (3-fold dilution) to make **1x Immuno Buffer 1**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

3) Dilute **PD-1-FLAG-Avi-His** in **1x Immuno Buffer 1** to 10 ng/µl. Keep diluted protein on ice until ready to use. Discard any remaining unused diluted protein after use.

4) Prepare the master mixture: N wells × (2 µl **3x Immuno Buffer 1** + 2 µl diluted **PD-1-FLAG +** 2 µl distilled water). Add 6 µl of master mixture to every well.

	Blank	Positive Control	Test Inhibitor
3x Immuno Buffer 1	2 µl	2 µl	2 µl
PD-1-FLAG-Avi-His (10 ng/µl)	2 µl	2 µl	2 µl
Distilled water	2 µl	2 µl	2 µl
Test Inhibitor	–	–	2 µl
Inhibitor buffer (no inhibitor)	2 µl	2 µl	–
1x Immuno Buffer 1	2 µl		
PD-L2-biotin (3 ng/µl)	–	2 µl	2 µl
Total	**10 µl**	**10 µl**	**10 µl**

5) Add 2 µl of inhibitor solution to each well designated “Test Inhibitor”. For the “Positive Control” and “Blank”, add 2 µl of the same solution without inhibitor (inhibitor buffer). *Note: If possible, keep final DMSO concentration below 0.5%.*

6) Add 2 µl of **1x Immuno Buffer 1** to the well designated “Blank”.

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

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8) Dilute **PD-L2-biotin** in **1x Immuno Buffer 1** to 3 ng/µl. Keep diluted proteins on ice until use. Discard any remaining unused diluted protein after use.

9) Initiate reaction by adding 2 µl of diluted **PD-L2-biotin** prepared as described above to each well designated "Positive Control" and "Test Inhibitor". Incubate at room temperature for 60 minutes.

Step 2:
Note: Protect your samples from direct exposure to light!

1) Dilute FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with **1x Immuno Buffer 1**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:
Note: Protect your samples from direct exposure to light!

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x Immuno Buffer 1**. Add 10 µl per well. Incubate at room temperature for 30 minutes.

2) Read Alpha-counts.

Due to lot to lot variability in AlphaScreen® *bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to PD-1 or PD-1L concentrations may improve signal-to-noise ratio.*

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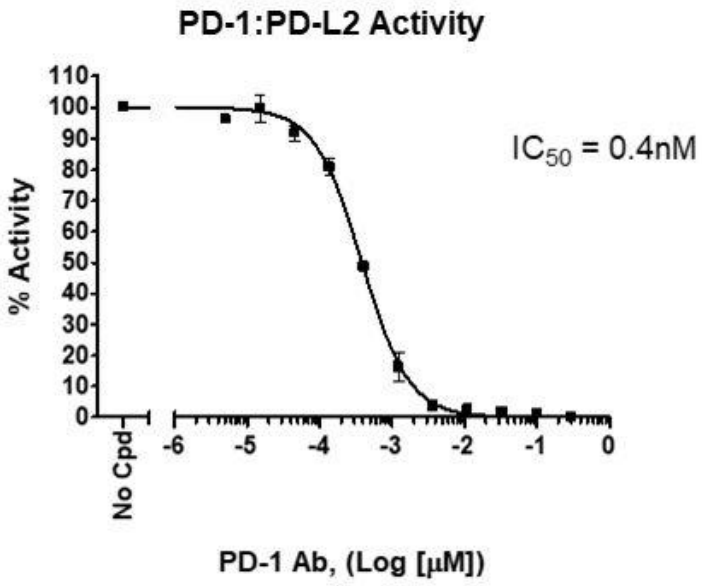
Example of Assay Results:



PD-1:PD-L2 inhibition, measured using the PD-1:PD-L2 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #72015 and PD-1 neutralizing antibody, Catalog #71213. *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	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-L2[Biotinylated] Inhibitor Screening Kit	72004	96 rxns
PD-1:PD-L1[Biotinylated] Inhibitor Screening Kit	72003	96 rxns
PD-L1 Inhibitor Screening Kit	72005	96 rxns
PD-L2 Inhibitor Screening Kit	72006	96 rxns
PD-1 Neutralizing Antibody	71120	50 µg
PD-L1 Neutralizing Antibody	71213	50 µg
PD-1:PD-L1 Homogenous Assay Kit	72014	384 rxns