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Data Sheet

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

Catalog #w82027
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. The *PD-1:PD-L1[Biotinylated] Inhibitor Screening Colorimetric Assay Kit* is designed for screening inhibitors of PD-L1 ligand binding to PD-1 receptor. This kit comes in a convenient 96-well format, with biotin-labeled PD-L1, 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-L1 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-L1 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	
w81116	PD-L1, Biotin-labeled	5 µg	-80℃	***(Avoid freeze/ thaw cycles!)***
w81117	PD-1	10 µg	-80℃	
	Streptavidin-HRP	15 µl	+4℃	
	3x PD-1 Assay Buffer	50 ml	-20℃	
	Blocking Buffer	50 ml	+4℃	
	Colorimetric HRP substrate	10 ml	+4℃	
	White 96-well microplate	1	+4℃	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
PBS (Phosphate buffered saline)
2 M sulfuric acid (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 useful for screening for inhibitors of PD-1 binding to PD-L1.

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

REFERENCES:

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-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 "Substrate Control" (see below).

4) Decant 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.

5) 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 PD-1 Assay Buffer** + 15 µl H_2O).

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

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

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

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x 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 PD-1 Assay Buffer	20 µl	–	–	–
PD-L1-biotin (2 ng/µl)	–	20 µl	20 µl	20 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**

5) Dilute **PD-L1-biotin** in **1x PD-1 Assay Buffer** at 2 ng/µl (40 ng/20 µl). Keep diluted protein on ice until use. Discard any unused diluted protein after use.

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

7) Initiate reaction by adding 20 µl of diluted **PD-L1-biotin** (see Step 1-5) to wells labeled “Positive Control”, “Substrate Control” and “Test Inhibitor”. Incubate at room temperature for two hours.

8) Decant to remove supernatant. Wash the plate 3 times with 100 µl/well **1x PD-1 Assay Buffer**. 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**.

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

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

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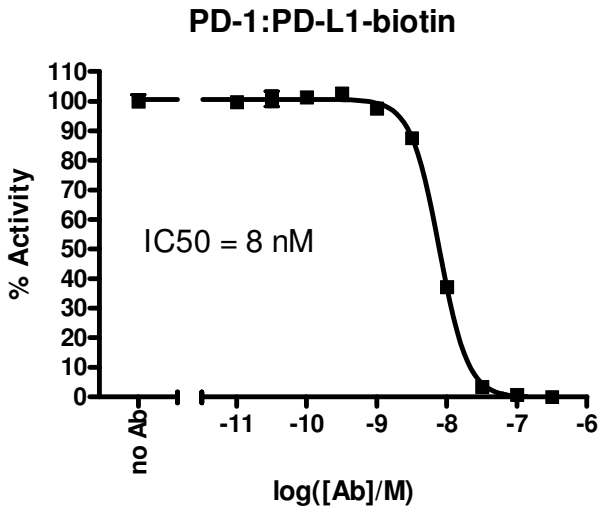
4) Block wells by adding 100 µl of **Blocking Buffer** to each well. Incubate for 10 minutes at room temperature. Decant to remove supernatant. Tap plate onto clean paper towels to remove liquid.

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 2 M sulfuric acid 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 2 M sulfuric acid, but the Signal-to-Background ratio will be decreased.*

Example of Assay Results:



Inhibition of PD-1:PD-L1 binding by PD-1 Neutralizing Antibody (WestBio Cat. #w81131) measured using the PD-1:PD-L1[Biotinylated] Inhibitor Screening Colorimetric Assay Kit (WestBio Cat.#72016). *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com.*

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TROUBLESHOOTING GUIDE

Problem	Possible Cause	Solution
Colorimetric signal of positive control reaction is weak	PD-1 or PD-L1 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.