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Data Sheet

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

Catalog # w82014
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 Assay Kit* is designed for screening and profiling inhibitors of this signaling. 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 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	
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℃	
	HRP chemiluminescent substrate A (transparent bottle)	6 ml	+4℃	
	HRP chemiluminescent substrate B (brown bottle)	6 ml	+4℃	
	White 96-well microplate	1	+4℃	

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

APPLICATIONS: This kit is useful for screening for inhibitors of PD1 binding to PD-L1.

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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. Remove supernatant as described in step 4.

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 enzyme to recover full contents of the tube. Aliquot **PD-L1-biotin** into single use aliquots. Immediately store remaining undiluted enzyme in aliquots at -80℃. *Note: PD-L1-biotin is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

	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. Remove supernatant as in Step 1-8.

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**, incubate in **Blocking Buffer**, and remove supernatant as described in Steps 1-8 and 1-9.

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4) Just before use, mix on ice 50 µl **HRP Chemiluminescent Substrate A** and 50 µl **HRP Chemiluminescent Substrate B**, then add 100 µl to each well. Discard any unused chemiluminescent reagent after use.

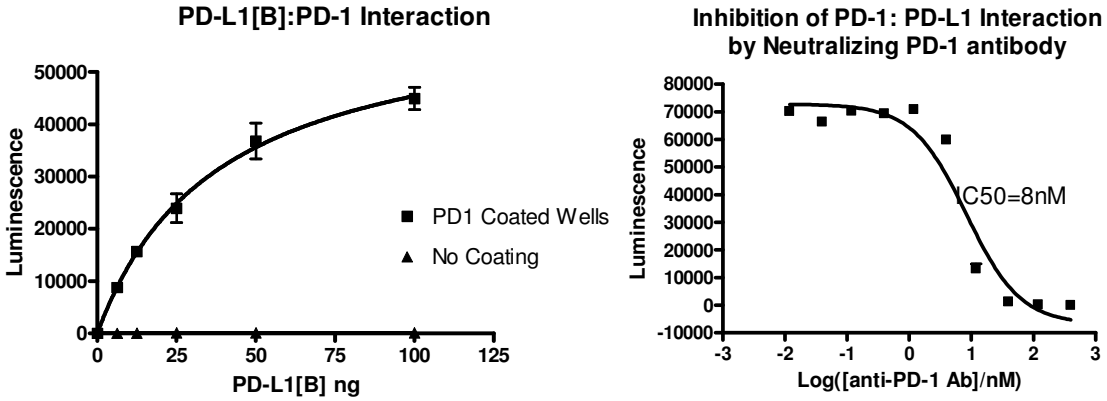
5) Immediately read sample in a luminometer or microtiter-plate capable of reading chemiluminescence. "Blank" value is subtracted from all readings.

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).

Example of Assay Results:



(Left) PD-1-PD-L1 binding activity, measured using the using the *PD-1:PD-L1[Biotinylated] Inhibitor Screening Assay Kit*, (WestBio Cat. #w82014) and (Right) Inhibition of PD-1-PD-L1 binding by PD-1 Neutralizing Antibody (WestBio Cat. #w81131). Luminescence was measured using a Bio-Tek fluorescent microplate reader. *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
Luminescence signal of positive control reaction is weak	PD-1 or PD-L1 has lost activity	Enzyme loses activity upon repeated freeze/thaw cycles. Use fresh PD-L1-biotin, (West Bioscience #w81116) and fresh PD-1 (West Bioscience #w81117). Store proteins in single-use aliquots. Increase time of enzyme incubation. Increase enzyme concentration.
	Antibody reaction is insufficient	Increase time for primary antibody incubation. Avoid freeze/thaw cycles of antibodies.
	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 PD-L1-biotin (West Bioscience #w81116) to create a standard curve.