

Data Sheet ***PD-1:PD-L2 Homogeneous Assay Kit*** Catalog #w82026

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	
w81126	PD-1-FLAG	10 µg	-80 °C	(Avoid freeze/ thaw cycles!)
w81119	PD-L2-biotin	5 µg	-80 °C	
	3x PD-1 assay buffer	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 (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). 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** 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 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
- 2) Dilute one part **3x PD-1 assay buffer** with 2 parts of distilled water (3-fold dilution) to make **1x PD-1 assay buffer**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.
- 3) Dilute **PD-1-FLAG** in **1x PD-1 assay buffer** 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 PD-1 assay buffer** + 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 PD-1 assay buffer	2 μl	2 μl	2 μl
PD-1-FLAG (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 PD-1 assay buffer	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 PD-1 assay buffer** 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 PD-1 assay buffer** 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 #AL109C) 250-fold with **1x PD-1 assay buffer**. Add 10 μl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

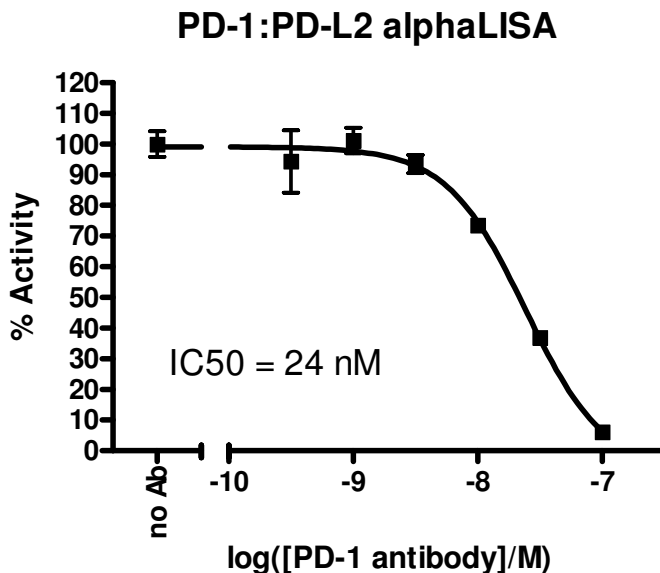
Note: Protect your samples from direct exposure to light!

- 1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x PD-1 assay buffer**. 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, West Bioscience, Catalog #w82026 and PD-1 neutralizing antibody, Catalog #w81224. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com.*

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