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Data Sheet
PD-1:PD-L2 TR-FRET Assay Kit
Catalog # w82023

DESCRIPTION:
The PD-1:PD-L2 TR-FRET Assay Kit is designed to measure the inhibition of PD-1 binding to PD-L2 in a homogeneous 384 reaction format. Cell signaling through the PD-1 receptor upon binding the PD-L2 ligand attenuates immune responses and is exploited by both tumors and viruses. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; a sample containing europium-labeled (Eu) PD-1, dye-labeled acceptor, biotin-labeled PD-L2, and an inhibitor is incubated for two hours. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	PD-1-Eu	2 µg	-80°C	***(Avoid freeze/thaw cycles!)***
w81119	PD-L2, Biotinylated	20 µg	-80°C	
	Dye-labeled acceptor	20 µl	-20°C	
	3x PD-1 TR FRET Assay Buffer	4 ml	-20°C	
	White, non-binding, low volume, 384-well microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: At least 6 months from date of receipt when stored as directed.

REFERENCES:
1. Molnar, E., *et al. Proc Natl Acad Sci USA*. 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.

Protocol for PD-1 assay

1) Dilute one part **3x PD-1 TR-FRET Assay Buffer** with 2 parts 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℃.

2) Dilute **Dye-labeled acceptor** 100-fold in **1x PD-1 Assay Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20℃.

3) Thaw **PD-1-Eu** on ice. Upon first thaw, briefly spin tube containing **PD-1-Eu** to recover the full contents of the tube. Aliquot into single-use aliquots. Store remaining undiluted **PD-1-Eu** at –80℃ immediately. *Note: **PD-1-Eu** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*

4) Dilute **PD-1-Eu** in **1x PD-1 Assay Buffer** to 0.2 µg/ml. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20℃.

5) Prepare the master mixture: N wells x (5 µl diluted **PD-1-Eu** + 5 µl diluted **Dye-labeled acceptor** + 3 µl **1x PD-1 Assay Buffer**). Add 13 µl to every well.

6) Add 2 µl of inhibitor solution to each well designated “Test Inhibitor”. Add 2 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled “Negative Control” and “Positive Control”.

7) Add 5 µl **1x PD-1 Assay Buffer** to wells designated for “Negative Control.”

	Positive Control	**Negative Control**	**Test Inhibitor**
PD-1 -Eu	5 µl	5 µl	5 µl
Dye-labeled acceptor	5 µl	5 µl	5 µl
1x PD-1 Assay Buffer	3 µl	8 µl	3 µl
Test Inhibitor	–	–	2 µl
Inhibitor Buffer (no inhibitor)	2 µl	2 µl	–
PD-L2-biotin	5 µl	–	5 µl
Total	**20 µl**	**20 µl**	**20 µl**

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

9) Dilute **PD-L2, biotinylated** in **1x PD-1 Assay Buffer** to 5 µg/ml. Initiate reaction by adding 5 µl of diluted **PD-L2, biotinylated** to wells designated for the "Positive Control" and "Test Inhibitor." Discard any remaining diluted PD-L2 protein after use.

10) Incubate at room temperature for 2 hours.

11) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	320±10 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:
Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

If desired, data can be normalized to percent inhibition. Typically for inhibitor screens the FRET value from the positive control is set to zero percent inhibition and the FRET value from the negative control is set to one hundred percent inhibition.

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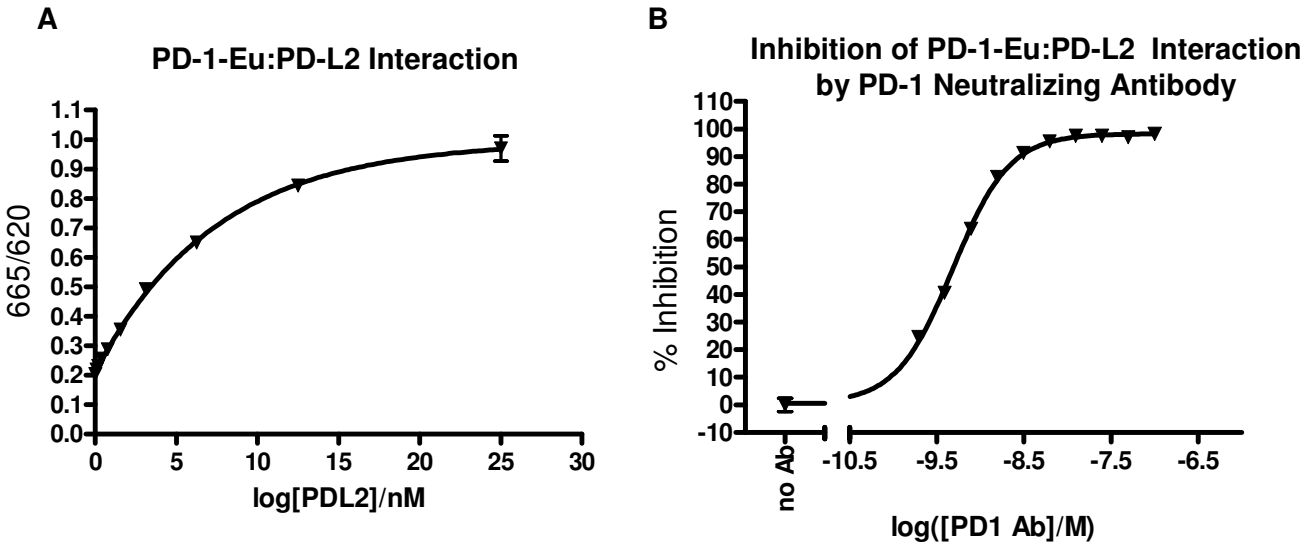
EXAMPLE OF ASSAY RESULTS:



(A) Interaction of PD-1 with PD-L2, data are expressed as raw 665/620 ratios. **(B)** Inhibition of PD-1:PD-L2 interaction using PD-1 neutralizing antibody (WestBio Cat. #w81131). *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com*

Note: The dye-labeled acceptor used in this assay is a product of Cisbio Bioassays.