6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet
PD-1:PD-L1 TR-FRET Assay (384 well)
Catalog # 72038

DESCRIPTION:
The PD-1:PD-L1 TR-FRET Assay is designed to measure the inhibition of PD-1 binding to PD-L1 in a homogeneous 384 reaction format. 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-L1, 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!)***
71105	PD-L1, Biotinylated	30 µg	-80°C	
	Dye-labeled acceptor	2 x 10 µl	-20°C	
79311	3x Immuno Buffer 1	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.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Protocol for PD-1 assay

1) Dilute one part **3x Immuno Buffer 1** with 2 parts 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.

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

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°C 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 Immuno Buffer 1** to 0.2 µg/ml. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

5) Prepare the master mixture: N wells x (5 µl diluted **PD-1-Eu** + 5 µl diluted **Dye-labeled acceptor**). Add 10 µl to every well.

6) Add 5 µl of inhibitor solution to each well designated “Test Inhibitor”. Add 5 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled “Negative Control” and “Positive Control”.

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

	Positive Control	Negative Control	Test Inhibitor
PD-1 –Eu (0.2 µg/ml)	5 µl	5 µl	5 µl
Dye-labeled acceptor	5 µl	5 µl	5 µl
1x Immuno Buffer 1	-	5 µl	-
Test Inhibitor	–	–	5 µl
Inhibitor Buffer (no inhibitor)	5 µl	5 µl	–
PD-L1-biotin (11 µg/ml)	5 µl	–	5 µl
Total	**20 µl**	**20 µl**	**20 µl**

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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

9) Dilute **PD-L1, biotinylated** in **1x Immuno Buffer 1** to 11 µg/ml. Initiate reaction by adding 5 µl of diluted **PD-L1, biotinylated** to wells designated for the "Positive Control" and "Test Inhibitor." Discard any remaining diluted PD-L1 protein after use.

10) Incubate at room temperature for 1.5 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	320±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

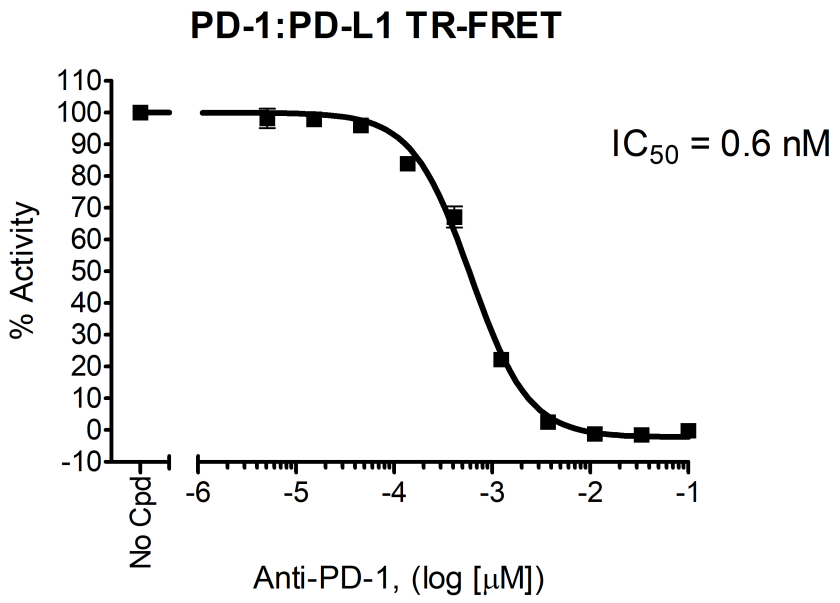
CALCULATING RESULTS:
Two sequential measurements should be conducted. Tb-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

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.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

EXAMPLE OF ASSAY RESULTS:



Inhibition of PD-1:PD-L1 interaction using PD-1 neutralizing antibody (BPS Cat. #71120). *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-L1[Biotinylated] Inhibitor Screening Assay Kit	72003	96 rxns
PD-1:PD-L2[Biotinylated] Inhibitor Screening Assay Kit	72004	96 rxns
PD-1[Biotinylated]:PD-L1 Inhibitor Screening Assay Kit	72005	96 rxns
PD-1[Biotinylated]:PD-L2 Inhibitor Screening Assay Kit	72006	96 rxns
PD-1 Neutralizing Antibody	71120	50 µg

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

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.