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# Data Sheet
## *PD-1:PD-L1 TR-FRET Assay (96 well)*
**Catalog # 72032**

**DESCRIPTION:**
The PD-1:PD-L1 TR-FRET Assay is designed to measure the inhibition of Human PD-1 binding to Human PD-L1 in a homogeneous 96 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 (Human) | 2 µg | -80°C | ***(Avoid freeze/thaw cycles!)*** |
| 71105 | PD-L1, Biotinylated (Human) | 30 µg | -80°C | |
| | Dye-labeled acceptor | 2 x 10 µl | -20°C | |
| 79311 | 3x Immuno Buffer 1 | 4 ml | -20°C | |
| 79685 | Black, non-binding, low volume, 96-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 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 (12.5 µl diluted **PD-1-Eu** + 12.5 µl diluted **Dye-labeled acceptor**). Add 25 µl to every well.

6) Add 12.5 µl of inhibitor solution to each well designated “Test Inhibitor”. Add 12.5 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled “Negative Control” and “Positive Control”.

7) Add 12.5 µl **1x Immuno Buffer 1** to wells designated for “Negative Control.”

| | Positive Control | Negative Control | Test Inhibitor |
|---|---|---|---|
| PD-1 –Eu (0.2 µg/ml) | 12.5 µl | 12.5 µl | 12.5 µl |
| Dye-labeled acceptor | 12.5 µl | 12.5 µl | 12.5 µl |
| 1x Immuno Buffer 1 | - | 12.5 µl | - |
| Test Inhibitor | – | – | 12.5 µl |
| Inhibitor Buffer (no inhibitor) | 12.5 µl | 12.5 µl | – |
| PD-L1-biotin (11 µg/ml) | 12.5 µl | – | 12.5 µl |
| **Total** | **50 µl** | **50 µl** | **50 µl** |

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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 12.5 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.

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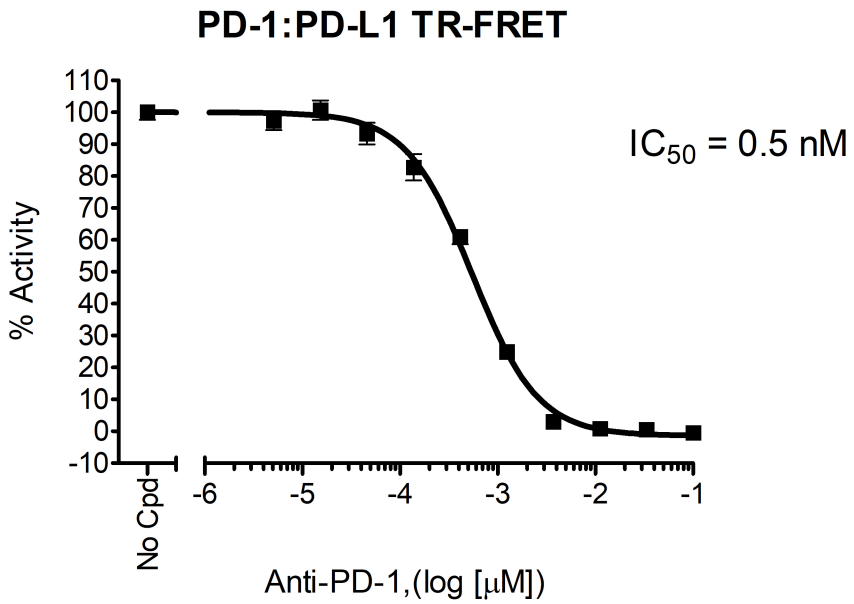
**EXAMPLE OF ASSAY RESULTS:**




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.

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