CAT.&Size: A1090003S (1,000 tests)
A1090003L (10,000 tests)
Storage at: -60 ℃ or Below

VKEYBIO-01-2026
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Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET
PD1/PD-L1 Binding Assay Kit
Technical Manual

1. Introduction

KeyTec® TR-FRET PD1/PD-L1 Binding Assay Kit is designed for the quantitative measurement of the interaction between human PD1 and PD-L1 proteins. This kit provides a Tag1-PD-L1 protein, a Tag2-PD1 protein, an anti-tag1 antibody labeled with KeyTec® TR-FRET Solar Eu*[1] (mAb anti-Tag1 - Solar Eu) ,an anti-tag2 antibody labeled with KeyTec® TR-FRET LA*[2] (mAb anti-Tag2 - LA) and other detection reagents. These components enable the high-throughput screening of small molecule inhibitors, peptides, or antibody blockers.

This assay is based on a competitive immunoassay method using KeyTec® TR-FRET technology, offering a simple, rapid, highly specific and sensitive, as well as reproducible detection process. The principle, outlined in Figure 1:

The binding of the mAb anti-Tag1 - Solar Eu, the Tag1-PD-L1 protein, the mAb anti-Tag2 - LA and the Tag2-PD1 protein brings the TR-FRET donor and acceptor into close proximity, enabling resonance energy transfer (RET) upon excitation and generating a TR-FRET signal. Compounds, peptides, or antibodies that block the PD1/PD-L1 interaction will cause a decrease in the TR-FRET signal.

*[1] KeyTec® TR-FRET Solar Eu: TR-FRET Donor

*[2] KeyTec® TR-FRET LA: TR-FRET Acceptor

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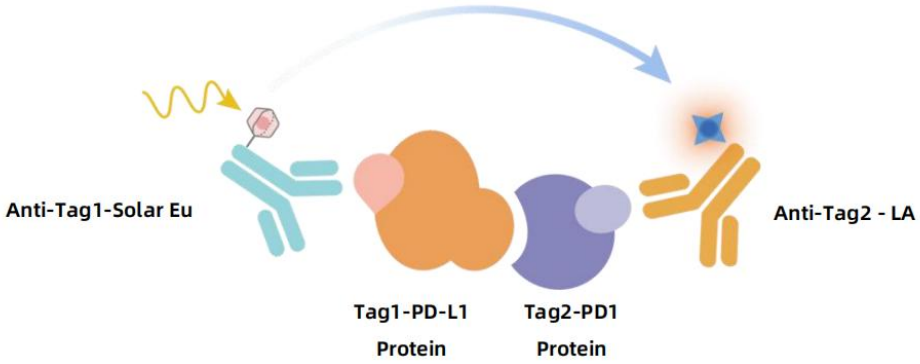

Figure1. The principle of KeyTec® TR-FRET human PD1/PD-L1 Binding detection

2. Components

Components	Storage	A1090003S (1,000 tests[*3])	A1090003L (10,000 tests[*3])
Tag1-PD-L1 NP_054862.1, Met 1 - Thr 239	≤ -60 ℃	1 vial of 80 μL 1,000 tests/vial	1 vial of 800 μL 10,000 tests/vial
Tag2-PD1 NP_005009.2, Met 1 - Gln 167	≤ -60 ℃	1 vial of 80 μL 1,000 tests/vial	1 vial of 800 μL 10,000 tests/vial
Anti-Tag1-Solar Eu	≤ -60 ℃	1 vial of 50 μL 1,000 tests/vial	1 vial of 500 μL 10,000 tests/vial
Anti-Tag2-LA	≤ -60 ℃	1 vial of 50 μL 1,000 tests/vial	1 vial of 500 μL 10,000 tests/vial
Binding Assay Diluent Buffer	2-8 ℃	1 bottle of 50 mL A1010001S	1 bottle of 200 mL A1010001L
Solar Eu Detection Buffer	2-8 ℃	1 bottle of 30 mL A1010002S	1 bottle of 120 mL A1010002L

*[3] Tests refer to the number of assay wells that can be performed in 96-well or 384-well plates with 20 μL in total reaction volume. The reagents of the kit are suggested to use as recommended.

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3. Storage

- Store all reagents according to the recommended conditions. The products are stable for one year from the date of receipt.
- After thawing, aliquot the stock into single-use volumes (recommended minimum: 10 μL) to avoid repeated freeze-thaw cycles. Store these aliquots at -60 °C or below.

4. Required Components (Not Supplied)

Material	Brand	Catalog
Microplate	VKEY-BIO	M2000102N
Top sealing film	VKEY-BIO	M1000102N
Microplate Reader with TR-FRET module	TECAN	Infinite® 200 PRO

5. Procedure

5.1 Reaction system

Components	Volume*4	Stock Conc.	Working Conc.	Final Conc.	Diluent Buffer
Test samples	2 μL	\	\	\	Diluent Buffer
Tag1-PD-L1	4 μL	250X	5X	1X	
Tag2-PD1	4 μL	250X	5X	1X	
Anti-Tag1-Solar Eu	5 μL	400X	4X	1X	Detection Buffer
Anti-Tag2-LA	5 μL	400X	4X	1X	

*4 Recommended Format: Shallow-well 384-well microplate; For 96-well or 1536-well microplates, proportionally scale the reaction system.

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5.2 Reagent preparation

- After thawing on ice, Aliquot the stock into single-use volumes (recommended minimum: 10μL) to avoid repeated freeze-thaw cycles. Store these aliquots at -60 ℃ or below. The buffers (Binding Assay Diluent Buffer and Solar Eu Detection Buffer) can be stored at 2-8 ℃.
- Before use, equilibrate all reagents to RT.
- Use the provided/recommended buffers to prepare sample and detection reagents.
- Prepare sample and detection reagent according to the kit technical manual.
- Prepare all reagents immediately before use, unless otherwise specified in the "Working Solution Preparation" section.
- Avoid vigorous mixing of all reagents.

6. Working Solution Preparation

6.1 Test Samples Preparation

- Dilute the test samples with Binding Assay Diluent Buffer. If the sample stock is in DMSO, ensure the DMSO concentration is consistent across the assay and the final total concentration is < 0.5%.

6.2 Protein Preparation

- **Tag1-PD-L1 Working Solution(5X)**: Dilute 10 volume of 250X Tag1-PD-L1 stock solution with 490 volume of Diluent Buffer and mix gently.
- **Tag2-PD1 Working Solution(5X)**: Dilute 10 volume of 250X Tag2-PD1 stock solution with 490 volume of Diluent Buffer and mix gently.
- Prepare Tag1-PD-L1 and Tag2-PD1 separately. Do not pre-mix these two proteins before adding to the assay plate.

6.3 Conjugates Preparation

- **Anti-Tag1-Solar Eu(4X)**: Dilute 5 volume of 400X Anti-Tag1-Solar Eu stock solution with 495 volume of Detection buffer and mix gently .
- **Anti-Tag2-LA(4X)**: Dilute 5 volume of 400X Anti-Tag2-LA stock solution with 495 volume of Detection buffer and mix gently .

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- **Pre-mixed Detection Antibody Pair(2X)**: Mix the 4X Anti-Tag1-Solar Eu and 4X Anti-Tag2-LA solutions at a 1:1 ratio prior to use[*5].

Reagent	Final Conc.	Working Conc.	Stock Conc.	Dilution Factor	Stock	Buffer	Total Vol.
Anti-Tag1-Solar Eu	1X	2X[*5]	400X	200	5 µL	990 µL	1,000 µL
Anti-Tag2-LA	1X	2X[*5]	400X	200	5 µL		

[*5] The pre-mixed solution contains each antibody at 2X. Add 10 µL per well of this pre-mixed solution to achieve a final 1X concentration of each antibody in a 20 µL assay volume.

7. Procedure

- Follow the steps in the table below.

	Negative Control	Positive Control	Test samples
Step 1	2 µL Diluent Buffer[*6]	2 µL Diluent Buffer[*6]	2 µL Test sample
Step 2	4 µL Tag1-PD-L1 Working Solution(5X)		
Step 3	4 µL Diluent Buffer	4 µL Tag2-PD1 Working Solution (5X)	
Step 4	Incubate (RT, 25 °C) for 15 min		
Step 5	10 µL Pre-mixed Detection Antibody Pair[*7]		
Step 6	Seal the microplate to prevent evaporation. Incubate (RT, 25 °C) for 2 hours		
Step 7	Read on a TR-FRET compatible reader without removing the sealing film		

[*6] If the test sample contains DMSO, the Negative and Positive controls must contain the same concentration of DMSO.

[*7] It is recommended to use the pre-mixed detection antibody pair, which reduces operational steps and minimizes deviations caused by manual handling.

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- Follow the steps in the table below.

Components	Diluent Buffer	Anti-Tag1-Solar Eu	Detection Buffer
Solar Eu Control[*8]	10 μL	5 μL	5 μL
Buffer Control[*9]	10 μL	\	10 μL

[*8] Solar Eu Control: Used to evaluate the TR-FRET donor signal level at 615 nm (or 620 nm). It is recommended to include this control in the initial assay.

[*9] Buffer Control: Used to evaluate the background fluorescence level of the assay system. It is recommended to include this control in the initial assay.

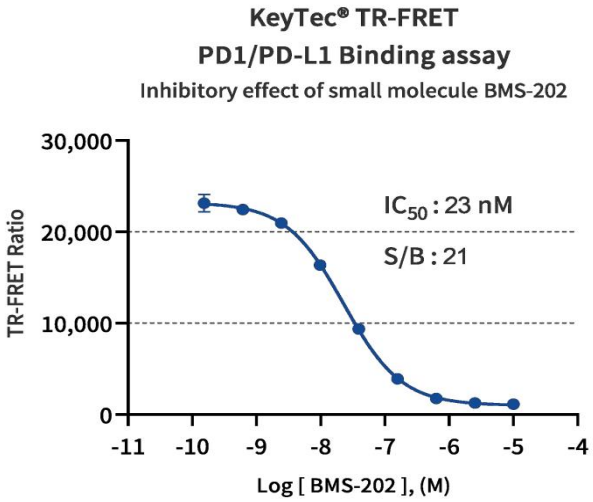
8. Data Analysis

- Calculate the 665 nm/620 nm Ratio (TR-FRET Ratio) and the percentage coefficient of variation (CV %) for each well.

$$\text{TR-FRET Ratio} = \frac{\text{Signal 665 nm}}{\text{Signal 620 nm}} \times 10{,}000$$

9. Results

- Concentration optimization results for the small molecule inhibitor BMS-202.



Note: Exemplary data shown. Results are instrument-dependent.

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