CAT.&Size: A1090012D(100 tests) A1090012S(500 tests) **VKEYBIO-01-2026**
A1090012L(5,000 tests) **For Research Use Only**
Storage at: -60℃ or Below

KeyTec® TR-FRET
BRD4 / VHL PROTAC Binding Assay Kit
Technical Manual

1. Introduction

KeyTec® TR-FRET BRD4 / VHL PROTAC Binding Assay Kit is designed to screen PROTAC molecules that mediate the formation of a ternary complex between the BRD4 protein and the VHL/Elongin C/Elongin B protein complex (hereinafter referred to as VCB complex). This kit utilizes a binding immunoassay developed based on KeyTec® TR-FRET technology, characterized by its simplicity, rapidity, high accuracy, and excellent reproducibility.

The principle is outlined in Figure 1: The KeyTec® TR-FRET Solar Eu*[1] labeled anti-Tag1 antibody specifically binds to the Tag1-VCB Complex protein, while the KeyTec® TR-FRET LA*[2] labeled anti-Tag2 antibody targets the Tag2-BRD4 protein. The Tag1-VCB Complex protein and the Tag2-BRD4 protein are brought together by a positive reference PROTAC compound, MZ 1, to form a ternary complex. This interaction brings the Solar Eu donor and the LA acceptor into close proximity. Upon excitation by an external light source, fluorescence resonance energy transfer (FRET) occurs between the donor and acceptor. By detecting the specific signal intensity at 665 nm, the PROTAC-mediated binding between BRD4 and VCB Complex can be quantitatively evaluated.

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

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

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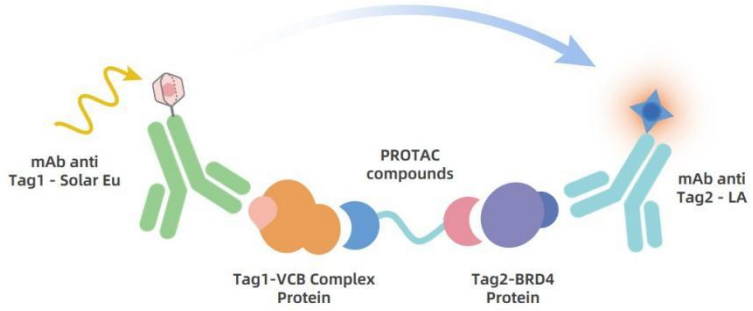

Figure1. The principle of BRD4 / VHL PROTAC Binding Assay Kit

2. Components

Components	Storage	A1090012D (100 tests[*3])	A1090012S (500 tests[*3])	A1090012L (5,000 tests[*3])
VHL binding standard 1 MZ 1 (20 mM in DMSO)	≤ -60 ℃	1 vial 5 μL/vial	1 vial 10 μL/vial A1010037S	1 vial 50 μL/vial
Tag1-VCB Complex 250X[*4] VHL, P40337-2, Pro 2 - Asp 213/ Elongin B, Q15370-1, Asp 2 - Gln 118/ Elongin C, Q15369-1, Asp 2 - Cys 112	≤ -60 ℃	1 vial 8 μL/vial	1 vial 40 μL/vial	1 vial 400 μL/vial
Tag2-BRD4 250X[*4] BRD4 (BD1/BD2), O60885-1, Glu 49 - Glu 460	≤ -60 ℃	1 vial 8 μL/vial	1 vial 40 μL/vial	1 vial 400 μL/vial
mAb anti-Tag1 - Solar Eu 400X[*4]	≤ -60 ℃	1 vial 5 μL/vial	1 vial 25 μL/vial	1 vial 250 μL/vial
mAb anti-Tag2 - LA 400X[*4]	≤ -60 ℃	1 vial 5 μL/vial	1 vial 25 μL/vial	1 vial 250 μL/vial
TCEP (1 M)	2-8 ℃	1 vial 5 μL/vial	1 vial 120 μL/vial A1010023S	2 vials 120 μL/vial A1010023S
PROTAC Diluent Buffer #2 Ready-to-Use	2-8 ℃	2 vials 2 mL/vial	1 bottle 50 mL/bottle A1010046S	1 bottle 200 mL/bottle A1010046L
PROTAC Detection Buffer #2 Ready-to-Use	2-8 ℃	1 vial 2 mL/vial	1 bottle 30 mL/bottle A1010047S	1 bottle 120 mL/bottle A1010047L

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*[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.

*[4] Stock concentration, final concentration is 1X in the 20 μL reaction system.

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(96-Well White Flat Low-Volume Microplates)	VKEY-BIO	M2000702N
Microplate(384-Well White Flat Microplates)	VKEY-BIO	M2000102N
Top sealing film	VKEY-BIO	M1000102N
Microplate Reader with TR-FRET module	TECAN	Infinite® 200 PRO (model)

5. Reagent Preparation

5.1 Reaction system

Components	Addition Volume[*5]	Stock Conc.	Working Conc.	Final Conc. (20 μL total vol.)	Diluent Buffer
Test Sample or Standard	2 μL	As sample	As sample	\	PROTAC Diluent buffer #2 containing 1 mM TCEP
Tag1-VCB Complex	4 μL	250X	5X	1X	
Tag2-BRD4	4 μL	250X	5X	1X	
mAb anti-Tag1 - Solar Eu[*6]	5 μL	400X	4X	1X	PROTAC Detection Buffer #2
mAb anti-Tag2 - LA[*6]	5 μL	400X	4X	1X	

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

*[6] For convenience, mAb anti-Tag1 - Solar Eu and mAb anti-Tag2 - LA can be pre-mixed; each well requires 10 µL.

5.2 Reagent preparation

- Thaw buffers at room temperature. Equilibrate to RT before use. After thawing, buffers and the TCEP stock solution can be stored at 2-8°C for long-term storage.
- Thaw all reagents except buffers on ice. Equilibrate to RT before use. These reagents should avoid repeated freeze-thaw cycles. Aliquot into small volumes as needed and store at -60°C or below.
- Use buffers provided in the kit for dilution and preparation to ensure assay accuracy and stability.
- Prepare and formulate reagents according to the relevant instructions for the purchased kit size.
- Prepare all working solutions immediately before use.
- The working concentrations of KeyTec® TR-FRET detection reagents Tag1-VCB Complex and Tag2-BRD4 have been optimized. Incorrect concentrations affect assay performance, result stability, and reproducibility.
- Mix all reagents gently; do not vortex.

5.3 Working Solution Preparation

5.3.1 PROTAC Diluent Buffer Preparation

- PROTAC Diluent Buffer #2 can be supplemented with TCEP to a final concentration of 1 mM for use, which provides a better signal-to-noise ratio and does not affect the EC_{50} of PROTAC molecule MZ 1. The TCEP stock concentration is 1 M. Prepare by adding it to PROTAC Diluent Buffer #2 (Ready-to-Use) at 1:1,000. For example, add 1 volume of 1 M TCEP solution to 1,000 volumes of PROTAC Diluent Buffer #2 (Ready-to-Use) and mix thoroughly. To ensure assay stability, PROTAC Diluent Buffer #2 supplemented with 1 mM TCEP must be prepared fresh immediately before use.

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5.3.2 Test Samples or Standards Preparation

- Use the freshly prepared PROTAC Diluent Buffer (containing 1 mM TCEP) for all sample and standard dilutions. If the test compounds are dissolved in DMSO, ensure the final DMSO concentration is uniform across all assay wells and remains below 1% in the final 20 µL reaction volume. Determine the total volume needed for your standard curve. The preparation volumes are for reference only.

Standard Curve	Final Conc. (nM)	Working Conc. (nM)	Preparation Method
STD-12	100,000	1,000,000	3 µL standard stock solution + 57 µL Diluent Buffer
STD-11	25,000	250,000	20 µL STD-12 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-10	6,250	62,500	20 µL STD-11 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-9	1,563	15,625	20 µL STD-10 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-8	390.6	3,906	20 µL STD-9 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-7	97.7	976.6	20 µL STD-8 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-6	24.4	244.1	20 µL STD-7 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-5	6.10	61.04	20 µL STD-6 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-4	1.53	15.26	20 µL STD-5 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-3	0.38	3.81	20 µL STD-4 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-2	0.10	0.95	20 µL STD-3 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-1	0.03	0.30	20 µL STD-2 + 60 µL Diluent Buffer (contain 5% DMSO)
STD-0 (NC)	0.00	0.00	60 µL Diluent Buffer (contain 5% DMSO)

5.3.3 Proteins Preparation

- **Tag1-VCB Complex Working Solution (5X):** Dilute the 250X stock solution 50-fold using the PROTAC Diluent Buffer (containing 1 mM TCEP). Each well requires 4 µL. For example, add 1 volume of Tag1-VCB Complex stock to 49 volumes of supplemented PROTAC Diluent Buffer #2.

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- **Tag2-BRD4 Working Solution (5X):** Dilute the 250X stock solution 50-fold using the PROTAC Diluent Buffer (containing 1 mM TCEP). Each well requires 4 µL. For example, add 1 volume of Tag2-BRD4 stock to 49 volumes of supplemented PROTAC Diluent Buffer #2.

  Note: Tag1-VCB Complex and Tag2-BRD4 proteins are sensitive; prepare working solutions immediately before dispensing. Aliquot remaining stocks promptly after the first thaw and store at the recommended temperature.

5.3.4 Preparation of Conjugate Pre-mixed Working Solution

- **mAb anti-Tag1 - Solar Eu Working Solution (4X):** Dilute the 400X stock 100-fold using PROTAC Detection Buffer #2 to prepare 4X mAb anti-Tag1 - Solar Eu Working Solution. Each well requires 5 µL. For example, add 1 volume of stock to 99 volumes of PROTAC Detection Buffer #2.

- **mAb anti-Tag2 - LA Working Solution (4X):** Dilute the 400X stock 100-fold using PROTAC Detection Buffer #2 to prepare 4X mAb anti-Tag2 - LA Working Solution. Each well requires 5 µL. For example, add 1 volume of stock to 99 volumes of PROTAC Detection Buffer #2.

- **Pre-mixed Working Solution (2X):** Mix the 4X mAb anti-Tag1 - Solar Eu and 4X mAb anti-Tag2 - LA working solutions at a 1:1 volume ratio. Each well requires 10 µL. Rapid Pre-mix Calculation: Mix 1 volume of Solar Eu stock and 1 volume of LA stock directly with 198 volumes of PROTAC Detection Buffer #2. Each well requires 10 µL. Using the pre-mixed antibody combination streamlines the operational workflow and mitigates experimental deviations introduced during separate pipetting steps.

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6. Procedure

- Follow the steps in the table below.

	Negative Control	Standard Curve	Test samples
Step 1	2 μL Diluent Buffer[*7]	2 μL serially diluted standards	2 μL prepared test samples
Step 2	4 μL Diluent Buffer	4 μL Tag1-VCB Complex Working Solution(5X)	
Step 3	4 μL Tag2-BRD4 Working Solution(5X)		
Step 4	10 μL Premixed Detection Antibody Pair[*8] (2X)		
Step 5	Seal the microplate to prevent evaporation. Incubate 2 hours at room temperature (25°C).		
Step 6	Read on a TR-FRET compatible reader (no need to remove the seals).		

*[7] If test samples are dissolved in DMSO, ensure that the negative control and positive control wells contain an identical background concentration of DMSO.

*[8] Using the pre-mixed antibody combination streamlines the operational workflow and mitigates experimental deviations introduced during separate pipetting steps.

7. Data Analysis

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

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

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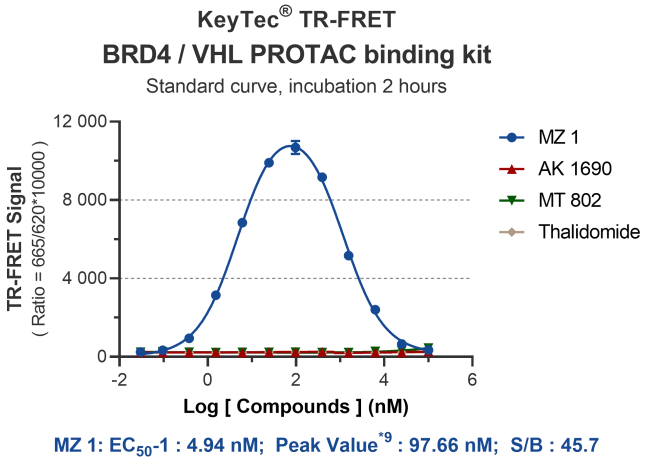
8. Results

- Standard Curve

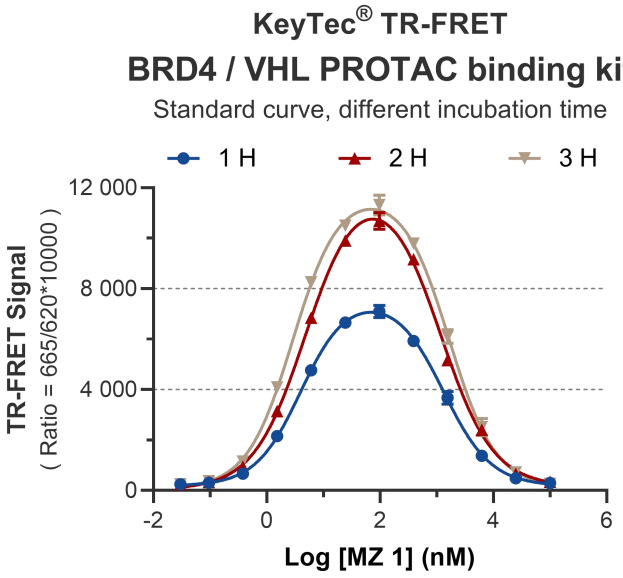
Standard Curve	MZ 1 (nM)	TR-FRET Ratio	CV%
STD-12	100,000	346	3.1
STD-11	25,000	653	2.8
STD-10	6,250	2,217	5.9
STD-9	1,563	5,183	3.7
STD-8	390.6	9,153	1.1
STD-7	97.7	10,486	4.5
STD-6	24.41	9,865	0.9
STD-5	6.10	6,804	2.9
STD-4	1.53	3,142	3.2
STD-3	0.38	958	5.2
STD-2	0.10	334	6.5
STD-1	0.03	247	0.9
STD-0	0	234	4.9



*[9] The PROTAC concentration at peak ternary complex formation.

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

- Comparison of Different Incubation Times and TCEP Supplementation



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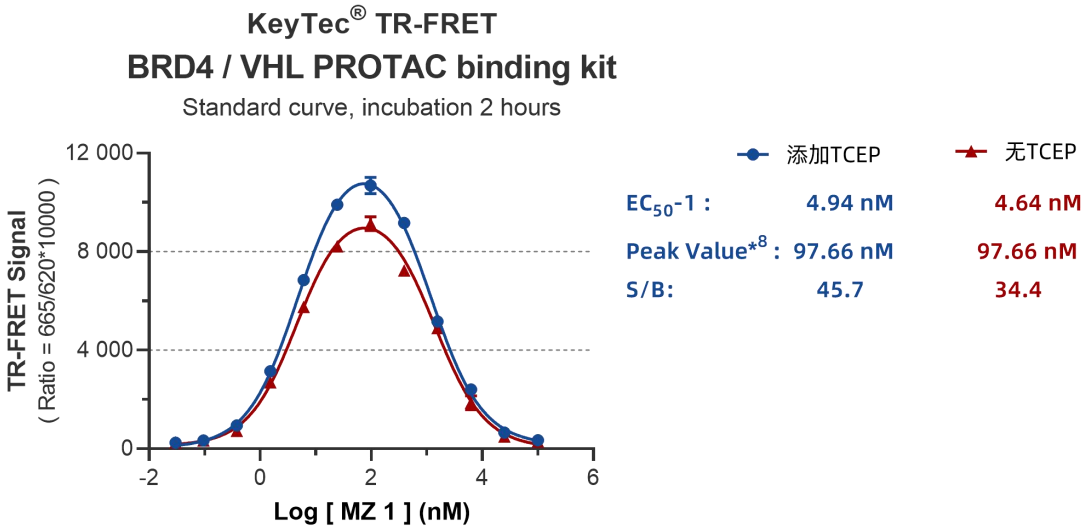

9. Instrument Model and Setting

Vender	TECAN
Instrument model	Infinite® 200 PRO [Ref. 30050303]
Mode	Fluorescence Top Reading
Excitation filter	320 (25) nm [Ref. 30094454]
Emission filter 1	665 (8.5) nm [Ref. 30094518]
Emission filter 2	620 (10) nm [Ref. 30094505]
Mirror	Dichroic 510
Lag time	150 µs
Integration Time	500 µs
Number of reads	5 or user-defined
Gain	150 or optimal
Z -focus (mm)	Can be calculated on the well giving the highest signal

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