

KeyTec® TR-FRET

Human cereblon binding kit



CAT.&Size: A1090004S (1,000 tests)
A1090004L (10,000 tests)

Storage at: -60 °C or Below

VKEYBIO-01-2025

For Research Use Only

Not For Diagnostic Or Therapeutic Use

KeyTec® TR-FRET

Human Cereblon Binding Kit

Technical Manual

1. Introduction

KeyTec® TR-FRET human cereblon binding kit is designed for the binding analysis of cereblon ligands/inhibitors to wild-type human cereblon. This kit provides a tag1-cereblon/DNA damage-binding protein 1 complex (Tag1-CRBN/DDB1), a Thalidomide-HX^{*1} (a cereblon inhibitor), an anti-tag1 antibody labeled with KeyTec® TR-FRET Solar Eu^{*2} (mAb anti-Tag1 - Solar Eu) and other detection reagents. These components enable the high-throughput screening of cereblon ligands/inhibitors.

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-CRBN/DDB1 and the Thalidomide-HX^{*1} brings the TR-FRET donor and acceptor into close proximity, enabling resonance energy transfer (RET) upon excitation and generating a TR-FRET signal. Cereblon ligands/inhibitors compete with Thalidomide-HX^{*1} for binding to the Tag1-CRBN/DDB1. The intensity of TR-FRET signal is inversely proportional to the apparent affinity of Cereblon ligands/inhibitors.

*¹ KeyTec® TR-FRET HX: TR-FRET Acceptor

*² KeyTec® TR-FRET Solar Eu: TR-FRET Donor

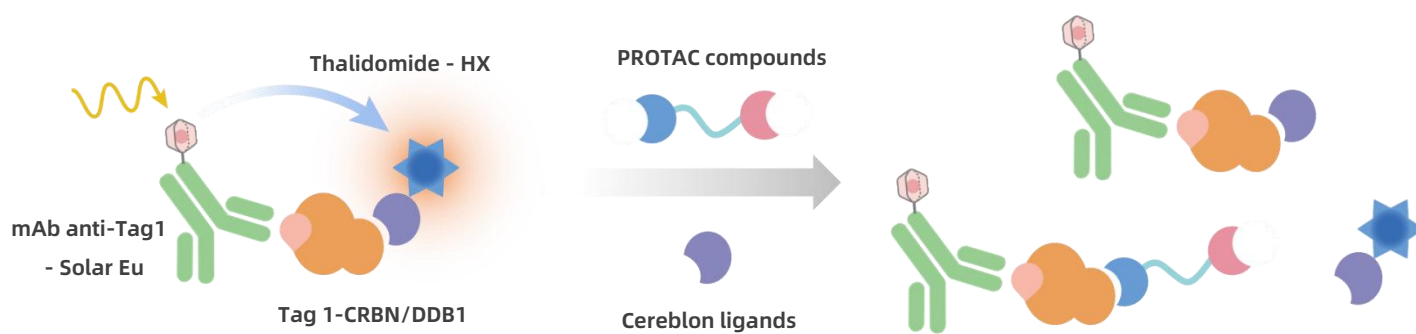


Figure1. The principle of KeyTec® TR-FRET human cereblon binding detection

2. Components

Components	Storage	A1090004S (1,000 tests ^{*3})	A1090004L (10,000 tests ^{*3})
Cereblon binding standard 1 Thalidomide (20 mM in DMSO)	≤ -60 °C	1 vial of 10 µL A1010027S	1 vial of 100 µL A1010027L
Tag1-CRBN/DDB1 (50X)	≤ -60 °C	1 vial 100 µL/vial	1 vial 1 mL/vial
Thalidomide - HX (100X)	≤ -60 °C	1 vial 50 µL/vial	1 vial 500 µL/vial
mAb anti-Tag1 - Solar Eu Lyophilized	2-8 °C	1 vial 1,000 tests/vial	1 vial 10,000 tests/vial
DTT Lyophilized	2-8 °C	1 vial 120 µmole/vial A1010022S	1 vial 600 µmole/vial A1010022L
PROTAC Diluent Buffer	2-8 °C	1 bottle 20 mL/bottle A1010025S	1 bottle 200 mL/bottle A1010025L
PROTAC Detection Buffer	2-8 °C	1 bottle 20 mL/bottle A1010026S	1 bottle 120 mL/bottle A1010026L

^{*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.

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 μ L) 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.
Test samples or standards	5 μ L	\	\	\
Tag1-CRBN/DDB1	5 μ L	50X	1X	\
Thalidomide - HX ^{*5}	5 μ L	100X	1X	\
mAb anti-Tag1 - Solar Eu ^{*5}	5 μ L	100X	1X	\

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

^{*5} It is recommended to premix Thalidomide - HX and mAb anti-Tag1 - Solar Eu, add 10 μ L pre-mix solution in TR-FRET detection.

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 °C or below. The buffers (PROTAC diluent buffer and PROTAC detection buffer) can be stored at 2-8 °C.
- ◆ 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 PROTAC Diluent buffer

- ◆ **DTT stock solution**: Before reconstitution, centrifuge DTT to pellet the powder to the bottom (850×g, 1-2 minutes). Add the recommended volume of Ultra-pure water to the powder and mix gently as below. Aliquot DTT stock solution into single-use tubes to avoid repeated freeze-thaw cycles. These aliquots can be stored at -60°C.

DTT Powder	Buffer	Volume	Stock Concentration
120 µmole/vial	Ultra-pure water	120 µL/vial	1 M
600 µmole/vial	Ultra-pure water	600 µL/vial	1 M

- ◆ **PROTAC Diluent buffer (1x, 1mM DTT)**: Dilute 1 volume of 1M DTT with 1000 volume of PROTAC diluent buffer and mix gently .

6.2 Test sample or positive control preparation (1X)

- ◆ Dilute the test samples/standards with PROTAC Diluent buffer (1x, 1mM DTT). The percentage of DMSO must not exceed 2% for serial dilution.

Standard	Working Conc. (1X,nM)	Final Conc. (0.25X, nM)	Dilution
STD-11	360 000	90 000	2.7 µL standard stock solution + 147.3 µL Diluent Buffer
STD-10	120 000	30 000	50 µL STD-11 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-9	40 000	10 000	50 µL STD-10 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-8	13 333	3333.3	50 µL STD-9 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-7	4 444	1111.1	50 µL STD-8 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-6	1 481	370.4	50 µL STD-7 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-5	493.8	123.5	50 µL STD-6 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-4	164.6	41.15	50 µL STD-5 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-3	54.87	13.72	50 µL STD-4 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-2	18.29	4.57	50 µL STD-3 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-1	6.10	1.52	50 µL STD-2 + 100 µL Diluent Buffer (contain1.8% DMSO)
STD-0 (PC)	0.00	0	100 µL Diluent Buffer (contain1.8% DMSO)

6.3 mAb anti-Tag1 - Solar Eu and Thalidomide - HX pre-mix solution

- ♦ **Thalidomide - HX working solution(1X):** Dilute 1 volume of 100X Thalidomide - HX stock solution with 99 volume of PROTAC detection buffer and mix gently .
- ♦ **mAb anti-Tag1 - Solar Eu stock solution:** Before reconstitution, centrifuge mAb anti-Tag1 - Solar Eu to pellet the powder to the bottom (850×g,1-2 minutes). Add the recommended volume of Ultra-pure water to the powder and mix gently as below. Aliquot mAb anti-Tag1 - Solar Eu stock solution into single-use tubes to avoid repeated freeze-thaw cycles. These aliquots can be stored at -60°C.

Size	Buffer	Volume	Stock Concentration
1,000 tests/vial	Ultra-pure water	50 µL/vial	100X
10,000 tests/vial	Ultra-pure water	500 µL/vial	100X

- ♦ **mAb anti-Tag1 - Solar Eu working solution (1X):** Dilute 1 volume of 100X mAb anti-Tag1 - Solar Eu stock solution with 99 volume of PROTAC detection buffer and mix gently .
- ♦ **(Optional) mAb anti-Tag1 - Solar Eu and Thalidomide - HX pre-mix solution (0.5X):** The pre-mix solution should be combined in a 1:1 volume ratio of mAb anti-Tag1 - Solar Eu working solution and Thalidomide - HX working solution.

Component	Pre-mix Solution Conc.	Working Solution Conc.	Stock Solution Conc.	Dilution	Stock Vol. µL	1X PROTAC Detection Buffer µL	Total Vol. µL
Thalidomide - HX	0.5X	1X	100X	200	5	990	1,000
mAb anti-Tag1 - Solar Eu	0.5X	1X	100X	200	5		

6.4 Protein solution (1X)

- Dilute 1 volume of 50X Tag1-CRBN/DDB1 stock solution with 49 volume of PROTAC Diluent buffer (1x, 1mM DTT) and mix gently. **After thawing on ice, Aliquot the stock into single-use volumes (recommended minimum: 10µL) to avoid repeated freeze-thaw cycles.**

7. Procedure

- Follow the steps in the table below.

	Negative Control	Standard curve	Test samples
Step 1	5 μL Diluent Buffer	5 μL Standards (1X)	5 μL Test sample (1X)
Step 2	5 μL Diluent Buffer	5 μL Tag1-CRBN/DDB1 (1X)	
Step 3	10 μL Thalidomide - HX and mAb anti-Tag1 - Solar Eu pre-mix solution(0.5X)		
Step 4	Seal the microplate to prevent liquid evaporation , Incubation (RT, 25 °C) for 1 to 3 hours		
Step 5	Record the data in microplate reader with top sealing film		

8. Data Analysis

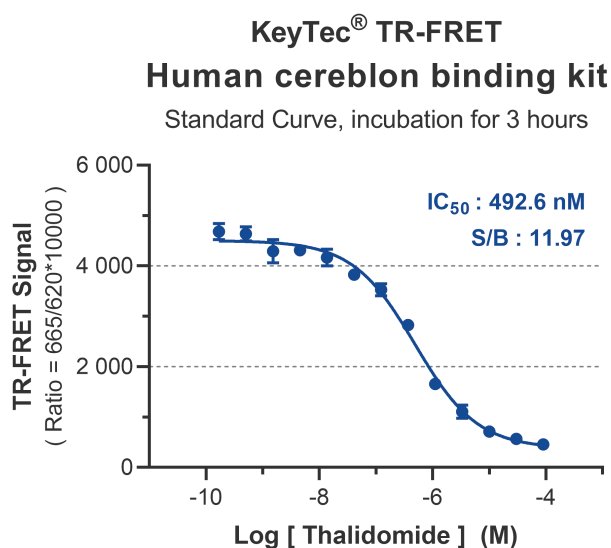
- Calculate the 665 nm/620 nm Ratio (TR-FRET Ratio) and the percentage coefficient of variation (CV %) for each well.

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

9. Summary

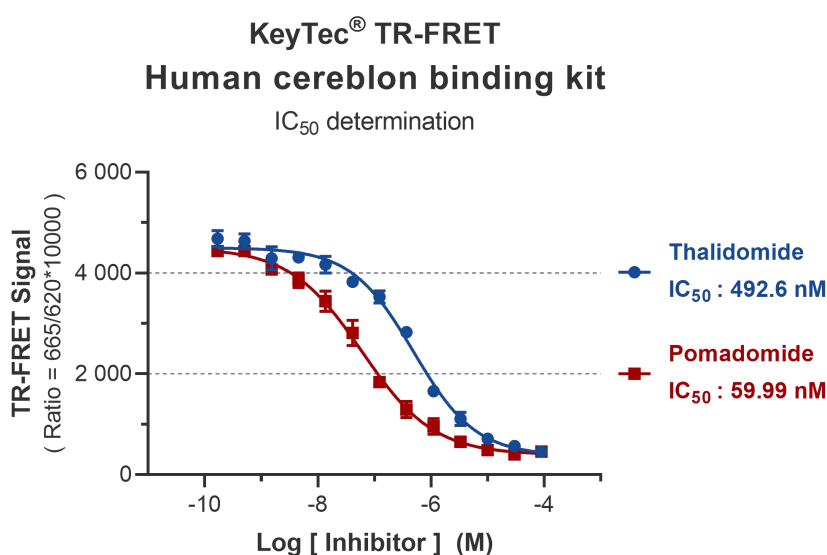
9.1 Standard Curve

Standard	Thalidomide (nM)	TR-FRET Ratio	CV%
STD-11	90 000	456	12.9
STD-10	30 000	566	3.5
STD-9	10 000	714	9.6
STD-8	3333.3	1,109	11.9
STD-7	1111.1	1,658	3.1
STD-6	370.4	2,829	2.2
STD-5	123.5	3,529	3.4
STD-4	41.15	3,825	0.5
STD-3	13.72	4,169	4.0
STD-2	4.57	4,310	2.2
STD-1	1.52	4,292	5.4
STD-0	0	4,637	3.0



9.2 Results

- ◆ IC₅₀ determination of cereblon ligands (Thalidomide and pomadomide) with KeyTec® TR-FRET human cereblon binding kit .



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

9.3 Parameter

Parameter	
Thalidomide - HX K_d	25.94 nM
Thalidomide - HX concentration	50.00 nM
Thalidomide IC_{50}	492.6 nM
Thalidomide K_i	168.3 nM

10. Instrument Model and Setting

Vendor	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 μ s
Integration Time	500 μ s
Number of reads	5 or user-defined
Gain	150 or optimal
Z -focus (mm)	Can be calculated on the well giving the highest signal