Aurora Biolabs
High Purity Protein for Research

TR-FRET Cereblon-4C Binding Assay Kit

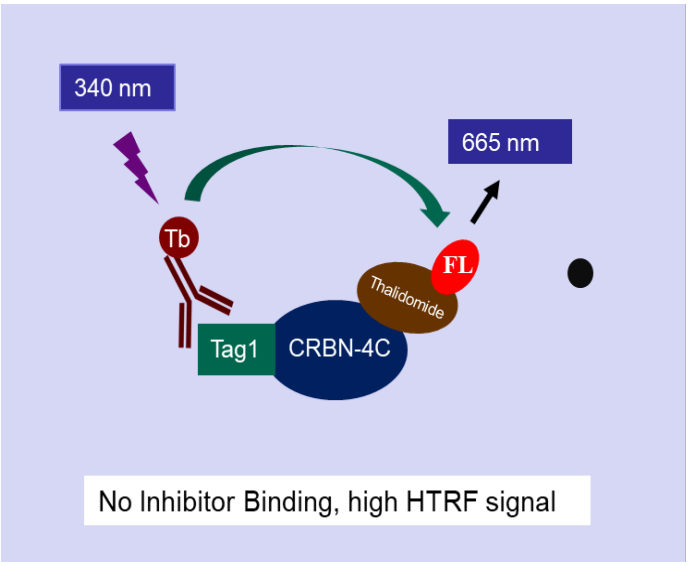
Catalog Number: 272625-384Rx

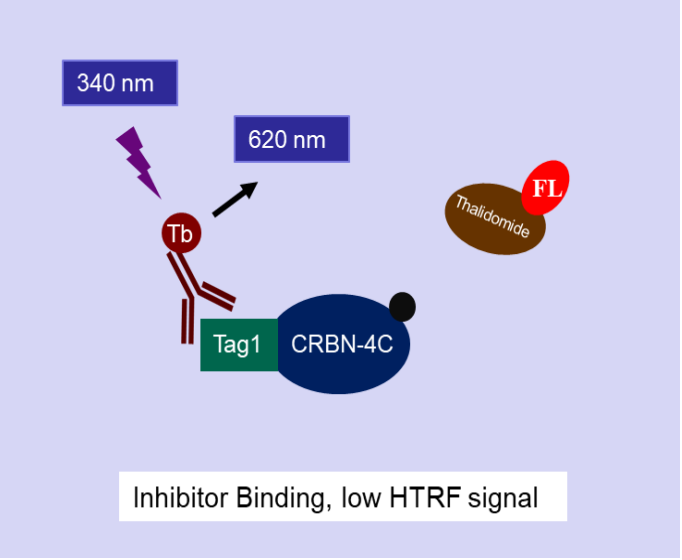
Background

E3 ubiquitin ligases are a large family of enzymes that catalyze the critical final step in the ubiquitination cascade. Cereblon (CRBN) serves as the substrate-recognition component of the E3 ubiquitin ligase complex CRBN/DDB1/CUL4A/RBX1 (CRBN-4C). CRBN is one of the most widely utilized E3 ligases in the design of PROTACs (Proteolysis-Targeting Chimeras) for targeted protein degradation (TPD) in drug discovery.

Assay Principle

The TR-FRET CRBN Binding Assay Kit is designed to measure the binding affinity between CRBN and its ligands. The kit includes Tag1-CRBN-4C (a complex of CRBN/CUL4A/DDB1/RBX1), a terbium-labeled anti-Tag1 antibody, and a fluorescently labeled CRBN ligand, thalidomide. When CRBN binds to thalidomide, the terbium donor (on the anti-Tag1 antibody) is brought into close proximity to the fluorescent acceptor (fluorophore-labeled thalidomide), resulting in fluorescence resonance energy transfer (FRET). The binding interaction can be quantitatively measured as an HTRF signal, calculated from the ratio of the emission intensities at 665 nm (acceptor) and 620 nm (donor). If a test compound competes with thalidomide for CRBN binding, the HTRF signal will decrease, indicating inhibition of CRBN–thalidomide interaction.





Application

High throughput screening of compounds that bind to Cereblon for drug discovery.

Plate Reader

A HTRF® certified microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET) is required.

Components

Catalog number	Item	Amount	Storage
272625-B	CRBN Binding Assay Buffer	20 mL	-20°C
272625-M	Recombinant human CRBN-4C	80 µL	-80°C
852461	Fluorescence-labeled Thalidomide	15 µL	-80°C
447852	Fluorescence labeled anti-Tag1 antibody	20 µL	-80°C
	384-well microplate	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader (such as Tecan M1000 or Tecan Spark, etc.)
2. Adjustable micro-pipettor
3. Sterile Tips

Please thaw all component tubes on ice. Briefly spin the tubes for 30 seconds to collect all contents at the bottom before opening them.

Assay protocol

1. Prepare the inhibitor compound solution

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in assay buffer (since you will add 2 µl to the 20 µl reaction).

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound than the highest concentration you want to test in DMSO. Then make a 10-fold dilution in assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

2. Prepare CRBN-4C solution

   Thaw CRBN-4C protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C.

   Note: CRBN-4C protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the CRBN-4C protein 20-fold (1µL CRBN-4C + 19 µL assay buffer).

   Add 4 µl of diluted protein solution to each positive control well and inhibitor test well.

   Add 4 µl of assay buffer to each of negative control well.

3. Add inhibitor

   Add 2 µl of diluted compound solution to each inhibitor test well.

   Add 2 µl of assay buffer to each of negative and positive control wells.

   If the compound is diluted in 10% DMSO, add 2 µl of assay buffer containing 10% DMSO to each of negative and positive control wells.

4. Prepare FL-Thalidomide solution

   Dilute FL-Thalidomide 125-fold (1 µL FL-Thalidomide + 124 µL of assay buffer).

   Add 4 µl of diluted FL-Thalidomide solution to each well.

5. Prepare dye solution

   Dilute Terbium-labeled anti-Tag1 antibody 1:200 in assay buffer. For example: 1 µl of Terbium-labeled anti-Tag1 antibody + 199 µl of assay buffer.

   Add 10 µl of this dye mixture to each well.

6. Incubate the reaction at room temperature for 20 minutes.
7. Measure fluorescent intensity

   HTRF compatible microplate reader is needed to measure fluorescent intensity of the samples.

   Fluorescent intensity should be measured twice:

   1. Excitation wavelength at 340 nm and emission at 620 nm.
   2. Excitation wavelength at 340 nm and emission at 665 nm.

Protocol Summary

Component	Negative Control	Positive Control	Inhibitor Test
assay buffer	4 µl		
CRBN-4C protein		4 µl	4 µl
compound dilution Buffer (Assay buffer or 10% DMSO)	2 µl	2 µl	
Inhibitor solution			2 µl
FL-Thalidomide	4 µl	4 µl	4 µl
Dye solution	10 µl	10 µl	10 µl
Total Volume	**20 µl**	**20 µl**	**20 µl**
Incubate at room temperature for 60 minutes.			

Data Analysis

1. Calculate sample HTRF signal of each well.

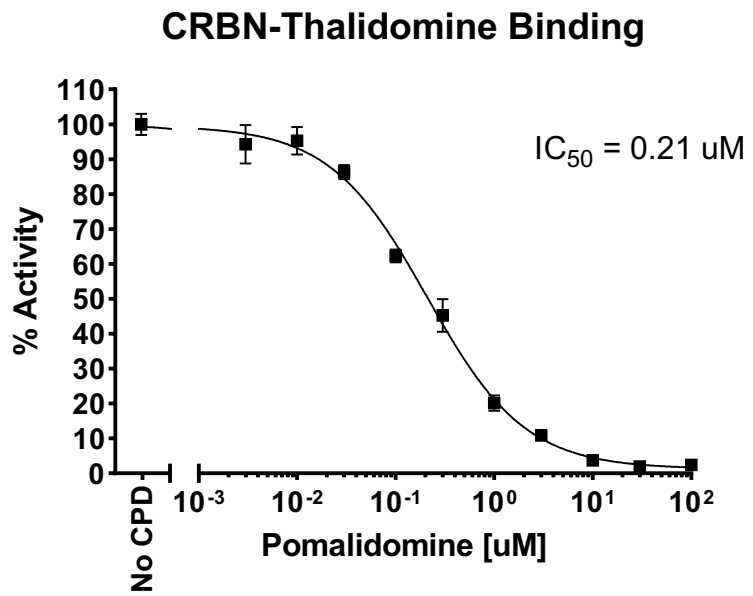
$$HTRF = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}} X10{,}000$$

2. Calculate percentage activity

   In the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\%\ \text{Activity} = \frac{S - N}{\text{P} - \text{N}} X100$$

Assay result



Related products:

Catalog #	Product Name	Size
845225	TR-FRET VHL Binding Assay Kit	40, 100, 384 reactions
2369401	OX40/OX40L Inhibitor Binding Assay Kit	40, 100, 384 reactions
2373532	PD-1/PD-L1 Inhibitor Binding Assay Kit	40, 100, 384 reactions
72771TAK	TR-FRET PARP1 Trapping Assay Kit	40, 100, 384 reactions
72772TAK	TR-FRET PARP2 Trapping Assay Kit	40, 100, 384 reactions
5727-4121BK	Kras WT–cRAF Binding Assay Kit	40, 100, 384 reactions
5727-4122BK	Kras G12C–cRAF Binding Assay Kit	40, 100, 384 reactions
5727-4123BK	Kras G12D–cRAF Binding Assay Kit	40, 100, 384 reactions
5727-4133BK	Kras G13D–cRAF Binding Assay Kit	40, 100, 384 reactions
5727-4127BK	Kras G12R–cRAF Binding Assay Kit	40, 100, 384 reactions
5727-4128BK	Kras G12V–cRAF Binding Assay Kit	40, 100, 384 reactions
5727-4121CK	Kras WT/cRAF/CYPA/Inhibitor Assay Kit	40, 100, 384 reactions
5727-4122CK	Kras G12C/cRAF/CYPA/Inhibitor Assay Kit	40, 100, 384 reactions
5727-4123CK	Kras G12D/cRAF/CYPA/Inhibitor Assay Kit	40, 100, 384 reactions
5727-4133CK	Kras G13D/cRAF/CYPA/Inhibitor Assay Kit	40, 100, 384 reactions
5727-4128CK	Kras G12V/cRAF/CYPA/Inhibitor Assay Kit	40, 100, 384 reactions
5727-4121NK	Kras WT Nucleotide Exchange Assay Kit	40, 100, 384 reactions
5727-4122NK	Kras G12C Nucleotide Exchange Assay Kit	40, 100, 384 reactions
5727-4123NK	Kras G12D Nucleotide Exchange Assay Kit	40, 100, 384 reactions
5727-4133NK	Kras G13D Nucleotide Exchange Assay Kit	40, 100, 384 reactions
5727-4127NK	Kras G12R Nucleotide Exchange Assay Kit	40, 100, 384 reactions

5727-4128NK	Kras G12V Nucleotide Exchange Assay Kit	40, 100, 384 reactions
362101	DNA Polymerase Theta Activity Assay Kit	96, 384 reactions
K777627	T7 High Yield RNA Synthesis Kit	25, 50, 100 reactions
756981BK	PKMYT1 Binding Assay Kit	384 reactions
34343BK	eIF4E/eIF4G Binding Assay Kit	384 reactions
810030	Caspase-3 Activity Assay Kit	384 reactions
910010	IDO1 Activity Assay Kit for Inhibitor Screening	96 reactions
728203	SARS-CoV-2 Mpro (3CL Protease) Assay Kit	96 reactions
728253	SARS-CoV-2 Papain-like Protease Assay Kit	96 reactions
728263	SARS-CoV-2 Nucleocapsid Protein Binding Kit (For mouse antibody)	384 reactions
728273	SARS-CoV-2 Nucleocapsid Protein Binding Kit (For rabbit antibody)	384 reactions
190001AK	TEV Protease Activity Assay Kit	96 reactions
190001	TEV Protease	1,000;10,000 Units
190001-R	TEV Protease-His	50 µg, 200 µg
190004-R	TEV Protease-GST	50 µg, 200 µg
190005-R	AiTEV™ Protease	50 µg, 200 µg
190002	PreScission Protease (HRV 3C)	1,00; 5,000 Units
190003	Recombinant SUMO Protease (Ulp1)	1,000; 5,000 Units
200100	Recombinant YopH (CLP)	10 µg, 20 µg, 100 µg, 1mg
90101	Recombinant Biotin Protein Ligase (BirA)	100 µg
90201	Recombinant Sortase A - 5M	50 ug
777627	Recombinant T7 RNA Polymerase	5,000. 25,000.100,000 Units
AB28234022	Anti-CD47 antibody	50 µg, 100 µg
56781	Recombinant Full-length Human MST1	10 µg, 50 µg, 100 µg, 500 µg
52352-FL	Recombinant Human CDK2	50 µg, 500 µg
5756981-FL	Recombinant Human Full Length PKMYT1	10 µg, 100 µg
5756981-CDD	Recombinant Human PKMYT1, catalytic domain – dephosphorylated	10 µg, 50 µg, 100 µg, 500 µg
5756981-CDP	Recombinant Human PKMYT1, catalytic domain – phosphorylated	10 µg, 50 µg, 100 µg, 500 µg
5727-4121G	Kras Wild Type (WT), GST-tag	50 µg, 100 µg
5727-WTG-G	Kras WT, GST-tag, GDP Loaded	50 µg, 100 µg
5727-WTG-GP	Kras WT, GST-tag, GppNHp loaded	50 µg, 100 µg
5727-4122G	Kras G12C, GST-tag	50 µg, 100 µg
5727-4122G -G	Kras G12C, GST-tag, GDP Loaded	50 µg, 100 µg
5727-4122G -GP	Kras G12C, GST-tag, GppNHp loaded	50 µg, 100 µg
5727-4123G	Kras G12D, GST-tag	50 µg, 100 µg
5727-4123G -G	Kras G12D, GST-tag, GDP Loaded	50 µg, 100 µg
5727-4123G -GP	Kras G12D, GST-tag, GppNHp loaded	50 µg, 100 µg
7671	SOS1	100 µg, 1 mg
7237231	Human RBD-RAF1, N-His tag, C-FLAG tag	100 µg

Products are for research use only and are not intended for human use. We do not sell to patients.