Aurora Biolabs
High Purity Protein for Research

TR-FRET VHL Binding Assay Kit

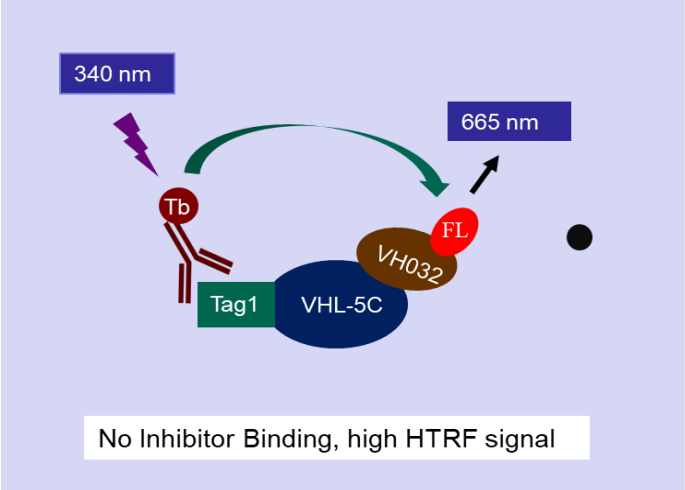
Catalog Number: 845225

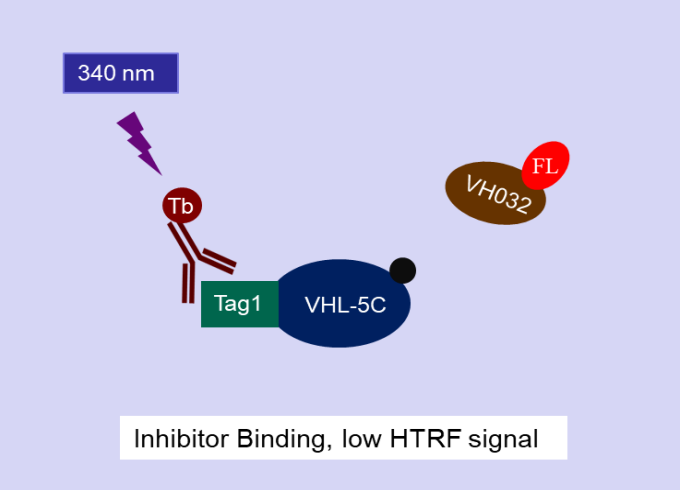
Background

Von Hippel–Lindau (VHL) is a member of an E3 ubiquitin ligase, a five-component complex including VHL, Cullin 2 (CUL2), Elongin B, Elongin C and RBX1 (RING-box protein 1). It is one of the most widely used E3 ligase recruiters in the design of PROTACs (Proteolysis-Targeting Chimeras) for targeted protein degradation (TPD) drug discovery. VHL plays a critical role in bringing the target protein and the ubiquitination machinery together for protein degradation via the proteasome.

Assay Principle

The TR-FRET VHL Binding Assy kit is designed to measure the binding affinity of VHL and its ligand, and it includes Tag1-VHL-5C (VHL/CUL2/EloC/EloB/RBX1 complex), Terbium-labeled Anti-Tag1 antibody and fluorescent labeled VHL ligand VH032. The binding of VHL to the ligand brings Terbium (fluorescence donor) on the anti-Tag1 antibody in close proximity to the fluorophore (FL) on VH032 (fluorescent receptor), which results in fluorescence resonance energy transfer (FRET). Thus, the binding status of VHL and VH032 can be quantitively determined using HTRF signal by calculating the ratio of the emission fluorescence intensity of the acceptor (665 nm) and donor (620 nm). If an compound binds to the VHL and blocks VH032 binding, the HTRF signal will be reduced.





Application

High throughput screening of compounds that bind to VHL 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
845225-B	VHL Binding assay buffer	25 mL	-20°C
845225-M	Recombinant human VHL-5C	40 µL	-80°C
84032	Fluorescence-labeled VH032 (FL-VH032)	200 µL	-80°C
44732	Fluorescence labeled anti-Tag1 antibody	40 µL	-80°C
	384-well microplate	1	Room temperature

Materials needed but not supplied

1. Microplate reader, HTRF® certified microplate reader
2. Adjustable micro-pipettor
3. Sterile Tips

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 VHL-5C solution

   Thaw VHL-5C 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: VHL-5C protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

   Dilute the VHL-5C protein 40-fold (1µL VHL-5C + 39 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-VH032 solution

   Dilute FL-VH032 10-fold (1 µL FL-VH032 + 9 µL of assay buffer).

   Add 4 µl of diluted protein solution to each well.

5. Prepare dye solution

   Dilute Terbium-labeled anti-Tag1 antibody 1:100 in assay buffer. For example: 1 µl of Terbium-labeled anti-Tag1 antibody + 99 µl of assay buffer.

   Add 10 µl of this dye mixture to each well.

6. Incubate the reaction at room temperature for 60 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		
VHL-5C protein		4 µl	4 µl
compound dilution Buffer (Assay buffer or 10% DMSO)	2 µl	2 µl	
Inhibitor solution			2 µl
FL-VH032	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.

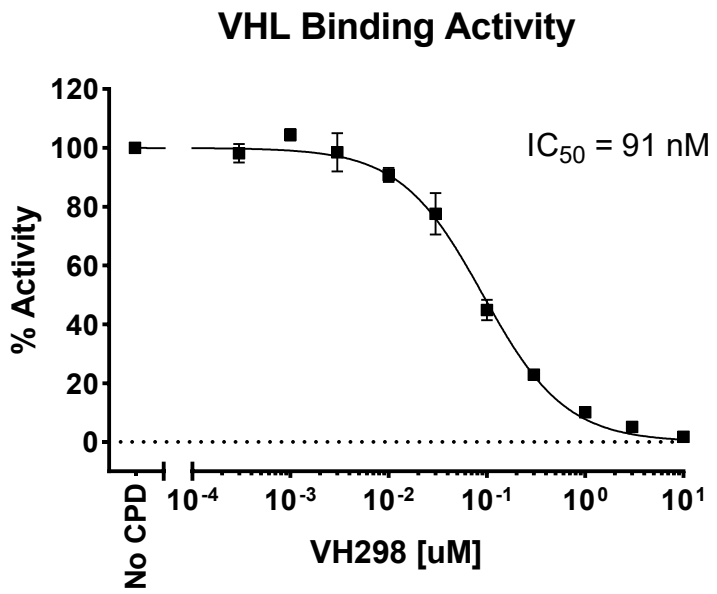
$$HTRF = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}} X10{,}000$$

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:

Product Name	Catalog #	Size
TR-FRET PARP1 Trapping Assay Kit	72771TAK	384 reactions
TR-FRET PARP2 Trapping Assay Kit	72772TAK	384 reactions
Kras WT Nucleotide Exchange Assay Kit	5727-4121NK	384 reactions
Kras G12C Nucleotide Exchange Assay Kit	5727-4122NK	384 reactions
Kras G12D Nucleotide Exchange Assay Kit	5727-4123NK	384 reactions
Kras G13D Nucleotide Exchange Assay Kit	5727-4133NK	384 reactions
Kras G12R Nucleotide Exchange Assay Kit	5727-4127NK	384 reactions
Kras G12V Nucleotide Exchange Assay Kit	5727-4128NK	384 reactions
Kras WT–cRAF Binding Assay Kit	5727-4121BK	384 reactions
Kras G12C–cRAF Binding Assay Kit	5727-4122BK	384 reactions
Kras G12D–cRAF Binding Assay Kit	5727-4123BK	384 reactions
Kras G12R–cRAF Binding Assay Kit	5727-4127BK	384 reactions
Kras G12V–cRAF Binding Assay Kit	5727-4128BK	384 reactions
Kras G13D–cRAF Binding Assay Kit	5727-4133BK	384 reactions
Kras WT/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4121CK	384 reactions
Kras G12C/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4122CK	384 reactions
Kras G12D/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4123CK	384 reactions
Kras G12V/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4128CK	384 reactions
Kras G13D/cRAF/CYPA/Inhibitor Binding Assay Kit	5727-4133CK	384 reactions
DNA Polymerase Theta Activity Assay Kit	362101	96 reactions, 384 reactions

OX40/OX40L Inhibitor Binding Assay Kit	2369401	384 reactions
PD-1/PD-L -1Inhibitor Binding Assay Kit	237352	384 reactions
T7 High Yield RNA Synthesis Kit	K777627	25, 50, 100 reactions
PKMYT1 Binding Assay Kit	756981BK	384 reactions
eIF4E/eIF4G Binding Assay Kit	34343BK	384 reactions
Caspase-3 Activity Assay Kit	810030	384 reactions
IDO1 Activity Assay Kit for Inhibitor Screening	910010	96 reactions
TEV Protease Activity Assay Kit	190001AK	96 reactions
SARS-CoV-2 Mpro (3CL Protease) Assay Kit	728203	96 reactions
SARS-CoV-2 Papain-like Protease Assay Kit	728253	96 reactions
SARS-CoV-2 Nucleocapsid Protein Binding Kit (For mouse antibody)	728263	384 reactions
SARS-CoV-2 Nucleocapsid Protein Binding Kit (For rabbit antibody)	728273	384 reactions
TEV Protease	190001	1,000 Units, 10,000 Units
TEV Protease- His-tag	190001-R	50 ug, 200 ug, 1 mg
PreScission Protease (HRV 3C)	190002	1,000 units, 10,000 units
Recombinant SUMO Protease (Ulp1)	190003	1,000 units, 10,000 units
Recombinant YopH	200100	10 ug, 20 ug, 100 ug, 1 mg
Recombinant Biotin Protein Ligase (BirA)	90101	100 ug
Recombinant SortaseA-5M	90201	50 ug, 200ug
Recombinant Mouse Leukemia Inhibitory Factor	11-0001	10 ug, 100 ug
Recombinant Human LIF	12-0002	10 ug, 100 ug, 1 mg
Recombinant Human FGF-basic, Carrier-free	12-0005CFR	50 ug, 100 ug, 500 ug, 1 mg
Human SOS1, Avi-His tag	7671HA	50 µg, 100 µg
Human RBD-RAF1, N-His tag, C-FLAG tag	7237231	100 µg
Recombinant Human PD-1	23731	100 µg
Recombinant Human PD-L1	237351	100 µg
Recombinant Human LAG3	235243	100 µg
Recombinant Human FGL1	233451	100 µg
Recombinant Human CD40	232340	100 µg
Recombinant Human CD40L	2323405	100 µg
Recombinant Human CD27	2323155	100 µg
Recombinant Human CD70	232370	100 µg
Recombinant Human OX40	236940	100 µg
Recombinant Human OX40L	2369405	100 µg
Recombinant Human GITR	234487	100 µg
Recombinant Human GITRL	2344875	100 µg
Recombinant Human CD40	232340	100 µg
Recombinant Human CD40L	2323405	100 µg
Recombinant Human CD155	2323155	100 µg
Recombinant Human TIGIT	2384448	100 µg

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