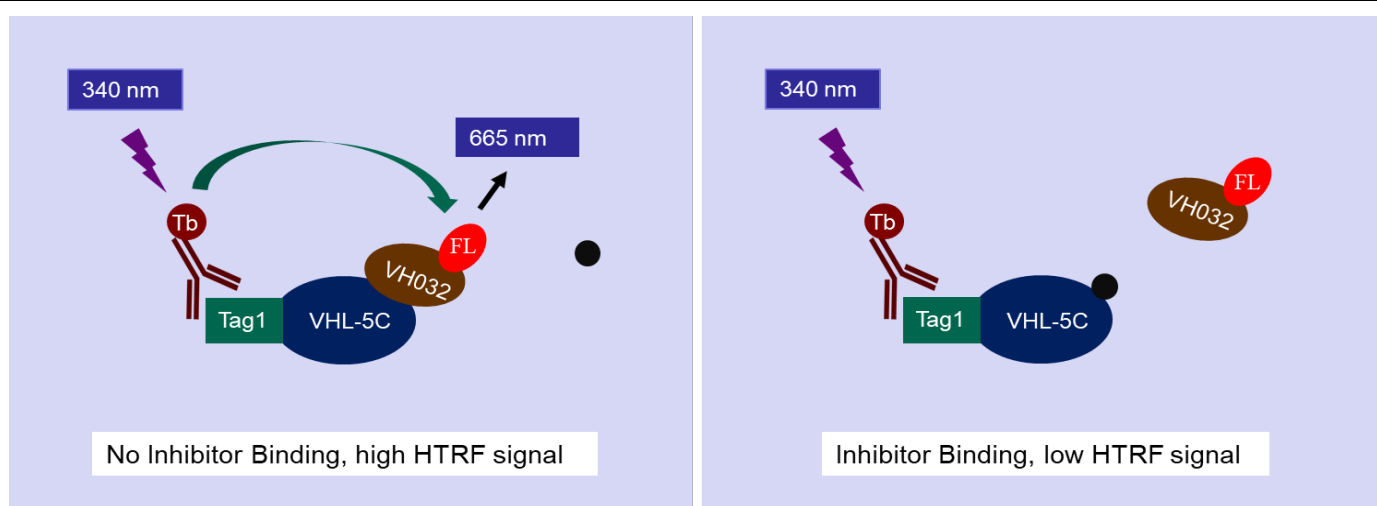


Background

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 Assay 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 quantitatively 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	20 mL	-20°C
845225-P	Recombinant human VHL-5C	40 µL	-80°C
853146	Fluorescence-labeled VH032 (FL-VH032)	170 µL	-80°C
447852	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 (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 μ L to the 20 μ L 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 a 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 (1 μ L VHL-5C + 39 μ L assay buffer).

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

Add 4 μ L of assay buffer to each of negative control well.

3. Add inhibitor

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

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

If the compound is diluted in 10% DMSO, add 2 μ L 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 μ L FL-VH032 + 9 μ L of assay buffer).

Add 4 μ L 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 μ L of Terbium-labeled anti-Tag1 antibody + 99 μ L of assay buffer.

Add 10 μ L 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.

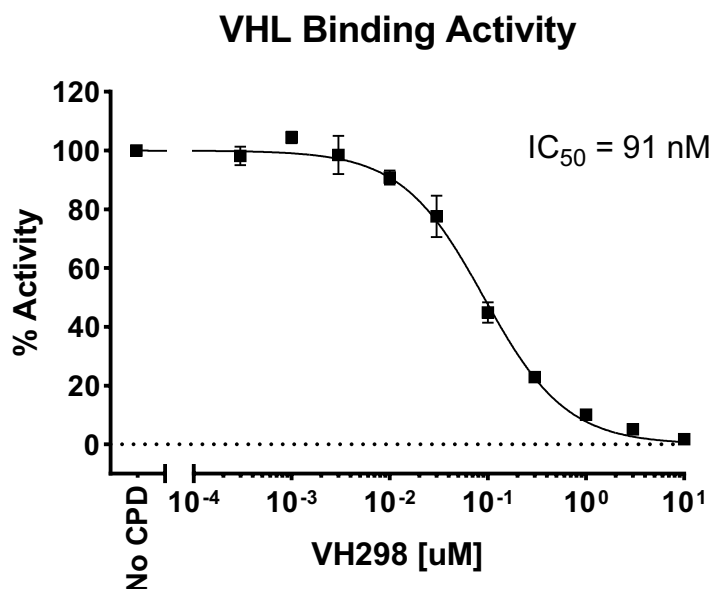
$$HTRF = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}} \times 10,000$$

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.

$$\% \text{ Activity} = \frac{S - N}{P - N} \times 100$$

Assay result



Related products:

<u>Catalog #</u>	<u>Product Name</u>	<u>Size</u>
272625	TR-FRET Cereblon-4C 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	<u>TEV Protease Activity Assay Kit</u>	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	<u>AiTEV™ Protease</u>	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 µg
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.