Aurora Biolabs
High Purity Protein for Research

# TR-FRET VHL Binding Assay Kit

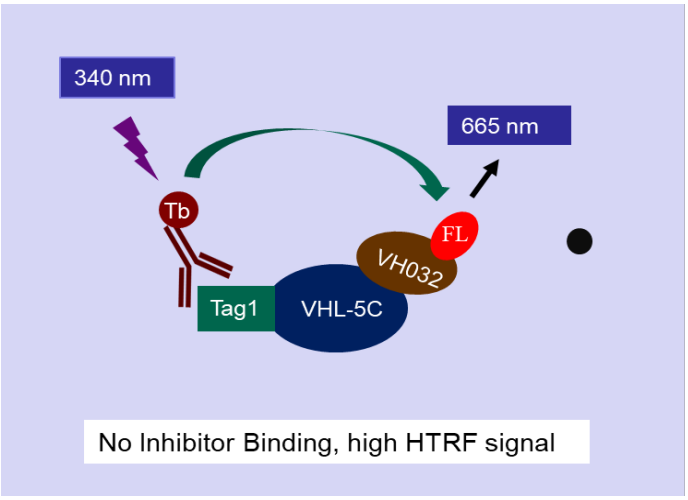
Catalog Number: 845225

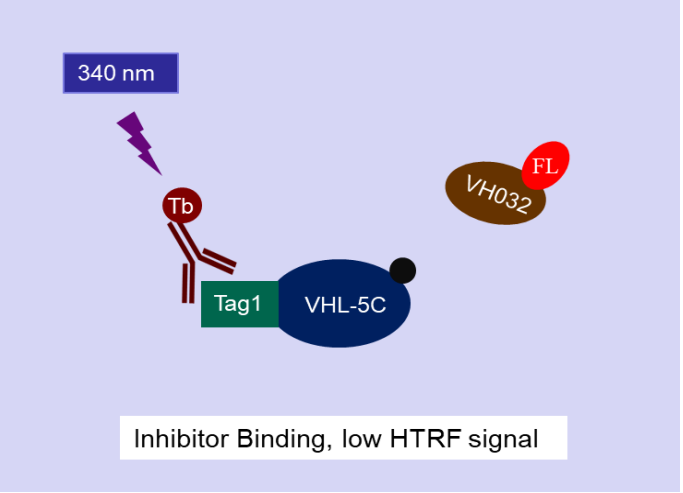
## Background

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 | 20 mL | -20°C |
| 845225-P | Recombinant human VHL-5C | 40 µL | -80°C |
| 853146 | Fluorescence-labeled VH032 (FL-VH032) | 170 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.

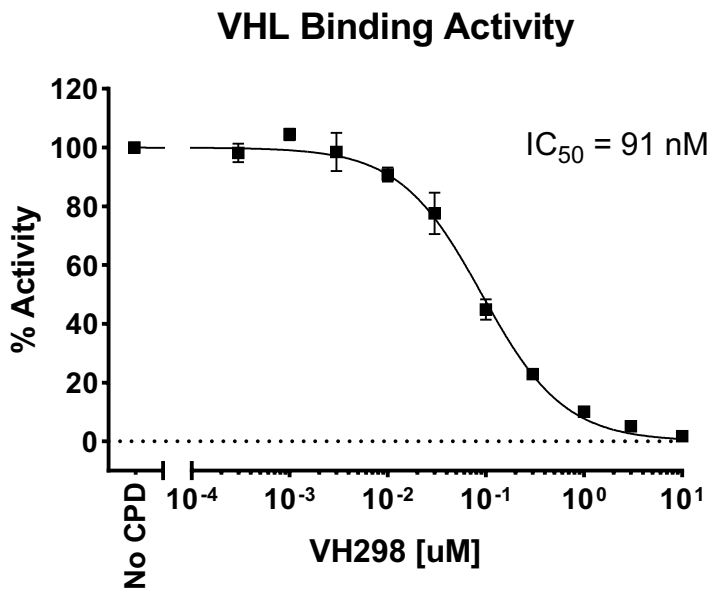
$$HTRF = \frac{\text{Fluorescent intensity at 665 nm}}{\text{Fluorescent intensity at 620 nm}} X10{,}000$$

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:

| Catalog # | Product Name | Size |
|---|---|---|
| 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 | 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.