

Description

The Von Hippel–Lindau tumor suppressor (VHL) Binding Assay Kit is a homogeneous assay which measures the binding of VHL to the high-affinity VHL fluorescent probe (BDY FL VH032) using Fluorescence Polarization (FP). The kit provides enough purified ELOB/ELOC/VHL complex, VHL fluorescent probe (BDY FL VH032), and assay buffer for 100 enzyme reactions. In addition, the kit includes the VHL inhibitor VH298 for use as an inhibitor control. This assay takes advantage of the fluorescent substrate BDY FL VH032. Using this kit, only one step on a 96-well plate is required. The BDY FL VH032 is incubated with the VHL complex in the presence of a compound of interest to produce a change in fluorescent polarization. The FP signal is measured using a fluorescent microplate reader *capable of measuring fluorescence polarization*.

Background

VHL is a tumor suppressor that functions as the substrate recognition subunit of the Cullin2 RING E3 ligase complex (CRL2^{VHL}). CRL2^{VHL} plays important roles in oxygen sensing by targeting hypoxia-inducible factor- α (HIF- α) subunits for ubiquitination and degradation. Elongin B (ELOB) and Elongin C (ELOC) are heterodimers that bind to the BC-box motif present in VHL. In this specific E3 ligase complex, the ELOB/ELOC works as an adapter between target proteins and Cullin-2/Rbx1 in VHL-box E3 ubiquitin ligases.

VHL is targeted by bifunctional molecules such as proteolysis-targeting chimeras to induce degradation of proteins of interest. Design and characterization of VHL binders is an important step in the design of VHL-targeting molecular degraders.

Application(s)

Titrate or screen small molecule inhibitors of VHL binding to the probe in drug discovery and high throughput (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
100361	ELOB/ELOC/VHL Complex*	150 μ g	-80°C
	BDY FL VH032, 250 μ M	10 μ l	-80°C
	VHL Assay Buffer (FP)	10 ml	-20°C
	VH298, 50 mM	15 μ l	-20°C
79685	Black, low binding, microtiter plate	1	Room Temperature

*The concentration of the protein is lot-specific and will be indicated on the tube

Materials Required but Not Supplied

Adjustable micropipettor and sterile tips

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Assay Principle

The VHL Binding Assay Kit (FP) is designed for testing and profiling of VHL inhibitors using Fluorescence Polarization. The assay is a competitive binding assay, based on the binding of fluorescent-labeled substrate to a purified recombinant VHL complex, in the presence of a competing binder.

Contraindications

The final concentration of DMSO in the assay should not exceed 1%.

Assay Protocol

- All samples and controls should be tested in duplicate.
- The assay should include a “Blank”, a “Positive control”, and a “Negative control.”

Step 1:

1. Thaw **ELOB/ELOC/VHL Complex** on ice. Briefly spin the tube containing **ELOB/ELOC/VHL Complex** to recover the full contents.

If the assay plate is going to be used more than once, prepare enough **ELOB/ELOC/VHL Complex** for this portion of the assay and aliquot the remaining into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C immediately. Refer to the protocol below to calculate how much of each protein is needed.

2. Dilute **ELOB/ELOC/VHL Complex** in **VHL Assay Buffer (FP)** to 37.5 ng/μl (1500 ng/well). Aliquot any remaining protein and store undiluted at -80°C. Keep diluted protein on ice. Discard any remaining diluted protein after use. *VHL is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

Note: The concentration of the protein complex varies with each lot and will be indicated on the tube.

- a. Add 40 μl of diluted **ELOB/ELOC/VHL Complex** to each well designated “Positive Control,” “Test Inhibitor,” and “VH298.”
 - b. Add 40 μl of **VHL Assay Buffer (FP)** to each well designated “Negative Control.”
 - c. Add 45 μl of **VHL Assay Buffer (FP)** to each well designated “Blank.”
3. Prepare the Test Inhibitor (5 μl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 50 μl.
 - a. If the Test Inhibitor is water-soluble, prepare serial dilutions in the VHL Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use VHL Assay Buffer (Diluent Solution).

OR

- b. If the Test Inhibitor is soluble in DMSO, prepare it in DMSO at a concentration 100-fold higher than the highest desired concentration, then dilute the Test Inhibitor 10-fold in VHL Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 10%. Prepare serial dilutions of the Test Inhibitor at concentrations 10-fold higher than the desired final concentrations using 10% DMSO in VHL Assay Buffer to keep the concentration of DMSO constant. For the controls, prepare 10% DMSO in VHL Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

4. Add 5 μ l of diluted Test Inhibitor to each well designated "Test Inhibitor."
Add 5 μ l of Diluent Solution to each well designated "Blank," "Positive Control," and "Negative Control."
 5. Dilute **VH298** (50 mM) 100-fold with **VHL Assay Buffer (FP)** to obtain a 500 μ M solution.
Note: To create an IC50 curve, prepare serial dilutions using the same diluent solution used to dilute the test inhibitor (typically 10% DMSO in VHL Assay buffer).
 6. Add 5 μ l of diluted **VH298** to each well designated "VH298."
 7. Incubate for 60 minutes at room temperature with slow shaking on a rotating platform.
 8. During the incubation, thaw **BDY FL VH032** on ice. Briefly spin the tube containing **BDY FL VH032** to recover its full contents.
 9. Dilute **BDY FL VH032 (250 μ M stock)** 100-fold with **VHL Assay Buffer (FP)** to make a 2.5 μ M solution.
If the assay plate is going to be used more than once, prepare enough BDY FL VH032 for this portion of the assay and aliquot the remaining into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C immediately.
- Note: BDY FL VH032 is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.**
10. After 60 minutes, start the binding reaction by adding 5 μ l of diluted **BDY FL VH032** to each well designated "Positive Control," "Negative Control," "Test Inhibitor," and "VH298." Do not add to the "Blank."
 11. Incubate at room temperature for 30-45 minutes with slow shaking.

Component	Blank	Negative Control (Reference)	Positive Control	Test Inhibitor	VH298
VHL Assay Buffer (FP)	45 μ l	40 μ l	-	-	-
VHL Complex (37.5 ng/ μ l)	-	-	40 μ l	40 μ l	40 μ l
Test Inhibitor	-	-	-	5 μ l	-
Diluent Solution	5 μ l	5 μ l	5 μ l	-	-
Diluted VH298	-	-	-	-	5 μ l
Incubate 60 minutes at room temperature					
BDY FL VH032 (2.5 μ M)	-	5 μ l	5 μ l	5 μ l	5 μ l
Incubate 30-45 minutes at room temperature					
Total	50 μl	50 μl	50 μl	50 μl	50 μl

Step 2:

Read fluorescent polarization in a plate reader capable of excitation at wavelength 485 nm (bandwidth 10-20 nm) and detection of emitted light at wavelength 528 nm (bandwidth 10-20 nm). Ensure that the instrument is set to read the type of plate used in the experiment. Blank value is subtracted from all other values.

Calculating Results; Definition of Fluorescence Polarization:

$$P = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}}$$

Where I_{\parallel} = Intensity with polarizers parallel and I_{\perp} = Intensity with polarizers perpendicular. Most instruments display fluorescence polarization in units of mP.

$$mP = \left(\frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + I_{\perp}} \right) \times 1000$$

The equation above assumes that light is transmitted equally well through both parallel and perpendicular oriented polarizers. In practice, this is generally not true and a correction must be made to measure the absolute polarization state of the molecule. This correction factor is called the "G Factor".

$$mP = \left(\frac{I_{\parallel} - G(I_{\perp})}{I_{\parallel} + G(I_{\perp})} \right) \times 1000 \quad \text{OR} \quad mP = \left(\frac{G(I_{\parallel}) - I_{\perp}}{G(I_{\parallel}) + I_{\perp}} \right) \times 1000$$

The G-factor is instrument-dependent and may vary slightly depending upon instrument and conditions. Please check the manual of your instrument to obtain the information about the establishment of the G-factor.

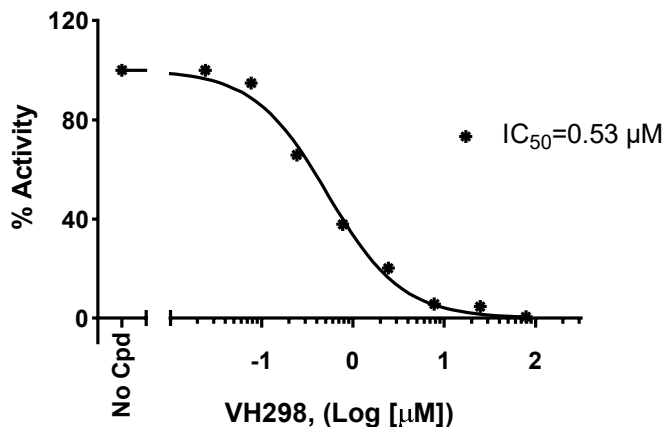
EXAMPLE OF ASSAY RESULTS**Inhibition of VHL binding to BDY FL VH032**

Figure 1. Inhibition of VHL binding to fluorescent probe BDY FL VH032 by VH298, measured using the VHL Binding Assay kit (BPS Bioscience #78805).

The assay was performed as described in the protocol. Results are expressed as % of activity with the "no compound" control set at 100%. Fluorescence was measured at λ_{ex} 485 nm, λ_{em} 528 nm using a Bio-Tek fluorescent microplate reader.

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

Troubleshooting Guide

Visit [Assay Kits FAQ \(bpsbioscience.com\)](https://www.bpsbioscience.com) for detailed troubleshooting instructions and information about FP assays. Read our eBook "[Fluorescence Polarization Assays](#)" to learn more about principles of FP.

For further questions, please email support@bpsbioscience.com.

References

1. Lee J, *et al.* Discovery of E3 Ligase Ligands for Target Protein Degradation. *Molecules* 2022; **27 (19)**: 6515.
2. Diehl CJ, Ciulli A. Discovery of small molecule ligands for the von Hippel-Lindau (VHL) E3 ligase and their use as inhibitors and PROTAC degraders. *Chem Soc Rev* 2022; **51 (19)**: 8216-8257.
3. Frost K, *et al.* Von Hippel-Lindau (VHL) small-molecule inhibitor binding increases stability and intracellular levels of VHL protein, *J Biol Chem* 2021; **297 (2)**: 100910.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
VHL/CUL2/ELOB/ELOC/RBX1 Complex Recombinant	100373	10 µg/50 µg
Cereblon/DDB1/Cul4A/Rbx1 Complex Recombinant	100329	10 µg/50 µg
Cereblon Ubiquitination Homogeneous Assay Kit	79881	384 reactions
ELOB/ELOC/VHL Complex Recombinant	100361	10 µg/50 µg
PROTAC® Optimization Kit for BET Bromodomain-Cereblon Binding	79770	384 reactions
PROTAC® Optimization Kit for BET Bromodomain-Von Hippel Lindau (VHL) Binding	79790	384 reactions