

## VHL BINDING ASSAY

Celtarys' VHL binding assay is a **fluorescent polarization-based assay** that uses CELT-504 as the fluorescent tracer, alongside the ElonginB/ElonginC-VHL (VBC) complex to study the binding affinity of different ligands for the VHL E3 ligase.

**CELT-504** has been developed with FP in mind starting with its design. The affinity of this compound is in the low nanomolar range, allowing for the calculation of the binding affinity of a wide range of compounds. Indeed, both  $IC_{50}$  and  $K_i$  (Chen-Prusoff equation)<sup>1</sup> can be obtained.

Obtaining **binary VHL interaction data** is a key step in **PROTAC** development, supporting both the design of **novel VHL ligands** and the understanding of PROTAC behaviour in binary complex formation.

### Why this assay?

This assay provides the following advantages:

- **Quantitative binding affinity** is obtained in a robust binary biochemical format using the intact, unmodified VHL-containing protein complex.
- As an FP assay, it involves a **limited number of reagents and steps** simplifying implementation.
- **Homogeneous** fluorescence polarization **readout** with **no washing or separation steps**.
- **High-affinity fluorescent tracer**: CELT-504 has a low nanomolar affinity for the VBC complex.
- **Expert-run** fluorescence polarisation screening service for **fast and reliable data generation**.
- The **fluorescent tracer has been extensively validated**, both using reference ligands (such as VH 298, MZ1) and newly developed ones. As seen in Table 1, the reference compounds used have led to results similar to those reported in literature, maintaining a similar range of affinity

constants. Differences of up to two-fold are considered method-concordant due to inter-assay variability.

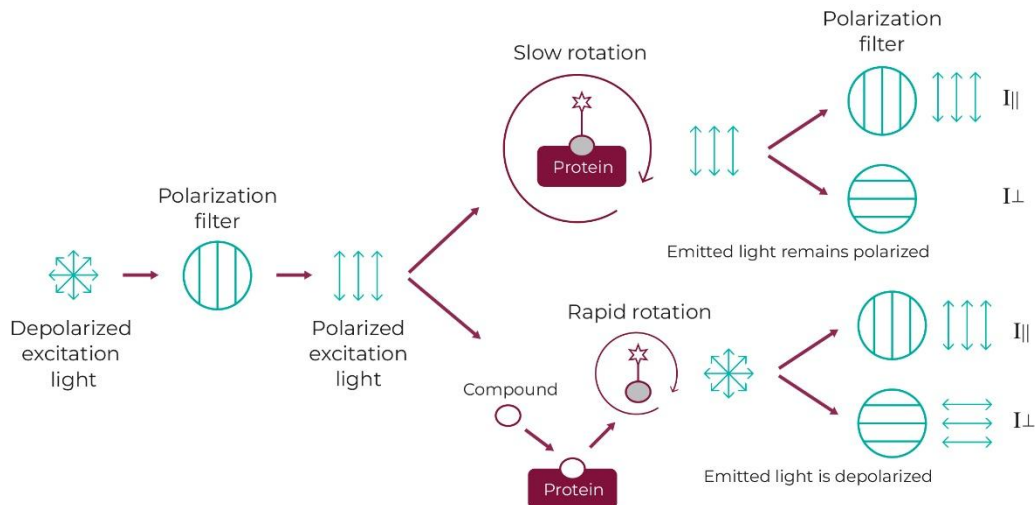
Compound	Type	K <sub>i</sub> VCB complex (nM)	
		JC9 FP competition or ITC	VHL BINDING ASSAY KIT
<b>VH 298</b>	Small Molecule	80 nM <sup>2</sup> (FP)	51.6 nM
<b>MZI</b>	PROTAC	70 nM <sup>3</sup> (ITC)	115 nM

**Table 1. Comparison of VBC complex affinity (K<sub>i</sub>, nM) between different assay formats.** FP KIT K<sub>i</sub> values were obtained from concentration-response displacement curves using Cheng-Prusoff<sup>1</sup> correction, with the fluorescent tracer CELT-504 and following Kit Protocol. **Cross-assay agreement:** K<sub>i</sub> values within ~5× of literature/reference are considered concordant given expected inter-method variability.

## How does FP (fluorescence polarization) work?

The principle of the assay is based on the **change in rotational mobility** that occurs when a **fluorescent ligand binds to a larger target protein**. Free CELT-504 rotates rapidly in solution and, when excited by plane-polarised light, produces a low fluorescence polarisation signal. When CELT-504 binds to the target protein VHL, its effective molecular volume increases, rotational relaxation slows down and the **emitted light** retains a **higher degree of polarisation**, resulting in a higher FP signal. Compounds that interact with the same binding site **compete with the tracer** and displace it in a concentration-dependent manner, resulting in a progressive **reduction in polarisation**. Thus, **the assay allows direct monitoring of binding events and the generation of concentration-response curves from which quantitative binding parameters can be derived**.

As a homogeneous format, FP offers a **simple and effective approach to compound evaluation**, as it requires no washing steps and can be carried out with a limited number of reagents. This makes this type of assay particularly attractive for rapid screening of large compound libraries and comparative profiling in drug discovery, where robust, quantitative and easy-to-perform binding assays are of great value.



**Figure 1. Depiction of the basis of FP assays.**

## What you receive

- PDF report with binding data affinity (IC<sub>50</sub>, K<sub>i</sub>)/ % inhibition, methodology, controls and curves of reference compounds.

## Assay formats

- Binding assay (single-concentration profiling):** qualitative detection of ligand binding based on fluorescence signal. Up to **16 compounds/plate** (final concentration of 1 μM, and 10 μM if desired) in duplicate plus controls.
- Displacement assay (concentration curve-binding):** to calculate IC<sub>50</sub> and estimate K<sub>i</sub> values for your compounds. Up to **4 compounds/plate × 7 concentration-points** (e.g., 10<sup>-5</sup>–10<sup>-10</sup> M) or **6 compounds/plate × 5 concentration-points** in duplicate plus controls. You can choose which concentrations to test.

## Workflow & timelines

- Scope & quote → proposal signature → PO/payment.
- Ship samples with the Celtarys Shipping Label ([Annex II](#)) and the Sample Shipping Workbook ([Annex III](#)).
- Conducting the assay.
- Delivery: report with the results.

Note: Results will be available within an estimated 7 to 10 working days after receiving the sample. Up to two 96-well plates can be processed per experiment.

## Sample requirements (summary)

- Format: **DMSO 10 mM**; minimum **50** µL per compound or **lyophilized solid**.
- Labelling: **unique ID on the Eppendorf tube** plus printed inventory; Compound IDs must match the workbook ([Annex II](#), [Annex III](#)).
- Stability/handling: specify storage on arrival (–20 °C / 4 °C / ambient), light protection, temperature in transit, max freeze–thaw cycles.
- If lyophilized: include MW and mass (mg); must allow reconstitution to ≥ 50 µL at 10 mM in DMSO ([Annex II](#), [Annex III](#)).

Note: Responsibility for transport and storage

Celtarys Research will handle samples strictly in accordance with the instructions provided on the sample submission form ([Annex II](#), [Annex III](#)). Celtarys is not responsible for any deterioration, loss of activity or delays resulting from inadequate packaging, non-compliant transport or failure to specify optimal storage/handling conditions. The customer must clearly indicate all special requirements (e.g., light protection: shipping in amber vials and/or wrapped in aluminum foil; temperature control with a validated carrier and recorder; inert atmosphere; humidity/pH limits; required diluent/solvent; maximum freeze-thaw cycles) and ensure that the product is packaged in accordance with them. Samples arriving outside the specified conditions or without complete documentation may be rejected or require a new shipment at the customer's expense and within the timeframe determined by the customer.

## Scientific References

- (1) Yung-Chi, C.; Prusoff, W. H. Relationship between the Inhibition Constant (KI) and the Concentration of Inhibitor Which Causes 50 per Cent Inhibition (I50) of an Enzymatic Reaction. *Biochemical Pharmacology* **1973**, *22*, 3099–3108.
- (2) Soares, P.; Gadd, M. S.; Frost, J.; Galdeano, C.; Ellis, L.; Epemolu, O.; Rocha, S.; Read, K. D.; Ciulli, A. Group-Based Optimization of Potent and Cell-Active Inhibitors of the von Hippel–Lindau (VHL) E3 Ubiquitin Ligase: Structure–Activity Relationships Leading to the Chemical Probe (2*S*,4*R*)-1-((*S*)-2-(1-Cyanocyclopropanecarboxamido)-3,3-Dimethylbutanoyl)-4-Hydroxy-*N*-(4-(4-Methylthiazol-5-Yl)Benzyl)Pyrrolidine-2-Carboxamide (VH298). *J. Med. Chem.* **2018**, *61*, 599–618.
- (3) Chan, K.-H.; Zengerle, M.; Testa, A.; Ciulli, A. Impact of Target Warhead and Linkage Vector on Inducing Protein Degradation: Comparison of Bromodomain and Extra-Terminal (BET) Degraders Derived from Triazolodiazepine (JQ1) and Tetrahydroquinoline (I-BET726) BET Inhibitor Scaffolds. *J. Med. Chem.* **2018**, *61*, 504–513.