Celtarys RESEARCH

CELT-050 VHL E3 Ligase

TR-FRET protocol

Protocol for E3 ubiquitin ligase VHL affinity binding assay using TR-FRET

CELT-050, a potent VHL E3 ligase fluorescent ligand, was used as fluorescent probe in a TR- FRET assay to study the affinity of a known inhibitor of E3 ubiquitin ligase VHL, VH298.

Equipment, Materials and Reagents:

- Plate Reader with a TR-FRET optic module capable of emitting in the 390nm range and detecting in the 650nm range.
- TR-FRET compatible plates such as CulturPlate-384.
- Centrifuge with adaptor compatible with 384 plates.
- Recombinant His Tagged Human VBC complex.
- Anti-His Europium labeled Antibody.
- CELT-050, VHL E3 ligase fluorescent ligand

Assay Buffer:

- 20 mM HEPES pH 7.5
- 150 mM NaCl
- 1 mM TCEP
- 0.01 % TWEEN20

Master Mix:

In order to streamline the process and run as many compounds as possible, preparation of a master mix is recommended. This mixture should have 2 times the concentration of the final concentration in the well:

- 400 nM CELT-050.
- 100 nM His-VBC complex.
- 1 nM AntiHis-EuAb.
- Assay Buffer.

Assay Procedure

The assay was performed using 384 Microplates with 15 µL as a final assay volume.

The assay was performed using Europium (Anti-His-EuAb) as donor and His-VBC complex as protein of interest to be degraded.

A 2X compound concentration template plate is recommended diluting the compounds in the assay buffer. The final DMSO concentration in the wells should be 2 %.

7.5 µL of each 2X template well are deposited in their corresponding wells, and once all compounds are ready the plate is centrifuged.

Afterwards, 7.5 µL of the Master Mix are added to each well, totaling 15 µL in each well. The plate is centrifuged again.

It is recommended to cover the plate from the light while incubating. Measures were taken every half an hour for 3 hours, as the signal stabilizes around the 90 minute mark.

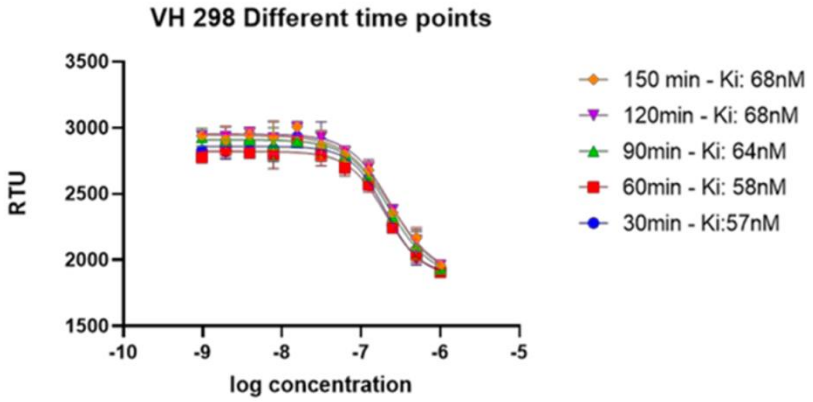
Assay Performance

·Z´prime of 0.87.

·Different percentages of DMSO were tested, from 0.1 % to 2 % (final concentration in the wells). Up to 2 % is acceptable although the assay window is reduced with the increasing percentages of DMSO.

Results

The figure below shows how increasing concentrations of VH298 compete with CELT-050 ligand and thereby prevent TR-FRET from occurring.



VH298 displayed the expected potency in good correlation with the literature (Frost et al., 2016, Potent and selective chemical probe of hypoxic signalling downstream of HIF-α hydroxylation via VHL inhibition. Nature Communications volume 7, Article number: 13312).