

CRBN BINDING ASSAY

Celtarys' CRBN binding assay is a **fluorescent polarization-based assay** that uses CELT-077 as the fluorescent tracer (Ex ~590 nm / Em ~616 nm), alongside the CUL4A/RBX1/DDB1/CRBN complex, to study the binding affinity of different ligands for CRBN.

CELT-077 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 IC50 and Ki can be obtained using the Cheng–Prusoff equation¹.

Obtaining **binary CRBN interaction data** is a key step in targeted protein degradation programs, supporting both the design of **novel CRBN ligands** and the understanding of degrader 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 CRBN 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-077 supports competitive binding measurements in the low nanomolar range.
- **Expert-run** fluorescence polarisation screening service for **fast and reliable data generation**.
- The **fluorescent tracer has been extensively validated**, using reference ligands (such as pomalidomide, thalidomide, cemsidomide and iberdomide) 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 _i CRBN complex (nM)	
		Literature reference	CRBN BINDING ASSAY KIT
Pomalidomide	Small Molecule	159.6 nM ² (FP)	300 nM
Thalidomide	Small Molecule	249 nM ² (ITC)	238 nM
Cemsiidomide	Molecular Glue	0.9 ³ nM	0.68 nM
Iberdomide	Molecular Glue	9-48 ⁴ nM (IC ₅₀ =60 nM)	17.9 nM

Table 1. Comparison of CRBN complex affinity (K_i, nM) between different assay formats. FP KIT K_i values were obtained from concentration-response displacement curves using Cheng-Prusoff¹ correction, with the fluorescent tracer CELT-077 and following Kit Protocol. **Cross-assay agreement:** K_i 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-077 rotates rapidly in solution and, when excited by plane-polarised light, produces a low fluorescence polarisation signal. When CELT-077 binds to the target protein CRBN, 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.

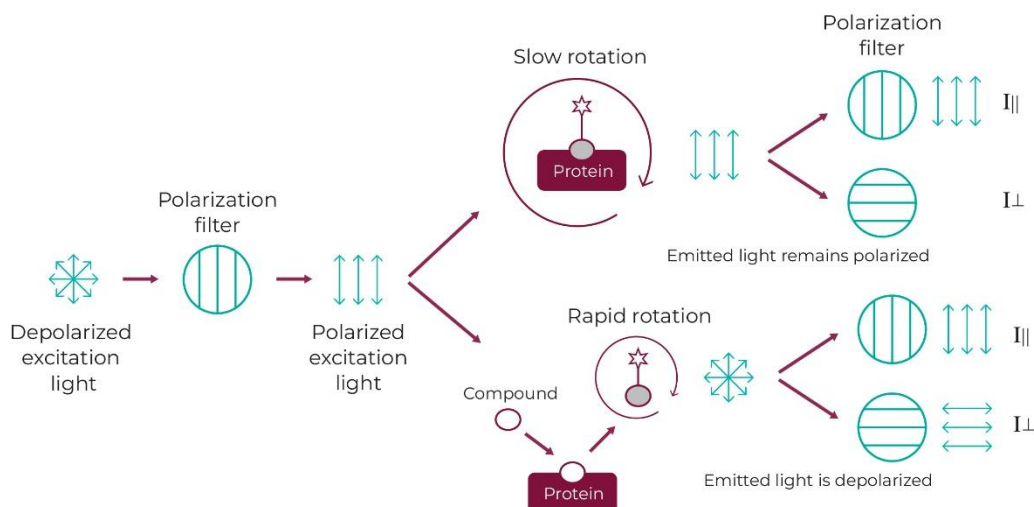


Figure 1. Depiction of the basis of FP assays.

What you receive

- PDF report with binding data affinity (IC_{50} , K_i)/ % 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 μ M, and 10 μ M if desired) in duplicate plus controls.
- Displacement assay (concentration curve-binding):** to calculate IC_{50} and estimate K_i values for your compounds. Up to **4 compounds/plate × 7 concentration-points** (e.g., 10^{-5} – 10^{-10} 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.
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3. Henderson, J. A., et al. (2022, April). *The discovery and characterization of CFT7455: A potent and selective degrader of IKZF1/3 for the treatment of relapsed/refractory multiple myeloma* [Poster presentation]. AACR Annual Meeting 2022. C4 Therapeutics.
4. Matyskiela, M. E., Zhang, W., Man, H. W., Muller, G., Khambatta, G., Baculi, F., Hickman, M., LeBrun, L., Pagarigan, B., Carmel, G., Lu, C. C., Lu, G., Riley, M., Satoh, Y., Schafer, P., Daniel, T. O., Carmichael, J., Cathers, B. E., & Chamberlain, P. P. (2018). A Cereblon Modulator (CC-220) with Improved Degradation of Ikaros and Aiolos. *Journal of medicinal chemistry*, 61(2), 535–542. <https://doi.org/10.1021/acs.jmedchem.6b01921>