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Data Sheet

Cereblon Binding Assay Kit

Catalog #79899
Size: 96 reactions

DESCRIPTION: The *Cereblon Binding Assay Kit (FP)* is designed for testing and profiling of Cereblon (CRBN) inhibitors using Fluorescence Polarization. The assay is a competitive binding assay, based on the binding of fluorescently-labeled Thalidomide, a CRBN binder, to purified recombinant CRBN.

CRBN is the substrate recognition component of a DCX (DDB1-CUL4-X-box) E3 protein ligase complex that mediates the ubiquitination and subsequent proteasomal degradation of target proteins. CRBN is a direct protein target for the immunomodulatory and antiproliferative activities of thalidomide analogues such as lenalidomide and pomalidomide.

The *Cereblon Binding Assay Kit (FP)* comes in a convenient 96-well format, with enough purified CRBN, Cy5-labeled Thalidomide, and assay buffer for 100 enzyme reactions. In addition, the kit includes the CRBN inhibitor Pomalidomide for use as an inhibitor control.

The key to the *CRBN Assay Kit* is the fluorescently-labeled Thalidomide. Using this kit, only one simple step on a microtiter plate is required for CRBN reactions. The Cy5-labeled Thalidomide is incubated with a sample containing CRBN to produce a change in fluorescent polarization. The FP signal is measured using a fluorescent microplate reader capable of measuring fluorescence polarization.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100255	FLAG-CRBN	60 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
	Cy5-labeled Thalidomide*, 10 µM	15 µl	-80°C	
79972	CRBN Assay buffer (FP)	10 ml	-80°C	
	Pomalidomide, 50 mM	15 µl	-20°C	
79685	Black, low binding, microtiter plate	1	Room temp.	

Thalidomide is known to cause severe birth defects in humans. It is very important to use all appropriate precautions when handling this compound, especially by pregnant women.

MATERIALS REQUIRED BUT NOT SUPPLIED:

Adjustable micropipettor
Sterile tips

APPLICATIONS: Great for studying enzyme kinetics and screening small molecular inhibitors for drug discovery and HTS applications.

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STABILITY: 6 months when stored as recommended.

REFERENCE(S):

1. Lopez-Girona A , *et al., Leukemia* 2012; **26:** 2326-35.
2. Fischer ES, *et al., Nature* 2014; **3512** (7512)**:** 49-53.

ASSAY PROTOCOL:

Immediately prior to assay:

1) Thaw **Cy5-labeled thalidomide** on ice. Upon first thaw, briefly spin tube containing Cy5-labeled thalidomide to recover full content of the tube. Aliquot into single use aliquots. Store remaining **Cy5-labeled thalidomide** in aliquots at -80°C immediately. *Note:* **Cy5-labeled thalidomide** *is sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

2) Thaw **CRBN** on ice. Upon first thaw, briefly spin tube containing **CRBN** to recover full content of the tube. Aliquot **CRBN** into single use aliquots. Store remaining CRBN in aliquots at -80°C immediately. *Note:* ***CRBN*** *is very sensitive to freeze/thaw cycles. Avoid multiple freeze/thaw cycles.*

Step 1:

All samples and controls should be tested in duplicate.

1) Dilute **Cy5-labeled Thalidomide** (10 µM stock) 200-fold with **CRBN Assay buffer (FP)** to make a 50 nM solution. (Make only sufficient quantity needed for the assay; store remaining 10 µM stock solution in aliquots at -80°C.)

2) Dilute **CRBN** in **CRBN Assay buffer (FP)** to 15 ng/µl (600 ng/reaction). Aliquot any remaining protein and store undiluted at -80°C. Keep diluted protein on ice. Discard any remaining diluted protein after use. *^Note: optimal protein concentration may vary depending on lot.*

3) Prepare 10X test inhibitor in an aqueous-based solution using **CRBN Assay buffer (FP)**. *Note: Final DMSO concentration must be ≤1%. Higher DMSO levels can significantly decrease binding activity*. For example, to test an inhibitor dissolved in 100% DMSO at 10 µM, dilute 1 mM inhibitor with **CRBN Assay buffer** to make a 100 µM inhibitor in 10% DMSO/90% **CRBN Assay buffer (Inhibitor Buffer**). Then, add 5 µl of the 100 µM solution to the assay to make a 1% DMSO concentration in the final reaction mixture. Serial dilutions should be made in this same solution (**Inhibitor Buffer**) to maintain the same DMSO concentration.

4) Dilute Pomalidomide 100-fold with **CRBN Assay buffer (FP)** to get 500 µM solution. To create an IC_{50} curve, prepare serial diluted solutions using the same solution used to make dilutions of your test inhibitor (**Inhibitor Buffer**, typically 10% DMSO in **CRBN Assay buffer**). Add 5 µl to each well designated “Inhibitor Control”. Discard diluted solution after use.

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5) Preincubate diluted CRBN with your inhibitor for 60 minutes at room temperature with slow shaking on a rotating platform, as follows:

Add 40 µl of diluted **CRBN** to each well designated "Positive Control," "Test Inhibitor," and "Inhibitor Control."

Add 40 µl of **CRBN Assay buffer (FP)** to each well designated "Negative Control," and 45 µl of **CRBN Assay buffer (FP)** to each well designated "Blank."

Add 5 µl of diluted **Inhibitor** to each well designated "Test Inhibitor."

Add 5 µl of 10% DMSO in CRBN Assay buffer (Inhibitor Buffer) to each well designated "Blank," "Positive Control," and "Negative Control." Add 5 µl of diluted Pomalidomide to each well designated "Inhibitor Control."

6) After 60 min, start binding reaction by adding 5 µl of diluted **Cy5-Thalidomide** to each well designated "Positive Control," "Negative Control," "Test Inhibitor," and "Inhibitor Control." Incubate at room temperature for 1.5 hours with slow shaking.

	Blank	**Negative Control (Reference)**	**Positive Control**	**Test Inhibitor**	**Inhibitor Control**
CRBN Assay buffer (FP)	45 µl	40 µl	–	–	–
CRBN (15 ng/µl)	–	–	40 µl	40 µl	40 µl
Test Inhibitor	–	–	–	5 µl	–
10% DMSO in CRBN Assay buffer (Inhibitor buffer)	5 µl	5 µl	5 µl	–	–
Diluted Pomalidomide	–	–	–	–	5 µl
Cy5-Labeled Thalidomide (50 nM)	–	5 µl	5 µl	5 µl	5 µl
Total	**50 µl**	**50 µl**	**50 µl**	**50 µl**	**50 µl**

Step 2:
Read fluorescent polarization of the sample in a microtiter-plate reader capable of excitation at wavelengths ranging from 630-640 nm and detection of emitted light ranging from 672-692 nm. Ensure that the machine is set to read the type of plate used in the experiment. Blank value is subtracted from all other values.

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CALCULATING RESULTS:

Definition of Fluorescence Polarization:

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

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

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

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".

$$mP = \left(\frac{I_{II} - G(I_{\perp})}{I_{II} + G(I_{\perp})}\right) x\ 1000 \quad \textbf{OR} \quad mP = \left(\frac{G(I_{II}) - I_{\perp}}{G(I_{II}) + I_{\perp}}\right) x\ 1000$$

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.

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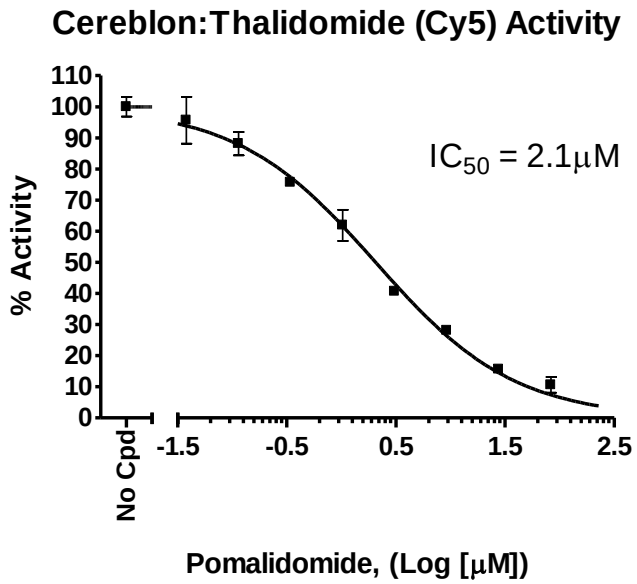
EXAMPLE OF ASSAY RESULTS



Inhibition of CRBN by Pomalidomide, measured using the CRBN Binding Assay kit, BPS Bioscience #79899. Fluorescence was measured at λex 635nm, λem 682 nm using a Bio-Tek fluorescent microplate reader. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Catalog #	Size
CRBN, FLAG-tag	100255	50 µg
Cereblon/DDB1/Cul4A/Rbx1 Complex	100329-1	10 µg
Cereblon/DDB1/Cul4A/Rbx1 Complex	100329-2	50 µg
MS-275 (Entinostat) inhibitor	27011	25 mg
Cereblon Ubiquitination Homogeneous Assay Kit	79881	384 rxns
PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding	50294	96 rxns
ELOB/ELOC/VHL Complex	100361-1	10 µg
ELOB/ELOC/VHL Complex	100361-2	50 µg
PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding	79770	384 rxns.
PROTAC Optimization Kit for BET Bromodomain-Von Hippel Lindau (VHL) Binding	79790	384 rxns.