SZABO SCANDIC

Part of Europa Biosite

Produktinformation

- Forschungsprodukte & Biochemikalien
- Zellkultur & Verbrauchsmaterial
- Diagnostik & molekulare Diagnostik
- Laborgeräte & Service

Weitere Information auf den folgenden Seiten!
See the following pages for more information!

Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

Cereblon Ubiquitination Homogeneous Assay Kit

Catalog #79881
Size: 384 reactions

BACKGROUND:

Cereblon (CRBN), a substrate receptor of Cullin-RING ligase 4 (CRL4), is a primary target of thalidomide and immunomodulatory imide drugs (IMiDs). CRBN binders are widely used in the design of heterobifunctional molecules called proteolysis-targeting chimeras (PROTACs) for degradation of the protein of interest. Several proteins, such as BRD4, CDK9, or Tau, can be successfully degraded by CRBN-based PROTACs. CRBN complex, $CRL4^{CRBN}$, is a unique E3 ubiquitin ligase because its substrate selectivity is altered by various ligands including IMiDs. IMiDs induce degradation of neosubstrates, such as Ikaros or CK1a. $CRL4^{CRBN}$ also promotes auto-ubiquitination of CRBN, which can be significantly inhibited by thalidomide.

DESCRIPTION:

The *Cereblon Ubiquitination Homogeneous Assay Kit* is designed to measure Cereblon auto-ubiquitination activity in a homogeneous 384 reaction format. It utilizes biotin-labeled ubiquitin and proper A-screen beads to complete the pairing. This homogeneous assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100402	UBE1 (E1)	4 µg	-80°C	***Avoid freeze/ thaw cycles!***
80313	UBCH5C (E2)	40 µg	-80°C	
100405	Human Cereblon complex (E3)	5 µg	-80°C	
11236	Biotin-Ubiquitin	12 µg	-80°C	
	ATP (40 mM)	20 µl	-80°C	
	Cereblon Assay buffer	4 ml	-20°C	
	Cereblon Detection buffer	4 mL	-20°C	
	100 mM (-)-Thalidomide	15 µl	-80°C	

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

AlphaLISA anti-FLAG acceptor beads, 5 mg/ml (PerkinElmer #AL112C)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
Optiplate 384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors or PROTACs for drug discovery and HTS applications.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). The presence of culture medium RPMI 1640 at >1% leads to signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least six months from date of receipt when stored as directed.

REFERENCES:

1) Chamberlain PP, Hamann LG. *Nat Chem Biol.* 2019 Oct;**15**(10):937-944.
2) Chen, Y.-A., Peng, Y.-J., Hu, M.-C., Huang, J.-J., *et al. Scientific Reports* 2015; **5** *(1).*

SAFETY WARNING: Thalidomide is known to cause severe birth defects in humans**. It is very important to use all appropriate precautions when handling this compound.**

ASSAY PROTOCOL

All samples and controls should be tested at least in duplicate. All incubations at room temperature are performed with slow shaking on a rotator platform.

Step 1:

1) Thaw UBE1, UBCH5C, Cereblon, Biotin-Ubiquitin, ATP, and Cereblon Assay buffer on ice. Aliquot each protein, assay buffer, and ATP into single-use aliquots and stored at -80°C immediately. *Note: UBE1, UBCH5C, Cereblon, Biotin-Ubiquitin, and assay buffer are sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

2) Carefully calculate the amount of proteins needed. Prepare appropriate amounts of diluted proteins and reagents as described below:

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Dilute the Cereblon in Cereblon Assay buffer at 17 ng/µl**;**
Dilute the UBE1 in Cereblon Assay buffer at 9 ng/µl**;**
Dilute the UBCH5C in Cereblon Assay buffer at 105 ng/µl**;**
Dilute the Biotin-Ubiquitin in Cereblon Assay buffer at 24 ng/µl**;**
Dilute the ATP in Cereblon Assay buffer at 2 mM (20-fold)**;**
Keep the diluted reagents on ice until use.

3) Add 4 µl of diluted Cereblon to each well designated "Positive Control" and "Test Sample". For the "Blank", add 4 µl of Cereblon Assay buffer.

4) Add 2 µl of inhibitor solution to each well designated "Test Sample". For the "Positive Control" and "Blank", add 2 µl of the same solution without inhibitor (Inhibitor buffer).

5) Preincubate Cereblon complex with the inhibitor(s) at room temperature for 30 min with slow shaking.

6) During the preincubation, prepare a master mixture using the diluted reagents prepared in step 2): N wells × (1 µl Biotin-Ubiquitin + 1 µl UBE1 + 1 µl UBCH5C + 1 µl ATP). After 30 minutes, start the reaction by adding 4 µl of master mixture to each well.

7) Incubate the reaction at 30°C for two hours. Cover the plate with a plate sealer if necessary.

	Blank	**Positive Control**	**Test Sample**
Cereblon	–	4 µl	4 µl
Cereblon Assay buffer	4 µl	–	–
Test Inhibitor/Activator	–	–	2 µl
Inhibitor buffer*	2 µl	2 µl	–
Biotin-Ubiquitin	1 µl	1 µl	1 µl
UBE1	1 µl	1 µl	1 µl
UBCH5C	1 µl	1 µl	1 µl
ATP (2 mM)	1 µl	1 µl	1 µl
Total	**10 µl**	**10 µl**	**10 µl**

*Inhibitor buffer typically represents Cereblon Assay buffer with proper concentration of DMSO.

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Note: Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:
Thaw Cereblon Detection Buffer on ice. Dilute anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with Cereblon Detection Buffer. Add 10 µl per well. Shake on a rotator platform for 30 minutes at room temperature.

Step 3:
1) Dilute Streptavidin-conjugated donor beads (PerkinElmer #6760002S) 125-fold with Cereblon Detection Buffer. Add 10 µl per well. Shake on a rotator platform for 15-30 minutes at room temperature.

2) Read Alpha-counts. "Blank" value should be subtracted from all readings.

Example of Assay Results:

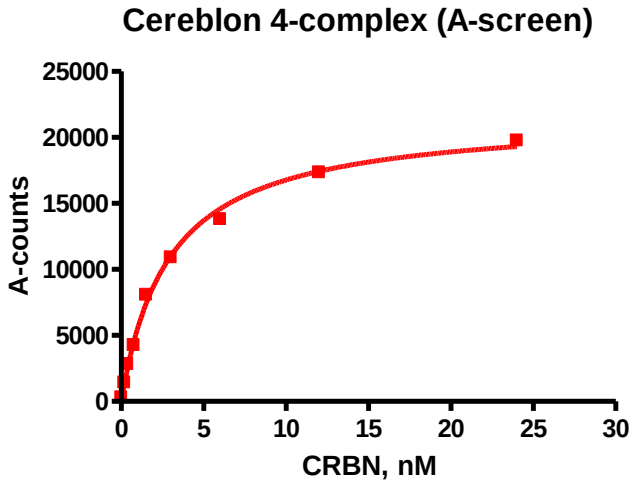

Figure 1: Titration of Cereblon ubiquitination using the *Cereblon Ubiquitination Homogeneous Assay Kit*, BPS Bioscience #79881. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

6042 Cornerstone Court W, Ste B
San Diego, CA 92121
Tel: 1.858.202.1401
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

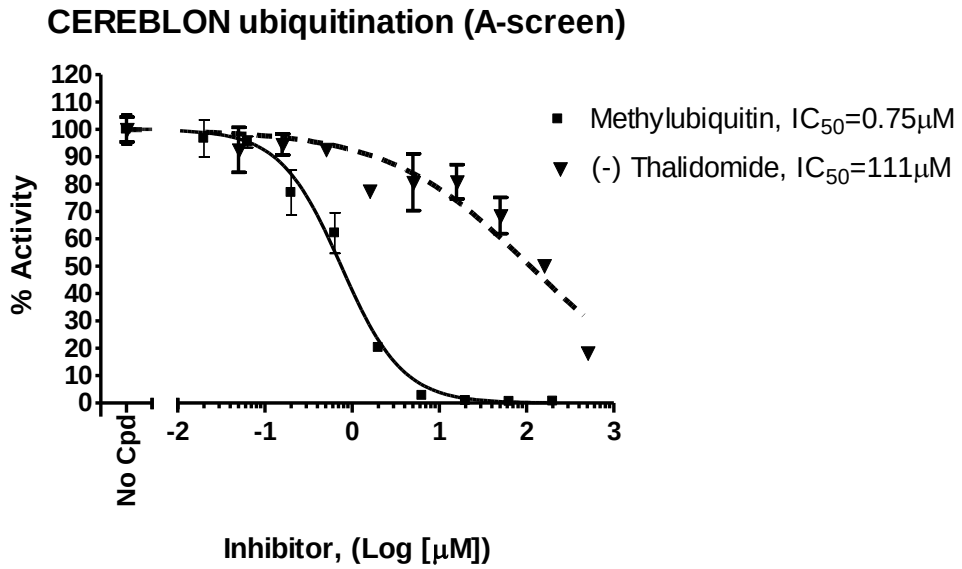

Figure 2: Inhibition of Cereblon ubiquitination by Methylated Ubiquitin or (-)Thalidomide, measured using the *Cereblon Ubiquitination Homogeneous Assay Kit*, BPS Bioscience #79881. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS

Product Name	**Catalog #**	**Size**
CBL-B, GST-Tag (Human)	#80415	100 µg
CBL-B, His-Avi-Tag	#80414	100 µg
CBL-B, Biotin-labeled (Human)	#80412	50 µg
CBL-B (Y363F), Biotin-labeled (Human)	#80413	50 µg
UBE1 (UBA1), FLAG-tag	#80301	100 µg
UBCH5b	#80314	100 µg
Cereblon/DDB1/Cul4A/Rbx1 Complex	#100329-1	10 µg
PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding	#79770	384 rxns.