



# SZABO SCANDIC

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## Produktinformation



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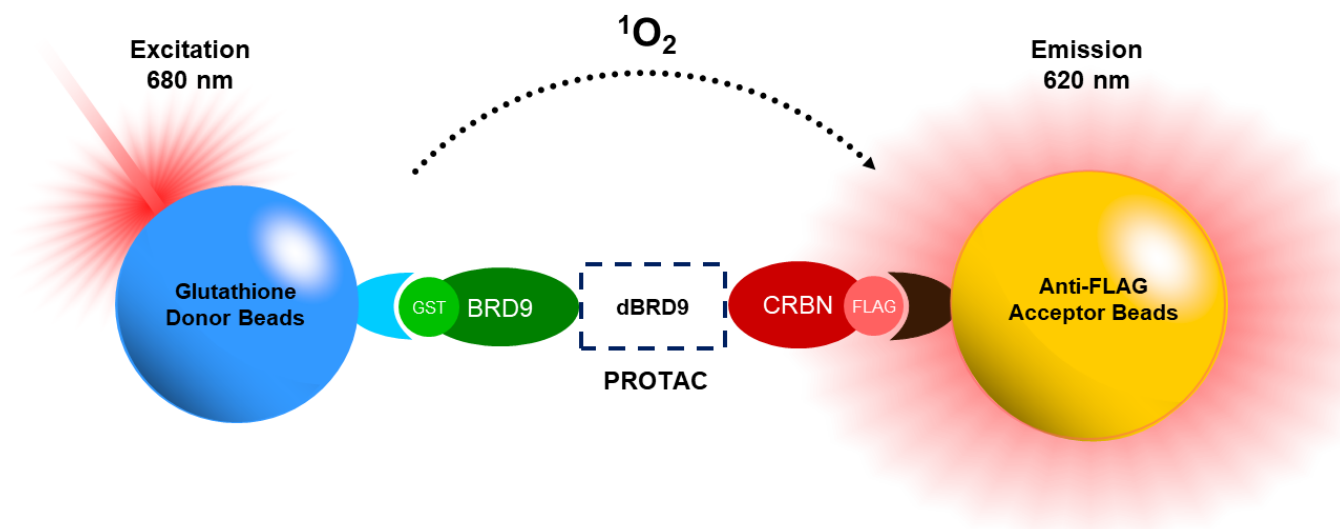
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**Description**

The PROTAC Optimization Kit for BRD9-Cereblon Binding is designed for the testing and profiling of PROTACs directed against BRD9 and Cereblon (CRBN). This Kit comes in a convenient AlphaLISA™ format, with the BRD9 Degradator dBRD9 (PROTAC) added as positive control, BRD9 buffer, and purified BRD9 and CRBN proteins for 384 reactions. The BRD9 inhibitor BI-7273 is included as a control inhibitor of PROTAC binding to BRD9. With this kit, three simple steps are required for the measurement of PROTAC activity. First, the PROTAC of interest is incubated with CRBN and BRD9. Next, acceptor beads are added, then donor beads, followed by reading of the Alpha-counts.



*Illustration of the assay principle:* a PROTAC of interest or positive control dBRD9 (PROTAC) interacts with both BRD9 and CRBN, bringing them in close proximity. BRD9 contains a GST tag, recognized by the GSH donor bead, while CRBN contains a FLAG tag that binds to the AlphaLISA™ acceptor bead conjugated with an anti-FLAG antibody. Upon excitation of the donor bead a singlet oxygen is generated by the donor bead, which excites the acceptor bead and emits light proportionally to the level of interaction. AlphaLISA™ immunoassays are a no-wash alternative to ELISA immunoassays. These assays are robust and ideal for a minimal hands-on approach.

**Background**

CRBN (cereblon) is the substrate-binding component of the E3 protein ligase complex DDB1-CUL4A-RBX1 involved in the ubiquitination and proteasomal degradation of target proteins. Cereblon binds to DDB1 (Damaged DNA binding protein 1), to the scaffolding protein CUL4A (Cullin 4A), and its regulator RBX1 (RING-Box protein 1). Binding of CRBN to a substrate protein engages the E3 ligase activity of the complex and results in the ubiquitination and ultimate degradation of the protein substrate. Many proteins are known targets of CRBN, including several transcription factors, growth factors, kinases and more. CRBN has become a target of choice for the development of many therapeutic PROTACs.

BRD9 (Bromodomain-containing protein 9) functions as a transcriptional regulator and is a component of a chromatin remodeling complex. It also regulates the formation of the RAD51-RAD54 complex, involved in homologous recombination. BRD9 plays a role in cancer and is a potential therapeutic target for cancer drugs.

## Applications

1. Discover and optimize PROTACs targeting BRD9
2. Design novel molecules targeting CRBN
3. Compare the activities of different PROTACs

## Supplied Materials

Catalog #	Name	Amount	Storage
100255	FLAG-Cereblon*	5 µg	-80°C
31091	GST-BRD9*	40 µg	-80°C
	1 mM dBRD9 PROTAC (MW=884 Da)	15 µl	-80°C
	3x BRD9 PROTAC Buffer**	4 ml	-20°C
	10 mM BI-7273 (MW=353 Da)	15 µl	-20°C

\*The concentration of the proteins is lot-specific and will be indicated on the tube.

\*\*Add 30 µl of 0.5 mM DTT to the assay buffer before experiment.

## Materials Required but Not Supplied

Name	Catalog #
AlphaLISA™ anti-FLAG acceptor beads, 5 mg/ml	PerkinElmer #AL112C
Alpha™ GSH donor beads, 5 mg/ml	PerkinElmer #6765300
Optiplate 384	PerkinElmer #6007290
AlphaScreen™ microplate reader	
Adjustable micropipettor and sterile tips	

## Storage Conditions



This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

## Safety



**dBRD9 is a pomalidomide-derivative, which is known to cause severe birth defects in humans. Use all appropriate precautions when handling this compound!**

Kit components should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly. This product is for research purposes only and not for human or therapeutic use.

### Contraindications

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

### Assay Protocols

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

### ASSAY PROTOCOL 1 -- Optimization of Bromodomain-Cereblon Binding

This protocol is designed to test the binding affinity of various PROTACs of interest to BRD9 or Cereblon.

*Prepare the reagents:*

1. Prepare 1x BRD9 PROTAC buffer by adding 1 part of stock 3x BRD9 buffer and 2 parts of distilled water. Add 30 µl of 0.5M DTT. Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 3x BRD9 buffer and store at -20°C.
2. Prepare dBRD9 PROTAC. Prepare an intermediate solution by diluting the 1 mM stock dBRD9 solution 25-fold with 1x BRD9 buffer to obtain a 40 µM solution. Dilute the 25-fold intermediate solution an additional 100-fold to obtain a 0.4 µM solution. Prepare only the amount needed for the experiment. Aliquot the remaining undiluted stock dBRD9 and store at -80°C.

*Note:* The final concentration of dBRD9 in the assay is 100 nM.

3. Thaw Cereblon and BRD9 on ice. Briefly spin the tubes containing the proteins to recover their full content. Use only the amount of protein required for your assay (as described in steps 4 and 5). Aliquot the remaining undiluted proteins for single use and store at -80°C immediately (example: if you will use the plate on 4 occasions, aliquot the remaining proteins in 3 aliquots each).

*Note:* Both BRD9 and Cereblon are sensitive to freeze/thaw cycles. Do not re-use thawed aliquots more than once and do not reuse the diluted proteins.

4. Dilute Cereblon in 1X BRD9 PROTAC buffer at 5 ng/µl. Keep the diluted protein on ice until use. Discard the diluted protein after use.
5. Dilute BRD9 in 1X BRD9 PROTAC buffer at 40 ng/µl. Keep the diluted protein on ice until use. Discard the diluted protein after use.

*Prepare the reaction:*

1. Prepare the master mix (7.5 µl/well): N wells × (2.5 µl Cereblon + 2.5 µl BRD9 + 2.5 µl 1x BRD9 PROTAC buffer). Add 7.5 µl of master mix to every well.

2. For the wells labeled as "Blank", add 2.5 µl of 1x BRD9 PROTAC buffer.
3. Prepare the Test PROTAC (2.5 µl/well): for a titration, prepare serial dilutions at concentrations 4-fold higher than the desired final concentrations. The final volume of the reaction is 10 µl.
  - 3.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x BRD9 PROTAC Buffer, 4-fold more concentrated than the desired final concentrations.
  - 3.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 25-fold in 1x PROTAC Buffer to prepare the highest concentration of the 4-fold intermediate dilutions. The concentration of DMSO is now 4%.

Prepare serial dilutions of the Test Inhibitor at 4-fold the desired final concentrations using 4% DMSO in 1x PROTAC Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 4% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO in the assay should not exceed 1%.

4. Add 2.5 µl of diluted Test PROTAC to each well designated "Test PROTAC".
5. Add 2.5 µl of diluted dBRD9 to each well designated "Positive Control".

Component	Blank	Positive Control	Test PROTAC
Master Mix	7.5 µl	7.5 µl	7.5 µl
1x BRD9 PROTAC buffer	2.5 µl	-	-
Test PROTAC	-	-	2.5 µl
dBRD9 (0.4 µM)	-	2.5 µl	-
<b>Total</b>	<b>10 µl</b>	<b>10 µl</b>	<b>10 µl</b>

6. Incubate at room temperature for 30 minutes.



Protect your samples from direct exposure to light for the remaining of the protocol. **Photobleaching will occur.**

7. Dilute the anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with 1x BRD9 PROTAC buffer. Add 10 µl per well.

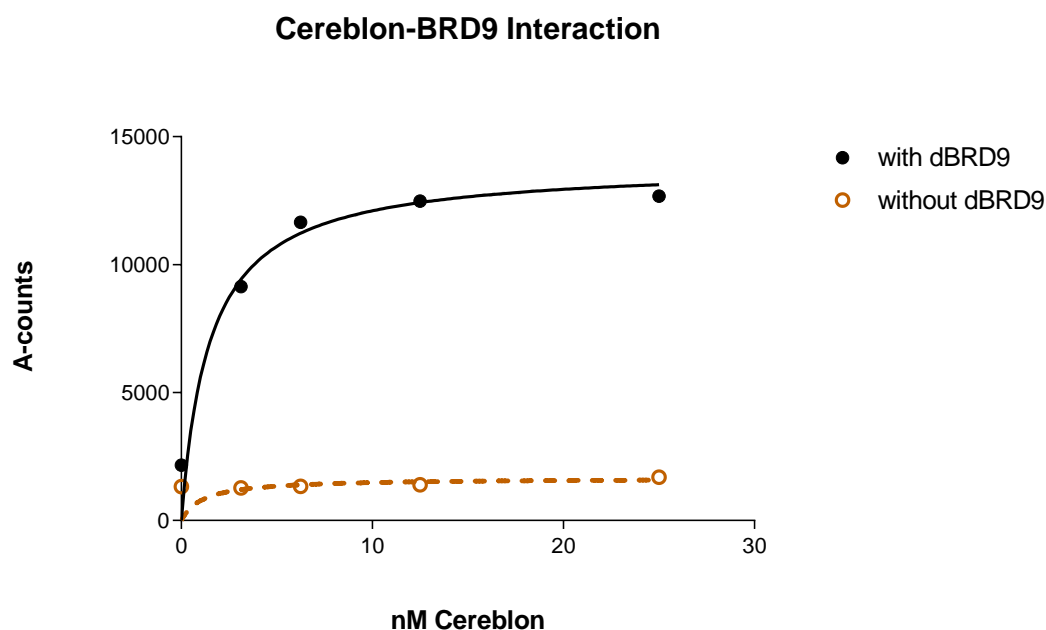
Shake on a rotator platform for 30-60 minutes at room temperature.

8. Dilute the GSH donor beads (PerkinElmer #6765300) 125-fold with 1x BRD9 PROTAC buffer. Add 10 µl per well.

Shake on a rotator platform for 30 minutes at room temperature.

9. Read the Alpha-counts. The "Blank" value should be subtracted from all readings.

## Example of Assay Results



*Figure 1: Titration of CRBN at fixed concentration of BRD9. A fixed amount of BRD9 was added to increasing concentrations of Cereblon in the presence or in the absence of dBRD9 (PROTAC). The dBRD9-mediated interaction of Cereblon with BRD9 was quantified using the PROTAC Optimization Kit for BRD9-Cereblon Binding (BPS Bioscience #78420).*

### ASSAY PROTOCOL 2 -- Competitive Inhibition of the test PROTAC

This protocol is designed to measure the inhibition of the test PROTAC binding to BRD9. The protocol can be easily modified to study inhibitors of PROTAC directed to Cereblon.

*Note:* All samples and controls should be tested in duplicate. All incubations are performed with slow shaking on a rotator platform.

#### Prepare the reagents:

1. Prepare 1x BRD9 PROTAC buffer by adding 1 part of stock 3x BRD9 buffer and 2 parts of distilled water. Add 30  $\mu$ l of 0.5M DTT. Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 3x BRD9 buffer and store at  $-20^{\circ}\text{C}$ .
2. Prepare dBRD9 PROTAC. Prepare an intermediate solution by diluting the 1 mM stock dBRD9 solution 25-fold with 1x BRD9 buffer to obtain a 40  $\mu$ M solution. Dilute the 25-fold intermediate solution an additional 100-fold to obtain a 0.4  $\mu$ M solution. Prepare only the amount needed for the experiment. Aliquot the remaining undiluted stock dBRD9 and store at  $-80^{\circ}\text{C}$ .

3. Thaw Cereblon and BRD9 on ice. Briefly spin the tubes containing the proteins to recover their full content. Use only the amount of protein required for your assay (as described in steps 4 and 5). Aliquot the remaining undiluted proteins for single use and store at -80°C immediately (example: if you will use the plate on 4 occasions, aliquot the remaining proteins in 3 aliquots each).
4. Dilute Cereblon in 1X BRD9 PROTAC buffer at 5 ng/μl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.
5. Dilute BRD9 in 1X BRD9 PROTAC buffer at 40 ng/μl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

*Prepare the reaction:*

1. Prepare the master mix (5 μl/well): N wells × (2.5 μl Cereblon (5 ng/μl) + 2.5 μl BRD9 (40 ng/μl)). Add 5 μl of master mix to every well.
2. For the wells labeled as "Blank", add 2.5 μl of 1x BRD9 PROTAC buffer.
3. Prepare the Test Inhibitor (2.5 μl/well): for a titration, prepare serial dilutions at concentrations 4-fold higher than the desired final concentrations. The final volume of the reaction is 10 μl.

3.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x BRD9 PROTAC Buffer, 4-fold more concentrated than the desired final concentrations.

3.2. If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 25-fold in 1x PROTAC Buffer to prepare the highest concentration of the 4-fold intermediate dilutions. The concentration of DMSO is now 4%.

Prepare serial dilutions of the Test Inhibitor at 4-fold the desired final concentrations using 4% DMSO in 1x PROTAC Buffer to keep the concentration of DMSO constant.

For positive and negative controls, prepare 4% DMSO in water (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

The final concentration of DMSO in the assay should not exceed 1%.

4. Add 2.5 μl of Test Compound to each well designated "Test Inhibitor".
5. For the "Positive Control" and "Blank", add 2.5 μl of the same solution without the test compound ("Compound buffer"). We recommend using 1x BRD9 PROTAC buffer with the same concentration of DMSO as in the Compound buffer.
6. Prepare a BI-7273 dilution: Resuspend 10 μl of BI-7273 (10 mM) with 240 μl of 1x BRD9 PROTAC buffer to obtain a 400 μM solution. For the wells labeled as "**BI-7273**", add 2.5 μl diluted BI-7273.
7. Preincubate for up to 30 minutes at room temperature with slow shaking.

- Initiate the reaction by adding 2.5 µl of diluted dBRD9 (0.4 µM) prepared as described above to the wells labeled “Positive Control”, “BI-7273” and “Test Inhibitor”. **Note that the “Blank” should not be added dBRD9.**
- Incubate at room temperature for one hour with slow shaking.

Component	Blank	Positive Control	BI-7273	Test Inhibitor
Master Mix	5 µl	5 µl	5 µl	5 µl
1x BRD9 PROTAC buffer	2.5 µl	-	-	-
Test Compound	-	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-	-
BI-7273	-	-	2.5 µl	-
dBRD9 (0.4 µM)	-	2.5 µl	2.5 µl	2.5 µl
<b>Total</b>	<b>10 µl</b>	<b>10 µl</b>	<b>10 µl</b>	<b>10 µl</b>



**Protect your samples from direct exposure to light for the remaining of the protocol. Photobleaching will occur.**

- Dilute the anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with 1x BRD9 PROTAC buffer. Add 10 µl per well.

Shake on a rotator platform for 30-60 minutes at room temperature.

- Dilute the GSH donor beads (PerkinElmer #6765300) 125-fold with 1x BRD9 PROTAC buffer. Add 10 µl per well.

Shake on a rotator platform for 30 minutes at room temperature.

- Read the Alpha-counts. The “Blank” value should be subtracted from all readings.



## Example of Assay Results

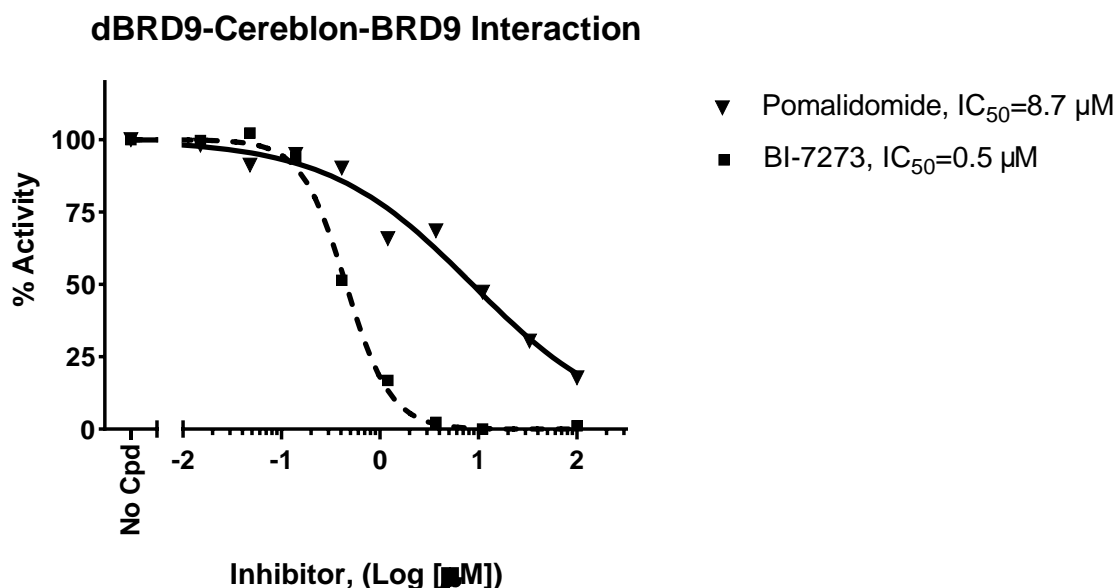


Figure 2: Effect of BRD9 or CRBN inhibitors. Inhibition of dBRD9-mediated interaction of Cereblon with BRD9 by increasing concentrations of BI-7273 (BRD9 inhibitor) or Pomalidomide (CRBN inhibitor), measured using the PROTAC Optimization Kit for BRD9-Cereblon Binding (BPS Bioscience #78420).

For lot-specific information, please contact BPS Bioscience, Inc. at [support@bpsbioscience.com](mailto:support@bpsbioscience.com)

## General Considerations

**Plates and Instruments:** A plate reader capable of Alpha technology detection is required. We recommend using PerkinElmer 384-Optiplate #6007290.

**“Blank” Control:** The “Blank” control is important to determine the background luminescence in the assay. We recommend doing these in duplicate.

**“Positive Control”:** The “Positive Control” is the maximum signal determined by the addition of a PROTAC molecule known to bind BRD9 and CRBN, i.e. dBRD9 (PROTAC).

## Troubleshooting Guide

Visit [bpsbioscience.com/assay-kits-faq](https://bpsbioscience.com/assay-kits-faq) for detailed troubleshooting instructions. For all further questions, please email [support@bpsbioscience.com](mailto:support@bpsbioscience.com)

## References

1. Remillard D. *et al. Angew Chem Int Ed Engl.* 2017; **56(21)**: 5738–5743.
2. Clark, P.G., *et al.* Development of chemical probes for the bromodomains of BRD7 and BRD9. *Drug Discov Today Technol.* 2016; **19**: 73-80.

**Related Products**

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding	79770	384 reactions
PROTAC Optimization Kit for CDK Kinase-Cereblon Binding	79924	384 reactions
Cereblon Intrachain TR-FRET Assay Kit	78301	384 reactions
Cereblon Ubiquitination Homogeneous Assay Kit	79881	384 reactions
Cereblon Binding Assay Kit	79899	96 reactions
BRD9 (BD1) Inhibitor Screening Assay Kit	32519	384 reactions
BRD9 TR-FRET Assay Kit	32621	384 reactions
BRD9, His-tag Recombinant	31090	100 µg
BRD9, GST-tag Recombinant	31091	100 µg