Description

The PROTAC® Optimization Kit for PARP1-Cereblon Binding is designed for the testing and profiling of PROTACs directed against PARP1 (Poly (ADP-ribose)-polymerase 1) and Cereblon. The PROTAC® Optimization Kit for PARP1-Cereblon Binding comes in a convenient AlphaLISA® format, with enough PARP1 Degrader iRucaparib-AP6 PROTAC®, assay buffer, recombinant purified PARP1 and CRBN (Cereblon)/ DDB1(damage specific DNA binding protein 1)/Cul4A (cullin 4A)/ Rbx1 (ring-box 1) complex for 384 reactions. The PARP1 inhibitor Rucaparib is included as a control inhibitor of PROTAC binding to PARP1 (internal control).

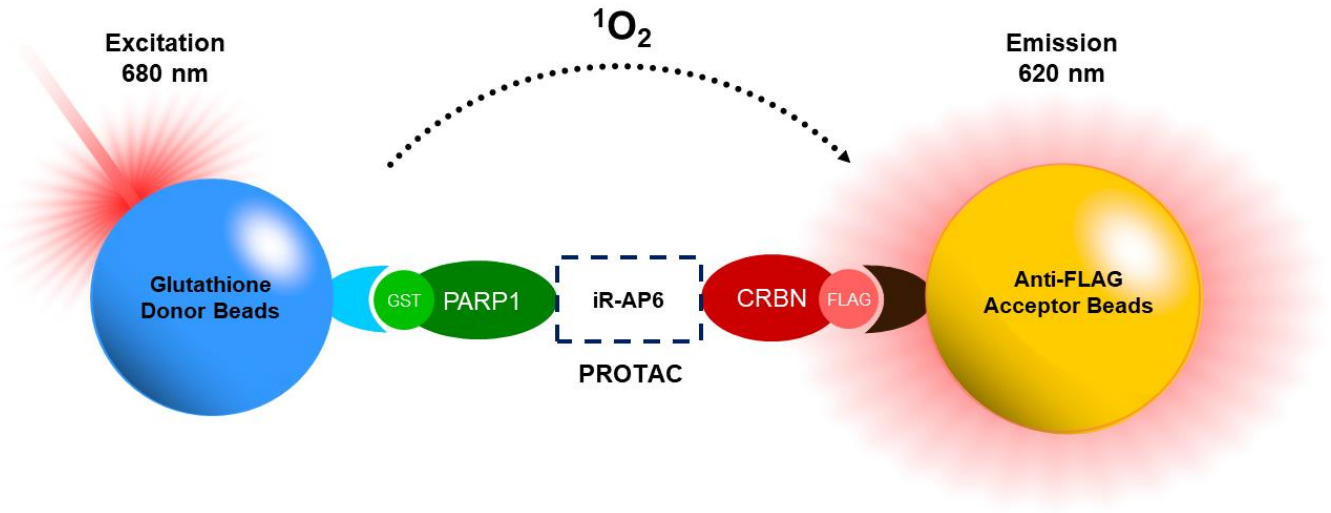

Figure 1. Schematic representation of PARP1-Cereblon complex formation via iRucaparib-AP6 (PROTAC®).
The PROTAC® of interest is incubated with CRBN and PARP1, bringing them in close proximity. PARP1 contains a GST tag, which is recognized by the GSH donor bead. CRBN contains a FLAG tag that binds to the AlphaLISA™ acceptor bead, which is conjugated with an anti-FLAG antibody. Upon excitation of the donor bead, a singlet oxygen is generated by the donor bead. The singlet oxygen excites the acceptor bead and emits light proportionally to the level of interaction.

Background

PARP1 (Poly (ADP-ribose) polymerase 1) acts as a first responder that detects DNA damage. PARP1 contributes to the efficiency of DNA repair by promoting the ADP-ribosylation of histones leading to the decompaction of chromatin structure, and by interacting with and modifying multiple DNA repair factors. PARP1 plays a role in cancer and is a well validated therapeutic target for cancer drugs.

Cereblon (CRBN) is the substrate-binding component of the E3 protein ligase complex DDB1-CUL4A-RBX1, which is involved in the ubiquitination and proteasomal degradation of target proteins. Binding of CRBN to a substrate engages its E3 ligase activity and results in the ubiquitination and ultimate degradation of the substrate. Many proteins are known targets of CRBN, including several transcription factors, growth factors and kinases. CRBN has become a target of choice for the development of many PROTACs of therapeutical interest.

Application(s)

- Identify and optimize PROTACs targeting PARP1.
- Design novel molecules targeting CRBN.
- Directly compare the activity of different PROTACs.

Supplied Materials

Catalog #	Name	Amount	Storage
100329	Cereblon/DDB1/Cul4A, Rbx 1 Complex*	20 µg	-80°C
80501	PARP1, GST-Tag*	20 µg	-80°C
	1 mM iRucaparib-AP6 PROTAC® (MW = 887 Da)	15 µl	-80°C
	5x PP-02 Buffer	4 ml	-20°C
	1mM Rucaparib (MW = 323 Da)	15 µl	-20°C

**The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*

Materials Required but Not Supplied

Component	Catalog #
AlphaLISA anti-FLAG acceptor beads, 5 mg/ml	PerkinElmer #AL112C
Alpha GSH donor beads, 1 mg	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

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

iRucaparib-AP6 PROTAC® is a pomalidomide-derivative, which is known to cause severe birth defects in humans.
It is very important to use all appropriate precautions when handling this compound.

Contraindications

- The final concentration of DMSO in the assay should not exceed 1%.
- Avoid green and blue dyes that absorb light in the AlphaScreen signal emission range (λ=520-620 nm), such as Trypan Blue.
- Avoid the use of 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 >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. Media like MEM, which lacks these components, does not affect AlphaScreen assays.

Assay Protocol 1 - Optimization of PARP1-Cereblon Binding

- This protocol is designed to test the binding affinity of various PROTACs to the PARP1 or Cereblon complex.
- All samples and controls should be performed in duplicate.

- The assay should include “Blank”, “Positive Control” and “Test PROTAC” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to.
- All incubations should be performed with slow shaking on a rotator platform.

STEP 1

1. Prepare Assay Buffer by diluting 5x PP-02 Buffer 5-fold with distilled water.

   Note: Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 5x PP-02 Buffer and store at -20°C.

2. Dilute 1 mM iRucaparib-AP6 PROTAC® 20-fold with Assay Buffer to make a 50 µM solution.

3. Dilute 50 µM iRucaparib-AP6 PROTAC® 100-fold to make a 0.5 µM solution.

   Note: The final concentration of iRucaparib-AP6 PROTAC in the assay will be 125 nM.

4. Thaw **Cereblon Complex** and **PARP1** on ice. Briefly spin the tubes containing the proteins to recover the full content of the tube.

5. Prepare the following dilutions (2.5 µl/well):

   a. Dilute **Cereblon Complex** to 17 ng/µl with Assay Buffer;

   b. Dilute **PARP1** to 14 ng/µl with Assay Buffer.

6. Prepare a **Master Mix** (7.5 µl/well): N wells × (2.5 µl of diluted Cereblon complex + 2.5 µl of diluted PARP1 + 2.5 µl of Assay Buffer).

7. Add 7.5 µl of Master Mix to every well.

8. 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.

   8.1 If the Test PROTAC is water-soluble, prepare serial dilutions 4-fold more concentrated than the desired final concentrations in Assay Buffer.

   For the positive and negative controls, use Assay Buffer (Diluent Solution).

   OR

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

   Using Assay Buffer in 4% DMSO, prepare serial dilutions of the Test PROTAC at 4-fold the desired final concentrations to keep the concentration of DMSO constant.

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

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

9. Add 2.5 µl of Test PROTAC to the "Test PROTAC" wells.
10. Add 2.5 µl of Diluent Solution to the "Blank" wells.
11. For the wells labeled as "Positive Control" add 2.5 µl of diluted iRucaparib-AP6 PROTAC® (0.5 µM).

Component	Blank	Positive Control	Test
Master Mix	7.5 µl	7.5 µl	7.5 µl
Diluent Solution	2.5 µl	-	-
Test PROTAC	-		2.5 µl
Diluted iRucaparib-AP6 PROTAC® (0.5 µM)	-	2.5 µl	-
Total	**10 µl**	**10 µl**	**10 µl**

12. Incubate at Room Temperature (RT) for 30 minutes.

STEP 2

Note: Protect your samples from direct exposure to light!

1. Dilute **anti-FLAG Acceptor beads** 250-fold with Assay Buffer (10 µl/well).
2. Add 10 µl per well.
3. Shake on a rotator platform for 30-60 minutes at RT.
4. Dilute **GSH donor beads** 125-fold with Assay Buffer (10 µl/well).
5. Add 10 µl per well. Shake on a rotator platform for 30 minutes at RT.
6. Read Alpha-counts.
7. The "Blank" value should be subtracted from all readings.

Assay Protocol 2 - Competitive Inhibition of the PROTAC

- This protocol is designed to measure inhibition of the PROTAC binding to PARP1. The protocol can be easily modified to study inhibitors of the binding of PROTAC to the cereblon complex.
- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control", 'Inhibitor Control" and "Test Compound" conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- All incubations should be performed with slow shaking on a rotator platform.

STEP 1

1. Prepare Assay Buffer by diluting 5x PP-02 Buffer 5-fold with distilled water.

   Note: Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 5x PP-02 Buffer and store at -20°C.

2. Dilute 1 mM iRucaparib-AP6 PROTAC® 20-fold Assay Buffer to make a 50 µM solution.

3. Dilute 50 µM iRucaparib-AP6 PROTAC® 100-fold to make a 0.5 µM solution.

   Note: The final concentration of iRucaparib-AP6 PROTAC in the assay will be 125 nM.

4. Thaw **Cereblon complex** and **PARP1** on ice. Briefly spin the tubes containing the proteins to recover the full content of the tube.

5. Prepare the following dilutions (2.5 µl/well):

   a. Dilute **Cereblon Complex** to 17 ng/µl with Assay Buffer;

   b. Dilute **PARP1** to 14 ng/µl with Assay Buffer.

6. Prepare a **Master Mix** (5 µl/well): N wells × (2.5 µl of diluted Cereblon complex + 2.5 µl of diluted PARP1).

7. Add 5 µl of Master Mix to every well.

8. Prepare the **Test Compound** (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.

   8.1 If the Test Compound is water-soluble, prepare serial dilutions 4-fold more concentrated than the desired final concentrations in Assay Buffer.

   For the positive and negative controls, use Assay Buffer (Diluent Solution).

   OR

   8.2 If the Test Compound is soluble in DMSO, prepare the test compound at 100-fold the highest desired concentration in 100% DMSO, then dilute the test compound 25-fold in Assay Buffer to prepare the highest concentration of the 4-fold intermediate dilutions. The concentration of DMSO is now 4%.

   Using Assay Buffer in 4% DMSO, prepare serial dilutions of the test compound at 4-fold the desired final concentrations to keep the concentration of DMSO constant.

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

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

9. Add 2.5 µl of diluted test compound to each well designated “Test Compound”.

10. Add 2.5 µl of Diluent Solution to the "Positive Control" and "Blank" wells.

11. Preincubate the test compound with the Cereblon and PARP1 for up to 30 minutes at RT with slow agitation.

12. Dilute Rucaparib 24-fold with Assay Buffer, to obtain a 40 µM solution (2.5 µl/well).

13. Add 2.5 µl of diluted Rucaparib to the "Inhibitor Control" wells.

14. Dilute 1 mM iRucaparib-AP6 PROTAC® 20-fold Assay Buffer to make a 50 µM solution.

15. Dilute 50 µM iRucaparib-AP6 PROTAC® 100-fold to make a 0.5 µM solution.

    Note: The final concentration of iRucaparib-AP6 PROTAC in the assay will be 125 nM.

16. Initiate the reaction by adding 2.5 µl of diluted iRucaparib-AP6 PROTAC® (0.5 µM) to wells labeled “Positive Control", “Inhibitor Control” and "Test Inhibitor".

17. Add 2.5 µl of Assay Buffer to the “Blank” wells.

Component	Blank	Positive Control	Inhibitor Control	Test Compound
Master Mix	5 µl	5 µl	5 µl	5 µl
Diluent Solution	2.5 µl	2.5 µl	-	-
Diluted Test Compound	-	-	-	2.5 µl
Diluted iRucaparib-AP6 PROTAC® (0.5 µM)	-	2.5 µl	2.5 µl	2.5 µl
Assay Buffer	2.5 µl	-	-	-
Diluted Rucaparib (40 µM)	-	-	2.5 µl	-
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

18. Incubate at RT for 30 minutes.

STEP 2

Note: Protect your samples from direct exposure to light!

1. Dilute **anti-FLAG Acceptor beads** 250-fold with Assay Buffer (10 µl/well).

2. Add 10 µl per well.

3. Shake on a rotator platform for 30 minutes at RT.

4. Dilute **GSH donor beads** 125-fold with Assay Buffer (10 µl/well).

5. Add 10 µl per well.

6. Shake on a rotator platform for 30 minutes minutes at RT.

7. Read Alpha-counts.

8. The "Blank" value should be subtracted from all readings.

Example Results

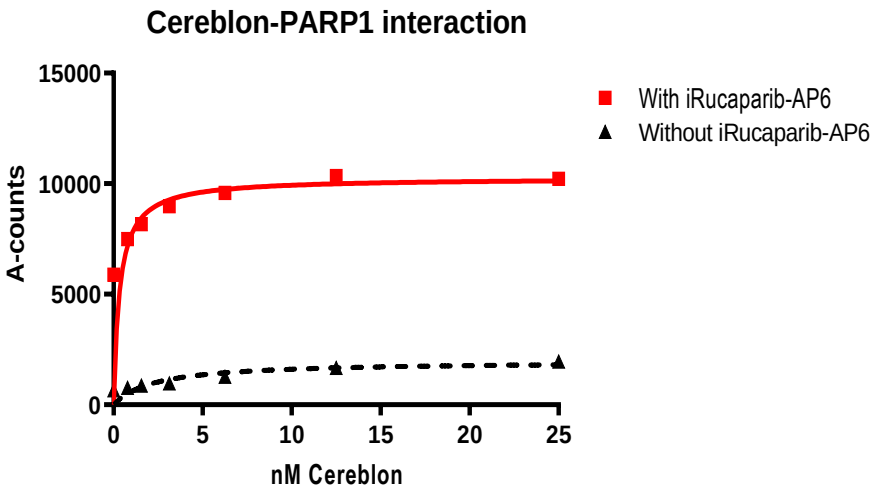

Figure 2: Interaction of Cereblon with PARP1 via iRucaparib-AP6 (PROTAC®).
A fixed amount of PARP1 was added to increasing concentrations of Cereblon in the presence or absence of a fixed concentration of iRucaparib-AP6 (PROTAC®).

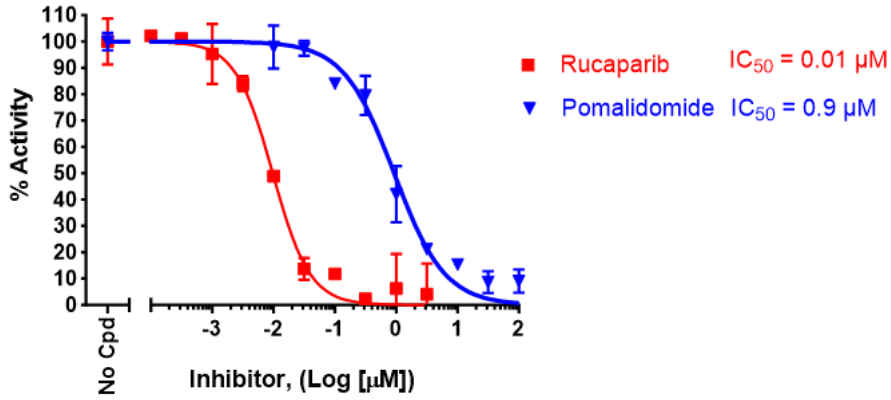

Figure 3: Inhibition by Rucaparib or Pomalidomide of iRucaparib-AP6 (PROTAC®)-mediated interaction of Cereblon with PARP1.
iRucaparib-AP6 (PROTAC®)-mediated interaction of Cereblon with PARP1 was measured in the presence of increasing concentrations of Rucaparib or Pomalidomide (BPS Bioscience #82026).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com

References

Chiho K., *et al.*, 2021 *Cell Chem Biol.* 28(4): 456–462.
Zhang, *et al.*, 2020 *J Enzyme Inhib Med Chem.* 35(1): 1606–1615.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

Related Products

Products	*Catalog #*	*Size*
PROTAC® Optimization Kit for BET Bromodomain-Cereblon Binding	79770	384 reactions
PROTAC® Optimization Kit for BET Bromodomain-Von Hippel Lindau (VHL) Binding	79790	384 reactions
PROTAC® Optimization Kit for BRD9-Cereblon Binding	78420	384 reactions
PROTAC® Optimization Kit for CDK Kinase-Cereblon Binding	79924	384 reactions
PROTAC® Optimization Kit for IRAK4-Cereblon Binding	78512	384 reactions
PROTAC® Optimization Kit for PARP1-Cereblon Binding	78441	384 reactions

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