Description

The PROTAC Optimization Kit for PARP1-Cereblon Binding is designed for the testing and profiling of PROTACs directed against PARP1 and Cereblon (CRBN). This Kit comes in a convenient AlphaLISA™ format, with the PARP1 Degrader iRucaparib-AP6 (PROTAC) added as positive control, PARP1 buffer, purified PARP1 and CRBN proteins for 384 reactions. The PARP1 inhibitor Rucaparib is included as a control that blocks PROTAC binding to PARP1. With this kit, three simple steps are required for the measurement of PROTAC activity. First, the PROTAC of interest is incubated with CRBN and PARP1. Next, acceptor beads are added, then donor beads, followed by reading of the Alpha-counts.

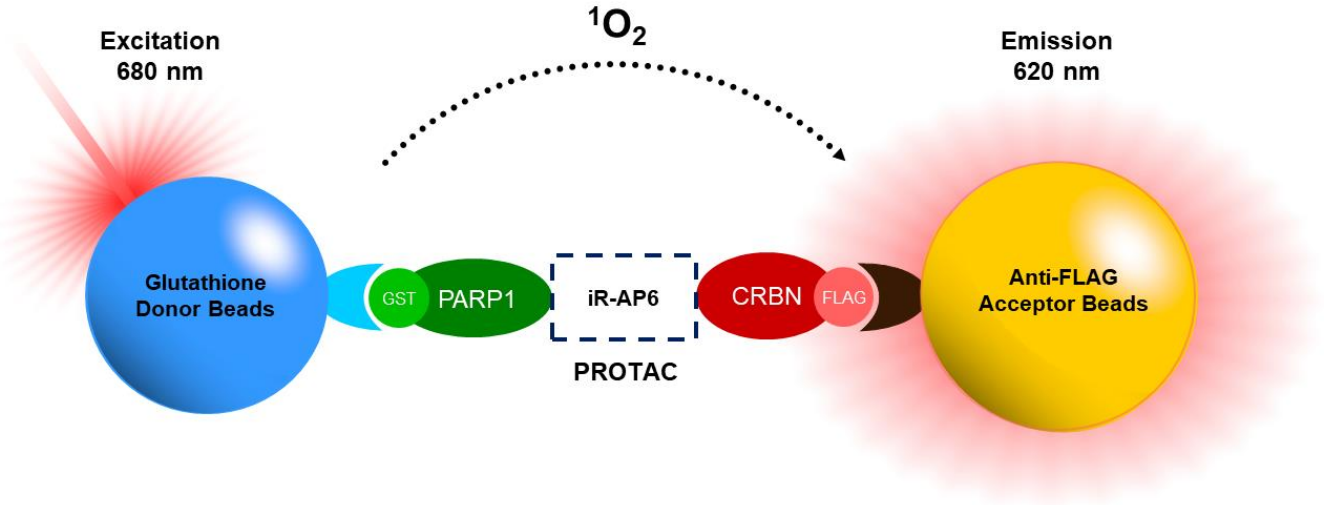

Figure 1: Illustration of the assay principle

A PROTAC of interest or positive control iRucaparib-AP6 (PROTAC) interacts with both PARP1 and CRBN, bringing them in close proximity. PARP1 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. The singlet oxygen 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. CRBN 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.

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.

Application(s)

- Discover and optimize PROTACs targeting PARP1
- Design novel molecules targeting CRBN
- Compare the activities of different PROTACs

Supplied Materials

Catalog #	Name	Amount	Storage
100329	FLAG-Cereblon*	20 µg	-80°C
80501	GST-PARP1*	20 µg	-80°C
	1 mM iRucaparib-AP6 PROTAC (MW=887 Da)	15 µl	-80°C
	5X PP-02 Buffer	4 ml	-20°C
	1 mM Rucaparib (MW=323 Da)	15 µl	-20°C

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

Materials Required but Not Supplied

Name	Catalog #
AlphaLISA™ anti-FLAG acceptor beads, 250 µg	PerkinElmer #AL112C
Alpha™ GSH donor beads, 1mg	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

iRucaparib-AP6 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 (NaN3) or metal ions (Fe2+, Fe3+, Cu2+, Zn2+ and Ni2+). 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.

Example of Assay Results

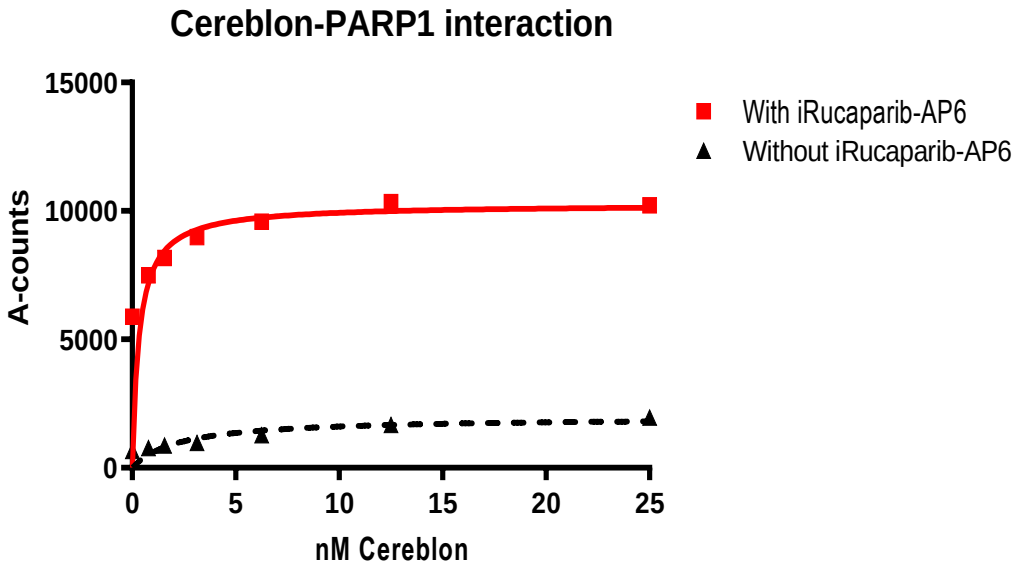

Figure 2: Titration of CRBN at fixed concentration of PARP1.
A fixed amount of PARP1 was added to increasing concentrations of Cereblon in the presence or in the absence of a fixed concentration of iRucaparib-AP6 (PROTAC). The iRucaparib-AP6-mediated interaction of Cereblon with PARP1 was quantified using the PROTAC Optimization Kit for PARP1-Cereblon Binding (BPS Bioscience #78441).

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 PARP1-Cereblon Binding

This protocol is designed to test the binding affinity of various PROTACs of interest to PARP1 or Cereblon.

Prepare the reagents:

1. Prepare 1x PARP1 PROTAC buffer by adding 1 part of stock 3x PARP1 buffer and 2 parts of distilled water.
    a. Prepare only the amount needed for the experiment.
    b. Aliquot the remaining undiluted 3x PARP1 buffer and store at -20°C.

2. Prepare iRucaparib-AP6 PROTAC.

    a. Prepare an intermediate solution by diluting the 1 mM stock iRucaparib-AP6 solution 20-fold with 1x PARP1 buffer to obtain a 50 µM solution.

    b. Dilute the 20-fold intermediate solution an additional 100-fold to obtain a 0.5 µM solution.

    c. Prepare only the amount needed for the experiment.

    d. Aliquot the remaining undiluted stock iRucaparib-AP6 and store at -80°C.

    Note: The final concentration of iRucaparib-AP6 in the assay is 125 nM.

3. Thaw Cereblon and PARP1 on ice.

    a. Briefly spin the tubes containing the proteins to recover their full content.

    b. Use only the amount of protein required for your assay (as described in steps 4 and 5).

    c. 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.

Both PARP1 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 PARP1 PROTAC buffer at 17 ng/µl. Keep the diluted protein on ice until use. Discard the diluted protein after use.

5. Dilute PARP1 in 1X PARP1 PROTAC buffer at 14 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 PARP1 + 2.5 µl 1x PARP1 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 PARP1 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.

    Without DMSO

    3.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x PARP1 PROTAC Buffer, 4-fold more concentrated than the desired final concentrations.

With DMSO

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%.

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

b. For positive and negative controls, prepare 4% DMSO in 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%.

4. Add 2.5 µl of diluted Test PROTAC to each well designated "Test PROTAC".

5. Add 2.5 µl of diluted iRucaparib-AP6 to each well designated "Positive Control".

Component	**Blank**	**Positive Control**	**Test PROTAC**
Master Mix	7.5 µl	7.5 µl	7.5 µl
1x PARP1 PROTAC buffer	2.5 µl	-	-
Test PROTAC	-	-	2.5 µl
iRucaparib-AP6 (0.5 µM)	-	2.5 µl	-
Total	**10 µl**	**10 µl**	**10 µl**

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 PARP1 PROTAC buffer.

8. Add 10 µl per well.

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

10. Dilute the GSH donor beads (PerkinElmer #6765300) 125-fold with 1x PARP1 PROTAC buffer.

11. Add 10 µl per well.

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

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

ASSAY PROTOCOL 2 -- Competitive Inhibition of the test PROTAC

This protocol is designed to measure the inhibition of the test PROTAC binding to PARP1. 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 PARP1 PROTAC buffer by adding 1 part of stock 3x PARP1 buffer and 2 parts of distilled water.
    a. Prepare only the amount needed for the experiment.
    b. Aliquot the remaining undiluted 3x PARP1 buffer and store at -20°C.
2. Prepare iRucaparib-AP6 PROTAC.
    a. Prepare an intermediate solution by diluting the 1 mM stock iRucaparib-AP6 solution 20-fold with 1x PARP1 buffer to obtain a 50 µM solution.
    b. Dilute the 20-fold intermediate solution an additional 100-fold to obtain a 0.5 µM solution.
    c. Prepare only the amount needed for the experiment.
    d. Aliquot the remaining undiluted stock iRucaparib-AP6 and store at -80°C.
3. Thaw Cereblon and PARP1 on ice. Briefly spin the tubes containing the proteins to recover their full content.
    a. Use only the amount of protein required for your assay (as described in steps 4 and 5).
    b. 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.

Both PARP1 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 PARP1 PROTAC buffer at 17 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.
5. Dilute PARP1 in 1X PARP1 PROTAC buffer at 14 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 (17 ng/µl) + 2.5 µl PARP1 (14 ng/µl). Add 5 µl of master mix to every well.

2. For the wells labeled as "Blank", add 2.5 µl of 1x PARP1 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.

   Without DMSO

   3.1. If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x PARP1 PROTAC Buffer, 4-fold more concentrated than the desired final concentrations.

   With DMSO

   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. Final concentration of DMSO is 4%.

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

      b. For positive and negative controls, prepare 4% DMSO in 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%.

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 PARP1 PROTAC buffer with the same concentration of DMSO as in the Compound buffer.

6. Prepare a Rucaparib dilution: Resuspend 10 µl of Rucaparib (1 mM) with 240 µl of 1x PARP1 PROTAC buffer to obtain a 40 µM solution. For the wells labeled as "**Rucaparib**", add 2.5 µl diluted Rucaparib.
   Note: The recommended final concentration of Rucaparib in the reaction is 10 µM.

7. Preincubate for up to 30 minutes at room temperature with slow shaking.

8. Initiate the reaction by adding 2.5 µl of diluted iRucaparib-AP6 (0.5 µM) prepared as described above to the wells labeled “Positive Control", “Rucaparib” and “Test Inhibitor”. **Note that the “Blank” should not be added iRucaparib-AP6.**

9. Incubate at room temperature for 30 minutes with slow shaking.

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

Component	Blank	Positive Control	Rucaparib	Test Inhibitor
Master Mix	5 µl	5 µl	5 µl	5 µl
1x PARP1 PROTAC buffer	2.5 µl	-	-	-
Test Compound	-	-	-	2.5 µl
Diluent Solution	2.5 µl	2.5 µl	-	-
Rucaparib	-	-	2.5 µl	-
iRucaparib-AP6 (0.5 µM)	-	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

7. Dilute the anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with 1x PARP1 PROTAC buffer.

8. Add 10 µl per well.

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

10. Dilute the GSH donor beads (PerkinElmer #6765300) 125-fold with 1x PARP1 PROTAC buffer. Add 10 µl per well.

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

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

Example of Assay Results

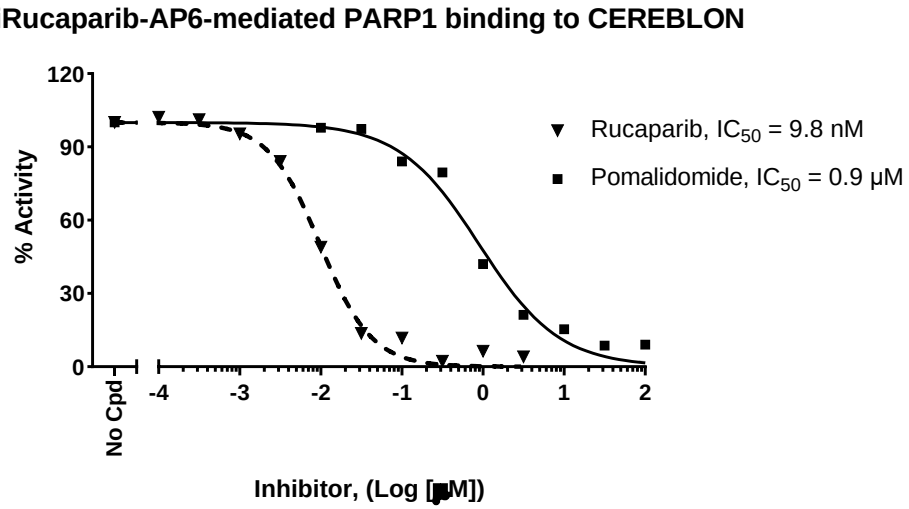

Figure 3: Effect of PARP1 or CRBN inhibitors.
Inhibition of iRucaparib-AP6-mediated interaction of Cereblon with PARP1 by increasing concentrations of Rucaparib (PARP1 inhibitor) or Pomalidomide (CRBN inhibitor), measured using the PROTAC Optimization Kit for PARP1-Cereblon Binding (BPS Bioscience #48441).

For lot-specific information, please contact BPS Bioscience, Inc. at 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 signal in the assay. We recommend doing these at least in duplicate.

"Positive Control": The "Positive Control" is the maximum signal determined by the addition of a PROTAC molecule known to bind PARP1 and CRBN, i.e. iRucaparib-AP6 (PROTAC).

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

References

1. Chiho Kim, Chuo Chen, Yonghao Yu. Avoid the trap: targeting PARP1 Beyond Human Malignancy. *Cell Chem Biol*. 2021; **28(4)**: 456–462.
2. Zhang, Zhimin, *et al.* Identification of probe-quality degraders for Poly(ADP-ribose) polymerase-1 (PARP-1). J Enzyme Inhib Med Chem. 2020; **35(1):** 1606–1615.

Related Products

Products	*Catalog #*	*Size*
PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding	79770	384 rxns.
PROTAC Optimization Kit for CDK Kinase-Cereblon Binding	79924	384 rxns.
PROTAC® Optimization Kit for BRD9-Cereblon Binding	78420	384 rxns.
PROTAC Optimization Kit for BET Bromodomain-Von Hippel Lindau (VHL) Binding	79790	384 rxns.
Cereblon Intrachain TR-FRET Assay Kit	78301	384 rxns.
Cereblon Ubiquitination Homogeneous Assay Kit	79881	384 rxns.
Cereblon Binding Assay Kit	79899	96 rxns.