Description

The Molecular Glue/PROTAC® Optimization Kit for CDK2/CDK9-Cereblon Binding is designed for the testing and profiling of Molecular Glues (MG) and PROTACs targeting CDK2 (cyclin dependent kinase 2) or CDK9 (cyclin dependent kinase 9) and Cereblon (CRBN). The Molecular Glue/PROTAC® Optimization Kit for CDK2/CDK9-Cereblon Binding comes in a convenient AlphaLISA® format, with enough PROTAC® buffer, purified CDK2/CyclinA2 and CDK9/CyclinK complexes, and CRBN for 384 reactions. This kit also contains the control PROTAC® (CDK2/9 Degrader) and CDK inhibitor FN-1501.

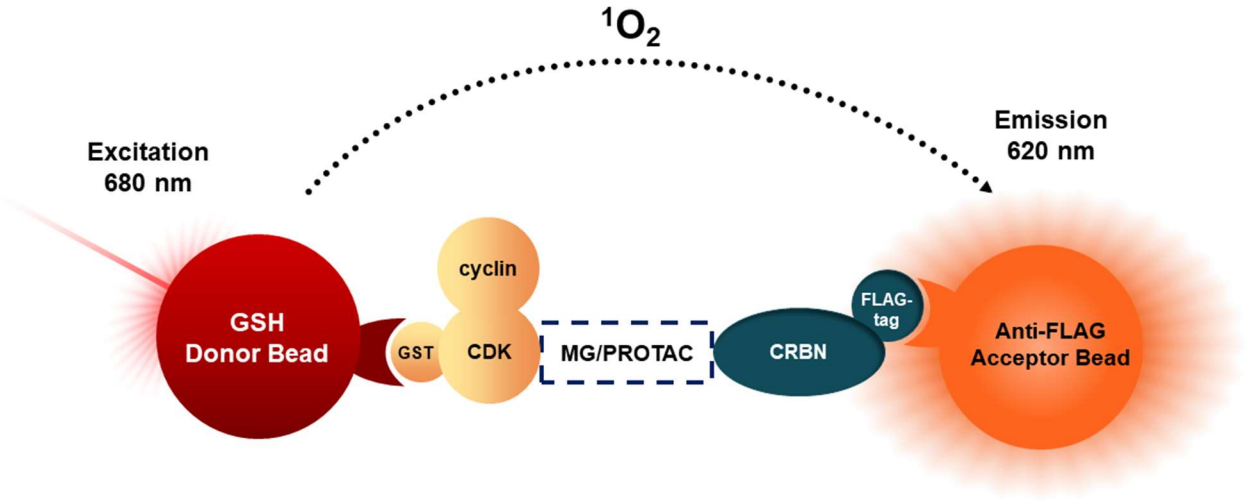

Figure 1. Schematic representation of the Molecular Glue/PROTAC® Optimization Kit for CDK2/CDK9-Cereblon Binding Assay Kit.

The Molecular Glue/PROTAC® of interest is incubated with Cereblon (CRBN) and CDK2 or CDK9, bringing them into proximity. CRBN contains a FLAG-tag, which is recognized by anti-FLAG AlphaLISA™ acceptor bead. CDK contains a GST-tag that binds to the donor bead. Upon excitation of the donor bead, a singlet oxygen is generated by the bead. The singlet oxygen excites the acceptor bead, which emits light proportionally to the level of interaction between CDK2/CDK9 and Cereblon.

Background

CDKs (cyclin-dependent kinases) are serine/threonine kinases involved in critical cellular functions. CDK2 (Cyclin-Dependent Kinase 2) is a cell cycle regulator specifically involved in the transition from G1 to S phase of the cell cycle. It forms complexes with Cyclin E and Cyclin A to phosphorylate key substrates involved in DNA replication and cell cycle progression. CDK9 (Cyclin-Dependent Kinase 9) primarily regulates transcription. It forms the catalytic core of the positive transcription elongation factor b (P-TEFb) complex and phosphorylates the C-terminal domain (CTD) of RNA polymerase II, playing a crucial role in promoting transcriptional elongation.

CDK9 is an emerging target for cancer therapy, especially in hematological malignancies. Since it is also involved in HIV replication, it is a potential target for anti-HIV therapies. CDK9 inhibitors are being investigated for their potential in treating inflammatory diseases and cardiovascular conditions. CDK2 is a well validated target in oncology, particularly in cancers with dysregulated cell cycle control. The protein may also play a role in certain neurodegenerative diseases. In cancer, CDK2 has emerged as a mechanism of resistance acquired after treatment of estrogen receptor-positive breast cancer patients with CDK4/6 inhibitors like Kisqali, Verzenio and Ibrance, a phenomenon driven by upregulation of CCNE1 (cyclin E1) which directly modulates CDK2 activity. These tumors may be activated by the amplification of Cyclin E1 or E2, loss of the AMBRA1 (activating molecule in Beclin-regulated autophagy) gene, or loss of retinoblastoma. Many CDK2 inhibitors are in development, however the

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protein is relatively difficult to drug, and toxic off-targets effects are an issue. Degraders are envisioned as an alternative to small molecule inhibitors. Both CDK2 and CDK9 are subjects of ongoing clinical research, with various inhibitors in different stages of clinical trials for various conditions, primarily cancer.

Application(s)

- Identify and optimize Molecular Glues/PROTACs targeting CDK2, CDK9, and CRBN.
- Design novel molecules targeting CDK2, CDK9, and CRBN.
- Directly compare the activity of different Molecular Glues/PROTACs.

Supplied Materials

Catalog #	Name	Amount	Storage
100255	Cereblon, FLAG-Tag*	10 µg	-80°C
40101	CDK2/Cyclin A2, His, GST-Tag*	2 x 5 µg	-80°C
102119	CDK9/Cyclin K, GST-Tag*	10 µg	-80°C
82649	CDK2/9 Degrader (MW=816 Da)	20 µg	-20°C
82620	5x PP-02 Assay Buffer	4 ml	-20°C
82650	FN-1501 (MW= 431 Da)	100 µg	-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	Ordering Information
AlphaLISA anti-FLAG Acceptor Beads, 250 µg	Revvity #AL112C
AlphaScreen Glutathione Donor Beads, 5 mg/ml	Revvity #6765300
Optiplate 384-well, white opaque	Revvity #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. **CDK2/9 Degrader is a thalidomide-derivative,** which is known to cause severe birth defects in humans. It is very important to use all appropriate precautions when handling this compound.

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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 CDK-Cereblon Binding

- This protocol is designed to test the binding affinity of various Molecular Glues/PROTACs to CDK2, CDK9 and Cereblon.
- All samples and controls should be performed in duplicate.
- The assay should include "Blank", "Positive Control" and "Test Molecular Glue/PROTAC" conditions.
- We recommend maintaining the diluted proteins on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- All incubations should be performed with slow agitation on a rotator platform.

STEP 1

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

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

2. Add 245 µl of DMSO to the vial of CDK2/9 Degrader. This makes a 0.1 mM stock solution.

3. Prepare a 0.4 µM CDK2/9 Degrader solution by diluting 0.1 mM CDK2/9 Degrader 250-fold with 1x Assay Buffer.

   Note: The final concentration of CDK2/9 Degrader in the assay will be 0.1 µM. The remaining undiluted stock CDK2/9 Degrader can be aliquoted and kept at -80°C.

4. Thaw **Cereblon** and **CDK2/CyclinA2** and/or **CDK9/CyclinK** on ice. Briefly spin the tubes containing the proteins to recover the full content of the tubes.

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

   a. Dilute **Cereblon** to 8.2 ng/µl with 1x Assay Buffer.

   b. Dilute **CDK2/CyclinA2** to 21.8 ng/µl with 1x Assay Buffer

      OR

   c. Dilute **CDK9/CyclinK** to 21.8 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (7.5 µl/well): N wells × (2.5 µl of diluted Cereblon + 2.5 µl of the diluted CDK2 or CDK9 complex + 2.5 µl of 1x Assay Buffer).

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

8. Prepare the **Test Molecular Glue/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 Molecular Glue/PROTAC is water-soluble, prepare serial dilutions 4-fold more concentrated than the desired final concentrations in 1 x Assay Buffer.

   For the positive and negative controls, use 1 x Assay Buffer (Diluent Solution).

   OR

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

   Using 1 x Assay Buffer with 4% DMSO, prepare serial dilutions of the Test Molecular Glue/PROTAC at 4-fold the desired final concentrations to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 4% DMSO in 1 x 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 Molecular Glue/PROTAC to the "Test Molecular Glue/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 0.4 µM CDK2/9 Degrader.

Component	Blank	Positive Control	Test
Master Mix	7.5 µl	7.5 µl	7.5 µl
Diluent Solution	2.5 µl	-	-
Test Molecular Glue/PROTAC	-		2.5 µl
Diluted CDK2/9 Degrader (0.4 µM)	-	2.5 µl	-
Total	**10 µl**	**10 µl**	**10 µl**

12. Incubate plate at room temperature (RT) for one hour.

STEP 2

Note: Protect your samples from direct exposure to light!

1. Dilute **Anti-FLAG Acceptor Beads** 250-fold with 1x Assay Buffer (10 µl/well).

2. Add 10 µl per well.

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

4. Dilute **Glutathione Donor Beads** 125-fold with 1x Assay Buffer (10 µl/well).

5. Add 10 µl per well. Shake on a rotator platform for 15-30 minutes at RT.

6. Read Alpha-counts.

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

Assay Protocol 2 – Molecular Glue/PROTAC Competitive Inhibition

- This protocol is designed to measure inhibition of Molecular Glue/PROTAC binding to CDK2/CyclinA2 or CDK9/CyclinK. The protocol can be easily modified to study inhibitors of the binding of Molecular Glue/PROTAC to CRBN.
- 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 1x Assay Buffer by diluting 5x PP-02 Assay Buffer with distilled water.

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

2. Add 245 µl of DMSO to the vial of CDK2/9 Degrader. This makes a 0.1 mM stock solution.

3. Prepare an 0.4 µM CDK2/9 Degrader solution by diluting 0.1 mM CDK2/9 Degrader 250-fold with 1x Assay Buffer.

   Note: The final concentration of CDK2/9 Degrader in the assay will be 0.1 µM. The remaining undiluted stock of CDK2/9 Degrader can be aliquoted and kept at -80°C (minimum 5 µl volume per aliquot).

4. Thaw **Cereblon** and **CDK2/CyclinA2** or **CDK9/CyclinK** 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** to 8.2 ng/µl with 1x Assay Buffer.
   b. Dilute **CDK2/CyclinA2** to 21.8 ng/µl with 1x Assay Buffer
      OR
   c. Dilute **CDK9/CyclinK** to 21.8 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (5 µl/well): N wells × (2.5 µl of diluted Cereblon + 2.5 µl of diluted CDK2 or CDK9 complex).

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

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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 in 1x Assay Buffer, 4-fold more concentrated than the desired final concentrations.

   For the positive and negative controls, use 1x 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 1x Assay Buffer to prepare the highest concentration of the 4-fold intermediate dilutions. The concentration of DMSO is now 4%.

   Using 1x 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 1x 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. Add 23.2 µl of DMSO to the vial with 100 µg of FN-1501, then dilute 25-fold with 1x Assay Buffer. This makes a 400 µM stock solution.

12. Add 2.5 µl of resuspended FN-1501 to the “Inhibitor Control” wells.

13. Preincubate the test compound with Master Mix for up to 30 minutes at RT with slow agitation.

14. Initiate the reaction by adding 2.5 µl of 0.4 µM CDK2/9 Degrader to wells labeled “Positive Control", “Inhibitor Control” and "Test Compound".

15. Add 2.5 µl of 1x 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 FN-1501 (400 µM)	-	-	2.5 µl	-
	Incubate 30 minutes at RT			
1x Assay Buffer	2.5 µl	-	-	-
Diluted CDK2/9 Degrader (0.4 µM)	-	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

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16. Incubate the plate at RT for one hour.

STEP 2

Note: Protect your samples from direct exposure to light!

1. Dilute **Anti-FLAG Acceptor Beads** 250-fold with 1x Assay Buffer (10 µl/well).
2. Add 10 µl per well.
3. Shake on a rotator platform for 30 minutes at RT.
4. Dilute **Glutathione Donor Beads** 125-fold with 1x Assay Buffer (10 µl/well).
5. Add 10 µl per well. Shake on a rotator platform for 15-30 minutes at RT.
6. Read Alpha-counts.
7. The "Blank" value should be subtracted from all readings.

Example Results

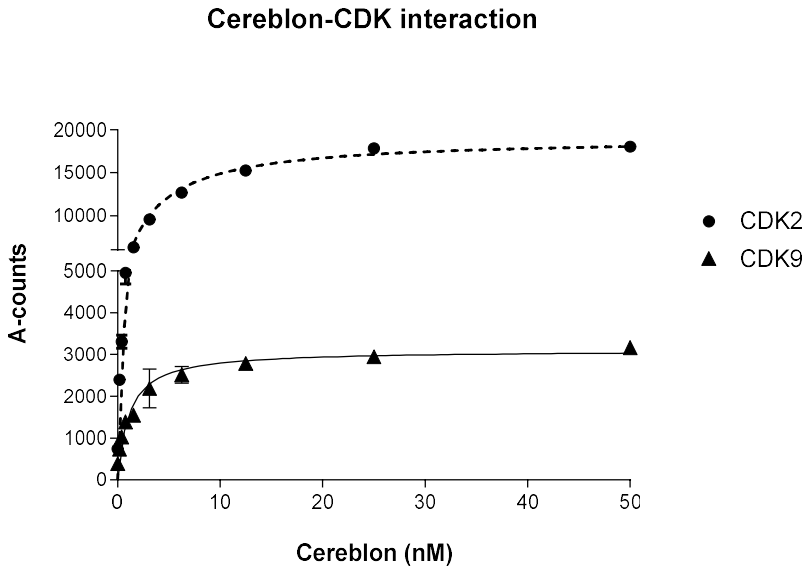

Figure 2: CDK2/9 Degrader-mediated interaction of Cereblon with CDK2 and CDK9.
CDK2/9 Degrader PROTAC® ability to bind CDK2 and CDK9 to Cereblon was measured in the presence of increasing concentrations of Cereblon.

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

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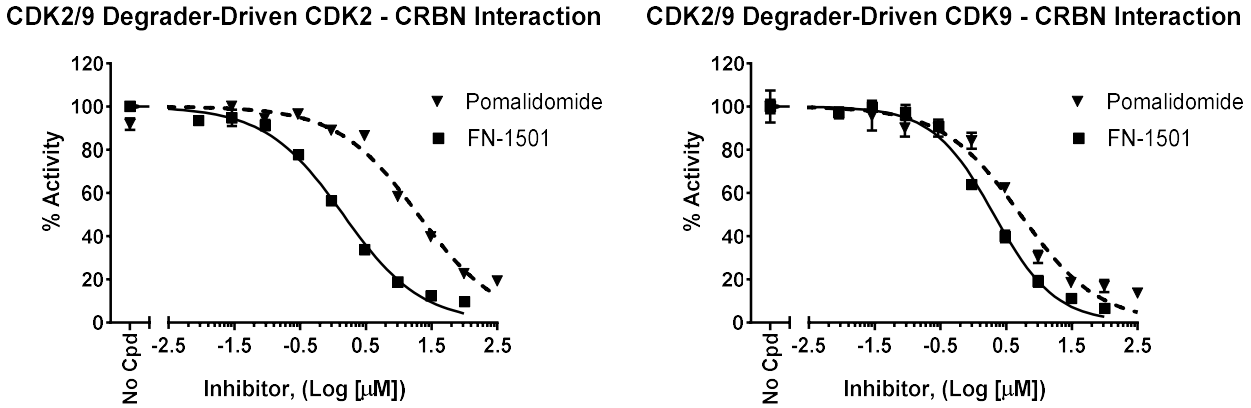

Figure 3: Inhibition of CDK2/9 Degrader-driven interaction of CDK2 and CDK9 with Cereblon by the inhibitors FN-1501 and Pomalidomide.
Inhibition of CDK2/9 Degrader-mediated interaction of CDK2 and CDK9 with Cereblon was measured in the presence of increasing concentrations of FN-1501 and Pomalidomide (#82026). The CDK inhibitor FN-1501 inhibits CDK2 and CDK9 binding with IC_{50}=1.4 µM and IC_{50}=2.1 µM, respectively. Pomalidomide inhibits CDK2 and CDK9 binding with IC_{50}=19.8 µM and IC_{50}=5.2 µM, respectively.

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

References

Gerosa R., *et al*., 2024 *Crit Rev Oncol Hematol.* 196: 104324.
Tadesse S., *et al*., 2020 *Drug Discov Today*. 25(2): 406-413.

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
CDK13/Cyclin K, GST-Tags Recombinant	101128	5 µg/10 µg
DCAF15/DDB1/DDA1/CUL4B/Rbx1 Complex Recombinant	101497	5 µg/10 µg

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