Description

The Molecular Glue/PROTAC® Optimization Kit for CDK/Cyclin K-DDB1 Containing Complex is an AlphaLISA® assay designed for the testing and profiling of Molecular Glues (MG) and PROTACs targeting CDK12 (cyclin dependent kinase 12) or CDK13 kinases and any complex containing DDB1 (DNA damage binding protein 1). The kit comes with enough recombinant CDK12/Cyclin K complex, Cereblon/DDB1/CUL4A (culin 4A)/Rbx1 (RING-box protein 1) as a DDB1-containing complex, and optimized PROTAC® buffer. This kit also contains the control molecular glue (R)-CR8, and the dual CDK12-CDK13 inhibitor THZ531.

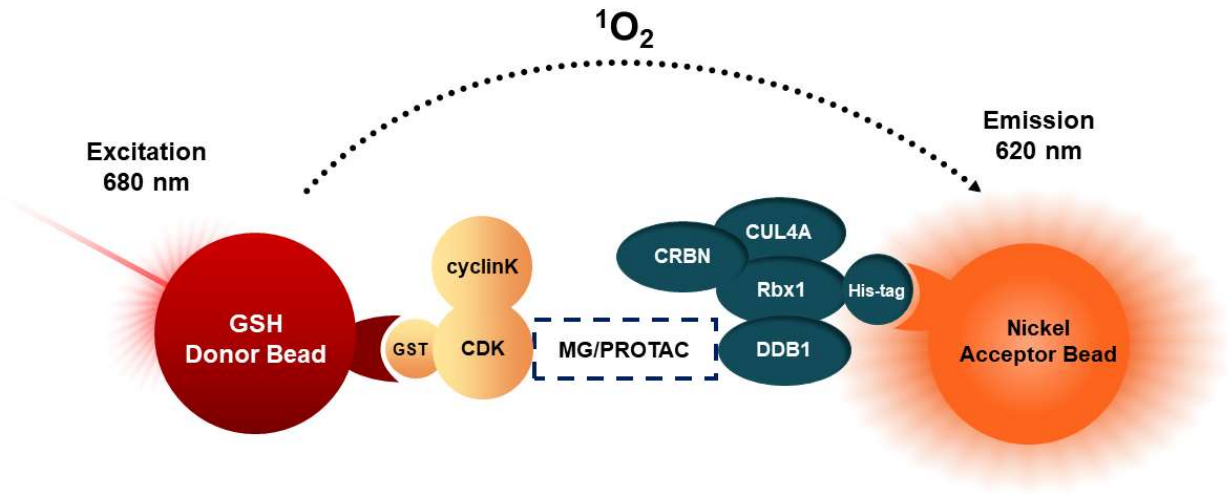

Figure 1. Schematic representation of the Molecular Glue/PROTAC® Optimization Kit for CDK/Cyclin K-DDB1 Containing Complex Assay Kit.

The Molecular Glue/PROTAC® of interest is incubated with DDB1 containing complex, such as DDB1/Cereblon, and CDK/Cyclin K, bringing them into proximity. The DDB1/Cereblon complex contains a His-tag, which is recognized by the Nickel- AlphaLISA™ acceptor bead. CDK12/Cyclin K 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 CDK/Cyclin K and DDB1/Cereblon complex.

Background

CDKs (cyclin-dependent kinases) are serine/threonine kinases involved in a myriad of critical celullar functions. CDK12 (cyclin-dependent kinase 12)/Cyclin K plays a critical pathophysiological role in cancer and other diseases through its involvement in transcription regulation, DNA damage repair, and genomic stability. CDK12/13 regulate transcription by phosphorylating the C-terminal domain of RNA polymerase II on Ser2, regulating the expression of DNA damage response (DDR) proteins. Understanding the mechanisms underlying CDK12/Cyclin K and how its inactivation can cause genomic instability, can lead to the development of novel targeted therapies and personalized treatment strategies for patients. DDB1 (DNA damage-binding protein 1) functions as a core component of the Cullin 4 (CUL4)-based E3 ubiquitin ligase complexes. DDB1 serves as a bridge or adaptor protein which interacts with Cereblon or with DCAFs (DDB1 and CUL4-associated factors), which are ubiquitin ligase substrates. Molecular glues such as (R)-CR8 induce the formation of a complex between CDK12/Cyclin K and the CUL4 adaptor protein DDB1, directly presenting CDK12/cyclin K for ubiquitination and degradation. CR8 is considered a pan-CDK inhibitor, by acting as a monovalent degrader of cyclin K. CR8 is derived from seliciclib, a CDK inhibitor with no solvent-exposed pyridine. The introduction of solvent exposure pyridine in CR8 created a new mechanism of action, highlighting the real impact of small molecular structure modifications to create innovative tools. The development of these molecular glues and PROTACS opens new avenues in cancer therapy.

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Application(s)

- Identify and optimize Molecular Glues/PROTACs targeting CDK12/Cyclin K or CDK13/Cyclin K and any DDB1-containing E3 ligase complex.
- Design novel molecules targeting CDK12(13)/Cyclin K and DDB1.
- Directly compare the activity of different Molecular Glues/PROTACs.

Supplied Materials

Catalog #	Name	Amount	Storage
100329	Cereblon/DDB1/CUL4A/Rbx1 Complex*	100 µg	-80°C
100998	CDK12/Cyclin K, GST-Tag*	10 µg	-80°C
	(R)-CR8 (MW=431.5 Da)	5.2 µg	-20°C
	5x PP-02 Assay Buffer	4 ml	-20°C
	THZ531 (MW=558 Da)	28 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 Nickel Chelate Acceptor Beads, 250 µg	Revvity #AL108C
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.

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.

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Assay protocol 1 - Optimization of CDK12 Binding to DDB1 Complex

- This protocol is designed to test the binding affinity of various Molecular Glues/PROTACs to CDK12/Cyclin K and the DDB1/Cereblon complex.
- 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.
- CDK12/Cyclin K can be replaced by CDK13/Cyclin K (#101128), although interaction efficiency is lower (contact BPS Bioscience, Inc. for more information).
- DDB1/Cereblon complex can be replaced by DCAF15/DDB1/DDA1/CUL4B/Rbx1 Complex (#101497), or any other DDB1-containing complex that has one of the proteins with an His-tag (contact BPS Bioscience, Inc. for more information).

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 15 µl of DMSO to the vial of (R)-CR8. This makes a 0.8 mM stock solution.

3. Prepare an 8 µM (R)-CR8 solution by diluting 0.8 mM (R)-CR8 100-fold with 1x Assay Buffer.

   Note: The final concentration of (R)-CR8 in the assay will be 2 µM. The remaining undiluted stock (R)-CR8 can be aliquoted and kept at -80°C (minimum 5 µl volume per aliquot).

4. Thaw **DDB1/Cereblon complex** and **CDK12/Cyclin K** 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 **DDB1/Cereblon complex** to 67 ng/µl with 1x Assay Buffer.

   b. Dilute **CDK12/Cyclin K** to 6.5 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (7.5 µl/well): N wells × (2.5 µl of diluted DDB1/Cereblon complex + 2.5 µl of the diluted CDK12/Cyclin K + 2.5 µl of 1x Assay Buffer).

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.

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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 8 µM (R)-CR8.

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 (R)-CR8 (8 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 **Nickel 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.

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Assay Protocol 2 – Molecular Glue/PROTAC Competitive Inhibition

- This protocol is designed to measure inhibition of Molecular Glue/PROTAC binding to CDK12/Cyclin K. The protocol can be easily modified to study inhibitors of the binding of Molecular Glue/PROTAC to DDB1 containing complexes.
- 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 15 µl of DMSO to the vial of (R)-CR8. This makes a 0.8 mM stock solution.

3. Prepare a 8 µM (R)-CR8 solution by diluting 0.8 mM (R)-CR8 100-fold with 1x Assay Buffer.

   Note: The final concentration of (R)-CR8 in the assay will be 2 µM. The remaining diluted stock of (R)-CR8 can be aliquoted and kept at -80°C (minimum 5 µl volume per aliquot).

4. Thaw **DDB1/Cereblon complex** and **CDK12/Cyclin K** 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 **DDB1/Cereblon complex** to 67 ng/µl with 1x Assay Buffer.

   b. Dilute **CDK12/Cyclin K** to 6.5 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (5 µl/well): N wells × (2.5 µl of diluted DDB1/Cereblon complex + 2.5 µl of diluted CDK12/Cyclin K).

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 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

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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 50 µl of DMSO to the vial with 28 µg of THZ531, then dilute 25-fold with 1x Assay Buffer. This makes a 40 µM stock solution.

12. Add 2.5 µl of resuspended THZ531 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 8 µM (R)-CR8 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 THZ531 (40 µM)	-	-	2.5 µl	-
	Incubate 30 minutes at RT			
1x Assay Buffer	2.5 µl	-	-	-
Diluted (R)-CR8 (8 µM)	-	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

16. Incubate the plate at RT for one hour.

STEP 2

Note: Protect your samples from direct exposure to light!

1. Dilute **Nickel 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.

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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

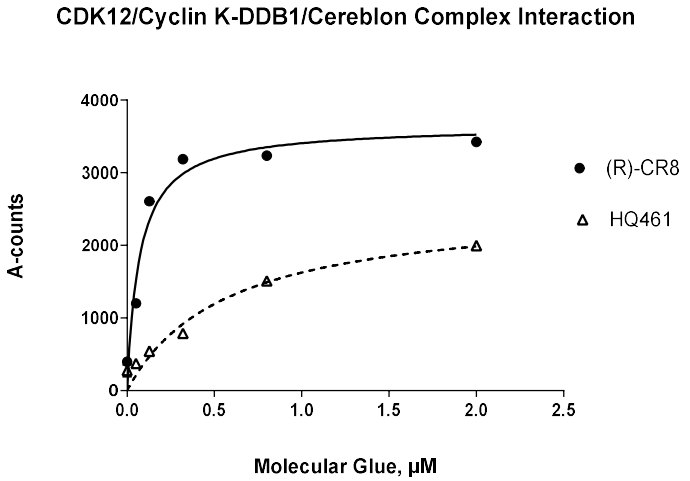

Figure 2: Molecular Glue-mediated interaction of CDK12 /Cyclin K and DDB1/Cereblon Complex. The binding of CDK12/Cyclin K to DDB1/Cereblon complex was measured in the presence of increasing concentrations of two molecular glues, (R)-CR8 and HQ461.

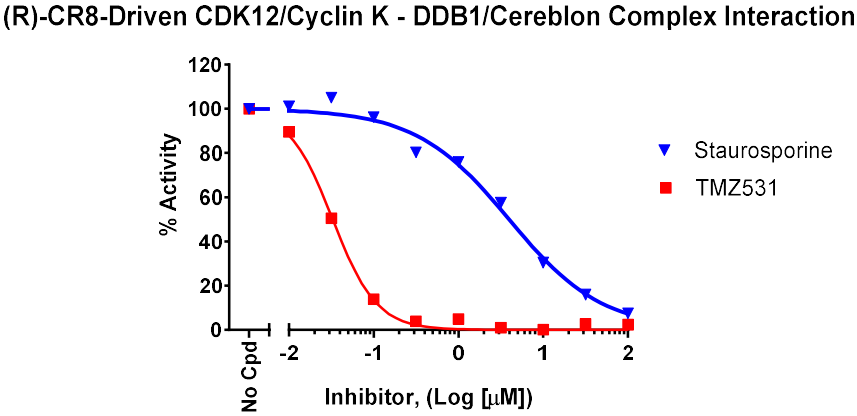

Figure 3: Inhibition of (R)-CR8-mediated interaction of CDK12/Cyclin K with DDB1/Cereblon complex by the inhibitors TMZ531 and staurosporine.
Inhibition of the molecular glue (R)-CR8 mediated interaction of CDK12/Cyclin K with DDB1/Cereblon complex was measured in the presence of increasing concentrations of Staurosporine (#27002) and TMZ531. The pan-kinase inhibitor Staurosporine inhibits the interaction with IC_{50}=4.0 µM. TMZ531 is a covalent inhibitor of CDK12 and CDK13. K_{inact}/K_i is not presented.

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

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References

Slabicki M., *et al.,* 2020 *Nature* 585(7824): 293-297.
Lv Lu, *et al.,* 2020 *eLife*; 9:e59994.
Thomas K., *et al.*, 2024 *ACS Chem Biol* 19(1):173-184.

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*
CDK13/Cyclin K	101128-2	10 µg
DCAF15/DDB1/DDA1/CUL4B/Rbx1	101497-1	10 µg
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

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