

Description

The Molecular Glue/PROTAC® Optimization Kit for GSPT1 is an AlphaLISA® assay designed for the testing and profiling of Molecular Glues (MG) and PROTACs (proteolysis targeting chimera) targeting GSPT1 (G1 to S phase transition 1). The kit comes with enough recombinant GSPT1, biotinylated Cereblon-DDB1 complex, and an optimized GSPT buffer. This kit also contains the GSPT degrader CC-885, and the Cereblon ligand pomalidomide.

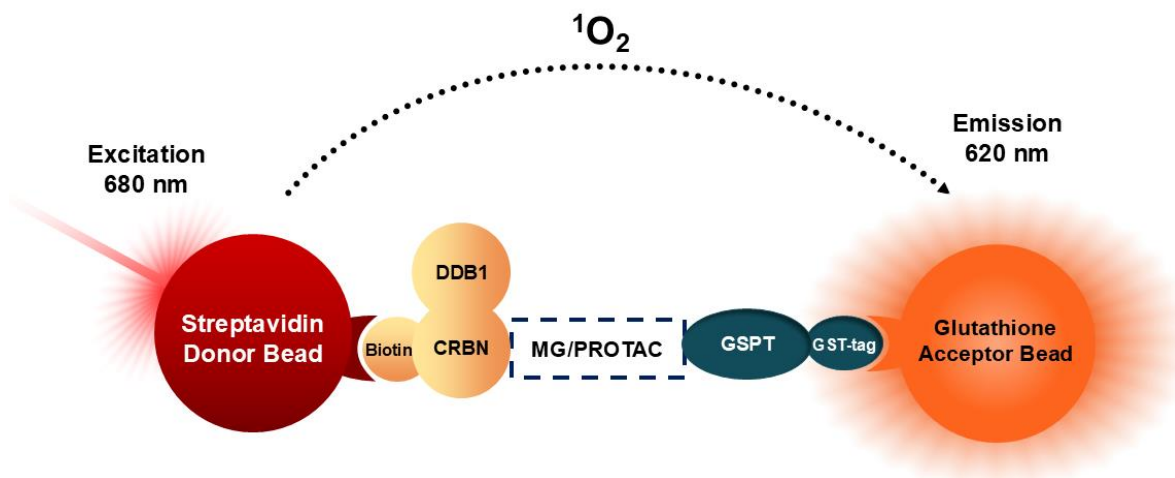


Figure 1. Schematic representation of the mechanism of Molecular Glue/PROTAC® Optimization Kit for GSPT1.

The Molecular Glue/PROTAC® of interest is incubated with biotinylated Cereblon/DDB1 complex and GSPT1, bringing them into proximity. The Cereblon/DDB1 complex is recognized by the Streptavidin donor bead. GSPT1 contains a GST-tag that binds to the Glutathione AlphaLISA™ acceptor 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 GSPT1 and Cereblon/DDB1 complex.

Background

GSPT1 (G1 to S phase transition 1), also known as eRF3a, is an essential translation-termination factor that became a key substrate for cereblon (CRNB)-based molecular glues (MGs) such as CC-885 and CC-90009. These compounds bind cereblon and trigger the E3 ligase to recruit and ubiquitinate GSPT1, leading to its rapid proteasomal degradation. Cereblon is the substrate-binding component of the E3 protein ligase complex DDB1 (damage specific DNA binding protein 1)-CUL4A (cullin 4)-RBX1 (ring-box 1), which is involved in the ubiquitination and proteasomal degradation of target proteins. CRBN has become a target of choice for the development of many PROTACs with therapeutical interest. Loss of GSPT1 disrupts translational fidelity and induces strong antiproliferative effects, particularly in AML (acute myeloid lymphoma) and other sensitive cancer types. As a result, GSPT1 is now considered a high-value target in the field of targeted protein degradation (TPD). The development of these molecular glues and PROTACs (proteolysis targeting chimeras) opens new avenues in cancer therapy.

Application(s)

- Identify and optimize Molecular Glues/PROTACs targeting GSPT1.
- Design novel molecules targeting CRBN.
- Directly compare the activity of different Molecular Glues/PROTACs.

Supplied Materials

Catalog #	Name	Amount	Storage
83883-KC10	Cereblon/DDB1 (Biotin)*	10 µg	-80°C
102693-KC10	GSPT1, GST-Tag*	4 x 10 µg	-80°C
83884-KC8.8	CC-885 (MW=431.5 Da)	8.8 µg	-20°C
83885-KC4	4x GSPT Assay Buffer	4 ml	-20°C
82735-KC200	0.5 M DTT	200 µl	-20°C
83886-KC15	50 mM Pomalidomide	15 µl	-20°C

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

Materials Required but Not Supplied

Component	Ordering Information
AlphaLISA Glutathione Acceptor Beads, 250 µg	Revvity #AL109C
AlphaScreen Streptavidine Donor Beads, 5 mg/ml	Revvity #67760002S
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.

Pomalidomide 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.

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 ($\lambda=520-620$ 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 lack these components, do not affect AlphaScreen assays.

Assay protocol 1 - Optimization of GSPT1-CRBN Binding

- This protocol is designed to test the binding of various Molecular Glues/PROTACs to GSPT1 and the Cereblon/DDB1 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 \(bpsbioscience.com\)](https://www.bpsbioscience.com/ProteinFAQs).
- All incubations should be performed with slow agitation on a rotator platform.

STEP 1

1. Prepare **1x Assay Buffer** by diluting **4x GSPT Assay Buffer** 4-fold with distilled water and adding **DTT** to the final concentration of 1 mM (for example, add 8 µl of 0.5M DTT per 1 ml of 4x GSPT Assay Buffer).

Note: Prepare only the amount needed for the experiment. Aliquot the remaining 4x GSPT Assay Buffer and store at -20°C.

2. Add 100 µl of DMSO to the vial of **CC-885**. This makes a 0.2 mM stock solution.
3. Prepare an **0.5 µM CC-885** solution by diluting 400-fold the 0.2 mM CC-885 solution with 1x Assay Buffer.

Note: The final concentration of CC-885 in the assay will be 125 nM. The remaining undiluted stock of CC-885 can be aliquoted and kept at -80°C (minimum 5 µl volume per aliquot).

4. Thaw **Cereblon/DDB1 complex** and **GSPT1** on ice. Briefly spin the tubes containing the proteins to recover their full content.
5. Prepare the following dilutions (2.5 µl/well):
 - a. Dilute **Cereblon/DDB1 complex** to 10 ng/µl with 1x Assay Buffer.
 - b. Dilute **GSPT1** to 38 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (7.5 µl/well): N wells × (2.5 µl of diluted Cereblon/DDB1 complex + 2.5 µl of the diluted GSPT1 + 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 1x Assay Buffer.

For the positive and negative controls, use 1x 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 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 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 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 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 0.5 µM CC-885.

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 CC-885 (0.5 µM)	-	2.5 µl	-
Total	10 µl	10 µl	10 µl

12. Incubate plate at 30°C for one hour.

STEP 2



Note: Protect your samples from direct exposure to light!

1. Dilute **Glutathione 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 Room Temperature (RT).
4. Dilute **Streptavidin 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 the inhibition of Molecular Glue/PROTAC binding to CRBN complex. The protocol can be easily modified to study effectors of the binding of Molecular Glue/PROTAC to GSPT1.
- 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 \(bpsbioscience.com\)](https://www.bpsbioscience.com/protein-faqs).
- All incubations should be performed with slow shaking on a rotator platform.

STEP 1

1. Prepare **1x Assay Buffer** by diluting **4x GSPT Assay Buffer** 4-fold with distilled water and adding **DTT** to the final concentration of 1 mM (for example, add 8 µl of 0.5M DTT per 1 ml of 4x GSPT Assay Buffer).

Note: Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 4x Assay Buffer and store at -20°C.

2. Add 100 µl of DMSO to the vial of **CC-885**. This makes a 0.2 mM stock solution.
3. Prepare a **0.5 µM CC-885** solution by diluting 400-fold the 0.2 mM CC-885 solution with 1x Assay Buffer.

Note: The final concentration of CC-885 in the assay will be 125 nM. The remaining diluted stock of CC-885 can be aliquoted and kept at -80°C (minimum 5 µl volume per aliquot).

4. Thaw **Cereblon/DDB1 complex** and **GSPT1** 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/DDB1 complex** to 10 ng/µl with 1x Assay Buffer.
 - b. Dilute **GSPT1** to 38 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (5 µl/well): N wells × (2.5 µl of diluted Cereblon/DDB1 complex + 2.5 µl of diluted GSPT1).

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

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. Take the vial with **50 mM Pomalidomide** solution and dilute 25-fold with 1x Assay Buffer. This makes a 2000 µM stock solution.
12. Add 2.5 µl of resuspended Pomalidomide to the "Inhibitor Control" wells.
13. Preincubate the test compound with the Master Mix for up to 30 minutes at RT with slow agitation.
14. Initiate the reaction by adding 2.5 µl of 0.5 µM CC-885 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 Pomalidomide (2000 µM)	-	-	2.5 µl	-
Incubate 30 minutes at RT				
1x Assay Buffer	2.5 µl	-	-	-
Diluted CC-885 (0.5 µM)	-	2.5 µl	2.5 µl	2.5 µl
Total	10 µl	10 µl	10 µl	10 µl

16. Incubate the plate at 30°C for one hour.

STEP 2



Note: Protect your samples from direct exposure to light!

1. Dilute **Glutathione 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 **Streptavidin Donor Beads** 125-fold with 1x Assay Buffer (10 µl/well).
5. Add 10 µl per well. Shake on a rotator platform for 10-30 minutes at RT.
6. Read Alpha-counts.
7. The “Blank” value should be subtracted from all readings.

Example Results

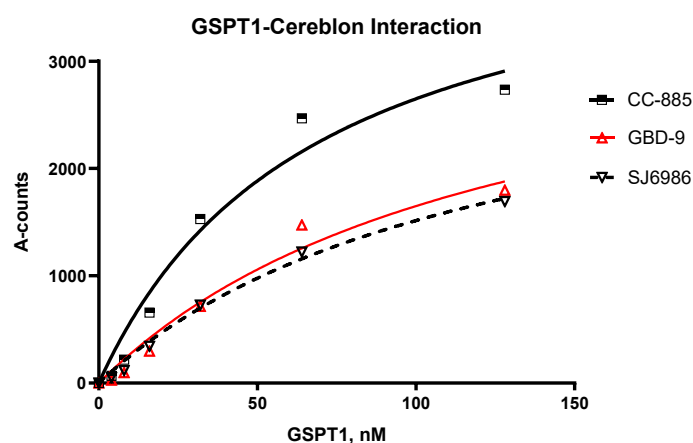


Figure 2: PROTAC/Molecular Glue-mediated interaction of GSPT1 and Cereblon/DDB1 Complex. The binding of GSPT1 to Cereblon/DDB1 complex was measured in the presence of equimolar concentrations of the PROTAC/Molecular Glues GBD-9, SJ6986, and CC-885.

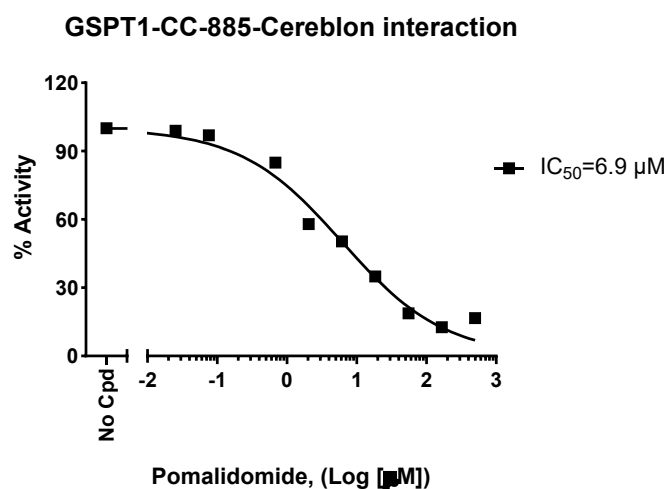


Figure 3: Inhibition of CC-885-mediated interaction of GSPT1 with DDB1/Cereblon complex by Pomalidomide.

Inhibition of the molecular glue CC-885 mediated interaction of GSPT1 with Cereblon/DDB1 complex was measured in the presence of increasing concentrations of Pomalidomide (#82026).

Data shown is representative.

References

Matyskiela M., *et al.*, 2016 *Nature* 535: 252-257.
 Surka C., *et al.*, 2021 *Blood*; 137(5): 661-677.
 Sellar R., *et al.*, 2022 *J Clin Invest* 132(16): p e153514.
 Nowak R., *et al.*, 2023 *RSC Med Chem* 14: 501-506.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For lot-specific information and all other questions, please visit <https://bpsbioscience.com/contact>.

Related Products

<i>Products</i>	<i>Catalog #</i>	<i>Size</i>
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

Version 121125