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Produktinformation

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Lieferung & Zahlungsart

siehe unsere Liefer- und Versandbedingungen

Zuschläge

- Mindermengenzuschlag
- Trockeneiszuschlag
- Gefahrgutzuschlag
- Expressversand

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Description

The Molecular Glue/PROTAC® Optimization Kit for RBM39-DCAF15 Binding is designed for the testing and profiling of Molecular Glues (MG)/PROTACs targeting the RBM39 (RNA-binding protein 39) and DCAF15 (DDB1 and CUL4 associated factor 15) interaction. The Molecular Glue/PROTAC® Optimization Kit for RBM39-DCAF15 Binding comes in a convenient AlphaLISA® format, with enough optimized assay and detection buffer, purified recombinant RBM39 (amino acids 245-530) and Rbx1/CUL4B/DDB1/DCAF15/DDA1 complex for 384 reactions. This kit also contains the molecular glue E7820 and Arginine as controls.

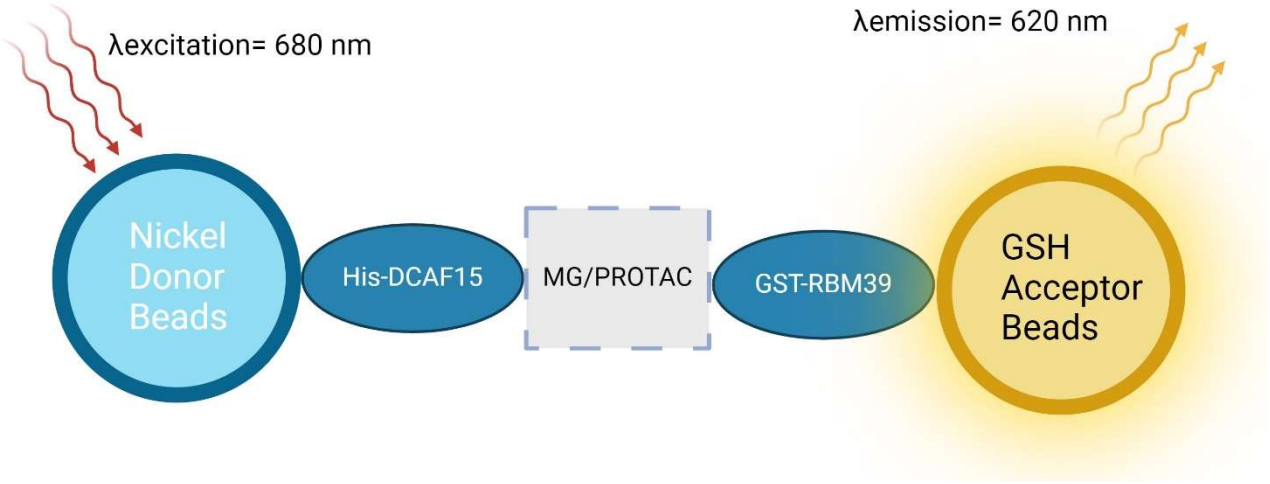

Figure 1. Schematic representation of the Molecular Glue/PROTAC® Optimization Kit for RBM39-DCAF15 Binding assay kit principle.

The Molecular Glue/PROTAC® of interest is incubated with DCAF15 complex and RBM39, bringing them in close proximity. DCAF15 contains a His-tag, which is recognized by the donor beads. RBM39 contains a GST tag that binds to the GSH- AlphaLISA™ acceptor beads. 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 RBM39 and DCAF15.

Background

DCAF15 is the substrate-binding component of the E3 protein ligase complex DDB1-DDA1-CUL4-Rbx1, involved in the ubiquitination and proteasomal degradation of target proteins. Binding of DCAF15 to a substrate protein engages the E3 ligase activity of the complex and results in the ubiquitination and ultimate degradation of the protein substrate. One of the most important targets of DCAF15 is RBM39 (RNA-binding motif protein 39).

RBM39 is a key factor in tumor-targeted mRNA and protein expression. Recent studies have revealed that RBM39 is the unexpected target of aryl sulphonamides, which act as molecular glues between RBM39 and the DCAF15-associated E3 ubiquitin ligase complex leading to selective degradation of the target. E7820 is an example of aryl sulfonamides that recruit RBM39 to Rbx-CUL4-DDA1-DDB1-DCAF15 E3 ligase complex, leading to its ubiquitination and degradation by the proteasome. Development of novel MG and PROTACS involved in the recruitment of RBM39 provides a framework for future efforts to utilize DCAF15 to degrade other proteins of interest.

Application(s)

- Identify and optimize Molecular Glues/PROTACs targeting RBM39.
- Design novel molecules targeting DCAF15.
- Directly compare the activity of different Molecular Glues/PROTACs.

Supplied Materials

Catalog #	Name	Amount	Storage
101497	Rbx1/CUL4B/DDB1/DCAF15/DDA1 Complex*	2 x 100 µg	-80°C
102059	RBM39, GST-Tag*	2 x 50 µg	-80°C
	E7820 (MW=336 Da)	100 µg	-20°C
	5x PP-02 Assay Buffer	4 ml	-20°C
	4x Detection Buffer 3D	2 ml	-20°C
	L-Arginine	10 mg	Room Temp
	Plate sealer	1	Room Temp

**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 GSH acceptor beads, 250 µg	PerkinElmer #AL109C
Alpha Nickel donor beads, 5 mg/ml	PerkinElmer #AS101D
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.

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 RBM39-DCAF15 Binding

- This protocol is designed to test the binding affinity of various Molecular Glues/PROTACs to RBM39 and the DCAF15 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 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 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 30 µl of DMSO to the vial of E7820. This makes a 10 mM stock solution.

3. Prepare a 16 µM E7820 solution by diluting 10 mM E7820 625-fold with 1x Assay Buffer.

   Note: The final concentration of E7820 in the assay will be 4 µM. The remaining undiluted stock E7820 can be aliquoted and kept at -80°C (minimum 5 µl volume per aliquot).

4. Thaw **DCAF15 complex** and **RBM39** 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 **DCAF15 complex** to 200 ng/µl with 1x Assay Buffer.

   b. Dilute **RBM39** to 92 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (7.5 µl/well): N wells × (2.5 µl of diluted DCAF15 complex + 2.5 µl of the diluted RBM39 + 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.

   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 16 µM E7820.

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 E7820 (16 µM)	-	2.5 µl	-
Total	**10 µl**	**10 µl**	**10 µl**

12. Seal the plate and incubate 37°C for one hour.

STEP 2

Note: Protect your samples from direct exposure to light!

1. Dilute 4x Detection Buffer 3D 4-fold with distilled water. This makes a 1x Detection Buffer 3D.

   Note: Prepare only the amount needed for the experiment. Aliquot the remaining 4x Detection Buffer 3D and store at -20°C.

2. Dilute **GSH Acceptor beads** 250-fold with 1x Detection Buffer 3D (10 µl/well).

3. Add 10 µl per well.

4. Shake on a rotator platform for 30-60 minutes at RT.

5. Dilute **Nickel donor beads** 125-fold with 1x Detection Buffer 3D (10 µl/well).

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

7. Read Alpha-counts.

8. 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 the Molecular Glue/PROTAC binding to RBM39. The protocol can be easily modified to study inhibitors of the binding of Molecular Glue/PROTAC to the DCAF15 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 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 30 µl of DMSO to the vial of E7820. This makes a 10 mM stock solution.

3. Prepare a 16 µM E7820 solution by diluting 10 mM E7820 625-fold with 1x Assay Buffer.

   Note: The final concentration of E7820 in the assay will be 4 µM. The remaining undiluted stock E7820 can be aliquoted and kept at -80°C (minimum 5 µl volume per aliquot).

4. Thaw **DCAF15 complex** and **RBM39** 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 **DCAF15 complex** to 200 ng/µl with 1x Assay Buffer.

   b. Dilute **RBM39** to 92 ng/µl with 1x Assay Buffer.

6. Prepare a **Master Mix** (5 µl/well): N wells × (2.5 µl of diluted DACf15 + 2.5 µl of diluted RBM39).

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. Resuspend 10 mg of Arginine with 1.15 ml of 1x Assay Buffer. This makes a 50 mM stock solution.

12. Add 2.5 µl of resuspended Arginine to the “Inhibitor Control” wells.

13. Preincubate the test compound with RBM39 and DCAF15 complex for up to 30 minutes at RT with slow agitation.

14. Initiate the reaction by adding 2.5 µl of 16 µM E7820 to wells labeled “Positive Control", “Inhibitor Control” and "Test Inhibitor".

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 E7820 (20 µM)	-	2.5 µl	2.5 µl	2.5 µl
1x Assay Buffer	2.5 µl	-	-	-
Diluted Arginine (50 mM)	-	-	2.5 µl	-
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

16. Seal the plate and incubate at 37°C for one hour.

STEP 2

Note: Protect your samples from direct exposure to light!

1. Dilute 4x Detection Buffer 3D 4-fold with distilled water. This makes a 1x Detection Buffer 3D.

   Note: Prepare only the amount needed for the experiment. Aliquot the remaining 4x Detection Buffer 3D and store at -20°C.

2. Dilute **GSH Acceptor beads** 250-fold with 1x Detection Buffer 3D (10 µl/well).

3. Add 10 µl per well.
4. Shake on a rotator platform for 30-60 minutes at RT.
5. Dilute **Nickel donor beads** 125-fold with 1x Detection Buffer 3D (10 µl/well).
6. Add 10 µl per well. Shake on a rotator platform for 15-30 minutes at RT.
7. Read Alpha-counts.
8. The "Blank" value should be subtracted from all readings.

Example Results

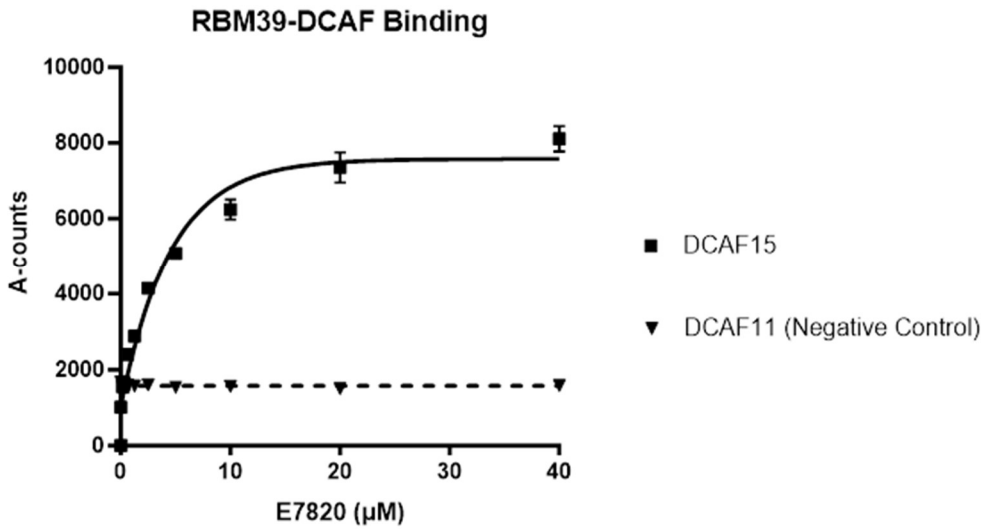

Figure 2: E7820-mediated interaction of DCAF15 and RBM39.
The binding of DCAF15 and DCAF11 (negative control) to RBM39 was measured in the presence of increasing concentrations of the molecular glue E7820.

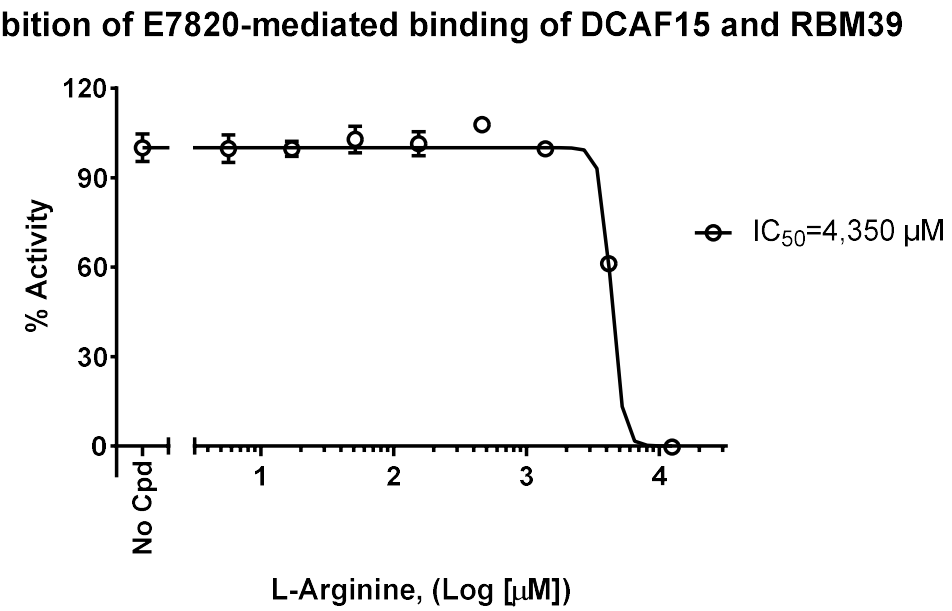

Figure 3: Inhibition by Arginine of the E7820-mediated interaction of DCAF15 with RBM39. Inhibition of the molecular glue E7820-mediated interaction of DCAF15 with RBM39 was measured in the presence of increasing concentrations of Arginine.

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

Reference

Nunes, J., *et al.* 2019 *ACS Med Chem Lett*; 10(7): 1081-1085.

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

Version 013024