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Data Sheet
PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding

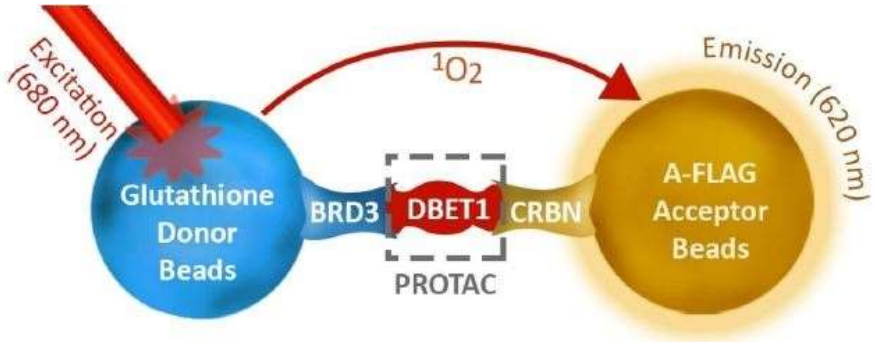
Catalog #79770
Size: 384 reactions

DESCRIPTION: The *PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding* is designed for testing and profiling of PROTACs directed against BET Bromodomain family and Cereblon complex. Cereblon (CRBN) is a Substrate recognition component of a DCX (DDB1-CUL44-Rbx1) E3 protein ligase complex that mediates the ubiquitination and subsequent proteasomal degradation of target proteins.

The *PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding* comes in a convenient AlphaLISA® format, with enough dBET1 PROTAC, Immuno Buffer 1, purified BRD3(BD2) and CRBN for 384 reactions. (+)JQ1 is included as a control inhibitor of PROTAC binding to BRD3(BD2). With this kit, only three simple steps on a microtiter plate are required for PROTAC activity detection. First, a sample containing PROTAC is incubated with CRBN and BRD3(BD2), one of the BET bromodomains. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
100329	Cereblon/DDB1/Cul4A/Rbx1 Complex	30 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
31033	BRD3(BD2), GST-tag	5 µg	-80°C	
	dBET1 (MW = 785 Da)	10 µg	-80°C	
79311	3x Immuno Buffer 1	4 ml	-20°C	
27403	(+)-JQ1 (10 mM)	100 µl	-20°C	



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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA anti-FLAG acceptor beads, 5 mg/ml (PerkinElmer #AL112C)
Alpha Glutathione donor beads, 5 mg/ml (PerkinElmer #6765300)
Optiplate 384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for the characterization of PROTACs targeting the BET family of bromodomains, design of novel molecules targeting CRBN, and comparison of the activities of different PROTACs.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the 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 culture medium RPMI 1640 at >1% leads to signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

STABILITY: At least six months from date of receipt when stored as directed.

REFERENCE: Winter, G.E., Paulk, J., Roberts, J.M., *et al. Science* 2015; **348**(6241):1376-81.

SAFETY WARNING: dBET1 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.**

ASSAY PROTOCOL 1 -- Optimization of Bromodomain-Cereblon Binding

This protocol is designed to test the binding affinity of various PROTAC samples to the bromodomain or cereblon complex.

All samples and controls should be tested in duplicate. All incubations are performed with slow shaking on a rotator platform.

Step 1:
1) Prepare 1x Immuno Buffer 1 by adding 1 part 3x Immuno Buffer 1 plus 2 parts distilled water. Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 3x Immuno Buffer 1 and store at -20°C.

2) Resuspend the tube with the dBET1 with 130 µl of DMSO to prepare a 100 µM stock solution. Gently pipet up and down to ensure the dBET1 is thoroughly dissolved. Store any unused dBET1 in single use aliquots at -80°C. Before the experiment, add 960 µl of 1x Immuno Buffer 1 to 40 µl of 100 µM stock to obtain a 4 µM solution. Note: final concentration of dBET1 in the assay may be in the range 0.1-1 µM.

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3) Thaw Cereblon and BRD3(BD2) on ice. Upon first thaw, briefly spin tubes containing proteins to recover full content of the tubes. Aliquot proteins into single use aliquots. Store remaining undiluted proteins in aliquots at -80°C immediately. Note: Both BRD3(BD2) and Cereblon are sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.

4) Dilute Cereblon in 1X Immuno Buffer 1 at 25 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

5) Dilute BRD3(BD2) in 1X Immuno Buffer 1 at 2.5 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

6) Prepare master mix: N wells × (2.5 µl Cereblon (25 ng/µl) + 2.5 µl BRD3(BD2) (2.5 ng/µl) + 2.5 µl 1x Immuno Buffer 1. Add 7.5 µl of master mixture to every well.

Reagent	Blank	Positive Control	Test PROTAC
Cereblon (25 ng/µl)	2.5 µl	2.5 µl	2.5 µl
BRD3(BD2) (2.5 ng/µl)	2.5 µl	2.5 µl	2.5 µl
1x Immuno Buffer 1	5.0 µl	2.5 µl	2.5 µl
Test PROTAC	–	–	2.5 µl
dBET1 (resuspended)	–	2.5 µl	–
Total	10 µl	10 µl	10 µl

7) For the wells labeled as "Blank", add 2.5 µl 1x Immuno Buffer 1. Dilute Test PROTAC in 1x Immuno Buffer 1. Add 2.5 µl of diluted Test PROTAC to each well designated “Test PROTAC”. Add 2.5 µl of diluted dBET1 to each well designated “Positive Control”.

8) Incubate at room temperature for one hour.

Note: Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:
Dilute anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with 1x Immuno Buffer 1. Add 10 µl per well. Shake on a rotator platform for 60 minutes at room temperature.

Step 3:
1) Dilute Glutathione donor beads (PerkinElmer #6765300) 125-fold with 1x Immuno Buffer 1. Add 10 µl per well. Shake on a rotator platform for 30-60 minutes at room temperature.

2) Read Alpha-counts. “Blank” value should be subtracted from all readings.

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ASSAY PROTOCOL 2 -- Competitive Inhibition of the PROTAC

This protocol is designed to measure inhibition of the PROTAC binding to the bromodomain. The protocol can be easily modified to study inhibitors of the PROTAC to the cereblon complex.

All samples and controls should be tested in duplicate. All incubations are performed with slow shaking on a rotator platform.

Step 1:

1) Prepare 1x Immuno Buffer 1 by adding 1 part 3x Immuno Buffer 1 plus 2 parts distilled water. Prepare only the amount needed for the experiment. Aliquot the remaining undiluted 3x Immuno Buffer 1 and store at -20°C.

2) Resuspend tube with the dBET1 as in Protocol 1.

3) Thaw Cereblon and BRD3(BD2) on ice. Upon first thaw, briefly spin tubes containing proteins to recover full content of the tubes. Aliquot proteins into single use aliquots. Store remaining undiluted proteins in aliquots at -80°C immediately. *Note: Both BRD3(BD2) and Cereblon are sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

4) Dilute Cereblon in 1X Immuno Buffer 1 at 5 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

5) Dilute BRD3(BD2) in 1X Immuno Buffer 1 at 2.5 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted enzyme after use.

6) Prepare master mix: N wells × (2.5 µl Cereblon (5 ng/µl) + 2.5 µl BRD3(BD2) (2.5 ng/µl). Add 5 µl of master mixture to every well.

7) For the wells labeled as "Blank", add 2.5 µl 1x Immuno Buffer 1.

8) Add 2.5 µl of test compound solution to each well designated “Test Inhibitor”. For the “Positive Control" and "Blank" add 2.5 µl of the same solution without the test compound (“Compound buffer”). We recommend using 1x Immuno Buffer 1 with the same concentration of DMSO as the Compound buffer. Preincubate the test compound with the cereblon and BRD3 for up to 30 minutes at room temperature at slow shaking.

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Reagent	Blank	Positive Control	Inhibitor Control	Test Inhibitor
1x Immuno Buffer 1	2.5 µl	–	–	–
Cereblon (5 ng/µl)	2.5 µl	2.5 µl	2.5 µl	2.5 µl
BRD3(BD2) (2.5 ng/µl)	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Test Compound	–	–	–	2.5 µl
Compound buffer*	2.5 µl	2.5 µl	–	–
(+)JQ1 (10 µM)	–	–	2.5 µl	–
dBET1 (resuspended)	–	2.5 µl	2.5 µl	2.5 µl
Total	10 µl	10 µl	10 µl	10 µl

*Typically, 1x Immuno Buffer 1 with proper concentration of DMSO.

9) Dilute JQ1 (10 mM) in Compound Buffer (see above) to 10 µM. For the wells labeled as "Inhibitor Control", add 2.5 µl diluted JQ1.

10) Initiate reaction by adding 2.5 µl of diluted dBET1 prepared as described above to wells labeled “Positive Control", “Inhibitor Control” and "Test Inhibitor". Incubate at room temperature for one hour.

Note: Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:

1) Dilute anti-FLAG Acceptor beads (PerkinElmer #AL112C) 250-fold with 1x Immuno Buffer 1. Add 10 µl per well. Shake on a rotator platform for 60 minutes at room temperature.

Step 3:

1) Dilute Glutathione donor beads (PerkinElmer #6765300) 125-fold with 1x Immuno Buffer 1. Add 10 µl per well. Shake on a rotator platform for 30-60 minutes at room temperature.

2) Read Alpha-counts. “Blank” value should be subtracted from all readings.

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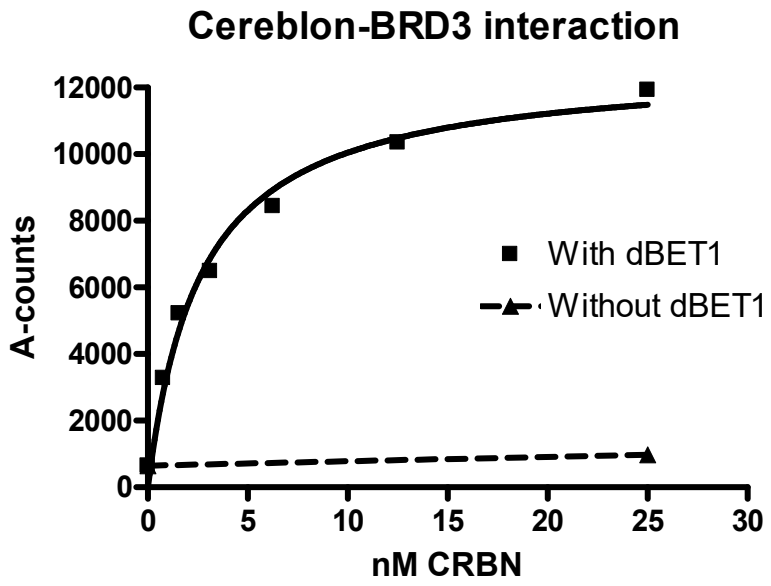
Examples of Assay Results:

Experiment 1. Titration of CRBN at fixed concentration of BRD3(BD2).



dBET1-mediated interaction of Cereblon with BRD3(BD2), measured using the *PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding*, BPS Bioscience #79770. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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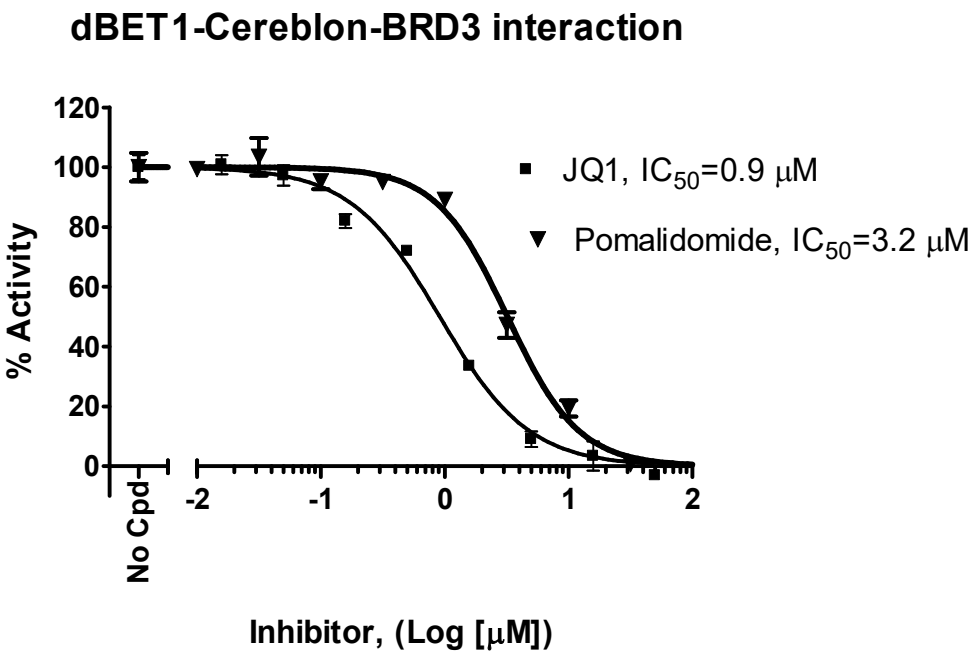
Experiment 2. Effect of BET bromodomain or CRBN inhibitors.



Inhibition by (+)-JQ1 (BPS Bioscience #27401) or Pomalidomide of dBET1-mediated interaction of Cereblon with BRD3(BD2), measured using the *PROTAC Optimization Kit for BET Bromodomain-Cereblon Binding*, BPS Bioscience #79770. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Cat. #	Size
BRD3(BD2), GST-tag	31033	100 µg
3x Immuno Buffer 1	79311	50 ml
RediSolution™ (+)-JQ1	27403	1 mg
(+)-JQ1	27401	1 mg
BRD3 (BD1+BD2), GST-tag	31035	100 µg
BRD3 (BD1), GST-tag	31032	100 µg
BRD2 (BD1+BD2), GST-tag	31024	100 µg
BRD4 (BD1+BD2), GST-tag	31044	100 µg
Cereblon/DDB1/Cul4A/Rbx1 Complex	100329-1	10 µg
ELOB/ELOC/VHL Complex	100361-1	10 µg
VHL/CUL2/ELOB/ELOC/RBX1 Complex	100373-1	10 µg

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