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Data Sheet
PROTAC Optimization Kit for BET Bromodomain-Von Hippel Lindau (VHL) Binding

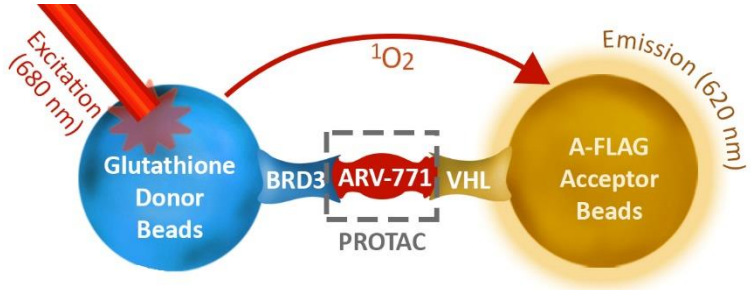
Catalog #79790
Size: 384 reactions

DESCRIPTION: The *PROTAC Optimization Kit for BET Bromodomain-Von Hippel Lindau (VHL) Binding* is designed for testing and profiling PROTACs directed against the BET Bromodomain family and VHL complex. VHL is a substrate recognition component of E3 protein ligase complex and is linked by Elongin C to a heterodimeric Cul2/Rbx1 module, which functions as a potent activator of the ubiquitination of target proteins by an E2 conjugating enzyme. Elongin B interacts with the complex through Elongin C and stabilizes the binding of Elongin C to VHL. Mutations in VHL are associated with the inherited von Hippel-Lindau cancer syndrome and numerous forms of renal cell carcinoma.

The *PROTAC Optimization Kit for BET Bromodomain-VHL Binding* comes in a convenient AlphaLISA® format, with ARV-771 PROTAC, purified BRD3(BD2) and VHL proteins, and buffer 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 VHL 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	
100373	VHL Complex, FLAG-tag	25 µg	-80°C	***Avoid Freeze/ Thaw Cycles***
31033	BRD3(BD2), GST-tag	10 µg	-80°C	
	ARV-771 (MW = 987 Da)	10 µg	-80°C	
79311	3x Immuno Buffer 1	4 ml	-80°C	
27403	(+)-JQ1 (10 mM)	20 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 identifying and optimizing PROTACs targeting BET family of bromodomains, design of novel molecules targeting VHL, and comparison of 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: Fisher, S.L., Phillips, A.J. *Curr Opin Chem Biol* 2018; **44**:47-55.

ASSAY PROTOCOL 1 -- Optimization of Bromodomain-VHL Binding

This protocol is designed to test the binding affinity of various PROTAC samples to the bromodomain or VHL 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 of 3x Immuno Buffer 1 plus 2 parts of 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 ARV-771 with 100 µl of DMSO to prepare 100 µM stock solution. Gently pipet up and down to ensure the ARV-771 is thoroughly dissolved. Store any unused ARV-771 in single use aliquots at -80°C. Before the experiment, add 980 µ l of 1x Immuno Buffer 1 to 20 µ l of 100 µM stock to make a 2 µM solution. Do not store or re-use diluted ARV-771. Note: the final concentration of ARV-771 in the assay may be in the range 0.1-0.5 µM.

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

4) Dilute VHL in 1X Immuno Buffer 1 at 24 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 8 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 VHL (24 ng/µl) + 2.5 µl BRD3(BD2) (8 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
VHL (24 ng/µl)	2.5 µl	2.5 µl	2.5 µl
BRD3(BD2) (8 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
ARV-771 (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 ARV-771 to each well designated “Positive Control”.

8) Incubate at room temperature for at least 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.

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Step 3:

1) Dilute Glutathione donor beads (PerkinElmer #6007290) 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.

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

2) Resuspend tube with the ARV-771 as in Protocol 1.

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

4) Dilute VHL in 1X Immuno Buffer 1 at 24 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 8 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 VHL (24 ng/µl) + 2.5 µl BRD3(BD2) (8 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.

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8) Add 2.5 µl of tested 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 proper concentration of DMSO as Compound buffer.* The final concentration of DMSO in the assay should be ≤1%.

Reagent	Blank	Positive Control	Inhibitor Control	Test Inhibitor
1x Immuno Buffer 1	2.5 µl	–	–	–
VHL (24 ng/µl)	2.5 µl	2.5 µl	2.5 µl	2.5 µl
BRD3(BD2) (8 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	–
ARV-771 (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.

Optional: If preincubation of the test compound with the VHL and BRD3 is desired, the plate can be incubated at this step for up to 30 minutes at room temperature with slow shaking.

10) Initiate reaction by adding 2.5 µl of diluted ARV-771 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.

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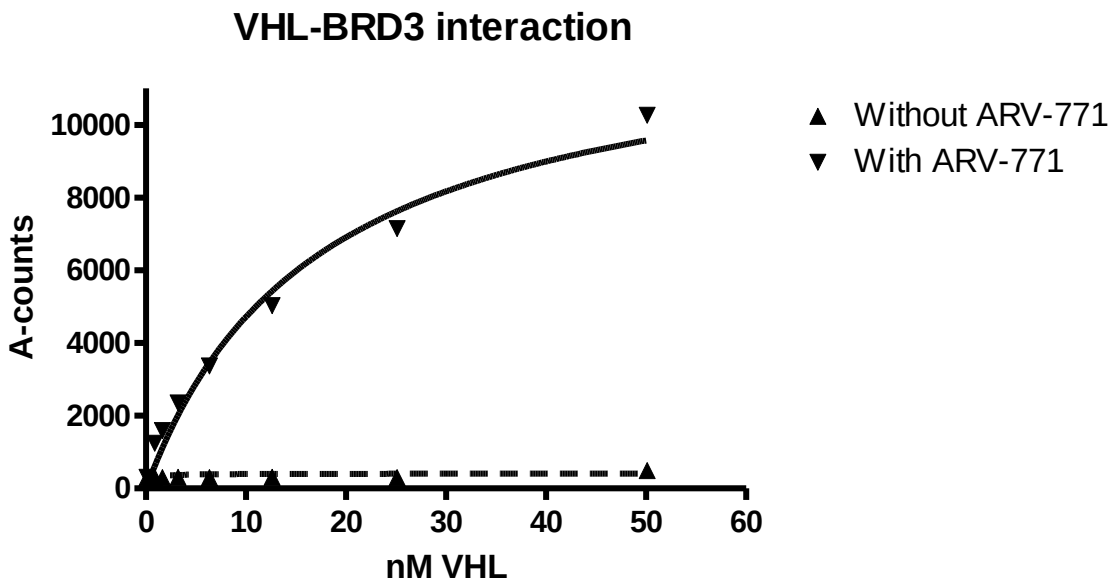
1) Dilute Glutathione donor beads (PerkinElmer #6007290) 125-fold with 1x Immuno Buffer 1. Add 10 µl per well. Shake on a rotator platform for 30-60 minutes at room temperature.

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

Examples of Assay Results:

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



ARV-771-mediated interaction of VHL with BRD3(BD2), measured using the *PROTAC Optimization Kit for BET Bromodomain-VHL Binding*, BPS Bioscience #79790. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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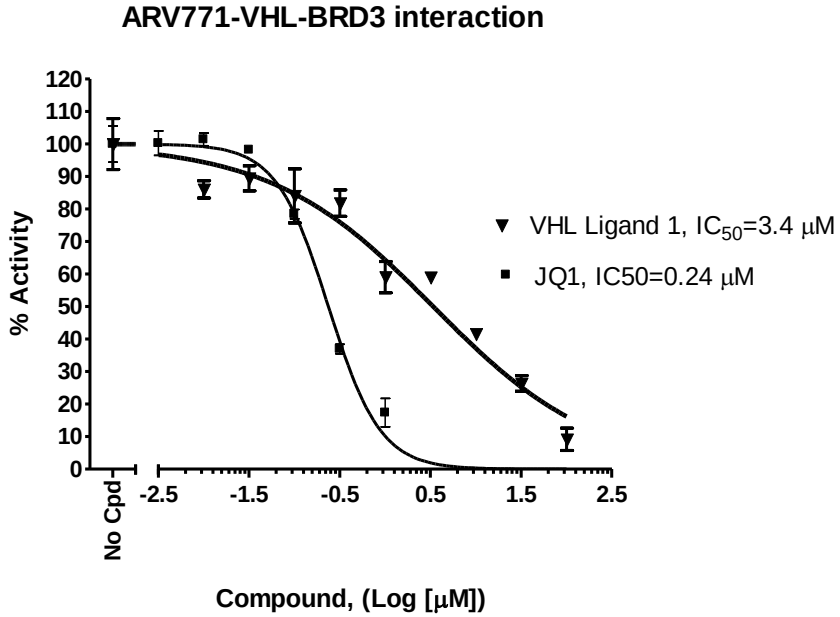
Experiment 2. Effect of BET bromodomain or VHL inhibitors.



Inhibition by (+)-JQ1 (BPS Bioscience #27401) or VHL Ligand 1 of the ARV-771-mediated interaction of VHL with BRD3(BD2), measured using the *PROTAC Optimization Kit for BET Bromodomain-VHL Binding*, BPS Bioscience #79790. *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
VHL/CUL2/ELOB/ELOC/RBX1 Complex	100373-1	10 µg
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
PROTAC Optimization Kit for BET BRD-Cereblon Binding	79770	384 rxns.

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