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Data Sheet

BRD1 Inhibitor Screening Assay Kit

Catalog # 32521
Size: 384 reactions

DESCRIPTION: The *BRD1 Inhibitor Screening Assay Kit* is designed to measure the inhibition of BRD1 binding to its substrate. The *BRD1 Inhibitor Screening Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone peptide substrate, assay buffer, detection buffer and purified GST-tagged BRD1 bromodomain to perform a total of 384 enzyme reactions. The key to the *BRD1 Inhibitor Screening Assay Kit* is the specific binding of the BRD1 bromodomain to the acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing BRD1 and an inhibitor of choice is incubated with the biotinylated substrate for thirty minutes. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
31011	BRD1 (561-668), GST-tag	24 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Ligand 1	400 µl	-80°C	
	Non-acetylated BET Ligand 1	200 µl	-80°C	
33007	3x BRD Homogeneous Assay Buffer 2	4 ml	-20°C	
33006	3x BRD Homogeneous Detection Buffer 2	3 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Glutathione AlphaLISA Acceptor Beads, 5 mg/ml (PerkinElmer #AL109C)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002)
Optiplate-384 (PerkinElmer #6007290)
AlphaScreen microplate reader
Adjustable micropipettor and sterile tips

APPLICATIONS: Useful for the study of bromodomain binding assays, screening inhibitors and selectivity profiling.

CONTRAINDICATIONS: Keep DMSO levels below 0.5%. 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 >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen assays.

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STABILITY: At least one year from date of receipt when stored as directed.

REFERENCE(S): Filippakopoulos, P., *et al., Cell* 2012; **149**:214.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate. Use slow shaking for all incubations.

Step 1:

1) Prepare the master mixture: N wells × (2.5 µl **3x BRD Homogeneous Assay Buffer 2** + 1 µl **BET Ligand 1** + 1.5 µl **H_2O**).

2) Add 5 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 2.5 µl **3x BRD Homogeneous Assay Buffer 2** + 1.5 µl **H_2O** +1 µl of **Non-acetylated BET Ligand 1.**

3) Thaw **BRD1** on ice. Upon first thaw, briefly spin tube containing protein to recover full content of the tube. Aliquot protein into single use aliquots. Store remaining undiluted protein in aliquots at -80°C immediately. *Note: **BRD1** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

4) Dilute **BRD1** in **1x BRD1 Homogeneous Assay Buffer 2** at 24 ng/µl. Keep diluted protein on ice until use. Discard any unused diluted protein after use.

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x BRD Homogeneous Assay Buffer 2	2.5 µl	2.5 µl	2.5 µl	2.5 µl
BET Ligand 1	1 µl	–	1 µl	1 µl
Non-acetylated BET Ligand 1	-	1 µl	-	-
H_2O	1.5 µl	1.5 µl	1.5 µl	1.5 µl
Test Inhibitor/Activator	–	–	–	2.5 µl
Inhibitor buffer (no inhibitor)	2.5 µl	2.5 µl	2.5 µl	–
1x BRD Homogeneous Assay Buffer 2	2.5 µl			
BRD1 (24 ng/µl)	–	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

5) Add 2.5 µl of **test inhibitor solution** to each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2.5 µl of the same solution without inhibitor (**inhibitor buffer**). *Note: Keep DMSO concentration below 0.5 %.*

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6) Add 2.5 µl of **1x BRD Homogeneous Assay Buffer 2** to the well designated "Blank".

7) Initiate reaction by adding 2.5 µl of diluted **BRD1** prepared as described above. Incubate at room temperature for 30 minutes.

Step 2:

Note: Protect your samples from direct exposure to light!

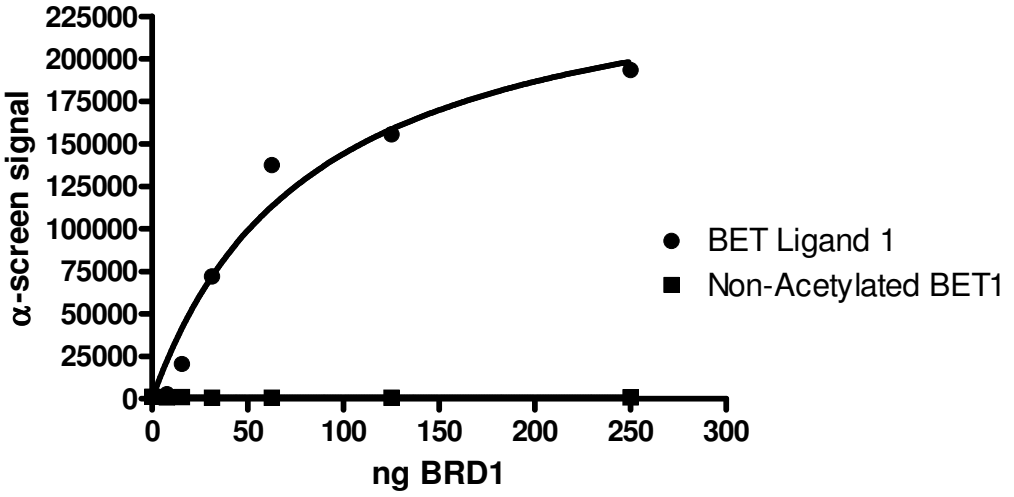
1) Dilute Glutathione AlphaLISA Acceptor Beads (PerkinElmer #AL109C) 250-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Incubate at room temperature for 15 - 30 minutes.

2) Read Alpha-counts.

Example of Assay Results:



BRD1 binding activity, measured using the *BRD1 Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog # 32521. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
BRD1 (561-668), GST-tag	31011	100 µg
BRD1 (561-668), His-tag	31010	100 µg
ATAD2A (981 – 1108), His-tag*	31109	100 µg
ATAD2B (953 – 1080), His-tag	31117	100 µg
BAZ2B (2054 – 2168), His-tag	31113	100 µg
BRD2 (339 – 459), His-tag*	31020	100 µg
BRD3 (29 – 145), His-tag*	31030	100 µg
BRD3 (306 – 417), His-tag*	31031	100 µg
BRD4 (49 – 170), His-tag*	31042	100 µg
BRD4 (342 – 460), His-tag*	31043	100 µg
BRD9 (135 – 242), His-tag	31090	100 µg
BRDT (22 – 138), His-tag*	31101	100 µg
BRDT (257 – 382), His-tag	31100	100 µg
BRD2 (BD2) Inhibitor Screening Kit	32522	384 rxns.
BRD3 (BD1) Inhibitor Screening Kit	32513	384 rxns.
BRD3 (BD2) Inhibitor Screening Kit	32523	384 rxns.
BRD4 (BD1) Inhibitor Screening Kit	32514	384 rxns.
BRD4 (BD2) Inhibitor Screening Kit	32524	384 rxns.
BRD9 Inhibitor Screening Kit	32519	384 rxns.
BAZ2B Inhibitor Screening Kit	32600	384 rxns.
(+)-JQ1 Bromodomain Inhibitor	27400	10 mg

*Also available with GST-tag