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Data Sheet

BRD2 (BD2) Inhibitor Screening Assay Kit

Catalog # 32522

DESCRIPTION: The *BRD2 (BD2) Inhibitor Screening Assay Kit* is designed to measure the inhibition of BRD2 bromodomain 2 (BD2) from binding to its substrate. The *BRD2* (BD2) *Inhibitor Screening Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone peptide substrate, assay buffer, detection buffer and purified, GST-tagged BRD2 BD2 to perform a total of 384 enzyme reactions. The key to the *BRD2 (BD2) Inhibitor Screening Assay Kit* is the highly specific binding of the BRD2 bromodomain 2 to the acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing BRD2 bromodomain 2 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	
31023	BRD2 (339-459), BD2, GST-tag	20 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand	400 µl	-80°C	
	Non-acetylated Ligand 1	200 µl	-80°C	
33001	3x BRD Homogeneous Assay Buffer 1	4 ml	-20°C	
33002	3x BRD Homogeneous Detection Buffer 1	3 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA® GSH acceptor beads, 5 mg/ml (PerkinElmer #AL109C)
AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002S)
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: Only limited amounts of DMSO can be included, as it has been shown to disrupt BRD-ligand interaction. Avoid 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.

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

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REFERENCE: McBride, A.A., *et al., Trends Microbiol*. 2004; **12**(12):527.

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

Step 1:

1) Dilute BET Bromodomain Ligand in H_2O at 1/4 (Add 100 µl BET Bromodomain Ligand to 300 µl H2O) and use it for the following steps.

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

3) Thaw **BRD2 (BD2)** on ice. Upon first thaw, briefly spin tube containing protein to recover full content of the tube. Aliquot both proteins into single use aliquots. Store remaining undiluted protein in aliquots at -80°C immediately. *Note: **BRD2** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

4) Dilute **BRD2 (BD2)** in **1x BRD Homogeneous Assay Buffer 1** at 4 ng/µl. Keep diluted proteins on ice until use. Discard any unused diluted protein after use.

   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 1** + 1 µl **Non-acetylated Ligand 1** + 1.5 µl **H_2O**.

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x BRD Homogeneous Assay Buffer 1	2.5 µl	2.5 µl	2.5 µl	2.5 µl
Diluted BET Bromodomain Ligand	1 µl	–	1 µl	1 µl
Non-acetylated 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	2.5 µl			
BRD2 (BD2) (4 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 **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 %.*
6) Add 2.5 µl of **1x BRD Homogeneous Assay Buffer 1** to the well designated "Blank".

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7) Initiate reaction by adding 2.5 µl of diluted **BRD2 (BD2)** prepared as described above to each well labeled "Positive Control", "Test Inhibitor", and "Substrate Control". Incubate at room temperature for 30 minutes.

Step 2:

Note: Protect your samples from direct exposure to light!

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

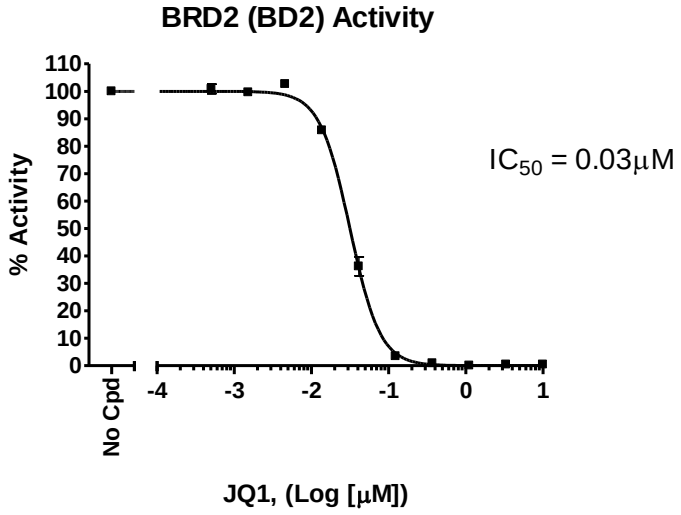
Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 250-fold with 1x BRD Homogeneous Detection Buffer 1. Add 10 µl per well. Incubate at room temperature for 15 – 30 minutes.

2) Read Alpha-counts.

Due to lot to lot variability in AlphaScreen® *bead performance, it may be necessary to optimize assay conditions. For example, slight adjustments to bromodomain or ligand concentrations may improve signal-to-noise ratio.*

Example of Assay Results:



BRD2 (BD2) binding activity, measured using the BRD2 (BD2) Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #32522 and (+)-JQ1 Inhibitor, Catalog #27400. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com.*

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RELATED PRODUCTS:

Product Name	Catalog	Size
BRD2 (339 – 459), GST-tag	31023	100 µg
BET Bromodomain Ligand	33000	0.5 mL
Bromodomain Non-acetylated Ligand 1	33005	0.5 mL
ATAD2A (981 – 1108), His-tag*	31109	100 µg
ATAD2B (953 – 1080), His-tag*	31117	100 µg
BAZ2B (2054 – 2168), His-tag	31113	100 µg
BRD1 (561 – 668), His-tag*	31010	100 µg
BRD2 (65 – 187), His-tag*	31022	100 µg
BRD2 (65 – 459), His-tag*	31025	100 µg
BRD3 (29 – 145), His-tag*	31030	100 µg
BRD3 (306 – 417), His-tag*	31031	100 µg
BRD3 (29 – 417), His-tag*	31034	100 µg
BRD4 (49 – 170), His-tag*	31042	100 µg
BRD4 (342 – 460), His-tag*	31043	100 µg
BRD4 (49 – 460), His-tag*	31045	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
BRDT (22 – 382), His-tag*	31061	100 µg
BRG1 (1480 – 1603), His-tag*	31102	100 µg
BRPF1 (627 – 746), His-tag	31112	100 µg
CREBBP (1081 – 1197), His-tag	31119	100 µg
GCN5 (727 – 837), His-tag	31114	100 µg
P300 (1046 – 1163), His-tag	31118	100 µg
PB1 (528 – 618), His-tag	31122	100 µg
PCAF (720 – 832), His-tag	31120	100 µg
SMARCA2 (1375 – 1511), His-tag	31111	100 µg
TAF1 (1400 – 1518), His-tag	31123	100 µg
TAF1 (1519 – 1657), His-tag*	31110	100 µg
TAF1L (1400 – 1651), GST-tag	31124	100 µg
TAF1L (1398 – 1516), His-tag*	31103	100 µg
TAF1L (1517 – 1649), His-tag*	31104	100 µg
TRIM24 (TIF1), 896 – 1014	31116	100 µg
WDR9 (1308 – 1436)	31115	100 µg
ATAD2A Inhibitor Screening Kit	32601	384 rxns.
ATAD2B Inhibitor Screening Kit	32605	384 rxns.
BAZ2B Inhibitor Screening Kit	32600	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.
TAF1 (BD1+BD2) Inhibitor Screening Kit	32604	384 rxns.
TAF1L (BD2) Inhibitor Screening Kit	32602	384 rxns.
TAF1L (BD1+BD2) Inhibitor Screening Kit	32603	384 rxns.
(+)-JQ1 Inhibitor	27401	1 mg

**Also available with GST-tag*