Data Sheet

BRD4 (BD1) Inhibitor Screening Assay Kit

Catalog # 32514

DESCRIPTION: The *BRD4 (BD1) Inhibitor Screening Assay Kit* is designed to measure the inhibition of BRD4 bromodomain 1 (BD1) from binding to its substrate. The *BRD4* (BD1) *Inhibitor Screening Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone peptide substrate, assay buffer, detection buffer and purified, GST-tagged BRD4 BD1 to perform a total of 384 enzyme reactions. The key to the *BRD4 (BD1) Inhibitor Screening Assay Kit* is the highly specific binding of the BRD4 bromodomain 1 to the acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing BRD4 bromodomain 1 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	
31040	BRD4 (49-170), BD1, GST-tag	20 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand	400 µl	-80°C	
	Non-acetylated Ligand	200 µl	-80°C	
33001	3x BRD assay buffer	4 ml	-20°C	
33002	3x Detection buffer	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: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: Only limited amounts of DMSO can be included, as it has been shown to disrupt BRD-ligand interaction (see **Assay Results**). 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.

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) Prepare the master mixture: N wells × (2.5 µl **3× BRD assay buffer** + 1 µl **BET Bromodomain Ligand** + 1.5 µl **H_2O**).

2) Thaw **BRD4 (BD1)** 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: **BRD4** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

3) Dilute **BRD4 (BD1)** in **1x BRD assay buffer** at 16 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 **3× BRD assay buffer** + 1 µl **Non-acetylated Ligand** + 1.5 µl **H_2O**.

	Blank	Substrate Control	Positive Control	Test Inhibitor
3x BRD assay buffer	2.5 µl	2.5 µl	2.5 µl	2.5 µl
BET Bromodomain Ligand	1 µl	–	1 µl	1 µl
Non-acetylated Ligand	-	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 assay buffer	2.5 µl			
BRD4 (BD1) (16 ng/µl)*	–	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

4) 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 %. See figure below.*

5) Add 2.5 µl of **1x BRD assay buffer** to the well designated "Blank".

6) Initiate reaction by adding 2.5 µl of diluted **BRD4 (BD1)** prepared as described above. Incubate at room temperature for 30 minutes.

Step 2:

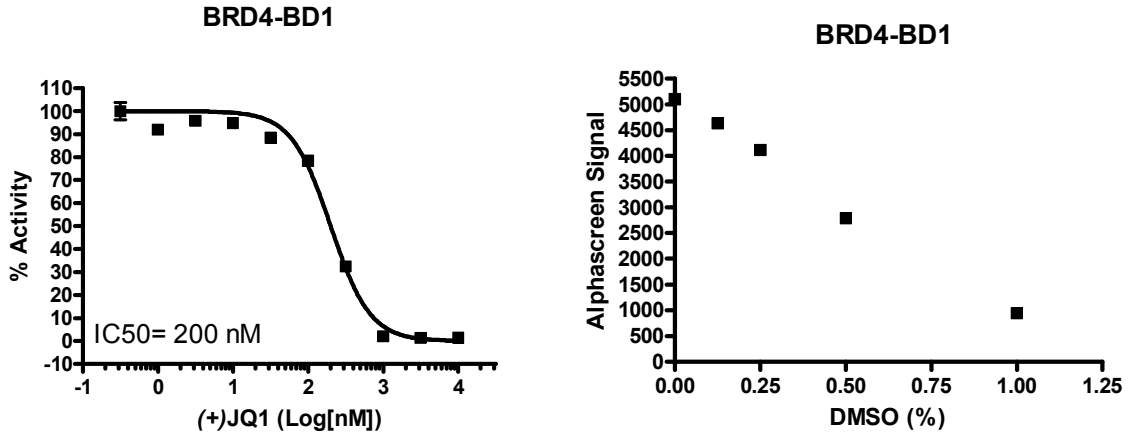
Note: Protect your samples from direct exposure to light!

1) Dilute GSH Acceptor beads (PerkinElmer #AL109C) 250-fold with 1x Detection buffer. 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 Detection buffer. Add 10 µl per well. Incubate at room temperature for 15 – 30 minutes.
2) Read Alpha-counts.

Example of Assay Results:



Left: BRD4 (BD1) binding activity, measured using the BRD4 (BD1) Inhibitor Screening Assay Kit, AMS Biotechnology, Cat #32514 and (+)-J Q1 Inhibitor, Catalog #27400. Right: Effect of DMSO in the assay mixture. *Data shown is lot-specific.*

RELATED PRODUCTS:

Product Name	Catalog #	Size
(+)-JQ1	27400	10 mg
BRD2 (339 – 459), His-tag	31020	100 µg
BRD2 (65 – 187), GST-tag	31021	100 µg
BRD3 (29 – 145), His-tag	31030	100 µg
BRD3 (29 – 145), GST-tag	31032	100 µg
BRD3 (306 – 417), His-tag	31031	100 µg
BRD3 (306 – 417), GST-tag	31033	100 µg
BRD4 (49 – 170), GST-tag	31040	100 µg
BRD4 (342 – 460), GST-tag	31041	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), GST-tag	31108	100 µg
BRDT (22 – 138), His-tag	31101	100 µg
BRDT (257 – 382), His-tag	31100	100 µg
BET Bromodomain Ligand	33000	0.5 mL
BRD4 (BD2) Inhibitor Screening Kit	32524	384 rxns.
BRD3 (BD1) Inhibitor Screening Kit	32513	384 rxns.
BRD3 (BD2) Inhibitor Screening Kit	32523	384 rxns.