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Data Sheet

BRD4 (BD1) Inhibitor Screening Assay Kit

Catalog # w42525

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	
w41051	BRD4 (49-170), BD1, GST-tag	20 µg	-80 °C	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand (BRD4-BD1)	400 µl	-80 °C	
	Non-acetylated Ligand	200 µl	-80 °C	
w43012	3x BRD assay buffer	4 ml	-20 °C	
w43013	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: 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) 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 1.6 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) (1.6 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 %.*

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

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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!

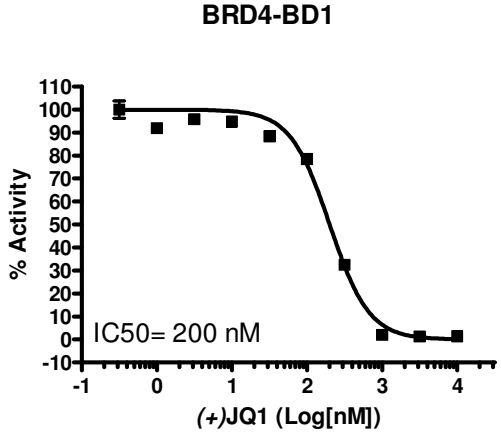
1) Dilute GSH Acceptor beads (PerkinElmer #AL109C) 250-fold with 1x Detection buffer. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

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.

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:



BRD4 (BD1) binding activity, measured using the BRD4 (BD1) Inhibitor Screening Assay Kit, West Bioscience, Catalog #w42525 and (+)-JQ1 Inhibitor, Catalog #w37411. *Data shown is lot-specific. For lot-specific information, please contact West Bioscience, Inc. at sale@westbioscience.com.*

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