9891 Irvine Center Dr. Suite 200
Irvine, CA 92618
United States
Tel: 1.800.831.1518
Fax: 1.800.831.1518
Email: sale@westbioscience.com

Data Sheet

BRD2 (BD2) Inhibitor Screening Assay Kit

Catalog # w42533

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	
w41034	*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*	
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) *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* **3× BRD assay buffer** *+ 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 assay buffer** *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 **3× BRD assay buffer** *+ 1 µl* **Non-acetylated Ligand 1** *+ 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*
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 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 %.*

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

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!

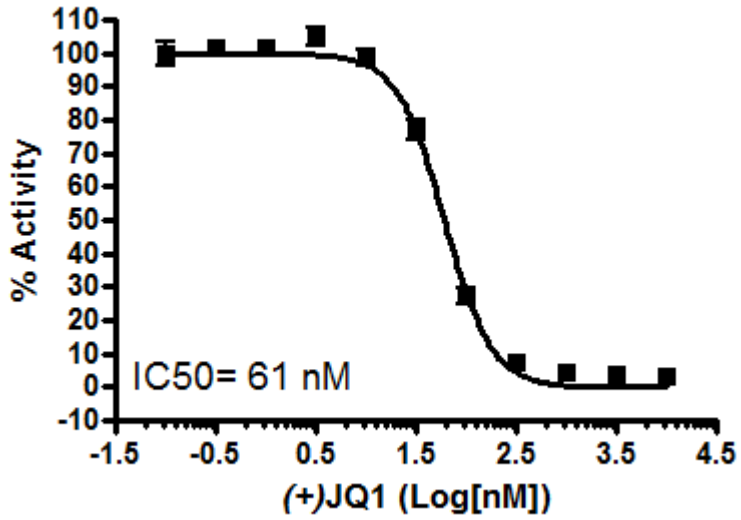
1) *Dilute GSH Acceptor beads (PerkinElmer #AL109C) 250-fold with 1x Detection buffer. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.*

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:



BRD2 (BD2) binding activity, measured using the BRD2 (BD2) Inhibitor Screening Assay Kit, West Bioscience, Catalog #w42533 and (+)-JQ1 Inhibitor, Catalog #w37411 . Data shown

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