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Data Sheet **BRD9 Inhibitor Screening Assay Kit** Catalog # 32519

DESCRIPTION: The *BRD9 Inhibitor Screening Assay Kit* is designed to measure the inhibition of BRD9 binding to its substrate. The *BRD9 Inhibitor Screening Assay Kit* comes in a convenient AlphaLISA[®] format, with biotinylated histone peptide substrate, assay buffer, detection buffer and purified GST-tagged BRD9 bromodomain to perform a total of 384 enzyme reactions. The key to the *BRD9 Inhibitor Screening Assay Kit* is the specific binding of the BRD9 bromodomain to an acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing BRD9 and an inhibitor of choice is incubated with the biotinylated substrate. Next, acceptor beads are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
31091	GST-BRD9 (135-242)	5 µg	-80°C	(Avoid freeze/ thaw cycles!)
33000	BET Bromodomain Ligand	400 µl	-80°C	
33005	Non-acetylated 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: DMSO concentrations above 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 (NaN₃) or metal ions (Fe²⁺, Fe³⁺, Cu²⁺, Zn²⁺ and Ni²⁺). 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: 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 Bromodomain Ligand** + 1.5 µl **H₂O**).
- 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 **H₂O** + 1 µl of **Non-acetylated Ligand 1**.
- 3) Thaw **BRD9** 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: **BRD9** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
- 4) Dilute **BRD9** in **1x BRD Homogeneous Assay Buffer 2** at 2 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 Bromodomain Ligand	1 µl	-	1 µl	1 µl
Non-acetylated Ligand 1	-	1 µl	-	-
H ₂ O	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			
BRD9 (2 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 μ l of **1x BRD Homogeneous Assay Buffer 2** to the well designated "Blank".
- 7) Initiate reaction by adding 2.5 μ l of diluted **BRD9** prepared as described above to the wells labeled "Positive Control", "Substrate Control", and "Test Inhibitor". Incubate at room temperature for 30 minutes.

Step 2:

Note: Protect your samples from direct exposure to light!

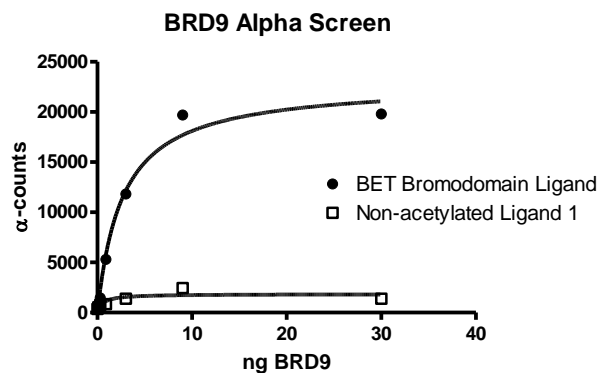
- 1) Dilute Glutathione AlphaLISA Acceptor Beads (PerkinElmer #AL109C) 250-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 μ l per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

- 1) Dilute Streptavidin-conjugated donor beads (PE #6760002) 125-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 μ l 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:



BRD9 binding activity, measured using the BRD9 Inhibitor Screening Assay Kit, BPS Bioscience, Catalog # 32519. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

<u>Product Name</u>	<u>Catalog #</u>	<u>Size</u>
BRD9 (135 – 242), GST-tag	31091	100 µg
BRD9 (135 – 242), His-tag	31090	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
BRDT (22 – 138), His-tag*	31101	100 µg
BRDT (257 – 382), His-tag	31100	100 µg
BET Bromodomain Ligand	33000	0.5 mL
BAZ2B Inhibitor Screening Kit	32600	384 rxns.
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.
(+)-JQ1 Bromodomain Inhibitor	27400	10 mg

*Also available with GST-tag

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