

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!) |
| | BET Bromodomain Ligand | 400 µl | -80 °C | |
| | Non-acetylated Ligand 1 | 200 µl | -80 °C | |
| | 3x BRD9 assay buffer | 4 ml | -20 °C | |
| | 3x BRD9 detection buffer | 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.

REFERENCE: Filippakopoulos, P., *et al.*, *Cell* 2012; **149**:214.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082**, Fax **1.858.481.8694**
Or you can Email us at: info@bpsbioscience.com
Please visit our website at: www.bpsbioscience.com

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× BRD9 assay buffer** + 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 **3× BRD9 assay buffer** + 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 **BRD9 assay buffer** 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 BRD9 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 | – | 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 BRD9 assay buffer | 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 %.*
- 6) Add 2.5 µl of **1x BRD9 assay buffer** to the well designated “Blank”.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082**, Fax **1.858.481.8694**
 Or you can Email us at: info@bpsbioscience.com
 Please visit our website at: www.bpsbioscience.com

- 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!

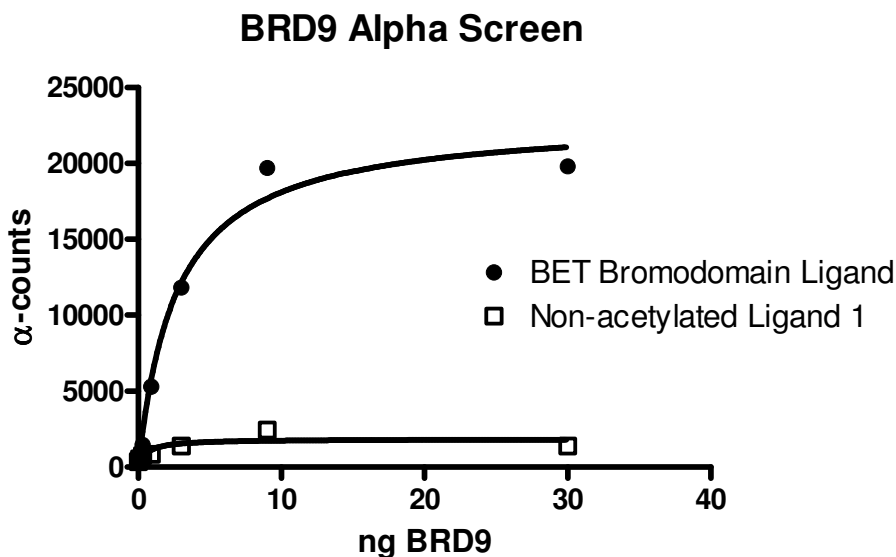
- 1) Dilute Glutathione AlphaLISA Acceptor Beads (PerkinElmer #AL109C) 250-fold with 1x BRD9 detection buffer. Add 10 μ l per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

- 1) Dilute Streptavidin-conjugated donor beads (PE #6760002) 125-fold with 1x BRD9 detection buffer. 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.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082**, Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com



6044 Cornerstone Ct. West, Ste. E
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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

AlphaScreen® and AlphaLISA® are registered trademarks of PerkinElmer, Inc.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

To place your order, please contact us by Phone **1.858.829.3082**, Fax **1.858.481.8694**

Or you can Email us at: info@bpsbioscience.com

Please visit our website at: www.bpsbioscience.com