

Data Sheet
ATAD2B Inhibitor Screening Assay Kit
Catalog # 32605

DESCRIPTION: The *ATAD2B Inhibitor Screening Assay Kit* is designed to measure the inhibition of ATAD2B binding to its substrate. The kit comes in a convenient AlphaLISA[®] format, with enough biotinylated histone peptide substrate, assay buffer, detection buffer and purified GST-tagged ATAD2B bromodomain to perform a total of 384 enzyme reactions. The key to the kit is the specific binding of the ATAD2B bromodomain to the acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing ATAD2B 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 | |
|-----------|---------------------------------------|--------|---------|------------------------------------|
| 31125 | GST-ATAD2B (953-1080) | 60 µg | -80 °C | (Avoid freeze/thaw cycles!) |
| | BET Bromodomain Ligand | 400 µl | -80 °C | |
| | 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 #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: 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.

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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 \times (2.5 μ l **3x BRD Homogeneous Assay Buffer 2** + 1 μ l **BET Bromodomain Ligand** + 1.5 μ l **H₂O**).
- 2) Thaw **ATAD2B** 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: ATAD2B is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
- 3) Dilute **ATAD2B** in **1x BRD Homogeneous Assay Buffer 2** to 50 ng/ μ l. Keep diluted protein on ice until use. Discard any unused diluted protein after use.
- 4) Add 5 μ l of master mixture to each well designated for the “Positive Control”, “Test Inhibitor”, and “Blank”. For the “Substrate Control”, add 2.5 μ l **3x BRD Homogeneous Assay Buffer 2** + 1.5 μ l **H₂O** + 1 μ l of **Non-acetylated Ligand 1**.

| | Blank | Substrate Control | Positive Control | Test Inhibitor |
|-----------------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| 3x BRD Homogeneous Assay Buffer 2 | 2.5 μ l | 2.5 μ l | 2.5 μ l | 2.5 μ l |
| BET Bromodomain Ligand | 1 μ l | – | 1 μ l | 1 μ l |
| Non-acetylated Ligand 1 | - | 1 μ l | - | - |
| H ₂ O | 1.5 μ l | 1.5 μ l | 1.5 μ l | 1.5 μ l |
| Test Inhibitor/Activator | – | – | – | 2.5 μ l |
| Inhibitor buffer (no inhibitor) | 2.5 μ l | 2.5 μ l | 2.5 μ l | – |
| 1x BRD Homogeneous Assay Buffer 2 | 2.5 μ l | | | |
| ATAD2B (50 ng/ μ l) | – | 2.5 μ l | 2.5 μ l | 2.5 μ l |
| Total | 10 μl | 10 μl | 10 μl | 10 μl |

- 5) Add 2.5 μ l of **inhibitor solution** to each well designated “Test Inhibitor”. For the “Positive Control”, “Substrate Control” and “Blank”, add 2.5 μ l of the same **solution without inhibitor** (inhibitor buffer). *Note: Keep DMSO concentration below 0.5 %.*
- 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 **ATAD2B** prepared as described above to the wells labeled “Positive Control”, “Test Inhibitor”, and “Substrate Control”. Incubate at room temperature for 30 minutes.

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Step 2:

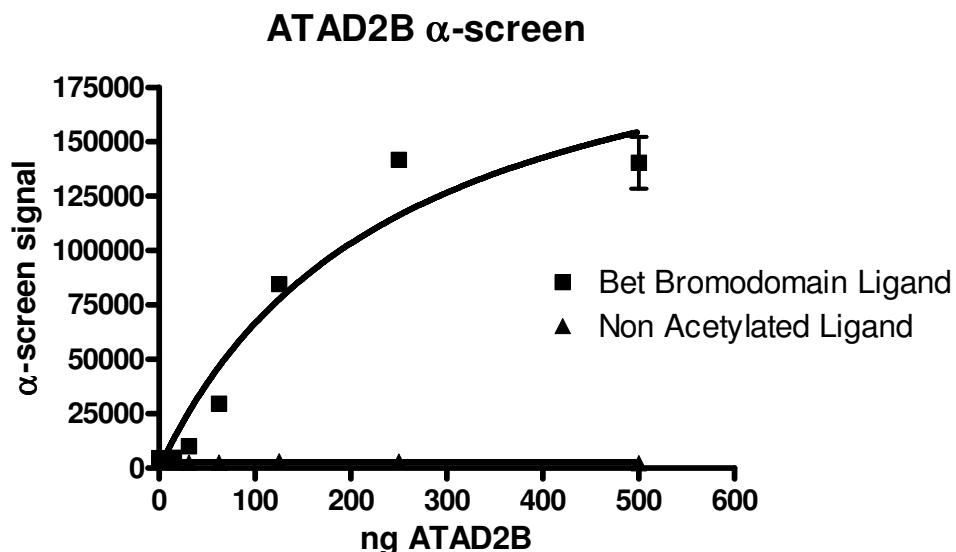
Note: Protect your samples from direct exposure to light!

- 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 #6760002S) 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:

ATAD2B binding activity, measured using the ATAD2B Inhibitor Screening Assay Kit, BPS Bioscience, Catalog #32605. *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> |
|--|-------------------------|--------------------|
| ATAD2B (953 – 1080), His-tag | 31117 | 100 µg |
| ATAD2A (981 – 1108), His-tag | 31109 | 100 µg |
| ATAD2A (981 – 1108), GST-tag | 31121 | 100 µg |
| BAZ2B (2054 – 2168), His-tag | 31113 | 100 µg |
| TAF1 (1400-1518), His-tag | 31123 | 100 µg |
| TAF1 (1519-1651), His-tag | 31110 | 100 µg |
| TAF1, BD1 and BD2 (1400-1651), GST-tag | 31124 | 100 µg |
| TAF1L (1398-1516), His-tag* | 31103 | 100 µg |
| TAF1L (1398-1649), GST-tag | 31107 | 100 µg |
| TAF1L (1517-1649), His-tag* | 31104 | 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 |
| BRD9 (135 – 242), His-tag | 31090 | 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 |
| TAF1 (BD1 + BD2) Inhibitor Screening Assay Kit | 32604 | 384 rxns |
| TAF1L (BD1 + BD2) Inhibitor Screening Assay Kit | 32603 | 384 rxns |
| TAF1L (BD2) Inhibitor Screening Assay Kit | 32602 | 384 rxns |
| ATAD2A Inhibitor Screening Kit | 32601 | 384 rxns. |
| 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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