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Data Sheet

TAF1 (BD2) Inhibitor Screening Assay Kit

Catalog # 32624
Size: 384 reactions

DESCRIPTION: The *TAF1 (BD2) Inhibitor Screening Assay Kit* is designed to measure the inhibition of TAF1 (BD2) binding to its substrate. The *TAF1 (BD2) Inhibitor Screening Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone peptide substrate, assay buffer, detection buffer and purified GST-tagged TAF1 bromodomain 2 to perform a total of 384 enzyme reactions. The key to the *TAF1 (BD2) Inhibitor Screening Assay Kit* is the specific binding of the TAF1 bromodomain 2 (a.a. 1519-1651) to the acetylated histone substrate. With this kit, only three simple steps on a microtiter plate are required. First, a sample containing *TAF1 (BD2)* 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	
31126	TAF1-BD2 (1519-1651), GST-tag	24 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Ligand 1	400 µl	-80°C	
	Non-acetylated BET 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: Keep DMSO levels below 0.5%. 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(S): 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 Ligand 1** + 1.5 µl **H_2O**).

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 **H_2O** +1 µl of **Non-acetylated Ligand 1.**

3) Thaw **TAF1-BD2** 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: **TAF1-BD2** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

4) Dilute **TAF1-BD2** in 1x **BRD Homogeneous Assay Buffer 2** at 24 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 Ligand 1	1 µl	–	1 µl	1 µl
Non-acetylated BET 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 Homogeneous Assay Buffer 2	2.5 µl			
TAF1-BD2 (24 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 **test 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 final DMSO concentration below 0.5%.*

6) Add 2.5 µl of **1x BRD Homogeneous Assay Buffer 2** to the well designated "Blank".

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7) Initiate reaction by adding 2.5 µl of diluted **TAF1-BD2** prepared as described above. Incubate at room temperature for 30 minutes.

Step 2:

Note: Protect your samples from direct exposure to light!

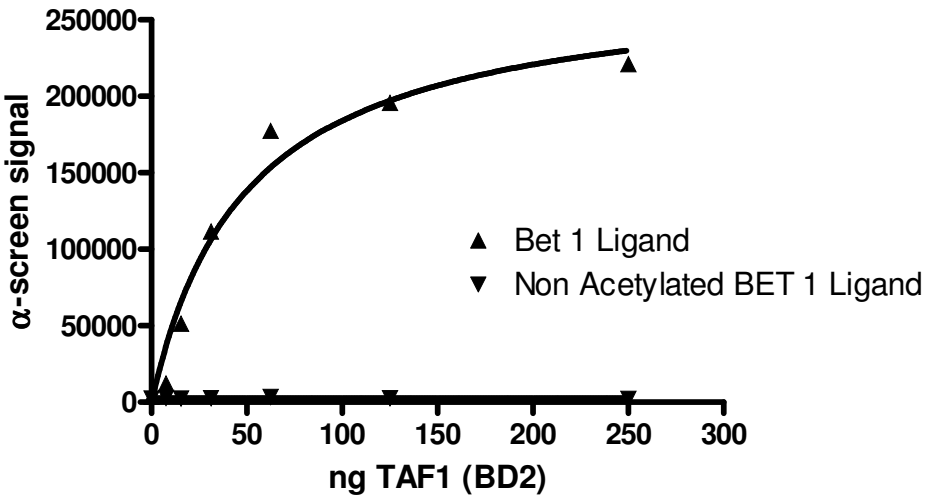
1) Dilute Glutathione AlphaLISA® Acceptor Beads (PerkinElmer #AL109C) 250-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Shake plate briefly. Incubate at room temperature for 30 minutes.

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PE #6760002S) 125-fold with **1x BRD Homogeneous Detection Buffer 2**. Add 10 µl per well. Incubate at room temperature for 15 - 30 minutes.

2) Read Alpha-counts.

Example of Assay Results:



TAF1-BD2 binding activity, measured using the *TAF1 (BD2) Inhibitor Screening Assay Kit*, BPS Bioscience, Catalog # 32624. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS:

Product Name	Catalog #	Size
TAF1 (1400-1518), His-tag	31123	100 µg
TAF1 (1519-1651), GST-tag	31126	100 µg
TAF1 (1519-1651), His-tag	31110	100 µg
TAF1, BD1 and BD2 (1400-1651), GST-tag	31124	100 µg
TAF1L (1398-1516), GST-tag	31105	100 µg
TAF1L (1398-1516), His-tag	31103	100 µg
TAF1L (1398-1649), GST-tag	31107	100 µg
TAF1L (1517-1649), GST-tag	31106	100 µg
TAF1L (1517-1649), His-tag	31104	100 µg
TAF1 (BD1 + BD2) Inhibitor Screening Kit	32604	384 rxns.
TAF1L (BD1 + BD2) Inhibitor Screening Kit	32603	384 rxns.
TAF1L (BD2) Inhibitor Screening Kit	32602	384 rxns.
(+)-JQ1 Bromodomain Inhibitor	27400	10 mg