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Data Sheet
TAF1 (BD1+BD2) TR-FRET Assay Kit
Catalog # 32628

DESCRIPTION:
The TAF1 (BD1+BD2) TR-FRET Assay Kit is designed to measure the inhibition of TAF1 (BD1+BD2) binding to its substrate in a homogeneous 384 reaction format. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The assay procedure is straightforward and simple; a sample containing trFluor^{Tm} europium (Eu)-labeled TAF1 (BD1+BD2), dye-labeled acceptor, substrate, and an inhibitor is incubated for 120 minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
	TAF1 (BD1+BD2)-Eu	3 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand	50 µl	-80°C	
	Non-acetylated Ligand 1	15 µl	-80°C	
	Dye-labeled acceptor	20 µl	-20°C	
33012	3x BRD TR-FRET Assay Buffer 1	4 ml	-20°C	
Fisher 07-200-330	White, Nonbinding Corning, low volume, microtiter plate	1	Room temp.	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
Adjustable micropipettor and sterile tips

APPLICATIONS: Great for screening small molecular inhibitors for drug discovery and HTS applications.

STABILITY: At least 6 months from date of receipt when stored as directed.

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

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

1) Dilute one part **3x BRD TR-FRET Assay Buffer 1** with 2 parts distilled water (3-fold dilution) to make **1x BRD TR-FRET Assay Buffer 1**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

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2) Dilute **Dye-labeled acceptor** 100-fold in **1x BRD TR-FRET Assay Buffer 1**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

3) Add 5 µl of **1x BRD TR-FRET Assay Buffer 1**, and 5 µl of diluted **Dye-labeled acceptor** to every well.

4) Add 2 µl of inhibitor solution to each well designated "Test Inhibitor". Add 2 µl of the same solution without inhibitor (inhibitor buffer) to the wells labeled "Substrate Control", and "Positive Control".

	Positive Control	Negative* Control	Test Inhibitor
Dye-labeled acceptor	5 µl	5 µl	5 µl
Test Inhibitor	–	–	2 µl
Inhibitor Buffer (no inhibitor)	2 µl	2 µl	–
BET Bromodomain Ligand	5 µl	–	5 µl
Non-acetylated Ligand 1	–	–	–
1x TAF1 (BD1+BD2) Buffer	5 µl	10 µl*	5 µl
TAF1 (BD1+BD2) (0.4 ng/µl)	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

*__Non-acetylated Ligand 1__ may be used as a substrate control in place of the negative control.

5) Thaw **BET Bromodomain Ligand** on ice. Upon first thaw, briefly spin tube containing enzyme to recover the full contents of the tube. Aliquot each ligand into single-use aliquots. Store remaining undiluted ligand at −80°C immediately. *Note: each ligand is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.*

6) Individually dilute **BET Bromodomain Ligand** 350-fold in **1x BRD TR-FRET Assay Buffer 1**. Add 5 µl of diluted **BET Bromodomain Ligand** to each well designated as "Positive Control" and "Test Inhibitor". Add 5 µl of **1x BRD TR-FRET Assay Buffer 1** to the wells labeled "Negative Control". *Note: if using **Non-acetylated Ligand 1**, dilute **Non-acetylated Ligand 1** 350-fold in **1x BRD TR-FRET Assay Buffer 1** and add 5 µl of diluted **Non-acetylated Ligand 1** to the "Negative Control" well in place of the 5 µl of **1x BRD TR-FRET Assay Buffer 1.***

7) Thaw **TAF1 (BD1+BD2)-Eu** bromodomain protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **TAF1 (BD1+BD2)-Eu** protein into single-use aliquots. Store remaining undiluted **TAF1 (BD1+BD2)-Eu** in aliquots at −80°C immediately. *Note: **TAF1 (BD1+BD2)-Eu** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

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8) Dilute **TAF1 (BD1+BD2)-Eu** in **1x BRD TR-FRET Assay Buffer 1** to 0.4 ng/µl (1.2 ng/reaction). Initiate reaction by adding 3 µl of diluted **TAF1 (BD1+BD2)-Eu** to every well. Discard any remaining diluted **TAF1 (BD1+BD2)-Eu** protein after use.

9) Incubate at room temperature for 2 hours.

10) Read the fluorescent intensity in a microtiter-plate reader capable of TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	320±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	320±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:

Two sequential measurements should be conducted. Eu-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

When percentage activity is calculated, the FRET value from the negative control can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\%\ Activity = \frac{FRET_S - FRET_{neg}}{FRET_P - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

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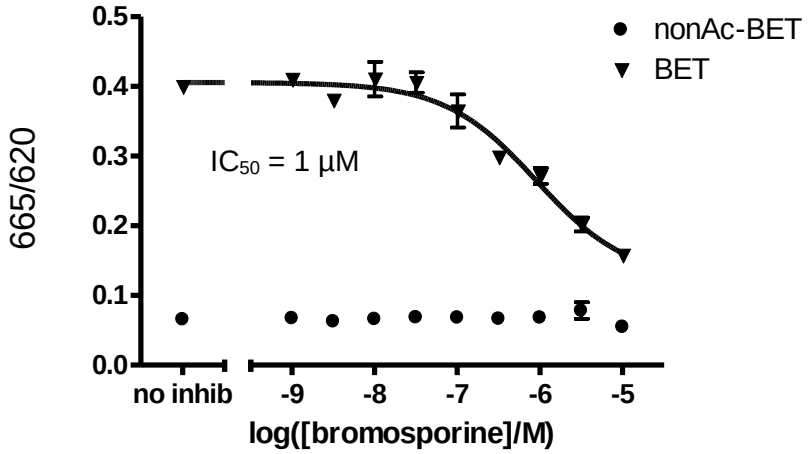
EXAMPLE OF ASSAY RESULTS:



Inhibition of TAF1 (BD1+BD2) with Bromosporine (BPS Bioscience Cat. #27612). Assay was done according to protocol for the TAF1 (BD1+BD2) TR-FRET Assay Kit (BPS Cat. #32628). *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
BET Bromodomain Ligand	33000	0.5 ml
Bromodomain Non-acetylated Ligand 1	33005	0.5 ml
TAF1L (1398-1649), GST-tag	31107	100 µg
TAF1L (1398-1516), GST-tag*	31105	100 µg
TAF1L (1517-1649), GST-tag*	31106	100 µg
TAF1 (1519-1651), GST-tag*	31126	100 µg
TAF1, BD1 and BD2 (1400-1651), GST-tag	31124	100 µg
TAF1 (1400-1518), His-tag	31123	100 µg
TAF1 (BD1+BD2) Inhibitor Screening Kit	32604	384 rxns.
TAF1 (BD2) Inhibitor Screening Kit	32624	384 rxns.
TAF1L (BD2) Inhibitor Screening Kit	32602	384 rxns.
TAF1L (BD1+BD2) Inhibitor Screening Kit	32603	384 rxns.
Bromosporine	27612	1 mg
(+)-JQ1 Inhibitor	27401	1 mg

*also available as His-tag

Note: Dye-labeled acceptor is a product of Cisbio Bioassays.