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Data Sheet
BPTF/FALZ TR-FRET Assay Kit
Catalog # 32632

DESCRIPTION:
The BPTF/FALZ TR-FRET Assay Kit is designed to measure the inhibition of the binding of BPTF, also known as FALZ, 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 terbium-labeled donor, dye-labeled acceptor, BPTF, substrate, and an inhibitor is incubated for 2 hours. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
31134	BPTF(FALZ), GST-tag	10 µg	-80°C	
	BET Bromodomain Ligand	50 µl	-80°C	
	Non-acetylated Ligand 1	15 µl	-80°C	***(Avoid freeze/ thaw cycles!)***
	Tb-labeleddonor	2x10 µl	-20°C	
	Dye-labeled acceptor	2x10 µl	-20°C	
	3x ATAD2A Assay Buffer	4 ml	-20°C	
	White Nonbinding 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):
1. Filippakopoulos, P., *et al.(2012). Cell* ; **149**:214.

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

1) Dilute one part **3x ATAD2A Assay Buffer** with 2 parts distilled water (3-fold dilution) to make **1x ATAD2A Assay Buffer**. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C.

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2) Dilute **Tb-labeled donor** and **Dye-labeled acceptor** 100-fold in **1x ATAD2A Assay Buffer**. Make only sufficient quantities needed for the assay; store remaining stock solution in aliquots at -20°C.

3) Add 5 µl of diluted **Tb-labeled donor**, 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**
Tb-labeled donor	5 µl	5 µl	5 µl
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 **ATAD2A** Buffer	–	5 µl*	–
BPTF (1 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 ligand 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** 40-fold in **1x ATAD2A Assay Buffer**. Add 5 µl of diluted **BET Bromodomain Ligand** to each well designated as "Positive Control" and "Test Inhibitor". Add 5 µl of **1x ATAD2A Assay Buffer** to the wells labeled "Negative Control". *Note: if using the **Non-acetylated Ligand 1**, dilute **Non-acetylated Ligand 1** 40-fold in **1x** **ATAD2A** **Assay Buffer** and add 5 µl of diluted **Non-acetylated Ligand 1** to the "Negative Control" well in place of the 5 µl of **1x** **ATAD2A** **Assay Buffer**.*

7) Thaw BPTF(FALZ), GST-tag on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot BPTF(FALZ), GST-tag into single-use aliquots. Store remaining undiluted **BPTF** in aliquots at −80°C immediately. *Note:* BPTF(FALZ), GST-tag *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
8) Dilute BPTF(FALZ), GST-tag in **1x ATAD2A Assay Buffer** to 1 ng/µl (3 ng/reaction). Initiate reaction by adding 3 µl of diluted BPTF(FALZ), GST-tag to every well. Discard any remaining diluted BPTF(FALZ), GST-tag after use.

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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	340±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:

Two sequential measurements should be conducted. Tb-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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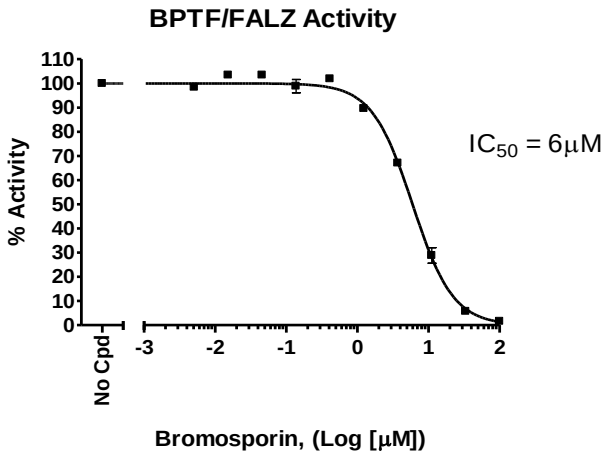
EXAMPLE OF ASSAY RESULTS:



Interaction of BPTF (BPS Bioscience Cat. #31134) with BET Ligand and inhibition by bromosporine (BPS Cat. #27612). Assay was done according to protocol for the BPTF TR-FRET Assay Kit (BPS Cat. #32632). *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Catalog #	Size
BET Bromodomain Ligand	33000	0.5 ml
Bromodomain Non-acetylated Ligand 1	33005	0.5 ml
BPTF (FALZ), GST-tag	31134	100 µg
BPTF (FALZ), His-tag	31131	100 µg
CECR2, GST-tag	31138	100 µg
CECR2, His-tag	31046	100 µg
PCAF (KAT2B), His-tag	31120	100 µg
GCN5 (727-837), His-tag	31114	100 µg
CECR2 Inhibitor Screening Kit	32611	384 rxns
CECR2 TR-FRET Assay Kit	32622	384 rxns
Bromosporine	27612	1 mg
(+)-JQ1 Inhibitor	27401	1 mg

Note: Tb-labeled donor and dye-labeled acceptor are products of Cisbio Bioassays.