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Data Sheet
TRIM24 TR-FRET Assay Kit
Catalog # 32630

DESCRIPTION:
The TRIM24 TR-FRET Assay Kit is designed to measure the inhibition of TRIM24 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 terbium-labeled donor, dye-labeled acceptor, TRIM24, substrate, and an inhibitor is incubated for 2 hours. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
31127	TRIM24 (TIF1), GST-tag	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	BET Bromodomain Ligand	75 µl	-80°C	
	Non-acetylated Ligand 1	15 µl	-80°C	
	Tb donor	20 µl	-20°C	
	Dye-labeled acceptor	20 µl	-20°C	
33013	3x BRD TR-FRET Assay Buffer 2	4 ml	-20°C	
	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.

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ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

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

2) Dilute **Tb-labeled donor** and **Dye-labeled acceptor** 100-fold in **1x BRD TR-FRET Assay Buffer 2**. 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 TRIM24 Buffer	–	5 µl*	–
TRIM24 (1 µg/ml)	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 BRD TR-FRET Assay Buffer 2**. 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 2** to the wells labeled "Negative Control". *Note: if using the **Non-acetylated Ligand 1**, dilute **Non-acetylated Ligand 1** 40-fold in **1x BRD TR-FRET Assay Buffer 2** and add 5 µl of diluted **Non-***

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acetylated Ligand 1 *to the "Negative Control" well in place of the 5 µl of* ***1x BRD TR-FRET Assay Buffer 2.***

7) Thaw **TRIM24** bromodomain protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **TRIM24** protein into single-use aliquots. Store remaining undiluted **TRIM24** in aliquots at −80°C immediately. *Note:* ***TRIM24*** *is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*
8) Dilute **TRIM24** in **1x BRD TR-FRET Assay Buffer 2** to 1 µg/ml (3 ng/reaction). Initiate reaction by adding 3 µl of diluted **TRIM24** to every well. Discard any remaining diluted TRIM24 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	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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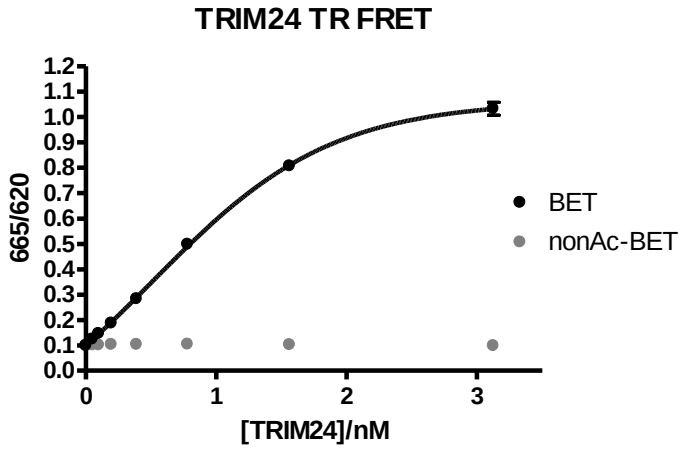
EXAMPLE OF ASSAY RESULTS:



Interaction of TRIM24 (BPS Bioscience Cat. #31127) with BET Ligand. Assay was done according to protocol for the TRIM24 Assay Kit (BPS Cat. #32630). *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
TRIM24, GST-tag	31127	100 µg
TRIM24, His-tag	31116	100 µg
TRIM28, GST-tag	31139	100 µg
TRIM28, His-tag	31146	100 µg
TRIM24 Inhibitor Screening Kit	32606	384 rxns.
Bromosporine	27612	1 mg
(+)-JQ1 Inhibitor	27401	1 mg

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