CREATIVE BIOMART®
Assay Kit

BPTF/FALZ TR-FRET Assay Kit

Product Information

Common Name

BPTF/FALZ

Cat.No.

Kit-1688

Description

The BPTF 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.

Applications

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

Storage

At least 6 months from date of receipt when stored as directed. Be sure to store each component at the proper temperature upon arrival.

Warning

Avoid freeze/thaw cycles.

Synonyms

BPTF; FALZ

UniProt ID

Q12830

Size

384 reactions

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Kit Components

BPTF(FALZ), GST-tag: 10 µg; -80°C
BET Bromodomain Ligand: 50 µl; -80°C
Non-acetylated Ligand 1: 15 µl; -20°C
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 Required but Not Supplied

Fluorescent microplate reader capable of measuring Time Resolved Fluorescence Resonance Energy Transfer (TR-FRET)
Adjustable micropipettor and sterile tips

Assay Protocol

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.
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*	–

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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), GSTtag 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.
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

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Lag Time 60 μs
Integration Time 500 μs

Analysis

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 = (FRETs - FRETneg)/(FRETp - FRETneg)
Where FRETs = Sample FRET, FRETneg = Negative control FRET, and FRETp = Positive control FRET.

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