CREBBP TR-FRET Assay Kit

#32619
384 reactions

Description

The CREBBP TR-FRET Assay Kit is designed to measure the inhibition of CREBBP 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: a terbium-labeled donor, dye-labeled acceptor, CREBBP protein, an acetylated BET Bromodomain Ligand, and test inhibitor are incubated together for 120 minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

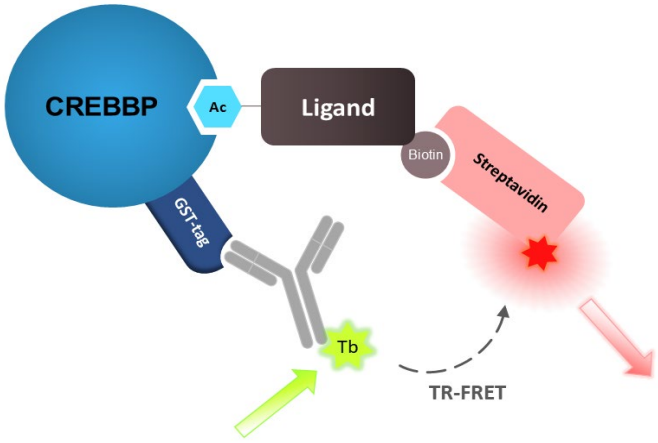

Illustration of the assay principle: The Donor Terbium-labeled donor binds to GST-tagged CREBBP. The acetylated (Ac) BET Bromodomain Ligand is labeled with biotin, which allows the dye-labeled streptavidin acceptor to bind to the ligand. The TR-FRET signal is generated by proximity induced upon interaction of CREBBP with the acetylated ligand. Inhibitors of the interaction will prevent TR-FRET from happening, decreasing the signal in a dose-dependent manner.

Background

Cyclic adenosine monophosphate Response Element Binding protein Binding Protein (CREB-binding protein, also known as CREBBP or CBP) is a transcriptional coactivator that possesses intrinsic acetyltransferase activity toward histones and transcription factors, thereby regulating gene transcription. CREBBP is implicated in cancer and represents an attractive therapeutic target. Interestingly, the association of CREBBP with β-catenin promotes cancer cell proliferation and correlates with disease aggressiveness. Due to the very large number of genes affected by CREBBP, it is also implicated in various neurological and psychiatric diseases, and in diabetes.

Applications

- Study enzyme kinetics
- Screen small molecular inhibitors in high throughput applications

Supplied Materials

Catalog #	Name	Amount	Storage
31128	CREBBP, GST*	10 µg	-80°C
33000	BET Bromodomain Ligand	50 µl	-80°C
33005	Non-acetylated Ligand 1	15 µl	-80°C
	Tb donor	2 x 10 µl	-20°C
	Dye-labeled acceptor	2 x 10 µl	-20°C
33012	3x BRD TR-FRET Assay Buffer 1	4 ml	-20°C
79969	White, Nonbinding, low volume, microtiter plate	1	Room Temperature

** The concentration of CREBBP is lot-specific and will be indicated on the tube containing the protein.*

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Materials Required but Not Supplied

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

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

Safety

This product is for research purposes only and not for human or therapeutic use. This product should be considered hazardous and is harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly.

Contraindications

The final concentration of DMSO in the assay should not exceed 0.2%. Higher DMSO concentrations can significantly decrease the quality of the results.

Assay Protocol

All samples and controls should be performed in triplicate.

1. Dilute 3x **BRD TR-FRET Assay Buffer** 3-fold by adding one part stock buffer to two parts distilled water. Prepare only the amount needed for the assay. Store the remaining stock solution at -20°C.

2. Dilute Tb-labeled donor and Dye-labeled acceptor 100-fold in 1x BRD TR-FRET Assay Buffer 1. Prepare only the amount needed for the assay. Store the remaining in aliquots at -20°C.

3. Add 5 µl of diluted Tb-labeled donor and 5 µl of diluted Dye-labeled acceptor to each well designated "Test Inhibitor," "Negative Control," and "Positive Control."

4. Prepare the Test Inhibitor (2 µl/well): for a titration, prepare serial dilutions at concentrations 10-fold higher than the desired final concentrations. The final volume of the reaction is 20 µl
   a) If the Test Inhibitor is water-soluble, prepare serial dilutions in the 1x BRD TR-FRET Assay Buffer, 10-fold more concentrated than the desired final concentrations. For the positive and negative controls, use 1x Assay Buffer (Diluent Solution).

   OR

   b) If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 100-fold the highest desired concentration in DMSO, then dilute the inhibitor 50-fold in 1x BRD TR-FRET Assay Buffer to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 2%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 2% DMSO in Assay Buffer to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 2% DMSO in Assay Buffer (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   The final concentration of DMSO in the assay should not exceed 0.2%.

5. Add 2 µl of Test Inhibitor serial dilutions to each well designated "Test Inhibitor."

6. Add 2 µl of Diluent solution (1x BRD TR-FRET Assay Buffer or 1x BRD TR-FRET Assay Buffer containing 2% DMSO) to the wells labeled "Negative Control" and "Positive Control."

7. Thaw **BET Bromodomain Ligand** and **Non-acetylated Ligand 1** on ice. Briefly spin the tubes containing the ligands to recover the full contents of the tubes. If the assay plate is going to be used more than once, prepare enough for this portion of the assay and aliquot the remaining into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

   Note: Each ligand is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots more than once.

8. Dilute the **BET Bromodomain ligand** 40-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."

9. Dilute the **Non-acetylated Ligand** 40-fold in **1x BRD TR-FRET Assay Buffer** and add 5 µl of diluted **Non-acetylated Ligand** to the "Negative Control" wells.

Component	Negative Control	Positive Control	Test Inbibitor
Tb-labeled donor	5 µl	5 µl	5 µl
Dye-labeled acceptor	5 µl	5 µl	5 µl
Test Inhibitor	-	-	2 µl
Diluent Solution	2 µl	2 µl	-
BET Bromodomain Ligand	-	5 µl	5 µl
Non-acetylated Ligand 1	5 µl	-	-
Total	**17 µl**	**17 µl**	**17 µl**

10. Thaw **CREBBP** protein on ice. Briefly spin the tube containing the protein to recover the full contents of the tube. If the assay plate is going to be used more than once, prepare enough for this portion of the assay and aliquot the remaining protein into single-use aliquots depending on how many times the assay plate will be used. Store the aliquots at -80°C.

    Keep the diluted protein on ice until use. Discard any unused diluted enzyme after use.

    Note: CREBBP is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.

11. Dilute **CREBBP** in 1x BRD TR-FRET Assay Buffer to 4 ng/µl (12 ng/reaction).

12. Initiate the reaction by adding 3 µl of diluted CREBBP to all the wells. The final volume of the reaction is now 20 µl.

13. Incubate at room temperature for 2 hours.

14. Read TR-FRET fluorescence using the following 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 the 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.

Example Results

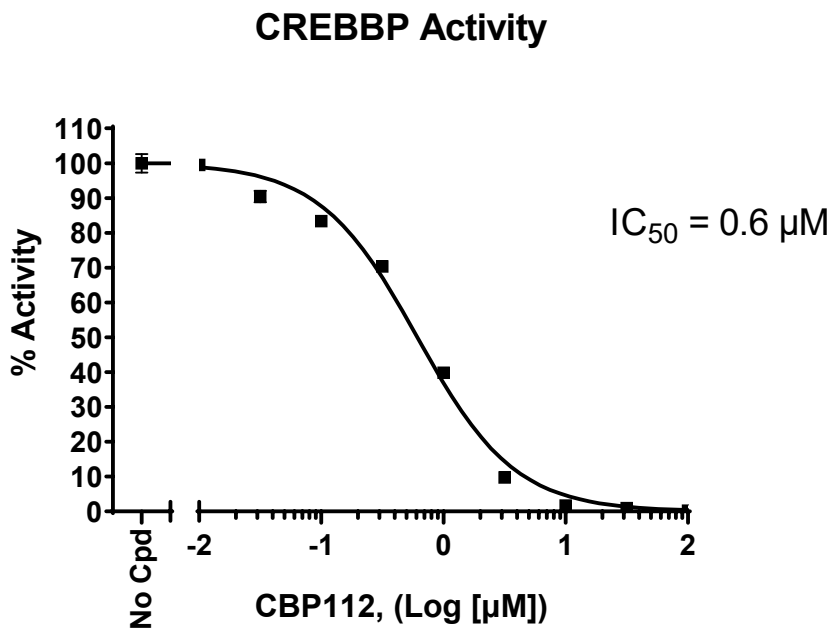

Figure 1: Inhibition of CREBBP by CBP112.
CREBBP was incubated with increasing concentrations of CBP112 and its binding to the ligand was measured as described in the protocol above.

Trouble Shooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

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

Related Products

Products	*Catalog #*	*Size*
ATAD2A TR-FRET Assay Kit	32618	384 reactions
BRD4 (BD1) TR-FRET Assay Kit	32613	384 reactions
P300 Homogeneous Assay Kit	50078	384 reactions
P300 Chemiluminescent Assay Kit	79705	384 reactions
(+)-JQ1 Inhibitor	27401	1 mg
BRD2 (65 – 459), GST-tag*	31024	100 µg
P300 (1046-1163), His-tag Recombinant	31118	100 µg