Description

The CREBBP TR-FRET Assay Kit is designed to measure binding activity of CREBBP (cAMP response element-binding protein binding-binding protein) to its substrate for screening and profiling applications using TR-FRET (Time-Resolved Fluorescence Resonance Energy Transfer). It utilizes Terbium-labeled donor and Cy5-labeled acceptor to complete the TR-FRET pairing. The CREBBP TR-FRET Assay Kit comes in a convenient 384-well format, with enough purified CREBBP (amino acids 1081-1197), Ligand, Tb-Labeled Donor and Dye-Labeled Acceptor, BET Bromodomain Ligand and assay buffer for 384 reactions. The assay also includes Non-Acetylated Ligand 1 as control for the specificity of binding.

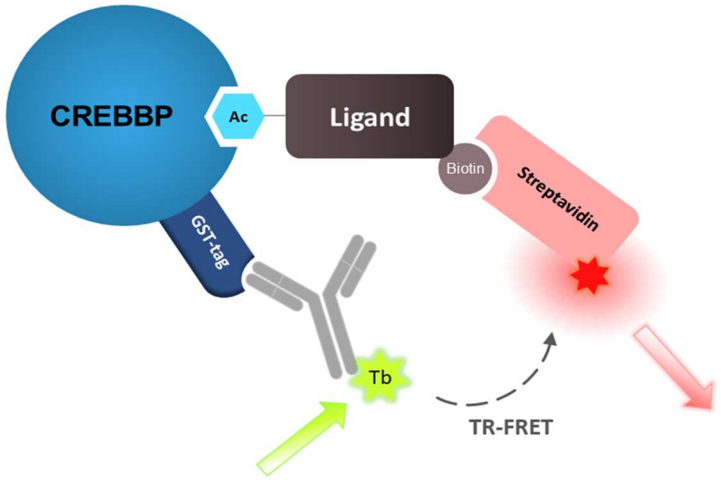

Figure 1: Illustration of the assay principle.

A sample containing terbium-labeled donor, dye-labeled acceptor, CREBBP, substrate, and an inhibitor is incubated for 2 hours. The fluorescence intensity is then measured using a fluorescence reader. In the presence of binding of CREBBP to its ligands, energy transfer occurs due to the proximity of the donor and acceptor. Disruption of the binding results in decrease of energy transfer. Fluorescence intensity at λ=665 nm corresponds directly to the binding of CREBBP to BET Bromodomain Ligand.

Background

CREBBP, also known as CREB-binding protein and cAMP response element-binding protein-binding protein, CBP or KAT3A, is a transcriptional coactivator. It has acetyltransferase activity, adding acetyl groups to histones and transcription factors, and serves as a protein scaffold in complex formation, thereby regulating gene transcription. It is ubiquitously expressed and interacts with multiple transcription factors. CREBBP has been implicated in several cancer types, including colorectal cancer and squamous cell carcinoma, but also diabetes, and neurological diseases such as Alzheimer's disease (AD). The broad spectrum of roles it performs by interacting with specific transcription factors has made CREBBP an attractive clinical target. CREBBP has also been identified as a radiosensitizer, with inhibition of this protein resulting in higher sensitivity to radiation in CREBBP mutant tumors and cell lines potentially due to homologous recombination impairment. A deeper understanding of the role of CREBBP in several pathways and diseases, combined with the development of inhibitors for each of the CREBBP/partner interactions, will result in significant advances in cancer therapy.

Applications

- Study enzyme kinetics.
- Screen small molecule inhibitors in high throughput screening (HTS) applications.

Supplied Materials

Catalog #	Name	Amount	Storage
31128	CREBBP (KAT3A), GST-Tag*	10 µg	-80°C
33000	BET Bromodomain Ligand	2.72 µg	-80°C
33005	Non-Acetylated Ligand 1	0.78 µg	-80°C
	Tb-Labeled 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 protein is lot-specific and will be indicated on the tube containing the protein.*

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 duplicate.
- The assay should include “Blank”, “Negative Control”, “Positive Control” and “Test Inhibitor” conditions.
- We recommend maintaining the diluted protein on ice during use.
- For detailed information on protein handling please refer to Protein FAQs (bpsbioscience.com).
- We recommend using CBP112 as internal control. If not running a dose response curve for the control inhibitor, we recommend running the control inhibitor at 0.1X, 1X and 10X the IC_{50} value shown in the validation data below.

1. Prepare **1x BRD TR-FRET Assay Buffer 1** by diluting 3-fold the **3x BRD TR-FRET Assay Buffer 1** with distilled water.

   Note: Make only the amount needed for the assay. The remaining 3x BRD TR-FRET Assay Buffer 1 can be stored as single use aliquots at -20°C.

2. Dilute Tb-Labeled Donor 100-fold with 1x BRD TR-FRET Assay Buffer 1 (5 µl/well).

3. Dilute Dye-Labeled Acceptor 100-fold with 1x BRD TR-FRET Assay Buffer 1 (5 µl/well).

   Note: Make only the amount needed of diluted Tb-Labeled Donor and Dye-Labeled Acceptor for the assay. The remaining solution can be stored as single use aliquots (minimum volume of 5 µl) at -20°C.

4. Add 5 µl of diluted Tb-Labeled Donor to all wells.

5. Add 5 µl of diluted Dye-Labeled Acceptor to all wells.

6. 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.

   6.1 If the Test Inhibitor is water-soluble, prepare 10-fold more concentrated serial dilutions of the inhibitor than the desired final concentrations in 1x BRD TR-FRET Assay Buffer 1.

   For the positive and negative controls, use 1x BRD TR-FRET Assay Buffer 1 (Diluent Solution).

   OR

   6.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at a concentration 500-fold higher than the highest desired concentration in 100% DMSO, then dilute the inhibitor 50-fold in 1x BRD TR-FRET Assay Buffer 1 to prepare the highest concentration of the 10-fold intermediate dilutions. The concentration of DMSO is now 2%.

   Using 1x BRD TR-FRET Assay Buffer 1 containing 2% DMSO to keep the concentration of DMSO constant, prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations.

   For positive and negative controls, prepare 2% DMSO in 1x BRD TR-FRET Assay Buffer 1 (vol/vol) so that all wells contain the same amount of DMSO (Diluent Solution).

   Note: The final concentration of DMSO should not exceed 0.2%.

7. Add 2 µl of diluted test inhibitor solution to the “Test Inhibitor” wells.

8. Add 2 µl of Diluent solution to the wells labeled “Blank”, “Negative Control” and “Positive Control”.

9. Resuspend **BET Bromodomain Ligand** in 50 µl of distilled water. This makes a 20 µM solution.

10. Resuspend **Non-acetylated Ligand** in 15 µl of distilled water. This makes a 20 µM solution.

    Note: Prepare enough for this portion of the assay and aliquot the remaining into single-use aliquots (minimum volume of 5 µl) and store aliquots at -80°C.

11. Dilute **BET Bromodomain Ligand** 320-fold with **1x BRD TR-FRET Assay Buffer 1** (5 µl/well). This makes a 62.5 nM solution.

12. Add 5 µl of diluted **BET Bromodomain Ligand** to the Control" and "Test Inhibitor" wells.

13. Add 5 µl of 1x BRD TR-FRET Assay Buffer 1 to the "Blank" wells.

14. Dilute the **Non-acetylated Ligand** to 320-fold with **1x BRD TR-FRET Assay Buffer 1** (5 µl/well). This makes a 62.5 nM solution.

15. Add 5 µl of diluted **Non-acetylated Ligand** to the "Negative Control" wells.

16. Thaw **CREBBP** protein on ice. Briefly spin the tube to recover the full content.

17. Dilute **CREBBP** to 4 ng/µl with 1x BRD TR-FRET Assay Buffer 1 (3 µl/well).

18. Initiate the reaction by adding 3 µl of diluted CREBBP to all the wells.

Component	**Blank**	**Negative Control**	**Positive Control**	**Test Inhibitor**
Diluted Tb-Labeled Donor	5 µl	5 µl	5 µl	5 µl
Diluted Dye-Labeled Acceptor	5 µl	5 µl	5 µl	5 µl
Test Inhibitor	-	-	-	2 µl
Diluent Solution	2 µl	2 µl	2 µl	-
Diluted BET Bromodomain Ligand (62.5 nM)	-	-	5 µl	5 µl
Diluted Non-acetylated Ligand 1 (62.5 nM)	-	5 µl	-	-
Diluted CREBBP (4 ng/µl)	3 µl	3 µl	3 µl	3 µl
1x BRD TR-FRET Assay Buffer 1	5 µl	-	-	-
Total	**20 µl**	**20 µl**	**20 µl**	**20 µl**

19. Incubate at room temperature for 2 hours.

20. Read fluorescence intensity of the samples in a microplate reader capable of measuring TR-FRET.

Instrument Settings

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

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: Calculate the FRET value by using the following formula:

$$FRET = \frac{S_{665} - \left(\frac{Tb_{665}}{Tb_{620}} \times S_{620}\right)}{S_{620}} \times 1000$$

S_{665} = Sample value measured at 665 nm, S_{620} = Sample value measured at 620 nm, Tb_{665} = Tb only or Blank value measured at 665 nm, Tb_{520} = Tb only or Blank value measured at 520 nm.

The FRET value calculated for the negative control should be subtracted from all other measurements and can be set as 0%. The FRET value from the "Positive Control" can be set as 100% activity.

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

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{Sub}$ = FRET value for the Substrate Control, and $FRET_p$ = FRET value for the Positive Control (no inhibitor).

Example Results

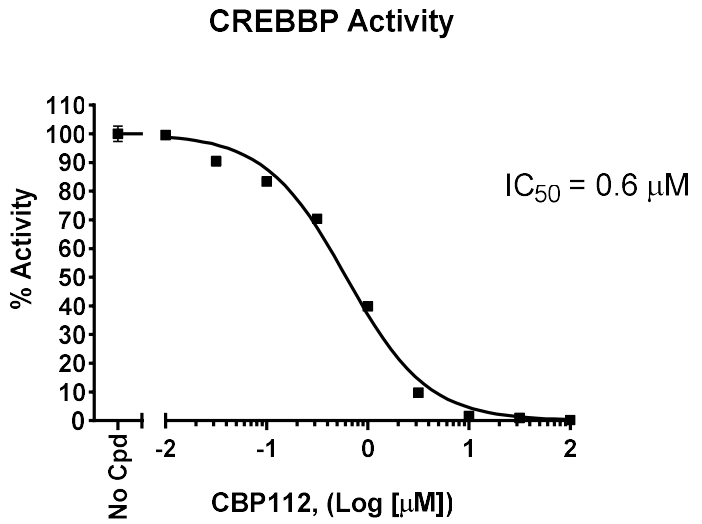

Figure 2: Inhibition of CREBBP by CBP112.
CREBBP activity was measured in the presence of increasing concentrations of CBP112. The "Blank" value was subtracted from all other values. Results are expressed as the percent of control (activity in the absence of inhibitor, set at 100%).

Data shown is representative. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

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.
Kumar M., *et al.*, 2021 *Nature Communications* 12: 6340.

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

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