Description

The SMARCA4/BRG1 TR-FRET Assay Kit is designed to measure the inhibition of SMARCA4 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4) binding to its substrate in a homogeneous 384-reaction format. This FRET (Time Resolved Fluorescence Resonance Energy Transfer)-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, SMARCA4 protein (amino acids 1480-1603), Bromodomain Ligand, and test inhibitor are incubated together for 120 minutes. Then, the fluorescence intensity is measured using a fluorescence reader capable of measuring TR-FRET.

Background

SMARCA4 (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4), also known as BRG1, has helicase and ATPase activity and is part of the SNF/SWI (Switch/Sucrose Non-Fermentable) ATP-dependent chromatin remodeling complex. SMARCA4 is a multidomain protein. One its domains, the BRD (bromodomain) binds to acetylated lysine in histone tails. It plays a role as tumor suppressor, but mutations in SWI/SNF protein accounts for about 20% of tumors. In NSCLC (non-small cell lung cancer) abnormal expression of this protein is responsible for about 10% of the cases, and contributes to progression of the disease and development of drug resistance. Interestingly, in cancers involving SMARCA4 mutations, inactivation of SMARCA2 results in synthetic lethality. The use of inhibitors could thus be of interest, however so far, most inhibitors have dual specificity for both SMARCA2 and SMARCA4 and have dose-limiting tolerability issues. Few inhibitors are also targeting the BRD domain, the main one being PIF3. Further studies on this protein and continued development of strategies to target it will open new doors in cancer treatment.

Applications

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

Supplied Materials

Catalog #	Name	Amount	Storage
31132	SMARCA4, GST-Tag*	10 µg	-80°C
33003	Bromodomain Ligand 2	1 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 0.5%. Higher DMSO concentrations can significantly decrease the quality of the results.
- Compounds that are fluorescent may interfere with the results, depending on their spectral excitation and emission properties.
- It is recommended that the compound alone is tested to determine any potential interference of the compound on the assay results.

Assay Protocol

- All samples and controls should be performed in triplicate.
- The assay should include "Positive Control", "Negative 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 PFI3 (#27332) 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.
- For instructions on how to prepare reagent dilutions please refer to Serial Dilution Protocol (bpsbioscience.com).

1. Dilute 3-fold the **3x BRD TR-FRET Assay Buffer 1** by adding one part stock buffer to two parts distilled water. This makes 1x BRD TR-FRET Assay Buffer 1.

   Note: 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 with 1x BRD TR-FRET Assay Buffer 1 (5 µl of each/well).

   Note: 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.

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

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

   OR

   4.2 If the Test inhibitor is soluble in DMSO, prepare the test inhibitor at 200-fold the highest desired concentration in DMSO, then dilute the inhibitor 20-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 5%.

   Prepare serial dilutions of the Test Inhibitor at 10-fold the desired final concentrations using 5% DMSO in 1x BRD TR-FRET Assay Buffer 1 to keep the concentration of DMSO constant.

   For positive and negative controls, prepare 5% 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 in the assay should not exceed 0.5%.

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

6. Add 2 µl of Diluent Solution to the wells labeled “Negative Control” and “Positive Control”.

7. Resuspend Bromodomain Ligand 2 in 60 µl of distilled water.

   Note: Bromodomain Ligand 2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots.

8. Dilute 80-fold the Bromodomain Ligand 2 with 1x BRD TR-FRET Assay Buffer 1 (5 µl/well).

9. Add 5 µl of diluted Bromodomain Ligand 2 to each well designated as “Positive Control” and “Test Inhibitor”.

10. Add 5 µl of 1x BRD TR-FRET Assay Buffer 1 to the wells labeled as “Negative Control”.

11. Thaw SMARCA4 protein on ice. Briefly spin tube containing protein to recover the full contents of the tube.

12. Dilute SMARCA4 with 1x BRD TR-FRET Assay Buffer 1 to 0.83 ng/µl (3 µl/well).

13. Initiate the reaction by adding 3 µl of diluted SMARCA4 to wells designated for the “Negative Control”, “Positive Control” and “Test Inhibitor”.

Component	Negative Control	Positive 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
Diluent Solution	2 µl	2 µl	-
Diluted Bromodomain Ligand 2	-	5 µl	5 µl
1x BRD TR-FRET Assay Buffer	5 µl		
Diluted SMARCA4 (0.83 ng/µl)	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

14. Incubate at room temperature for 2 hours.

15. Read the fluorescent intensity in a microtiter-plate reader capable of measuring TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	340 (20 nm bandwidth)
Emission Wavelength	620 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	340 (20 nm bandwidth)
Emission Wavelength	665 (10 nm bandwidth)
Lag Time	60 µs
Integration Time	500 µs

CALCULATING RESULTS:
Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

$$FRET = \frac{S_{665}}{S_{620}}$$

When percentage activity is calculated, the FRET value from the Blank (it is expected that Blank and Negative Control have a similar value) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

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

$FRET_S$ =FRET value for samples of Test Inhibitor, $FRET_{neg}$ = FRET value for the Negative Control, and $FRET_p$ = FRET value for the Positive Control.

Example Results

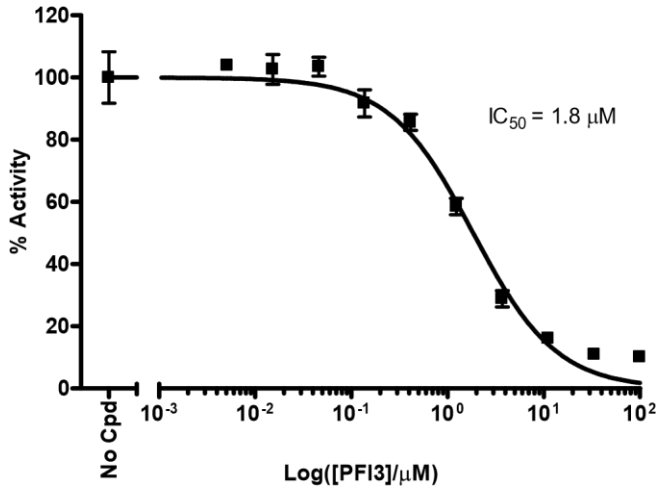

Figure 1: Inhibition of SMARCA4 by PFI3.
SMARCA4 was incubated with increasing concentrations of PIF3 (#27332) and its binding to the ligand was measured as described in the protocol above.

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

References
Schiaffino-Ortega S., *et al.*, 2014. J. Hematol. Oncol. 7:81.
Lu T., *et al.*, 2018 *Acta Pharmacologica Sinica* 39:1544-1552.
Tian Y., *et al.*, 2023 *Cancer Letters* 554: 216022.

Related Products

Products	*Catalog #*	*Size*
SMARCA2, His-Tag Recombinant	31111	100 µg
SMARCA2, His-Tag, Biotin-Labeled Recombinant	31129	50 µg
SMARCA2 TR-FRET Assay Kit	40342	384 reactions
SMARCA4 (BRG1), His-Tag Recombinant	31102	100 µg
SMARCA4, GST-Tag Recombinant	31132	100 µg

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