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Data Sheet
SMARCA2 TR-FRET Assay Kit
Catalog # 40342
Size: 384 reactions

DESCRIPTION: The SMARCA2 TR-FRET Assay Kit is designed to measure the inhibition of SMARCA2 (BRM) 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, SMARCA2, substrate, and an inhibitor is incubated for 120 minutes. Then, the fluorescence intensity is measured using a fluorescence reader.

COMPONENTS:

Catalog #	Component	Amount	Storage	
31141	SMARCA2	10 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
	Bromodomain Ligand 2	50 µl	-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	
	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

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: Schiaffino-Ortega, S., *et al.,* 2014. *J. Hematol. Onco*l. **7**:81.

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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 1** with 2 parts distilled water (3-fold dilution) to make **1x BRD TR-FRET Assay Buffer 1**. 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 1**. 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 each well designated "Test Inhibitor", "Negative Control", and "Positive Control".

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 "Negative Control", and "Positive Control". *Note: Keep DMSO concentration below 0.5%.*

	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
Inhibitor Buffer (no inhibitor)	2 µl	2 µl	–
Bromodomain Ligand 2	–	5 µl	5 µl
1x BRD TR-FRET Assay Buffer 1	5 µl	–	–
SMARCA2 (0.83 ng/µl)	3 µl	3 µl	3 µl
Total	**20 µl**	**20 µl**	**20 µl**

5) Thaw **Bromodomain Ligand 2** on ice. Upon first thaw, briefly spin tube containing enzyme 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 **Bromodomain Ligand 2 40**-fold in **1x BRD TR-FRET Assay Buffer 1**. Add 5 µl of diluted **Bromodomain Ligand 2** to each well designated as "Positive Control" and "Test Inhibitor". Add 5 µl of **1x BRD TR-FRET Assay Buffer 1** to the wells labeled as "Negative Control.

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7) **Thaw SMARCA2** protein on ice. Upon first thaw, briefly spin tube containing protein to recover the full contents of the tube. Aliquot **SMARCA2** protein into single-use aliquots. Store remaining undiluted **SMARCA2** in aliquots at −80°C immediately. *Note: SMARCA2 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted protein.*

8) Dilute **SMARCA2** in **1x BRD TR-FRET Assay Buffer 1** to 0.83 ng/µl (2.5 ng/reaction). Initiate reaction by adding 3 µl of diluted **SMARCA2** to wells designated for the "Negative Control" "Positive Control", and "Test Inhibitor". Discard any remaining diluted SMARCA2 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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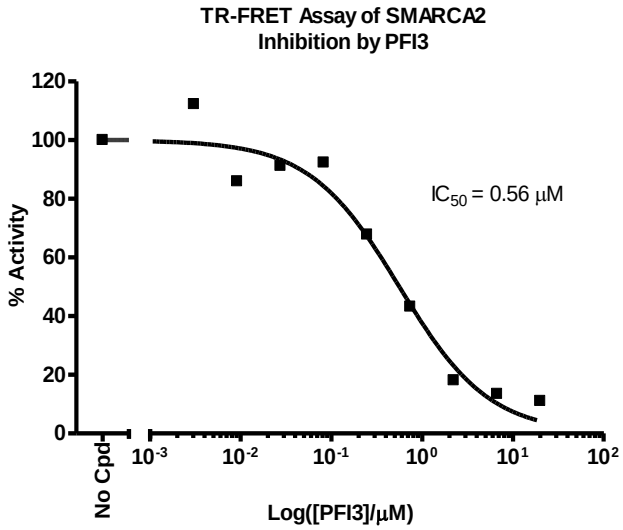
EXAMPLE OF ASSAY RESULTS:



Inhibition of SMARCA2 by PFI3 (BPS Cat. # 27332), measured using the *SMARCA2 TR-FRET Assay Kit*, BPS Bioscience # 40342. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

Product	Catalog #	Size
SMARCA2 (1375 – 1511), GST-tag	31141	100 µg
SMARCA2 (1375 – 1511), His-tag	31111	100 µg
BRG1 (SMARCA4) (1480 – 1603), GST-tag	31132	100 µg
BRG1 (SMARCA4) (1480 – 1603), His-tag	31102	100 µg
SMARCA2, His-Tag, Biotin-Labeled	31129	50 µg
Bromodomain Ligand 2	33003	0.5 ml
SMARCA2 Inhibitor Screening Assay Kit	32610	384 rxns.
SMARCA4/BRG1 Inhibitor Screening Assay Kit	32609	384 rxns.

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

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