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Data Sheet

EZH2(Y641F) TR-FRET Assay Kit

Catalog # 52680
Size: 384 reactions

DESCRIPTION: The *EZH2 (Y641F) TR-FRET Assay Kit* is designed to measure activity of the mutant EZH2 complex (EZH2 (Y641F)/EED/SUZ12/RbAp48/ AEBP) 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 *EZH2 (Y641F) TR-FRET Assay Kit* comes in a convenient format, with histone H3 peptide substrate, a Tb-labeled antibody against methylated K27 residue of Histone H3, S-adenosylmethionine, methyltransferase assay buffer, TR-FRET detection buffer, dye-labeled acceptor, and purified EZH2 (Y641F) complex for 384 enzyme reactions. The key to the EZH2 (Y641F) Assay Kit is a highly specific antibody that recognizes methylated Histone H3K27. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, S-adenosylmethionine is incubated with a sample containing assay buffer and methyltransferase enzyme for 240 minutes. Next, antibody is added. Finally, dye-labeled acceptor is added followed by fluorescence reading.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51017	EZH2 (Y641F)/EED/SUZ12/RbAp48/AEBP2	2 x 50 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120	20 µM S-adenosylmethionine	2 x 250 µl	-80°C	
52089	Tb-labeled antibody	5 µl	-80°C	
	Biotinylated histone H3 peptide substrate*	500 µl	-80°C	
52170-A	4x HMT Assay Buffer 2A	3 ml	-20°C	
	Dye-labeled acceptor	2 x 10 µl	-20°C	
	TR-FRET Detection Buffer	4 ml	-20°C	
	White, Nonbinding Corning, low volume, microtiter plate	1	Room temp.	

**Resuspend in 500 ul of distilled water.*

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MATERIALS OR INSTRUMENTS 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(S):

1. Dillon, S.C., *et al. Genome Biology* 2005; **6:**227.
2. Morin, R.D., *et al. Nat Genet*. 2010, **42**(2):181.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Thaw **S-adenosylmethionine** on ice. Upon first thaw, briefly spin tube containing **S-adenosylmethionine** to recover full content of the tube. Aliquot **S-adenosyl-methionine** into single use aliquots. Store remaining **S-adenosylmethionine** in aliquots at -80ºC immediately. *Note: S-adenosylmethionine is very sensitive to freeze/thaw cycles. Avoid multiple freeze-thaw cycles.*

2) Prepare the master mixture: N wells × (2.5 µl **4× HMT Assay Buffer 2A** + 1 µl **Histone Substrate** + 1 µl **20 µM S-adenosylmethionine**)

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	Blank	Substrate Control	Positive Control	Test Inhibitor
4× HMT assay buffer 2A	2.5 µl	2.5 µl	2.5 µl	2.5 µl
20 µM S-adenosylmethionine	1 µl	–	1 µl	1 µl
Histone substrate	1 µl	1 µl	1 µl	1 µl
H_2O	–	1 µl	–	–
Test Inhibitor/Activator	–	–	–	3 µl
Inhibitor buffer (no inhibitor)	3 µl	3 µl	3 µl	–
1× HMT assay buffer 2A	2.5 µl	–	–	–
EZH2(Y641F) (100 ng/µl)	–	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

3) Add 3.5 µl of master mixture to each well designated for the "Positive Control", "Test Inhibitor", and "Blank". For the "Substrate Control", add 2.5 µl **4× HMT Assay Buffer 2A** + 1 µl **Histone Substrate** + 1 µl **H_2O**.
4) Add 3 µl of inhibitor solution of each well designated "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 3 µl of the same solution without inhibitor (inhibitor buffer).

5) Dilute one part 4x HMT Assay Buffer 2A with 3 parts distilled water (4-fold dilution) to make 1x HMT assay buffer 2A. Make only a sufficient quantity needed for the assay; store remaining stock solution in aliquots at -20°C. Add 2.5 µl of **1 × HMT assay buffer 2A** to the well designated "Blank".

6) Thaw **EZH2(Y641F) enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **EZH2(Y641F) enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -70°C immediately. *Note: **EZH2(Y641F) enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

7) Dilute **EZH2(Y641F) enzyme** in **1× HMT assay buffer 2A** at 100 ng/µl (250 ng/2.5 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

8) Initiate reaction by adding 2.5 µl of diluted **EZH2(Y641F)** prepared as described above to wells labeled "Positive Control", "Test Inhibitor", and "Substrate Control". Incubate at room temperature for four hours.

9) Cover the plate with a plate sealer if necessary.

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Step 2:

1) Thaw **TR-FRET Detection Buffer** on ice.
2) Dilute **Tb-labeled antibody** 400-fold with **TR-FRET Detection Buffer**.
3) Add 5 µl per well. Incubate 30 minutes at room temperature with slow shaking.

Step 3:

1) Dilute **Dye-labeled acceptor** 100-fold with **TR-FRET Detection Buffer**.
2) Add 5 µl per well. Incubate for 30 min. at room temperature with slow shaking.

   (Alternatively, dilute Tb-labeled antibody (1:800) and Dye-labeled acceptor (1:200) with TR-FRET Detection Buffer in one step. Add 10 µL of Antibody/Acceptor mixture per well and incubate 1 hour.)
3) 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

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CALCULATING RESULTS:

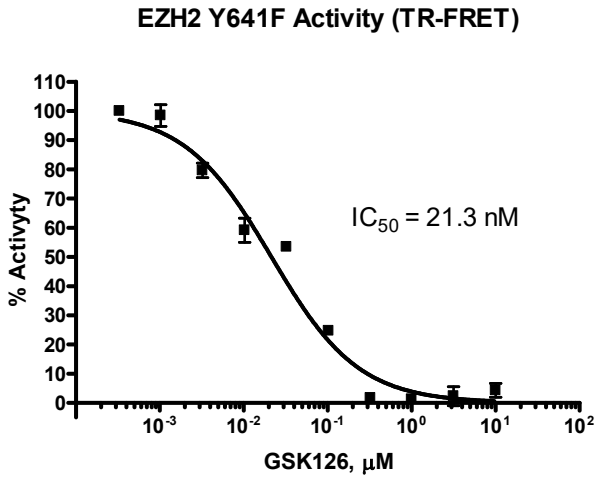
Two sequential measurements should be conducted. Eu-donor emission should be measured at 620 nm followed by dye-acceptor emission at 665 nm. Data analysis is performed using the TR-FRET ratio (665 nm emission/620 nm emission).

When percentage activity is calculated, the FRET value from the negative control (Blank or Substrate Control) can be set as zero percent activity and the FRET value from the positive control can be set as one hundred percent activity.

$$\% \text{ Activity} = \frac{FRET_s - FRET_{neg}}{FRET_p - FRET_{neg}} \times 100\%$$

Where $FRET_s$ = Sample FRET, $FRET_{neg}$ = negative control FRET, and $FRET_P$ = Positive control FRET.

Example of Assay Results:



Inhibition of EZH2(Y641F) enzyme activity by GSK126, measured using the *EZH2 (Y641F) TR-FRET Assay Kit*, BPS Bioscience #xxxxx. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

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RELATED PRODUCTS

Product Name	Catalog #	Size
EZH2 (Y641F)/EED/SUZ12/RbAp48/AEBP2	51017	20 µg
EZH2 (Y641C)/EED/SUZ12/RbAp48/AEBP2	51029	20 µg
EZH2 (Y641N)/EED/SUZ12/RbAp48/AEBP2	51028	20 µg
EZH2 (Y641S)/EED/SUZ12/RbAp48/AEBP2	51013	20 µg
EZH2 (Y641H)/EED/SUZ12/RbAp48/AEBP2	51011	20 µg
EZH2/EED/SUZ12/RbAp48/AEBP2	51004	50 µg
EZH2 (Y641F) TR-FRET Assay Kit	52078	384 rxns.
EZH2 Chemiluminescent Assay Kit	52009L	96 rxns.
EZH2 (Y641F) Chemiluminescent Assay Kit	52075	96 rxns.