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Data Sheet

PRMT5 TR-FRET Assay Kit

Catalog # 52171
Size: 384 reactions

DESCRIPTION: The *PRMT5 TR-FRET Assay Kit* is designed to measure PRMT5 activity in a homogeneous 384 reaction format. PRMT5 is a histone methyltransferase that exhibits methylation activity toward H4-R3. This FRET-based assay requires no time-consuming washing steps, making it especially suitable for high throughput screening applications. The *PRMT5 TR-FRET Assay Kit* comes in a convenient format, with histone H4 peptide substrate, a Eu-labeled antibody, S-adenosylmethionine, methyltransferase assay buffer, FRET detection buffer, dye-labeled acceptor, and purified PRMT5 for 384 enzyme reactions. The key to the *PRMT5 TR-FRET Assay Kit* is a highly specific antibody that recognizes methylated substrate.

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 120 minutes. Next, antibody is added. Finally, dye-labeled acceptor is added followed by fluorescence detection.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51048	PRMT5/MEP50	40 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120	60 µM S-adenosylmethionine	250 µl	-80°C	
	Eu-labeled antibody	5 µl	-80°C	
	Biotinylated histone H4 peptide substrate	1000 rxns	-80°C	
	4x PRMT5 assay buffer*	3 x 1 ml	-20°C	
	Dye-labeled acceptor	2 x 10 µl	-20°C	
	PRMT5 TR-FRET Detection Buffer	4 ml	-20°C	
	White, nonbinding, low volume, microtiter plate	1	Room temp.	

* Add 10 µl of 0.5 M DTT per vial before use.

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MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:

0.5M DTT (Dithiothreitol, Sigma Aldrich, Cat. #D0632)
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: Yang, Y., Bedford, M.T. 2013. *Nat Rev Cancer.* **13(1):**37-50.

ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

Step 1:

1) Re-suspend tube with **Biotinylated histone H4 peptide substrate** in 500 µL of distilled water.

2) Add 10 µl **0.5M DTT** (not provided) to a 1-ml tube of **4x PRMT5 Assay Buffer.** Prepare **1x PRMT5 Assay Buffer** by adding 1 part of **4x PRMT5 Assay Buffer** to 3 parts water (v/v). Dilute only enough **4x PRMT5 Assay Buffer** as required for the assay.

3) 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.*

4) Prepare the master mixture: N wells × (2.5 µl **4× PRMT5 Assay Buffer** + 0.5 µl **60 µM S-adenosylmethionine** + 0.5 µl **Histone Substrate** + 1 µl **H_2O**)

5) Add 4.5 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× PRMT5 Assay Buffer** + 0.5 µl **Histone Substrate** + 1.5 µl **H_2O**.

6) 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).

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7) Add 2.5 µl of **1 × PRMT5 assay buffer** to the well designated "Blank".

	Blank	Substrate Control	Positive Control	Test Inhibitor
4× PRMT5 assay buffer	2.5 µl	2.5 µl	2.5 µl	2.5 µl
60 µM S-adenosylmethionine	0.5 µl	–	0.5 µl	0.5 µl
Histone substrate	0.5 µl	0.5 µl	0.5 µl	0.5 µl
H_2O	1 µl	1.5 µl	1 µl	1 µl
Test Inhibitor/Activator	–	–	–	3 µl
Inhibitor buffer (no inhibitor)	3 µl	3 µl	3 µl	–
1× PRMT5 assay buffer	2.5 µl	–	–	–
PRMT5/MEP50 (20-40 ng/µl)	–	2.5 µl	2.5 µl	2.5 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

8) Thaw **PRMT5 enzyme** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT5 enzyme** into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C immediately. *Note: **PRMT5 enzyme** is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

9) Dilute **PRMT5 enzyme** in **1× PRMT5 assay buffer** at 20-40 ng/µl (50-100 ng/2.5 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

10) Initiate reaction by adding 2.5 µl of diluted **PRMT5** prepared as described above to the wells labeled "Positive Control", "Test Inhibitor", and "Substrate Control". Incubate at room temperature for two hours. Cover the plate with a plate sealer if necessary.

Step 2:

1) Thaw **PRMT5 TR-FRET Detection Buffer** on ice.
2) Dilute **Eu-labeled antibody** 1000-fold with **PRMT5 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 **PRMT5 TR-FRET Detection Buffer**.
2) Add 5 µl per well. Incubate for 30 min. at room temperature with slow shaking.

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(Alternatively, dilute Eu-labeled antibody (1:2000) and Dye-labeled acceptor (1:200) with PRMT5 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 measuring TR-FRET.

Instrument Settings

Reading Mode	Time Resolved
Excitation Wavelength	317±20 nm
Emission Wavelength	620±10 nm
Lag Time	60 µs
Integration Time	500 µs
Excitation Wavelength	317±20 nm
Emission Wavelength	665±10 nm
Lag Time	60 µs
Integration Time	500 µs

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.

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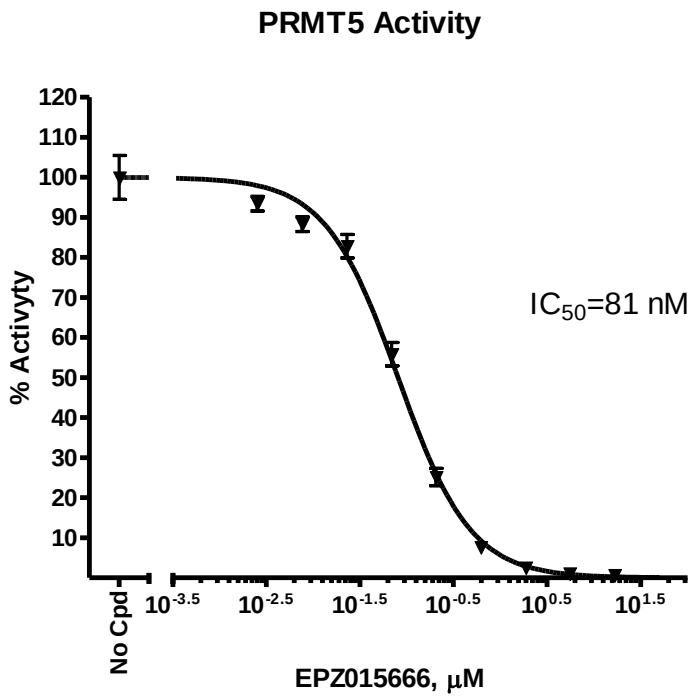
Example of Assay Results:



PRMT5 enzyme activity, measured using the *PRMT5 TR-FRET Assay Kit*, BPS Bioscience # …. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com.*

RELATED PRODUCTS

Product Name	Catalog #	Size
PRMT1 Homogeneous Assay Kit	#52054	384 reactions
PRMT3 Homogeneous Assay Kit	#52055	384 reactions
PRMT6 Homogeneous Assay Kit	#52056	384 reactions
PRMT8 Homogeneous Assay Kit	#52058	384 reactions
PRMT1 Chemiluminescent Assay Kit	#52004L	96 reactions
PRMT3 Chemiluminescent Assay Kit	#52005L	96 reactions
PRMT4 Chemiluminescent Assay Kit	#52041L	96 reactions
PRMT5 Chemiluminescent Assay Kit	#52002L	96 reactions
PRMT6 Chemiluminescent Assay Kit	#52046	96 reactions
PRMT5 recombinant protein (HEK293)	#51045	20 µg
MTAP, GST-tag	#50305	50 µg

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PRMT5/MEP50 recombinant protein (Sf9)	#51048	20 µg
PRMT1 recombinant protein (*E. coli*)	#51040	50 µg
PRMT1 recombinant protein (Sf9)	#51041	20 µg
PRMT3 recombinant protein	#51043	50 µg
PRMT4 (CARM 1) recombinant protein	#51047	20 µg
PRMT6 recombinant protein	#51049	20 µg
PRMT7 recombinant protein	#51054	20 µg
PRMT8 recombinant protein	#51052	20 µg
PRMT9 recombinant protein	#51053	20 µg

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