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Data Sheet

PRMT5 Homogeneous Assay Kit

Catalog #52052
Size: 384 reactions

DESCRIPTION: The *PRMT5 Homogeneous Assay Kit* is designed to measure PRMT5 activity for screening and profiling applications. PRMT5 is a histone methyltransferase that exhibits methylation activity toward H4-R3. The *PRMT5 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H4 peptide substrate, primary antibody, methylation assay buffer, and purified PRMT5 for 384 enzyme reactions. The key to the *PRMT5 Homogeneous 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, a sample containing PRMT5 enzyme is incubated with the biotinylated substrate for two hours. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51045	PRMT5/MEP50	2x 40 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120-A	60 µM S-adenosylmethionine	250 µl	-80°C	
52150-3	Primary antibody 4-3 *	100 µl	-80°C	
	Biotinylated histone H4 peptide substrate	500 rxns	-80°C	
	4x PRMT5 assay buffer (add DTT before experiment)	3x1 ml	-20°C	
79389	4x Detection buffer	2 ml	-20°C	

*The detection antibody for this kit was changed in January, 2017.

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA® anti-rIgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002)
Optiplate-384 (PerkinElmer #6007290)
DTT 0.5M

APPLICATIONS: Great for studying enzyme kinetics and HTS applications.

CONTRAINDICATIONS: Green and blue dyes that absorb light in the AlphaScreen® signal emission range (520-620 nm), such as Trypan Blue. Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN_3) or metal ions ($Fe2^+$, $Fe3^+$, $Cu2^+$, $Zn2^+$ and $Ni2^+$). The presence of >1% RPMI 1640 culture medium leads to a signal reduction due to the presence of excess biotin and iron in this medium. MEM, which lacks these components, does not affect AlphaScreen® assays.

STABILITY: At least one year from date of receipt when stored as directed.

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REFERENCE(S): Dillon SC, *et al.* 2005. *Genome Biology* **6:**227.

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 1-ml tube with **4x PRMT5 Assay Buffer.** Prepare **1x PRMT5 Assay Buffer** by adding 1 part of **4x PRMT5 Assay Buffer** to 3 parts water (v/v).

3) Prepare the master mixture: N wells x (2 µl **4x PRMT5 Assay Buffer** + 0.5 µl **S-adenosylmethionine** (60 µM) + 1 µl **Biotinylated substrate** + 1 µl water). Add 5 µl to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2 µl **4x PRMT5 Assay Buffer** + 1 µl **Biotinylated substrate** + 2 µl water.

4) Add 3 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 3 µl of the same solution without inhibitor (Inhibitor buffer).

5) Thaw **PRMT5** 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. *Note: PRMT5 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme. Depending on the nature of inhibitor, pre-incubation with the enzyme may be necessary.*

6) Dilute **PRMT5** in **1x PRMT5 Assay Buffer** at 100 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

	Positive Control	Test Sample	Substrate Control	Blank
4x PRMT5 Assay Buffer	2 µl	2 µl	2 µl	2 µl
60 µM S-adenosylmethionine	0.5 µl	0.5 µl	–	0.5 µl
Biotinylated substrate	1 µl	1 µl	1 µl	1 µl
H_2O	1.5 µl	1.5 µl	2 µl	1.5 µl
Test Inhibitor/Activator	–	3 µl	–	–
Inhibitor Buffer (no inhibitor)	3 µl	–	3 µl	3 µl
PRMT5 (100 ng/µl)	2 µl	2 µl	2 µl	–
1x PRMT5 Assay Buffer	–	–	–	2
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

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7) To the wells designated as "Blank", add 2 µl of 1x PRMT5 Assay buffer.

8) Initiate reaction by adding 2 µl of diluted **PRMT5** enzyme to the wells designated "Positive Control", "Substrate Control", and "Test Sample". Incubate at room temperature for 2 hours.

Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:

1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) 1:250-fold with **1x Detection Buffer** (made by diluting **4x Detection buffer** 1:4 in distilled water). Add 5 µl per well. Shake plate briefly.

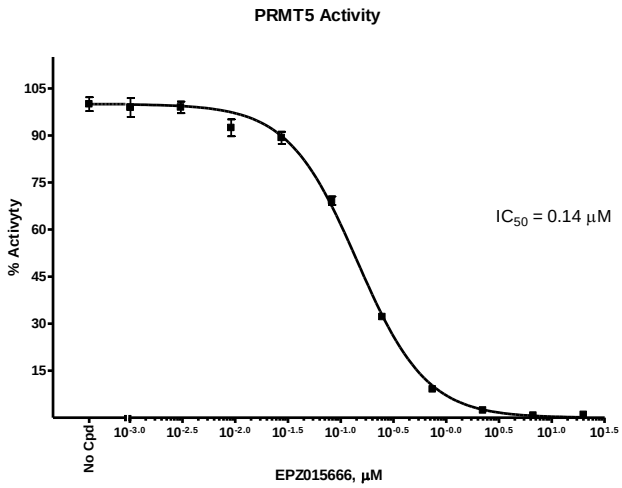
2) Dilute **Primary antibody 4-3** 30-fold with **1x Detection Buffer**. Add 5 µl per well. Shake plate. Incubate 30-60 min at room temperature.
(Alternatively, dilute anti-Rabbit Acceptor beads (1:500) and Primary antibody 4-3 (1:60) with 1x Detection buffer in one step. Add 10 µL of acceptor beads/antibody mixture per well.)

Step 3:

1) Dilute Streptavidin-conjugated donor beads (PerkinElmer #6760002) 125-fold with **1x Detection Buffer**. Add 10 µl per well. Incubate for 30 min. at room temperature.

2) Read Alpha-counts.

Example of Assay Results:



PRMT5 enzyme activity, measured using the *PRMT5 Homogeneous Assay Kit*, BPS Bioscience Cat. #52052. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

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
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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