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Data Sheet
PRMT1 Homogeneous Assay Kit
Catalog #52054

DESCRIPTION: The *PRMT1 Homogeneous Assay Kit* is designed to measure PRMT1 activity for screening and profiling applications. PRMT1 is a histone methyltransferase that exhibits methylation activity toward H4-R3. The *PRMT1 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with a 384-well plate, biotinylated histone H4 peptide substrate, primary antibody, methylation assay buffer, and purified PRMT1 for 384 enzyme reactions. The key to the *PRMT1 Homogeneous Assay Kit* is a highly specific antibody that recognizes methylated histone substrate. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, a sample containing PRMT1 enzyme is incubated with the biotinylated substrate for one hour. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Cat. #	Components	Amount	Storage	
51040	PRMT1	1 µg	-80°C	***(Avoid freeze/ thaw cycles!)***
52120	20 µM S-adenosylmethionine	500 µl	-80°C	
52150-3	Primary antibody 4-3	4 x 100 µl	-80°C	
	Biotinylated histone H4 peptide substrate	500 rxns	-80°C	
52170-A	4x HMT assay Buffer 2A	3 ml	-20°C	
	4x Detection Buffer	2 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA anti-rIgG acceptor beads, 5 mg/ml (Perkin Elmer #AL104C)
AlphaScreen Streptavidin-conjugated donor beads, 5 mg/ml (Perkin Elmer # 6760002)
OptiPlate-384 (Perkin Elmer #6007290)
AlphaScreen microplate reader

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 (Fe^{2+}, Fe^{3+}, Cu^{2+}, Zn^{2+} and Ni^{2+}). 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: One year from date of receipt when stored as directed.

REFERENCE(S): Li Y., et al., J. Neurosci. 2015; **35(37):** 12890-12902.

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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) Prepare the master mixture: N wells x (2.5 µl **4x HMT Buffer 2A** + 1 µl **S-adenosyl-methionine** (20 µM) + 1.0 µl **Biotinylated substrate**). Add 4.5 µl to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2.5 µl **4x HMT Buffer 2A** + 1.0 µl **Biotinylated substrate** + 1.0 µl water.

3) Add 2.5 µl of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2.5 µl of the same solution without inhibitor (Inhibitor buffer).

4) Prepare **1x HMT Buffer 2A** by adding 1 part of **4x HMT Buffer 2A** to 3 parts water (v/v).

5) Thaw **PRMT1** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT1** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PRMT1 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*

6) Dilute **PRMT1** in **1X HMT assay Buffer 2A** at 0.5 ng/µl. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

	Blank	Positive Control	Test Sample	Substrate Control
4x 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	–
Biotinylated substrate	1 µl	1 µl	1 µl	1 µl
H_2O	3 µl	–	–	1 µl
Test Inhibitor/Activator	–	–	2.5 µl	–
Inhibitor Buffer (no inhibitor)	2.5 µl	2.5 µl	–	2.5 µl
PRMT1 (0.5 ng/µl)	–	3 µl	3 µl	3 µl
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

7) To the wells designated as "Blank", add 3 µl of water.

8) Initiate reaction by adding 3 µl of diluted **PRMT1** enzyme to the wells designated "Positive Control", "Substrate Control", and "Test Sample". Incubate at room temperature for 1 hour.

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Step 2:
Note: Protect your samples from direct exposure to light!

1) Prepare **1x Detection Buffer** by adding 1 part of **4x Detection Buffer** to 3 parts water (v/v).

2) Dilute anti-Rabbit Acceptor beads (Perkin Elmer #AL104C) 1:250-fold with **1x Detection Buffer**. Add 5 µl per well. Shake plate briefly.

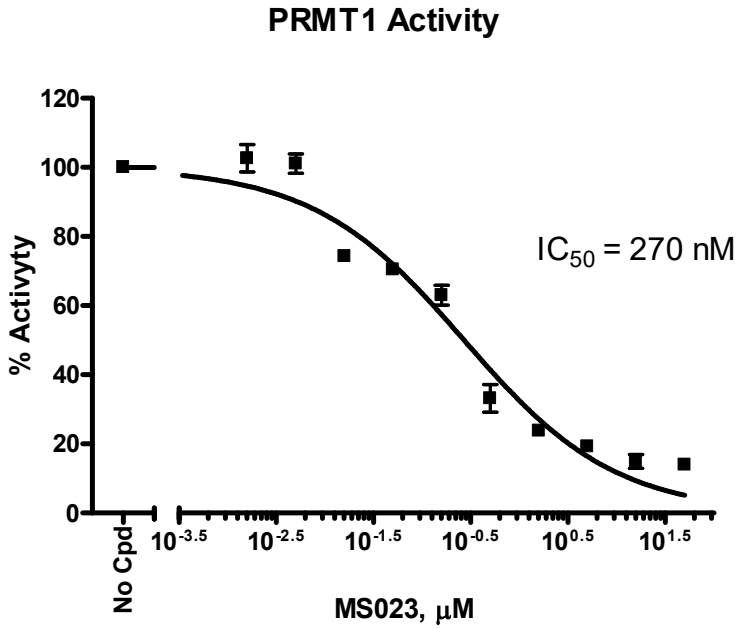
3) Dilute **Primary antibody 4-3** 10-fold with **1x Detection Buffer**. Add 5 µl per well. Shake plate. Incubate 30 min. at room temperature.

Step 3:

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

2) Read Alpha-counts. The "Blank" value is subtracted from all other values.

Example of Assay Results:



PRMT1 enzyme activity, measured using the *PRMT1 Homogeneous Assay Kit*, BPS Bioscience Cat. #52054. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

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RELATED PRODUCTS:

PRMT8 Homogeneous Assay Kit	#52058	384 reactions
PRMT3 Homogeneous Assay Kit	#52055	384 reactions
PRMT5 Homogeneous Assay Kit	#52052	384 reactions
PRMT6 Homogeneous Assay Kit	#52056	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
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
PRMT5 recombinant protein (HEK293)	#51045	20 µg
PRMT5/MEP50 recombinant protein (Sf9)	#51048	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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