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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	2 x 250 µl	-80°C	
52150-3	Primary antibody 4-3	4 x 100 µl	-80°C	
	Biotinylated histone H4 peptide substrate	1000 µl*	-80°C	
52170-A	4x HMT assay Buffer 2A	3 ml	-20°C	
52301	4x Detection Buffer	2 ml	-20°C	

*Solubilize in 1,000 µl of distilled water.

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 (NaN₃) 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: 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) Prepare **1x HMT Buffer 2A** by adding 1 part of **4x HMT Buffer 2A** to 3 parts water (v/v).
- 2) Add 2.5 μ l of Inhibitor solution of each well labeled as "Test Inhibitor." For the "Positive Control," "Substrate Control," and "Blank," add 2.5 μ l of 1x HMT assay Buffer 2A in 3.3% DMSO (Inhibitor buffer).
- 3) Add 1 μ l of **S-adenosyl-methionine** (20 μ M) to each well labeled "Blank," "Positive Control," and "Test Inhibitor."
- 4) 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.*
- 5) Dilute **PRMT1** in **1X HMT assay Buffer 2A** at 0.5 ng/ μ l. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.
- 6) Add 3 μ l of diluted **PRMT1** enzyme to the wells designated "Positive Control," "Substrate Control," and "Test Sample."
- 7) Preincubate **PRMT1**, **S-adenosyl-methionine**, and Test Inhibitor at room temperature for 30 minutes.
- 8) Re-suspend tube with **Biotinylated histone H4 peptide substrate** in 1,000 μ l of distilled water.
- 9) Prepare the master mixture: N wells x (3 μ l **4x HMT Buffer 2A** + 0.5 μ l **Biotinylated substrate**). Add 3.5 μ l to wells designated "Positive Control," "Test Sample," and "Blank." To wells labeled "Substrate Control," add 3 μ l **4x HMT Buffer 2A** + 0.5 μ l **Biotinylated substrate** + 1.0 μ l water.

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	Blank	Positive Control	Test Inhibitor	Substrate Control
4x HMT assay Buffer 2A	3 μ l	3 μ l	3 μ l	3 μ l
20 μ M S-adenosylmethionine	1 μ l	1 μ l	1 μ l	-
Biotinylated substrate	0.5 μ l	0.5 μ l	0.5 μ l	0.5 μ l
H ₂ O	3 μ l	-	-	1 μ l
Test Inhibitor/Activator	-	-	2.5 μ l	-
1x HMT assay Buffer 2A in 3.3% DMSO (inhibitor buffer)	2.5 μ l	2.5 μ l	-	2.5 μ l
PRMT1 (0.5 ng/ μ l)	-	3 μ l	3 μ l	3 μ l
Total	10 μl	10 μl	10 μl	10 μl

10) To the wells designated as "Blank," add 3 μ l of water.

11) Incubate at room temperature for 1 hour.

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 distilled water (v/v).
- 2) Dilute anti-Rabbit Acceptor beads (Perkin Elmer #AL104C) 1:250-fold with **1x Detection Buffer**. Add 5 μ l per well. Shake plate briefly.
- 3) Dilute **Primary antibody 4-3** 10-fold with **1x Detection Buffer**. Add 5 μ l per well. Shake plate. Incubate 30 min. at room temperature.

Step 3:

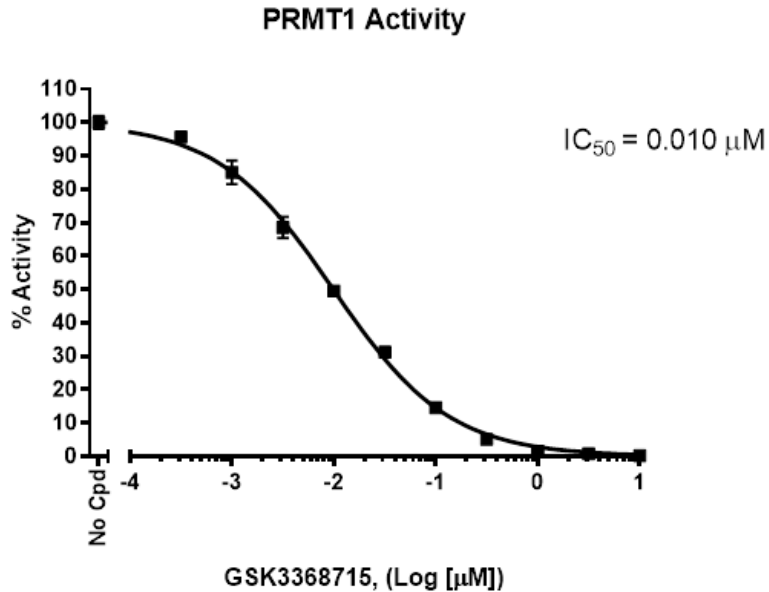
- 1) Dilute Streptavidin-conjugated donor beads (PE #6760002) 125-fold with **1x Detection Buffer**. Add 10 μ l per well. Incubate for 10 min. at room temperature.
- 2) Read Alpha-counts. The "Blank" value is subtracted from all other values.

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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 (<i>E. coli</i>)	#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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