



6044 Cornerstone Court W, Ste E  
San Diego, CA 92121  
**Tel:** 1.858.829.3082  
**Fax:** 1.858.481.8694  
**Email:** [info@bpsbioscience.com](mailto:info@bpsbioscience.com)

**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<sup>®</sup> 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	<b>(Avoid freeze/thaw cycles!)</b>
52120-A	20 µM S-adenosylmethionine	500 µl	-80 °C	
52150-A	Primary antibody 4	400 µl	-80 °C	
	Biotinylated histone H4 peptide substrate	400 µl	-80 °C	
52170-A	4x HMT assay Buffer 2	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<sub>3</sub>) or metal ions (Fe<sup>2+</sup>, Fe<sup>3+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup> and Ni<sup>2+</sup>). 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):** Dillon SC, *et al.* 2005. *Genome Biology* 6:227.

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## ASSAY PROTOCOL:

All samples and controls should be tested in duplicate.

### Step 1:

- 1) Prepare the master mixture: N wells x (2.5  $\mu$ l **4x HMT Buffer 2** + 1  $\mu$ l **S-adenosyl-methionine** (20  $\mu$ M) + 1.0  $\mu$ l **Biotinylated substrate**). Add 4.5  $\mu$ l to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2.5  $\mu$ l **4x HMT Buffer 2** + 1.0  $\mu$ l **Biotinylated substrate** + 1.0  $\mu$ l water.
- 2) Add 2.5  $\mu$ l of Inhibitor solution of each well labeled as "Test Inhibitor". For the "Positive Control", "Substrate Control" and "Blank", add 2.5  $\mu$ l of the same solution without inhibitor (Inhibitor buffer).
- 3) Prepare **1x HMT Buffer 2** by adding 1 part of **4x HMT Buffer 2** to 3 parts water (v/v).
- 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 2** at 0.25 ng/ $\mu$ l. 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 2	2.5 $\mu$ l	2.5 $\mu$ l	2.5 $\mu$ l	2.5 $\mu$ l
20 $\mu$ M S-adenosylmethionine	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l	–
Biotinylated substrate	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l
H <sub>2</sub> O	3 $\mu$ l	–	–	1 $\mu$ l
Test Inhibitor/Activator	–	–	2.5 $\mu$ l	–
Inhibitor Buffer (no inhibitor)	2.5 $\mu$ l	2.5 $\mu$ l	–	2.5 $\mu$ l
PRMT1 (0.25 ng/ $\mu$ l)	–	3 $\mu$ l	3 $\mu$ l	3 $\mu$ l
<b>Total</b>	<b>10 <math>\mu</math>l</b>	<b>10 <math>\mu</math>l</b>	<b>10 <math>\mu</math>l</b>	<b>10 <math>\mu</math>l</b>

- 6) To the wells designated as "Blank", add 3  $\mu$ l of water.
- 7) Initiate reaction by adding 3  $\mu$ l 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:**

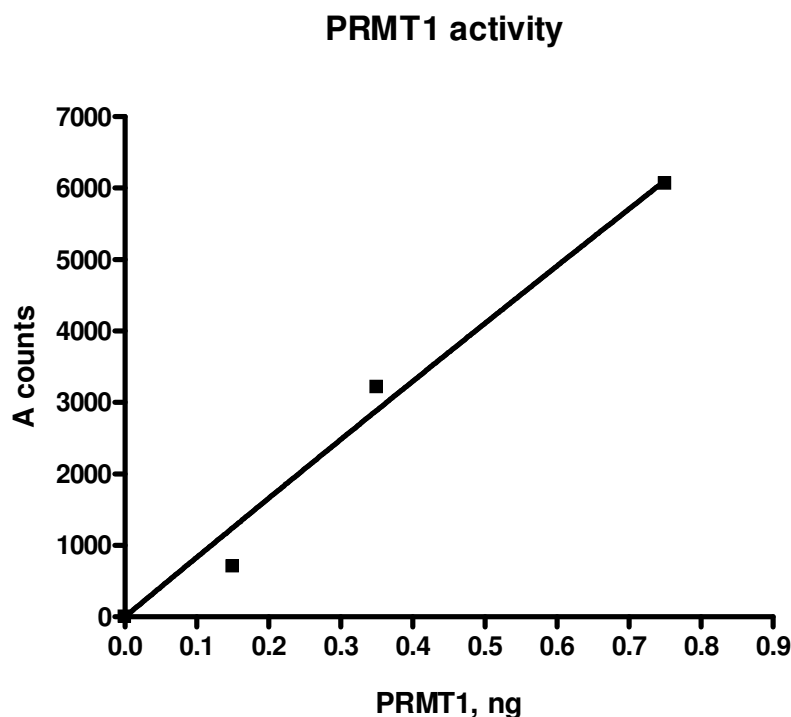
**Note: Protect your samples from direct exposure to light!**

- 1) Dilute anti-Rabbit Acceptor beads (Perkin Elmer #AL104C) 1:250-fold with **1x Detection Buffer**. Add 5 µl per well. Shake plate briefly.
- 2) Dilute **Primary antibody 4** 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](mailto: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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