

**Data Sheet**  
***PRMT6 Homogeneous Assay Kit***  
**Catalog #52056**  
**Size: 384 reactions**

**DESCRIPTION:** The *PRMT6 Homogeneous Assay Kit* is designed to measure PRMT6 activity for screening and profiling applications. PRMT6 is a histone methyltransferase that exhibits methylation activity toward histone H3-R2 and H4-R3. The *PRMT6 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with biotinylated histone H4 peptide substrate, primary antibody, methylation assay buffer, and purified PRMT6 for 384 enzyme reactions. The key to the *PRMT6 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 PRMT6 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:**

Catalog #	Component	Amount	Storage	
51049	PRMT6	20 µg	-80°C	<b>(Avoid freeze/thaw cycles!)</b>
52120	400 µM S-adenosylmethionine	250 µl	-80°C	
52150-3	Primary antibody 4-3	200 µl	-80°C	
	Biotinylated histone H4 peptide substrate	500 µl	-80°C	
52192-A	4x HMT assay Buffer 6A*	3 ml	-20°C	
	4x Detection Buffer	2 ml	-20°C	

\*Add 31 µl of 0.5M DTT before use.

*Since January of 2019, this kit has been improved with a more specific antibody and reformulated buffer. The previous version of PRMT6 kit #52056 can still be purchased upon special request*

**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  
Adjustable micropipettor and sterile tips

**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

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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.

**REFERENCE(S):** 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) Add 31  $\mu$ l of 0.5M DTT before use. Prepare the master mixture: N wells x (2.5  $\mu$ l **4x HMT Assay Buffer 6** + 0.5  $\mu$ l **S-adenosylmethionine** (400  $\mu$ M) + 1.0  $\mu$ l **Biotinylated substrate** + 1.0  $\mu$ l water). Add 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 6** + 1.0  $\mu$ l **Biotinylated substrate** + 1.5  $\mu$ 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 6** by adding 1 part of **4x HMT Buffer 6** to 3 parts water (v/v).
- 4) Thaw **PRMT6** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT6** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PRMT6 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme.*
- 5) Dilute **PRMT6** in **1X HMT assay Buffer 6** at 20 ng/ $\mu$ l. Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use

	Positive Control	Test Sample	Substrate Control	Blank
4x HMT Assay Buffer 6	2.5 $\mu$ l	2.5 $\mu$ l	2.5 $\mu$ l	2.5 $\mu$ l
400 $\mu$ M S-adenosylmethionine	0.5 $\mu$ l	0.5 $\mu$ l	–	0.5 $\mu$ l
Biotinylated substrate	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l	1 $\mu$ l
H <sub>2</sub> O	1 $\mu$ l	1 $\mu$ l	1.5 $\mu$ l	3.5 $\mu$ l
Test Inhibitor/Activator	–	2.5 $\mu$ l	–	–
Inhibitor Buffer (no inhibitor)	2.5 $\mu$ l	–	2.5 $\mu$ l	2.5 $\mu$ l
PRMT6 (20 ng/ $\mu$ l)	2.5 $\mu$ l	2.5 $\mu$ l	2.5 $\mu$ 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>

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6) To the wells designated as "Blank", add 2.5  $\mu$ l of water.

7) Initiate reaction by adding 2.5  $\mu$ l of diluted **PRMT6** enzyme to the wells designated "Positive Control", "Substrate Control", and "Test Sample". Incubate at room temperature for 1 hour.

**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  $\mu$ l per well. Shake plate briefly.

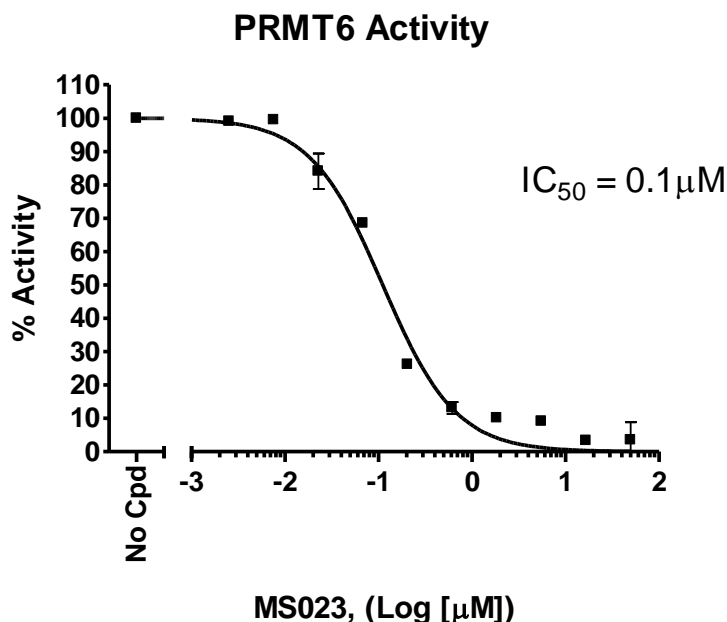
2) Dilute **Primary antibody 4** 10-fold with **1x Detection Buffer**. Add 5  $\mu$ l per well. Shake plate. Incubate 30 min at room temperature.

**Step 3:**

1) Dilute Streptavidin-conjugated donor beads (PerkinElmer #6760002) 125-fold with **1x Detection Buffer**. Add 10  $\mu$ l per well. Incubate for 5 min. at room temperature.

2) Read Alpha-counts.

**Example of Assay Results:**



PRMT6 enzyme activity, measured using the *PRMT6 Homogeneous Assay Kit*, BPS Bioscience Cat. #52056. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com*

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**RELATED PRODUCTS:**

PRMT1 Homogeneous Assay Kit	#52054	384 reactions
PRMT3 Homogeneous Assay Kit	#52055	384 reactions
PRMT5 Homogeneous Assay Kit	#52052	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
PRMT1 recombinant protein (Sf9)	#51041	20 µg
PRMT3 recombinant protein	#51043	50 µg
PRMT4 (CARM 1) recombinant protein	#51047	20 µg
PRMT5/MEP50 recombinant protein (Sf9)	#51048	20 µg
PRMT6 recombinant protein	#51046	20 µg
PRMT7 recombinant protein	#51054	20 µg
PRMT8 recombinant protein	#51052	20 µg
PRMT9 recombinant protein	#51053	20 µg

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