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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	***(Avoid freeze/ thaw cycles!)***
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_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

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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 µl of 0.5M DTT before use. Prepare the master mixture: N wells x (2.5 µl **4x HMT Assay Buffer 6** + 0.5 µl **S-adenosylmethionine** (400 µM) + 1.0 µl **Biotinylated substrate** + 1.0 µl water). Add 5 µl to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2.5 µl **4x HMT Buffer 6** + 1.0 µl **Biotinylated substrate** + 1.5 µl water.

2) 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).

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/µl. 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 µl	2.5 µl	2.5 µl	2.5 µl
400 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 µl	1 µl	1.5 µl	3.5 µl
Test Inhibitor/Activator	–	2.5 µl	–	–
Inhibitor Buffer (no inhibitor)	2.5 µl	–	2.5 µl	2.5 µl
PRMT6 (20 ng/µl)	2.5 µl	2.5 µl	2.5 µl	–
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

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6) To the wells designated as "Blank", add 2.5 µl of water.

7) Initiate reaction by adding 2.5 µl 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 µl per well. Shake plate briefly.

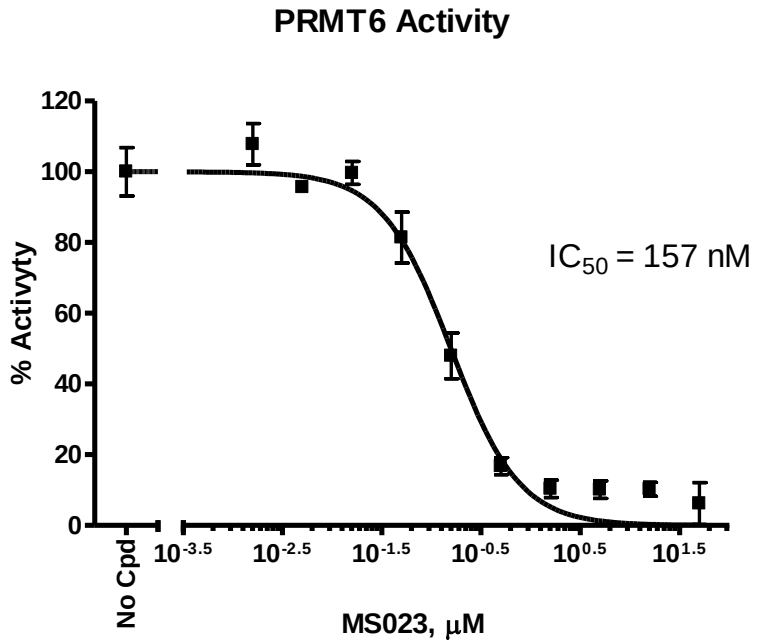
2) Dilute **Primary antibody 4** 10-fold with **1x Detection Buffer**. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature.

Step 3:

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

2) Read Alpha-counts.

Example of Assay Results:



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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 info@bpsbioscience.com*

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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