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Data Sheet

PRMT4 Homogeneous Assay Kit

Catalog #52068
Size: 384 reactions

DESCRIPTION: The *PRMT4 Homogeneous Assay Kit* is designed to measure PRMT4 activity for screening and profiling applications. PRMT4 (CARM1) is an arginine methyltransferase and dual functional coregulator that facilitates transcription initiation by methylation of Arg17 and Arg26 of histone H3. The *PRMT4 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with His-tagged PRMT4 substrate, primary antibody, methylation assay buffer, and purified PRMT4 for 384 enzyme reactions. The key to the *PRMT4 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 PRMT4 enzyme is incubated with the substrate. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51047	PRMT4/CARM1	60 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120-B	100 µM S-adenosylmethionine	250 µl	-80°C	
52140Z3	Primary antibody 28	20 µl	-80°C	
	PRMT4 substrate	400 µl	-80°C	
	4x PRMT4 assay buffer	3x1 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 (PerkinElmer #AL104C)
AlphaScreen Nickel donor beads, 5 mg/ml (PerkinElmer #AS101D)
Optiplate-384 (PerkinElmer #6007290)

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 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: Yang, Y., Bedford, M.T. 2013. *Nat Rev Cancer*. **13(1):**37-50.

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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 (2.0 µl **4x PRMT4 Assay buffer** + 0.5 µl **S-adenosylmethionine** (100 µM) + 1 µl **PRMT4 substrate** + 1.5 µl water). Add 5 µl to wells designated "Positive Control", "Test Sample", and "Blank". To wells labeled "Substrate Control", add 2 µl **4x PRMT4 Assay buffer** + 1 µl **PRMT4 substrate** + 2 µl water.

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

3) Prepare **1x PRMT4 buffer** by adding 1 part of **4x PRMT4 Assay buffer** to 3 parts water (v/v).

4) Thaw **PRMT4** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT4** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PRMT4 is very sensitive to freeze/thaw cycles. Do not re-use thawed aliquots or diluted enzyme. Depending on the nature of inhibitor, pre-incubation with the enzyme may be necessary.*

5) Dilute **PRMT4** in **1X PRMT4 Assay buffer** at 75 ng/µl (150 ng/2 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

	Positive Control	Test Sample	Substrate Control	Blank
4x PRMT4 Assay buffer	2 µl	2 µl	2 µl	2 µl
100 µM S-adenosylmethionine	0.5 µl	0.5 µl	–	0.5 µl
PRMT4 substrate	1 µl	1 µl	1 µl	1 µl
H_2O	1.5 µl	1.5 µl	2 µl	1.5 µl
Test Inhibitor/Activator	–	3 µl	–	–
Inhibitor Buffer (no inhibitor)	3 µl	–	3 µl	3 µl
PRMT4 (75 ng/µl)	2 µl	2 µl	2 µl	–
1x PRMT4 Assay buffer	–	–	–	2
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

6) To the wells designated as "Blank", add 2 µl of 1x PRMT4 Assay buffer.

7) Initiate reaction by adding 2 µl of diluted **PRMT4** enzyme to the wells designated "Positive Control", "Substrate Control", and "Test Sample". Incubate 1 hour with slow shaking at room temperature.

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Protect your samples from direct exposure to light for steps 2 and 3!

Step 2:

1) Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) 1:250-fold with **1x Detection buffer** (made by diluting **4x Detection buffer** 1:4 in distilled water). Add 5 µl per well. Shake plate briefly.

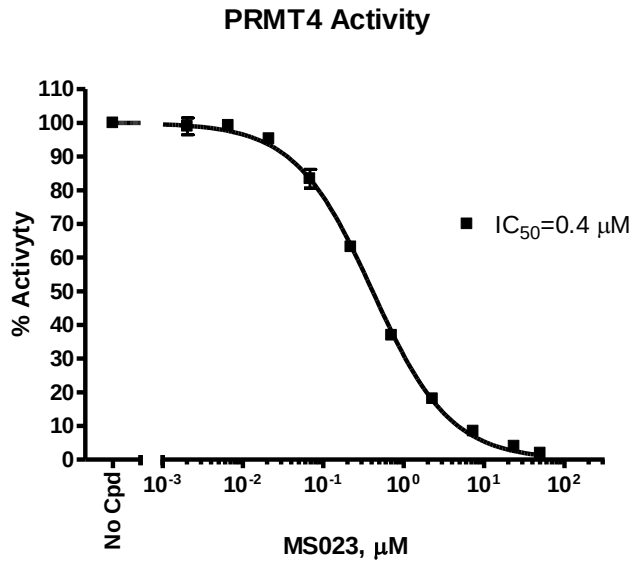
2) Dilute **Primary antibody 28** 100-fold with **1x Detection buffer**. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature.
(Alternatively, dilute anti-Rabbit Acceptor beads (1:500) and Primary antibody 28 (1:200) with 1x Detection buffer in one step. Add 10 µL of acceptor beads/antibody mixture per well.)

Step 3:

1) Dilute Nickel donor beads (PerkinElmer #6765300) 125-fold with **1x Detection buffer**. Add 10 µl per well. Incubate for 45-60 min. at room temperature.

2) Read Alpha-counts.

Examples of Assay Results:



PRMT4 enzyme activity, measured using the *PRMT4 Homogeneous Assay Kit*, BPS Bioscience Cat. #... *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@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
PRMT6 Homogeneous Assay Kit	#52056	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 (*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
PRMT4 peptide substrate, Biotin-labeled	#50352	80 nmole

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