6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

Data Sheet

PRMT9 Homogeneous Assay Kit

Catalog #52069
Size: 384 reactions

DESCRIPTION: The *PRMT9 Homogeneous Assay Kit* is designed to measure PRMT9 activity for screening and profiling applications. PRMT9 is a histone methyltransferase that exhibits methylation activity toward an arginine residue of SAP145. The *PRMT9 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with GST-tagged PRMT9 substrate, primary antibody, methylation assay buffer, and purified PRMT9 for 384 enzyme reactions. The key to the *PRMT9 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 PRMT9 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	
52057	PRMT9	20 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120-A	250 µM S-adenosylmethionine	400 µl	-80°C	
52140Z3	Primary antibody 28	20 µl	-80°C	
	PRMT9 substrate	400 µl	-80°C	
52170-A	4x HMT assay buffer 2A	3 ml	-20°C	
	4x PRMT9 detection buffer	2 ml	-20°C	
	Protective film (to seal the wells)			

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA® anti-rIgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen® Glutathione donor beads, 5 mg/ml (PerkinElmer #6765300)
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(S): Cook, J, et al., Biochem. Biophys. Res. Commun. 2006 Apr; 342(2):475-481.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 HMT assay buffer 2A** + 1.0 µl **S-adenosylmethionine** (250 µM) + 0.5 µl **PRMT9 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 HMT assay buffer 2A** + 0.5 µl **PRMT9 substrate** + 2.5 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 HMT buffer 2A** by adding 1 part of **4x HMT buffer 2A** to 3 parts water (v/v).

4) Thaw **PRMT9** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT9** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PRMT9 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 **PRMT9** in **1X HMT Assay buffer 2A** at 25 ng/µl (50 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 HMT assay buffer 2A	2 µl	2 µl	2 µl	2 µl
250 µM S-adenosylmethionine	1 µl	1 µl	–	1 µl
PRMT9 substrate	0.5 µl	0.5 µl	0.5 µl	0.5 µl
H_2O	1.5 µl	1.5 µl	2.5 µl	1.5 µl
Test Inhibitor/Activator	–	3 µl	–	–
Inhibitor Buffer (no inhibitor)	3 µl	–	3 µl	3 µl
PRMT9 (25 ng/µl)	2 µl	2 µl	2 µl	–
1x HMT assay buffer 2A	–	–	–	2
Total	**10 µl**	**10 µl**	**10 µl**	**10 µl**

6) To the wells designated as "Blank", add 2 µl of 1x PRMT9 Assay buffer.

7) Initiate reaction by adding 2 µl of diluted **PRMT9** enzyme to the wells designated "Positive Control", "Substrate Control", and "Test Sample". Seal the wells with the protective film. Incubate overnight with slow shaking at room temperature.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

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 PRMT9 Detection buffer** (made by diluting **4x PRMT9 Detection buffer** 1:4 in distilled water). Add 5 µl per well. Shake plate briefly.

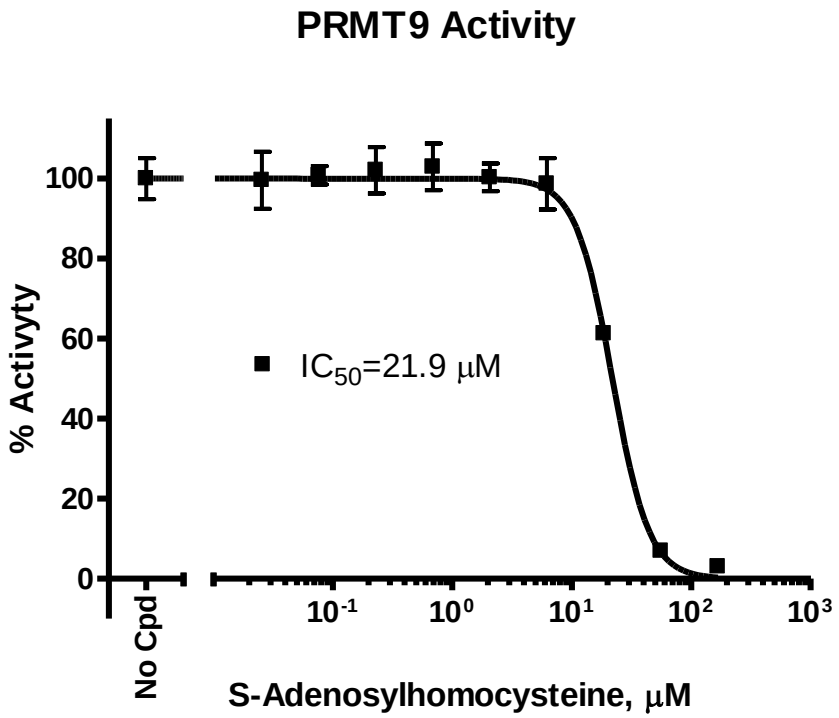
2) Dilute **Primary antibody 28** 100-fold with **1x PRMT9 Detection buffer**. Add 5 µl per well. Shake plate. Incubate 30 min at room temperature.
(Alternatively, dilute anti-Rabbit Acceptor beads (1:500) and Primary antibody 28 (1:200) with 1x Detection buffer in one step. Add 10 µL of acceptor beads/antibody mixture per well.)

Step 3:

1) Dilute Glutathione donor beads (PerkinElmer #6765300) 125-fold with **1x PRMT9 Detection buffer**. Add 10 µl per well. Incubate for 30-60 min. at room temperature.

2) Read Alpha-counts.

Examples of Assay Results:



OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.

6042 Cornerstone Ct. West, Ste. B
San Diego, CA 92121
Tel: 1.858.829.3082
Fax: 1.858.481.8694
Email: info@bpsbioscience.com

PRMT9 enzyme activity, measured using the *PRMT9 Homogeneous Assay Kit*, BPS Bioscience Cat. #... *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
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
PRMT5 recombinant protein (HEK293)	#51045	20 µg
PRMT5/MEP50 recombinant protein (Sf9)	#51048	20 µg
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
PRMT6 recombinant protein	#51049	20 µg
PRMT7 recombinant protein	#51054	20 µg
PRMT8 recombinant protein	#51052	20 µg
PRMT9 recombinant protein	#51053	20 µg

AlphaScreen® and AlphaLISA® are registered trademarks of PerkinElmer, Inc.

OUR PRODUCTS ARE FOR RESEARCH USE ONLY. NOT FOR DIAGNOSTIC OR THERAPEUTIC USE.