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Data Sheet

PRMT7 Homogeneous Assay Kit (Automethylation)

Catalog #79721
Size: 384 reactions

DESCRIPTION: The *PRMT7 Homogeneous Assay Kit (Automethylation)* is designed to measure PRMT7 automethylation for screening and profiling applications. Automethylation of PRMT7 plays a key role in inducing the epithelial–mesenchymal transition program and in promoting the migratory and invasive behavior of breast cancer cells. The *PRMT7 Homogeneous Assay Kit (Automethylation)* comes in a convenient AlphaLISA® format, with S-adenosylmethionine (SAM), primary antibody, methylation assay buffer, and purified Flag-tagged PRMT7 for 384 enzyme reactions. The key to the *PRMT7 Homogeneous Assay Kit (Automethylation)* is a highly specific antibody that recognizes methylated PRMT7. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, a sample containing PRMT7 enzyme is incubated with SAM. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

Note: To study methylation of histone proteins by PRMT7, we recommend using our PRMT7 Homogeneous Assay Kit (H2b), #79720.

COMPONENTS:

Catalog #	Component	Amount	Storage	
51054	PRMT7-FLAG-tag	60 µg	-80°C	***Avoid freeze/ thaw cycles!***
52120-A	20 µM S-adenosylmethionine	400 µl	-80°C	
52140Z3	Primary antibody 28	20 µl	-80°C	
	4x PRMT7 assay buffer (add DTT before experiment)	3x1 ml	-20°C	
	4x PRMT7 Detection buffer	2 ml	-20°C	

MATERIALS OR INSTRUMENTS REQUIRED BUT NOT SUPPLIED:
AlphaLISA® anti-rIgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
AlphaScreen® Flag donor beads, 5 mg/ml (PerkinElmer #AS103D)
Optiplate-384 (PerkinElmer #6007290)
Dithiothreitol (DTT), 0.5 M

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.

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STABILITY: At least one year from date of receipt when stored as directed.

REFERENCE(S):
1. You Feng, *et al.* 2014. *J Biol Chem* **289:**32604.
2. Pengyu Geng, *et al.* 2017. *FASEB J* **31:**2287.

ASSAY PROTOCOL:
All samples and controls should be tested in duplicate.

Step 1:

1) Add 10 µl 0.5M DTT (not provided) to 1-ml tube with **4x PRMT7 Assay Buffer**. Prepare **1x PRMT7 Assay Buffer** by adding 1 part of **4x PRMT7 Assay Buffer** to 3 parts water (v/v). Reserve at least 800 µl of undiluted **4x PRMT7 Assay Buffer** for preparing the master mixture.

2) Prepare the master mixture: N wells x (2.0 µl **4x PRMT7 Assay buffer** + 0.5 µl **S-adenosylmethionine** (20 µM) + 2.5 µl water). Add 5 µl to wells designated "Positive Control", "Test Sample", and "Blank".

3) Add 3 µl of Inhibitor solution of each well labeled as "Test Inhibitor". We recommend to use **1x PRMT7 Assay buffer** for intermediate dilution of inhibitors. For the "Positive Control", "Substrate Control" and "Blank", add 3 µl of the same solution without inhibitor (Inhibitor buffer).

4) Thaw **PRMT7** on ice. Upon first thaw, briefly spin tube containing enzyme to recover full content of the tube. Aliquot **PRMT7** enzyme into single use aliquots. Store remaining undiluted enzyme in aliquots at -80°C. *Note: PRMT7 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.*

	Positive Control	Test Sample	Blank
4x PRMT7 Assay buffer	2 µl	2 µl	2 µl
20 µM S-adenosylmethionine	0.5 µl	0.5 µl	0.5 µl
H_2O	2.5 µl	2.5 µl	2.5 µl
Test Inhibitor/Activator	–	3 µl	–
Inhibitor Buffer (1xPRMT7 Assay buffer)	3 µl	–	3 µl
1x PRMT7 Assay buffer	–	–	2 µl
PRMT7 (75 ng/µl)	2 µl	2 µl	–
Total	**10 µl**	**10 µl**	**10 µl**

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5) Dilute **PRMT7** in **1X PRMT7 Assay buffer** at 75 ng/µl (150 ng/2 µl). Keep diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

6) To the wells designated as "Blank", add 2 µl of **1x PRMT7 Assay buffer**.

7) Initiate reaction by adding 2 µl of diluted **PRMT7** enzyme to the wells designated “Positive Control”, “Substrate Control”, and "Test Sample". Incubate two hours at room temperature with slow shaking.

(Alternatively, preincubate diluted enzyme with inhibitor for 30 minutes, then initiate 2 hour reaction by addition of S-adenosylmethionine.)

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

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 FLAG donor beads (PerkinElmer #AS103D) 125-fold with **1x Detection buffer**. Add 10 µl per well. Incubate for 30-60 min. at room temperature.

2) Read Alpha-counts.

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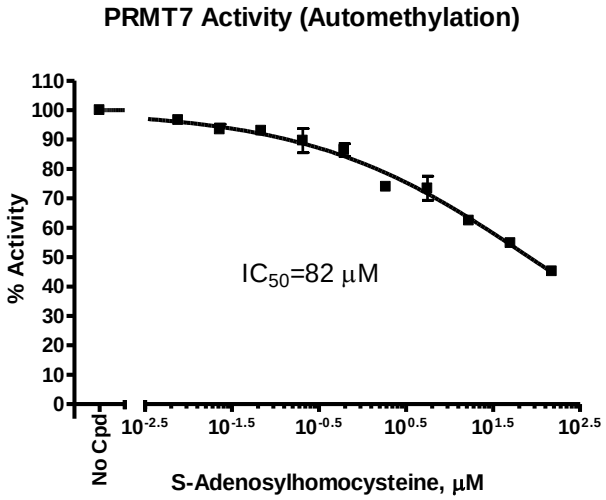
Example of Assay Results:



Inhibition of PRMT7 automethylation activity by S-adenosyl homocysteine, BPS Bioscience #52031, measured using the *PRMT7 Homogeneous Assay Kit (Automethylation)*, BPS Bioscience #79721. *Data shown is lot-specific. For lot-specific information, please contact BPS Bioscience, Inc. at info@bpsbioscience.com*

RELATED PRODUCTS:

PRMT7 recombinant protein	#51054	20 µg
PRMT7 Homogeneous Assay Kit (H2b)	#79720	384 reactions
S-adenosyl homocysteine	52031	250 µl
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
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

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