PRMT7 Homogeneous Assay Kit (Histone H2b)

#79720
384 reactions

Description

The *PRMT7 Homogeneous Assay Kit (Histone H2b)* is designed to measure PRMT7 activity for screening and profiling applications. PRMT7 methylates arginine residues on various protein substrates and is involved in DNA transcription, RNA splicing, DNA repair, cell differentiation, and metastasis. The *PRMT7 Homogeneous Assay Kit* comes in a convenient AlphaLISA® format, with His-tagged PRMT7 substrate, primary antibody, methylation assay buffer, and purified PRMT7 for 384 enzyme reactions. The advantage of the *PRMT7 Homogeneous Assay Kit* is a highly specific antibody that specifically recognizes the methylated substrate. With this kit, only three steps are required for methyltransferase detection. First, a sample containing PRMT7 enzyme is incubated with the substrate. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

Applications

- Study enzyme kinetics
- Screen compounds in high throughput applications

Supplied Materials

Catalog #	Name	Amount	Storage
51054	PRMT7*	40 µg	-80°C
52120	20 µM S-adenosylmethionine	2 x 250 µl	-80°C
52140Z3	Primary Antibody 28	20 µl	-80°C
	PRMT7 Histone substrate	200 µl	-80°C
	4x PRMT7 assay buffer (add fresh DTT before use)	3 x 1 ml	-20°C
	4x Detection Buffer 3	2 ml	-20°C

**The concentration of the proteins is lot-specific and will be indicated on the tube.*

Materials Required but Not Supplied

Name	Catalog #
AlphaLISA® anti-rIgG acceptor beads, 5 mg/ml	PerkinElmer #AL104C
AlphaScreen® Nickel donor beads, 5 mg/ml	PerkinElmer #AS101D
Optiplate-384	PerkinElmer #6007290
Adjustable micropipettor and sterile tips	
Dithiothreitol (DTT), 0.5 M	

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed.

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Safety

Kit components should be considered hazardous and harmful by inhalation, in contact with skin, eyes, clothing, and if swallowed. If contact occurs, wash thoroughly. This product is for research purposes only and not for human or therapeutic use.

Contraindications

Green and blue dyes, such as Trypan Blue, absorb light in the AlphaScreen™ signal emission range (520-620 nm). Avoid the use of the potent singlet oxygen quenchers such as sodium azide (NaN3) 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.

ASSAY PROTOCOL

All samples and controls should be tested in duplicate.

STEP 1:

1. Add 10 µl of 0.5M DTT (not provided) to 1 ml of stock 4x PRMT7 Assay Buffer. Prepare 1x PRMT7 Assay Buffer by adding 1 volume of stock PRMT7 Assay Buffer to 3 volumes of distilled water.

2. Prepare the Master Mix (5 µl/well): N wells x (2.0 µl of 4x PRMT7 **Assay buffer** containing DTT + 0.5 µl of **S-adenosylmethionine** (20 µM) + 0.5 µl of PRMT7 **substrate** + 2 µl of distilled **water**).

3. Add 5 µl to the wells designated “Positive Control”, “Test Compound”, and “Blank”.

4. To the wells labeled “No-Substrate Control”, add 2 µl of 4x PRMT7 **Assay buffer** containing DTT + 0.5 µl **S-adenosylmethionine** (20 µM) + 2.5 µl **water**.

5. Prepare the Test Compound **(3 µl/well)**. The final volume of the reaction is 10 µl.

   Without DMSO

   5.1. If the Test Compound is water-soluble, prepare serial dilutions in the PRMT7 Assay Buffer, 3.33-fold more concentrated than the desired final concentrations.

   With DMSO

   5.2. If the Test Compound is soluble in DMSO, prepare it at 100-fold the highest desired concentration in DMSO, then dilute 33-fold in **1x Assay Buffer** to prepare the highest concentration of the 3.33-fold intermediate dilutions. The concentration of DMSO is now 3.33%.

      a. Using **1x Assay Buffer** in 3.33% DMSO, prepare serial dilutions of the Test Inhibitor at 3.33-fold the desired final concentrations to keep the concentration of DMSO constant.

      b. For positive and negative controls, prepare 3.33% DMSO in **1x Assay Buffer** (vol/vol) so that all wells contain the same amount of DMSO (**Diluent Solution**).

      Note: The final concentration of DMSO in the assay should not exceed 1%.

6. Add 3 µl of Compound dilutions to each well labeled as “Test Compound”.

7. For the “Positive Control", “No-Substrate Control” and “Blank”, add 3 µl of the solution used to dilute the compound without inhibitor (**Diluent Solution**).

8. Thaw **PRMT7 protein** on ice. Briefly spin the tube containing the enzyme to recover its full contents. Dilute PRMT7 in 1X PRMT7 Assay buffer at **50 ng/µl** (100 ng/2 µl). Keep the diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

   Use enough enzyme for the assay. Aliquot unused, undiluted PRMT7 enzyme into single use aliquots and store 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 the compound, pre-incubation with the enzyme may be necessary.

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

10. Initiate the reaction by adding 2 µl of diluted PRMT7 enzyme to the wells designated “Positive Control”, “No-Substrate Control”, and "Test Sample". Incubate two hours at room temperature with slow shaking.

    Alternatively, preincubate the diluted enzyme with the Test Compound for 30 minutes, then initiate the 2 hour-reaction by addition of S-adenosylmethionine and Substrate.

Component	**No-Substrate control**
4 x Assay Buffer	2 µl
20 µM S-adenosylmethionine	0.5 µl
Distilled water	2.5 µl
Diluent Solution	3 µl
PRMT7 (50 ng/µl)	2 µl
Total	**10 µl**

Component	**Blank**	**Positive Control**	**Test Compound**
Master Mix	5 µl	5 µl	5 µl
Test Compound	-	-	3 µl
Diluent Solution	3 µl	3 µl	-
PRMT7 (50 ng/µl)	2 µl	2 µl	2 µl
Total	**10 µl**	**10 µl**	**10 µl**

Protect your samples from direct exposure to light during the next steps!

STEP 2:

1. Prepare the 1x Detection buffer 3 by dilution of 1 volume of stock 4x Detection buffer 3 in 3 volumes of distilled water.

2. Dilute anti-Rabbit Acceptor beads (PerkinElmer #AL104C) 1:250-fold with 1x Detection buffer 3.
3. Add 5 µl of the diluted Acceptor Beads to each well. Shake the plate briefly.

4. Dilute the Primary antibody 28 100-fold using 1x Detection buffer 3.

5. Add 5 µl of the diluted Primary Antibody to each well. Shake the plate.

6. Incubate 30 min at room temperature.

   Alternatively, dilute the anti-Rabbit Acceptor beads to 1:500 and the Primary antibody to 1:200 with 1x Detection buffer 3 in one step. Add 10 µl of the acceptor beads/antibody mixture per well.

STEP 3:

1. Dilute the Nickel donor beads (PerkinElmer #AS101D) 125-fold with 1x Detection buffer 3.

2. Add 10 µl of the diluted Nickel donor beads to each well.

3. Incubate for 30-60 minutes at room temperature.

   Alternatively, for an improved signal, we recommend sealing the assay plate and incubating overnight in a dark room at room temperature prior to reading the Alpha-counts. Please ensure the plate sealer is removed before obtaining the readings.

4. Read the Alpha-counts.

Example of Assay Results

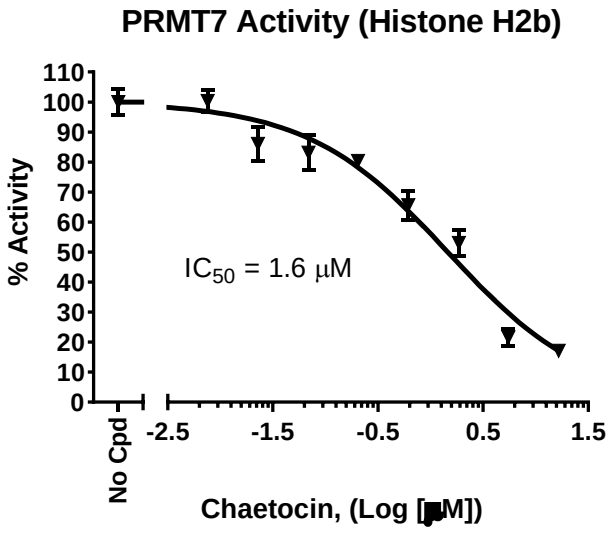

Figure 1: Effect of Chaetocin on PRMT7 activity.
Serial dilutions of chaetocin were incubated with PRMT7 to test enzymatic activity as described

in the protocol above. Results are expressed as percent of the no-compound control (set at 100%).

For lot-specific information, please contact BPS Bioscience, Inc. at support@bpsbioscience.com

General Considerations

Plates and Instruments: A plate reader capable of Alpha technology detection is required. We recommend using PerkinElmer 384-Optiplate #6007290.

"Blank" Control: The "Blank" control is important to determine the background signal in the assay. We recommend doing these at least in duplicate.

Troubleshooting Guide

Visit bpsbioscience.com/assay-kits-faq for detailed troubleshooting instructions. For all further questions, please email support@bpsbioscience.com

References

You Feng, *et al*. 2014. *J Biol Chem* **289**: 32604.

Related Products

Products	*Catalog #*	*Size*
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
PRMT3 recombinant protein	51043	50 µg
PRMT4 (CARM1) 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