Description

The PRMT5 Homogeneous Assay Kit is designed to measure PRMT5 activity for screening and profiling applications. PRMT5 is a histone methyltransferase that methylates histone H4-R3. The PRMT5 Homogeneous Assay Kit comes in a convenient AlphaLISA® format, with biotinylated histone H4 peptide substrate, primary antibody, methylation assay buffer, and purified PRMT5/MEP50 complex for 384 enzyme reactions. The Assay Kit relies on a highly specific antibody that recognizes the methylated form of the substrate. With this kit, only three simple steps on a microtiter plate are required for methyltransferase detection. First, a sample containing PRMT5 enzyme is incubated with the biotinylated substrate for two hours. Next, acceptor beads and primary antibody are added, then donor beads, followed by reading the Alpha-counts.

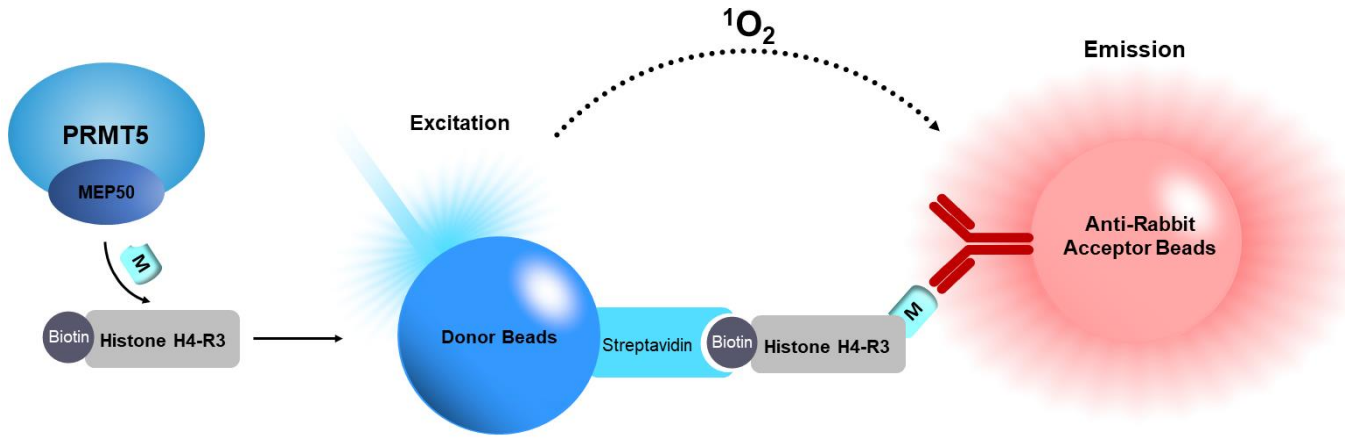

Illustration of the assay principle: The PRMT5/MEP50 enzyme methylates the biotinylated histone H4 peptide substrate. The methylated form of the peptide is recognized by a highly specific rabbit antibody, which binds to anti-Rabbit acceptor beads, while the biotin binds to streptavidin-conjugated donor beads, bringing the acceptor and donor beads in close proximity. Upon excitation of the donor bead a singlet oxygen is generated by the donor bead, which excites the acceptor bead and emits light proportionally to the level of interaction. AlphaLISA™ immunoassays are a no-wash alternative to ELISA immunoassays. These assays are robust and ideal for a minimal hands-on approach.

**NOTE: As of April 2023, this kit has been re-optimized for performance.*

Background

Protein arginine N-methyltransferase 5 (PRMT5, also known as ANM5) is part of the methylosome complex, composed of MEP50, PRMT5, WDR77 and CLNS1A. This complex methylates/demethylates specific arginine residues in histones H2AR3, H4R3, H3R2, and H3R8. It also methylates various proteins such as SUPT5H (Transcription elongation factor SPT5), Piwi proteins, RPS10, and more. MEP50 protein is the non-catalytic component of the complex and is a core component of the complex that mediates interactions with binding partners and substrates.

Applications

- Screen molecules in high-throughput applications
- Determine compound IC_{50}
- Perform real-time kinetic analyses

Supplied Materials

Catalog #	Name	Amount	Storage	
51045	PRMT5/MEP50 FLAG-Tag, His-Tag (HEK293-derived) *	10 µg	-80°C	***Avoid multiple freeze/ thaw cycles***
52120	S-adenosylmethionine (20 µM)	2 x 250 µl	-80°C	
52150-3	Primary Antibody 4-3 **	100 µl	-80°C	
	Biotinylated histone H4 peptide substrate	500 reactions	-80°C	
	PRMT5 Assay buffer (4x); **add DTT before use**	3 ml	-20°C	
52301	Detection Buffer (4x)	2 ml	-20°C	

** The initial concentration of enzyme is lot-specific and will be indicated on the tube containing the protein.*
*** The detection antibody for this kit was changed in January 2017.*

Materials Required but Not Supplied

- AlphaLISA® anti-rabbit IgG acceptor beads, 5 mg/ml (PerkinElmer #AL104C)
- AlphaScreen® Streptavidin-conjugated donor beads, 5 mg/ml (PerkinElmer #6760002)
- Optiplate-384 (PerkinElmer #6007290)
- Dithiothreitol, DTT (0.5M)

Storage Conditions

This assay kit will perform optimally for up to 6 months from date of receipt when the materials are stored as directed. ***Avoid multiple freeze/ thaw cycles!***

Safety

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

Contraindications

Green and blue dyes that absorb light in the AlphaScreen® signal emission range (520-620 nm), such as Trypan Blue, interfere with the assay. Avoid using 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 performed in duplicate
- The assay should include a “Blank”, and a “Positive Control”

Step 1:

1. Add 10 µl of **0.5M DTT** (not provided) per 1-ml **4x PRMT5 Assay Buffer**. Only prepare enough DTT-containing buffer as required for assay. Prepare **1x PRMT5 Assay Buffer** by adding 1 part of **4x PRMT5 Assay Buffer** to 3 parts of water (v/v).

2. Thaw **PRMT5** on ice. Briefly spin the tube containing the enzyme to recover the full content of the tube. If not using the entire plate at once, store the unused, undiluted enzyme in single-use aliquots at -80°C.

   Note: PRMT5 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.

3. Dilute PRMT5 in 1x PRMT5 Assay Buffer to 5 ng/µl. Keep the diluted enzyme on ice until use. Discard any unused diluted enzyme after use.

4. To the wells designated as "Blank", add 2 µl of 1x PRMT5 Assay Buffer.

5. Add 2 µl of diluted PRMT5 enzyme to the wells designated “Positive Control”, and "Test Inhibitor".

6. Prepare serial dilutions of the Test Inhibitor:

   Without DMSO
   a. If the Test Inhibitor is soluble in water, prepare dilutions of the compound in 1x PRMT5 Assay Buffer that are 3.33-fold higher than the final desired concentrations.

   Or
   With DMSO
   a. If the Test Inhibitor is dissolved in DMSO, prepare a solution of the compound in DMSO that is 100-fold higher than the highest desired concentration of the serial dilution. Then dilute 30-fold in PRMT5 Assay Buffer (at this step the compound concentration is 3.33-fold higher than the desired final concentration). The concentration of DMSO in the dilution is now 3.33%.

   b. Prepare serial dilutions of the Test Inhibitor at concentrations 3.33-fold higher than the desired final concentrations using 3.33% DMSO in 1x PRMT5 Assay Buffer to keep the concentration of DMSO constant

   c. For positive and negative controls, prepare 3.33% DMSO in 1x PRMT5 Assay Buffer (v/v) so that all wells contain the same amount of DMSO (Diluent Solution).

7. Add 3 µl of diluent solution to each well labeled as “Blank” and “Positive Control.”

8. Add 3 µl of the serial dilutions to each well labeled as “Test Inhibitor”. Preincubate diluted PRMT5 with diluted inhibitor(s) for up to 30 minutes at room temperature with slow shaking.

Component	Blank	Positive Control	Test Inhibitor
Test Inhibitor	-	-	3 µl
Diluent solution*	3 µl	3 µl	-
PRMT5 (5 ng/µl)	-	2 µl	2 µl
1x PRMT5 Assay Buffer	2 µl	-	-
Master Mix	5 µl	5 µl	5 µl
Total	**10 µl**	**10 µl**	**10 µl**

**The diluent solution contains the assay buffer with the same concentration of solvent (e.g. DMSO) as the test compound solution.*

9. Resuspend the lyophilized Biotinylated Histone H4 peptide substrate in 500 µl of distilled water.

10. Prepare the Master Mix (5 µl/well): N wells x (2 µl of **4x PRMT5 Assay Buffer** + 1 µl of **S-adenosylmethionine** (20 µM) + 1 µl of **Biotinylated substrate** + 1 µl of water).

11. Initiate the reaction by adding 5 µl of Master Mix to all wells. Incubate at room temperature for 1 hour with slow shaking.

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

Step 2:

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

2. Dilute the **Primary antibody 4-3** 30-fold with 1x Detection Buffer. Add 5 µl per well. Shake the plate.

3. Incubate 30-60 min at room temperature.

   Alternatively, dilute the anti-Rabbit Acceptor beads (1:500) and the Primary antibody 4-3 (1:60) with 1x Detection buffer in one step. Add 10 µL of acceptor beads/antibody mixture per well. Incubate 30-60 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 30 minutes at room temperature.

2. Read Alpha-counts.

Example Results

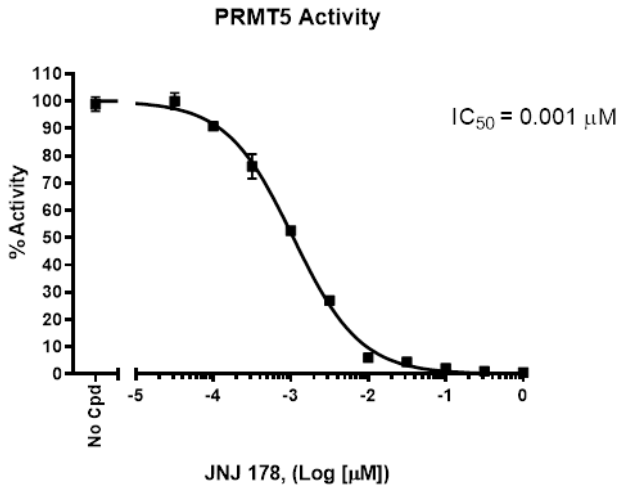

Figure 1: Inhibition of PRMT5 enzymatic activity.
PRMT5 enzymatic activity was measured in the presence of increasing concentrations of inhibitor JNJ 178 using the PRMT5 homogenous Assay Kit.

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

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

References
Dillon SC, *et al*. 2005. *Genome Biology* **6**:227.

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Related Products

Products	*Catalog #*	*Size*
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 TR-FRET Assay Kit	52171	384 reactions
PRMT4 Homogeneous Assay Kit	52068	384 reactions
PRMT1 Homogeneous Assay Kit	52054	384 reactions