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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
P365-380FG-05	5 µg
P365-380FG-10	10 µg

PRMT6, Active

Full length recombinant protein expressed in Sf9 cells

Catalog # P365-380FG
Lot # U1692-8

Product Description

Recombinant full-length human PRMT6 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The PRMT6 protein accession number is NM_018137.

Gene Aliases

HRMT1L6

Formulation

Recombinant protein stored in 50mM Tris-HCl, pH 7.5, 150mM NaCl, 10mM glutathione, 0.1mM EDTA, 0.25mM DTT, 0.1mM PMSF, 25% glycerol.

Storage and Stability

Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

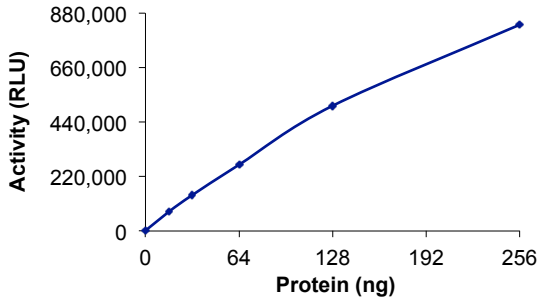
Scientific Background

PRMT6 (protein arginine methyltransferase 6), an arginine methyltransferase that exhibits type I PRMT activity and catalyzes the formation of both omega-N monomethylarginine (MMA) and asymmetrical dimethylarginine (aDMA), with a strong preference for the formation of aDMA. It can methylate itself. PRMT6 does not recognize the same substrates as PRMT4/CARM1 and displays limited substrate overlap with PRMT1. It resides in the nucleus along with PRMT1, PRMT2, and PRMT4, whereas PRMT3 and PRMT5 GFP fusions localize exclusively to the cytoplasm.

References

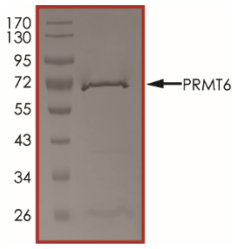
1. Frankel A, et al: The novel human protein arginine N-methyltransferase PRMT6 is a nuclear enzyme displaying unique substrate specificity. J Biol Chem. 277(5):3537-43, 2002.
2. Miranda TB, et al: Protein arginine methyltransferase 6 specifically methylates the nonhistone chromatin protein HMGA1a. Biochem Biophys Res Commun. 336(3):831-5, 2005.

Specific Activity



The specific activity of PRMT6 was determined to be **1.4 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PRMT6 was determined to be **>90%** by densitometry, approx. MW **69 kDa**.

PRMT6, Active

Full length recombinant protein expressed in Sf9 cells

Catalog #	P365-380FG
Specific Activity	1.4 nmol/min/mg
Lot #	U1692-8
Purity	>90%
Concentration	0.1 µg/µl
Stability	1yr at –70°C from date of shipment
Storage & Shipping	Store product at –70°C. For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

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Activity Assay Protocol

Reaction Components

Active Methyltransferase (Catalog #: P365-380FG)

Active PRMT6 (0.1 μg/μl) diluted with Methyltransferase Reaction Buffer and assayed as outlined in sample activity plot. (Note: these are suggested working dilutions and it is recommended that the researcher perform a serial dilution of Active PRMT6 for optimal results).

Methyltransferase Reaction Buffer

Buffer components: 20mM Tris-HCl, pH 8.0, 50 mM NaCl, 1 mM EDTA, 3 mM $MgCl_2$, 0.1 mg/ml BSA. Add 1mM DTT prior to use.

MTase-Glo™ Methyltransferase Assay (Promega, Catalog #: V7601)

S-Adenosyl-Methionine (SAM), 1mM
S-Adenosyl-Homocysteine (SAH), 15 μM
Methyltransferase-Glo™ Reagent, 10X
MTase-Glo™ Detection Solution, 1 bottle

Substrate (Catalog #: H12-58)

Histone H3 Peptide (1-21) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

The PRMT6 assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw the active PRMT6 and all Methyltransferase-Glo™ Assays kit reagents on ice.

Step 2. Prepare the following working solutions with Methyltransferase Reaction Buffer on ice:

- 2X final concentration of Active PRMT6 (Catalog # P365-380FG)
- 2X Substrate Cocktail: 40 μM of SAM and 100ng/μl of Histone H3 Peptide (1-21) (Catalog # H12-58) in water

Step 3. In a polystyrene 96-well plate, add the following components to bring the initial reaction volume to 20 μl:

Component 1. 10 μl of 2X Substrate Cocktail

Component 2. 10 μl of 2X Active PRMT6

Note: A blank control can be set up as outlined in step 3 by replacing the substrate working solution with an equal volume of Reaction Buffer.

Step 4. Mix the reaction on an orbital shaker for 2 minutes. Seal the plate with a plate seal and incubate at 37°C for 60 minutes

Step 5. Dilute 10X Methyltransferase-Glo™ Reagent with equal volume of nanopure water, and add 5 μl of the 5X Methyltransferase-Glo™ Reagent to all reaction wells

Step 6. Mix on an orbital shaker for 2 minutes and then incubate at room temperature for 30 minutes.

Step 7. Add 25 μl of MTase-GloTM Detection Solution to all reaction wells. Mix for 2 minutes and then incubate at room temperature for 30 minutes

Step 8. Read the plate using the KinaseGlo Luminescence Protocol on a GloMax plate reader (Promega; Cat# E7031)

Step 9. Using the SAH standard curve, determine the concentration of SAH produced (nM) and calculate the methyltransferase specific activity as outlined below. For a detailed protocol of how to determine SAH amount from RLUs, see MTase-Glo™ Methyltransferase Assay protocol at Promega's website: www.promega.com/protocols

Methyltransferase Specific Activity (SA) (nmol/min/mg)

$$= \frac{[SAH](nM) \times Reaction\ Volume(ul)}{Reaction\ Time\ (min) \times Enzyme\ Amount\ (mg)} \times 10^{-6}$$

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