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

PRMT4, Active

Full length recombinant protein expressed in Sf9 cells

Catalog # P365-380DG
Lot # P1545-10

Product Description

Recombinant full-length human PRMT4 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The PRMT4 protein accession number is NM_199141.

Gene Aliases

CARM1

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.

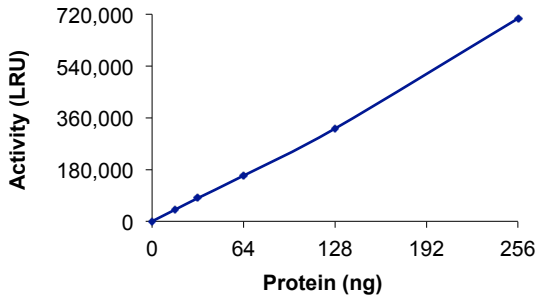
Scientific Background

Histone-arginine methyltransferase, Coactivator-associated arginine methyltransferase-1 (CARM1 or PRMT4) methylates (mono- and asymmetric dimethylation) the guanidino nitrogens of arginyl residues in several proteins involved in DNA packaging, transcription regulation, pre-mRNA splicing, and mRNA stability. It is known to enhance transcriptional activation by nuclear receptors through interactions with the coactivators p160 and cAMP response element binding protein-binding protein (CBP) and methylation of histone H3 at arginine 17 (H3-R17).

References

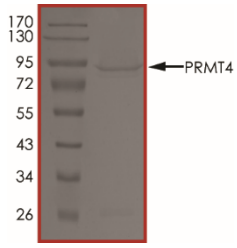
1. Miao F, Li S, Chavez V, Lanting L, Natarajan R. Coactivator-associated arginine methyltransferase-1 enhances nuclear factor-kappaB-mediated gene transcription through methylation of histone H3 at arginine 17. Mol Endocrinol. 20(7):1562-73. 2006.

Specific Activity



The specific activity of PRMT4 was determined to be **800 pmol /min/mg** as per activity assay protocol.

Purity



The purity of PRMT4 was determined to be **>90%** by densitometry, approx. MW **92 kDa**.

PRMT4, Active

Full length recombinant protein expressed in Sf9 cells

Catalog #	P365-380DG
Specific Activity	800 pmol/min/mg
Lot #	P1545-10
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 #: P365-380DG)

Active PRMT4 (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 PRMT4 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 PRMT4 assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw the active PRMT4 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 PRMT4 (Catalog # P365-380DG)
- 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 PRMT4

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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