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Catalogue #	Aliquot Size
P365-380G-05	5 µg
P365-380G-10	10 µg

PRMT1, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog # P365-380G
Lot # J606-2

Product Description

Recombinant full-length human PRMT1 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The PRMT1 protein accession number is Q99873-3.

Gene Aliases

ANM1; HCP1; IR1B4

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.

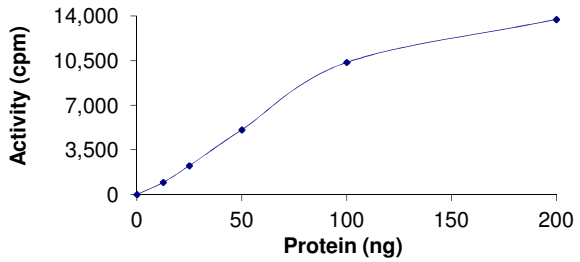
Scientific Background

PRMT1 is a protein arginine methyltransferase that functions as a histone methyltransferase specific for histone H4. PRMT1 specifically methylates arginine-3 of histone H4 in vitro and in vivo. Methylation of arg3 by PRMT1 facilitates subsequent acetylation of histone H4 tails by p300 (1). PRMT1 performs methylation of arg31 of STAT1 and this is required for transcription induced by IFN-alpha/IFN-beta (2). Arginine methylation of STAT1 is an additional posttranslational modification regulating transcription factor function, and alteration of arginine methylation might be responsible for the lack of interferon responsiveness observed in many malignancies.

References

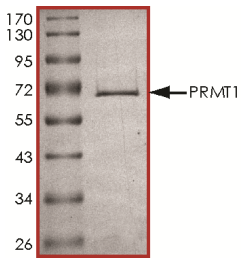
1. Wang, H. et al: Methylation of histone H4 at arginine 3 facilitating transcriptional activation by nuclear hormone receptor. Science 293: 853-857, 2001.
2. Mowen, K. A. et al: Arginine methylation of STAT1 modulates IFN-alpha/beta-induce d transcription. Cell 104: 731-741, 2001.

Specific Activity



The specific activity of PRMT1 was determined to be **6 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PRMT1 was determined to be **>90%** by densitometry, approx. MW **68 kDa**.

PRMT1, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog Number	P365-380G
Specific Activity	6 nmol/min/mg
Specific Lot Number	J606-2
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 #: P365-380G)

Active PRMT1 (0.1μg/μl) diluted with Methyltransferase Dilution Buffer II (Catalog #: M22-09) 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 PRMT1 for optimal results).

Methyltransferase Dilution Buffer II (Catalog#: M22-09)

Methyltransferase Assay Buffer II (Catalog #: M02-09) diluted at a 1:4 ratio (5X dilution) with distilled H_2O.

Methyltransferase Assay Buffer II (Catalog #: M02-09)

Buffer components: 250mM Tris-HCl, pH 8.0, 50 ng/μl BSA. Add 2mM DTT to Acetyltransferase Assay Buffer prior to use.

Adenosyl-L-methionine, S-[methyl-^{3}H] solution

The [^{3}H]-Adomet solution (0.54945μCi/μl and 10μCi/nmol) in 10mM H_2SO_4 : Ethenol (9:1) solution was purchased from PerkinElmer (Cat. # NET155250UC). The final concentration of [^{3}H]-Adomet is 54.945 μM or 54.945 pmol/μl.

Substrate (Catalog #: H13-58)

Histone H4 Peptide (1-21) substrate (SGRGKGGKGLGKGG-AKRHRKVGGKKC) diluted in distilled H_2O to a final concentration of 1mg/ml.

Assay Protocol

Step 1. Thaw [^{3}H]-Adomet solution in shielded container in a designated radioactive working area.

Step 2. Thaw the Active PRMT1, Methyltransferase Assay Buffer II, Substrate and Methyltransferase Dilution Buffer II on ice.

Step 3. In a pre-cooled microfuge tube, add the following reaction components bringing the initial reaction volume up to 20μl:

- **Component 1.** 10μl of diluted Active PRMT1 (Catalog #P365-380G)
- **Component 2.** 5μl of 1mg/ml stock solution of substrate (Catalog #H13-58)
- **Component 3.** 5μl of Methyltransferase Assay Buffer II (Catalog #: M02-09)

Step 4. Set up the blank control as outlined in step 3, excluding the addition of the substrate. Replace the substrate with an equal volume of distilled H_2O.

Step 5. Initiate the reaction by the addition of 5μl [^{3}H]-Adomet solution bringing the final volume up to 25μl and incubate the mixture in a water bath at 30°C for 30 minutes.

Step 6. After the 30 minute incubation period, terminate the reaction by spotting 20μl of the reaction mixture onto individual pre-cut strips of phosphocellulose P81 paper.

Step 7. Air dry the pre-cut P81 strip and sequentially wash in a 10% trichloroacetic acid solution with constant gentle stirring. It is recommended that the strips be washed a total of 3 intervals for approximately 10 minutes each.

Step 8. Count the radioactivity on the P81 paper in the presence of scintillation fluid in a scintillation counter.

Step 9. Determine the corrected cpm by removing the blank control value (see Step 4) for each sample and calculate the methyltransferase specific activity as outlined below.

Calculation of [^{3}H]-Adomet Specific Activity (SA) (cpm/nmol)

Specific activity (SA) = cpm for 5μl [^{3}H]-Adomet / nmoles of Adomet
5μl of a 54.945 μM Adomet solution gives 165,000cpm
Therefore 165,000cpm / 5μl*54.945 pmol/μl = 600 cpm/pmol

Methyltransferase Specific Activity (SA) (pmol/min/μg or nmol/min/mg)

Corrected cpm from reaction / [(SA of [^{3}H]-Adomet in cpm/pmol)*(Reaction time in min)*(Enzyme amount in μg or mg)]*[(Reaction Volume) / (Spot Volume)]

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