

PRMT3, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog # P365-380CG

Lot # J594-5

Product Description

Recombinant full-length human PRMT3 was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The PRMT3 gene accession number is [BC064831](#).

Gene Aliases

HRMT1L3

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.

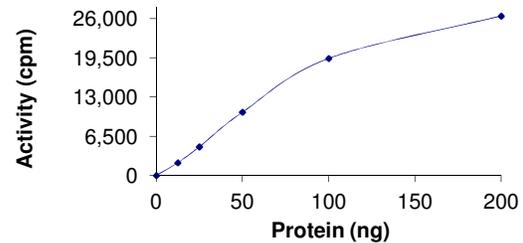
Scientific Background

PRMT3 is a protein arginine methyltransferase that catalyze the formation of asymmetric N(G),N(G)-dimethylarginine (ADMA) residues in proteins (1). 40S ribosomal protein S2 is a physiological substrate of PRMT3 and PRMT3 regulates ribosome biosynthesis at a stage beyond pre-rRNA processing. DAL-1 (differentially expressed in adenocarcinoma of the lung)/4.1B is a tumor suppressor gene that can significantly inhibits PRMT3 methylation of cellular substrates (2). Modulation of post-translational methylation may be an important mechanism through which DAL-1/4.1B affects tumor cell growth.

References

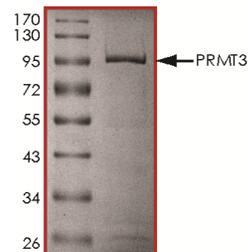
1. Tang, J. et al: PRMT 3, a type I protein arginine N-methyltransferase that differs from PRMT1 in its oligomerization, subcellular localization, substrate specificity, and regulation. *J. Biol. Chem.* 273: 16935-16945, 1998.
2. Singh V, DAL-1/4.1B tumor suppressor interacts with protein arginine N-methyltransferase 3 (PRMT3) and inhibits its ability to methylate substrates in vitro and in vivo. *Oncogene.* 2004 Oct 14;23(47):7761-71.

Specific Activity



The specific activity of PRMT3 was determined to be **12 nmol /min/mg** as per activity assay protocol.

Purity



The purity of PRMT3 was determined to be **>90%** by densitometry, approx. MW **97 kDa**.

PRMT3, Active

Recombinant full-length human protein expressed in Sf9 cells

Catalog Number	P365-380CG
Specific Activity	12 nmol/min/mg
Specific Lot Number	J594-5
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 #: P365-380CG)

Active PRMT3 (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 PRMT3 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 H₂O.

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-³H] solution

The [³H]-Adomet solution (0.54945µCi/µl and 10µCi/nmol) in 10mM H₂SO₄ : Ethenol (9:1) solution was purchased from PerkinElmer (Cat. # NET155250UC). The final concentration of [³H]-Adomet is 54.945 µM or 54.945 pmol/µl.

Substrate (Catalog #: H13-58)

Histone H4 Peptide (1-21) substrate (SGRGKGGKGLGKGG-AKRHRKVGKKC) diluted in distilled H₂O to a final concentration of 1mg/ml.

Assay Protocol

- Step 1.** Thaw [³H]-Adomet solution in shielded container in a designated radioactive working area.
- Step 2.** Thaw the Active PRMT3, 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 PRMT3 (Catalog #P365-380CG)
 - 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 H₂O.
- Step 5.** Initiate the reaction by the addition of 5µl [³H]-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 [³H]-Adomet Specific Activity (SA) (cpm/nmol)

Specific activity (SA) = cpm for 5µl [³H]-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 [³H]-Adomet in cpm/pmol)*(Reaction time in min)*(Enzyme amount in µg or mg)]*[(Reaction Volume) / (Spot Volume)]

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