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Catalog #	Aliquot Size
N355-380BH-05	5 µg
N355-380BH-10	10 µg

N6AMT2, Active

Full length recombinant protein expressed in Sf9 cells

Catalog # N355-380BH
Lot # F660-4

Product Description

Full-length recombinant human N6AMT2 was expressed by baculovirus in Sf9 insect cells using an N-terminal His tag. The gene accession number is NM_174928.

Gene Aliases

ESP13

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 7.0, 300mM NaCl, 150mM imidazole, 0.1mM PMSF, 0.25mM DTT, 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.

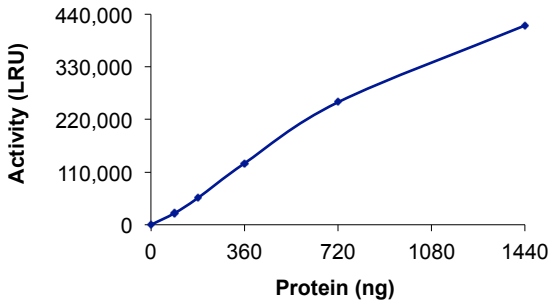
Scientific Background

N6AMT2 is a putative DNA methyltransferase, belongs to the methyltransferase superfamily, AML1 family. The N6AMT2 gene is conserved in human, mouse, rat, chimpanzee, dog, cow, chicken, zebrafish, fruit fly, mosquito, C.elegans, S.cerevisiae, K.lactis, N.crassa, A.thaliana, and rice (1). The N6AMT2 phosphorylation at Ser-2 has been reported (2). The function of this protein has not been widely studied yet.

References

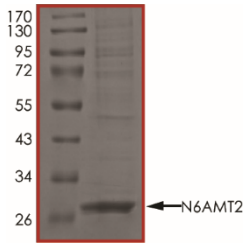
1. Albrecht M, et.al: Novel Sm-like proteins with long C-terminal tails and associated methyltransferases. FEBS Lett. 2004 Jul 2;569(1-3):18-26.
2. Gauci,S. et.al: Lys-N and trypsin cover complementary parts of the phosphoproteome in a refined SCX-based approach. Anal. Chem. 81 (11), 4493-4501 (2009).

Specific Activity



The specific activity of N6AMT2 was determined to be **110 pmol /min/mg** as per activity assay protocol.

Purity



The purity of N6AMT2 was determined to be **>90%** by densitometry, approx. MW **28 kDa**.

N6AMT2, Active

Full length recombinant protein expressed in Sf9 cells

Catalog #	N355-380BH
Specific Activity	110 pmol/min/mg
Lot #	F660-4
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 #: N355-380BH)

Active N6AMT2 (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 N6AMT2 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 #: H13-58)

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

Assay Protocol

The N6AMT2 assay is performed using the Methyltransferase-Glo™ Assays kit (Promega, Catalog #: V7601).

Step 1. Thaw the active N6AMT2 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 N6AMT2 (Catalog # N355-380BH)
- 2X Substrate Cocktail: 40 μM of SAM and 100ng/μl of Histone H4 Peptide (1-21) (Catalog # H13-58) 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 N6AMT2

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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