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1-866-9 KINASE (54-6273)

Catalog #	Aliquot Size
N330-380G-05	5 µg
N330-380G-10	10 µg

NNMT, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog # N330-380G

Lot # L1894-6

Product Description

Recombinant full-length human NNMT was expressed by baculovirus in Sf9 insect cells using an N-terminal GST tag. The NNMT protein accession number is NM_006169.

Gene Aliases

(None)

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.

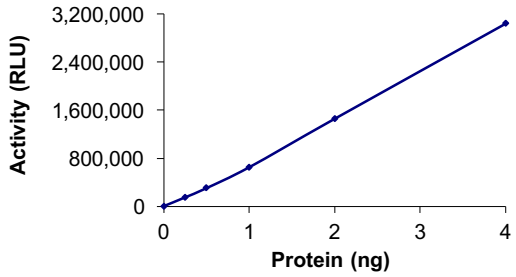
Scientific Background

Nicotinamide N-methyltransferase (NNMT) is expressed predominantly in the liver, and mediates the N-methylation of nicotinamide and other pyridines. The activity of NNMT is involved in drug and xenobiotic toxicity (1). NNMT has also been identified as serum tumor marker for colorectal cancer (2), as well as potential marker in urine-based diagnostic test for bladder cancer (3).

References

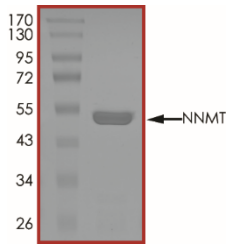
1. Aksoy, S., et al. Human liver nicotinamide N-methyl-transferase. cDNA cloning, expression, and biochemical characterization. J Biol Chem. 269(20):14835-40, 1994.
2. Roessler, M., et al. Identification of nicotinamide N-methyl-transferase as a novel serum tumor marker for colorectal cancer. Clin Cancer Res. 11(18):6550-7, 2005.
3. Sartini, D., et al. Upregulation of tissue and urinary nicotinamide N-methyltransferase in bladder cancer: potential for the development of a urine-based diagnostic test. Cell Biochem Biophys. 65(3):473-83, 2013.

Specific Activity



The specific activity of NNMT was determined to be **220 nmol /min/mg** as per activity assay protocol.

Purity



The purity of NNMT was determined to be **>95%** by densitometry, approx. MW **54 kDa**.

NNMT, Active

Full-length recombinant human protein expressed in Sf9 cells

Catalog #	N330-380G
Specific Activity	220 nmol/min/mg
Lot #	L1894-6
Purity	>95%
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 #: N330-380G)

Active NNMT (0.1μg/μl) diluted with Methyl-transferase 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 NNMT 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 (Sigma, Catalog #: N0636)

Nicotinamide diluted in Reaction Buffer to a final concentration of 80 μM.

Assay Protocol

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

Step 1. Thaw the active NNMT 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 NNMT (Catalog # N330-380G)
- 2X Substrate Cocktail: 40 μM of SAM and 80 μM of Nicotinamide in Reaction Buffer

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 NNMT

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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