

DESCRIPTION

Source *E. coli*-derived human Nicotinamide N-Methyltransferase/NNMT protein
Met1-Leu264, with a C-terminal 6-His tag
Accession # P40261

N-terminal Sequence Analysis Met1

Predicted Molecular Mass 30 kDa

SPECIFICATIONS

SDS-PAGE 25-29 kDa, reducing conditions

Activity Measured by its ability to methylate nicotinamide.
The specific activity is >65 pmol/min/μg, as measured under the described conditions.

Endotoxin Level <1.0 EU per 1 μg of the protein by the LAL method.

Purity >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 μm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 20 mM Tris, pH 8.5
- Hydrolysis Buffer: 100 mM HEPES pH 7.0
- Recombinant Human Nicotinamide N-Methyltransferase/NNMT (rhNNMT) (Catalog # 7736-MT)
- Glutathione, reduced (Amresco, Catalog # 399), 250 mM stock in deionized water
- Recombinant Human Adenosylhomocysteinase/AHCY (rhAHCY) (Catalog # 6466-AH)
- Recombinant Human Adenosine Deaminase/ADA, (rhADA) (Catalog # 7048-AD)
- S-adenosylmethionine (Sigma, Catalog # A7007), 10 mM stock in 50% DMSO in deionized water
- Nicotinamide (Sigma, Catalog # 72340), 100 mM stock in 50 mM Tris, 100 mM NaCl, 30% Isopropanol, pH 8.0
- ThioGlo® 3 Fluorescent Thiol Reagent (Covalent Associates, Inc., Catalog # T-003), 10 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

- Assay**
1. Dilute the stock of reduced glutathione to 40 μM (40 pmol/μL) in Assay Buffer. This is the first point of the standard curve.
 2. Continue standard curve by performing six ½ serial dilutions of the 40 μM glutathione in Assay Buffer. The standard curve has a range of 31.25 to 2000 pmol per well.
 3. Dilute rhNNMT to 2 μg/mL in Assay Buffer.
 4. Create Substrate Mixture containing 200 μM S-adenosylmethionine and 4 mM Nicotinamide in Assay Buffer.
 5. Combine equal volumes of 2 μg/mL rhNNMT and Substrate Mixture. As a Control, combine equal volumes of Substrate Mixture with Assay Buffer.
 6. Incubate reactions at 37 °C for 30 minutes.
 7. To stop the reaction, boil samples at 100 °C for 5 minutes. Then cool reactions on ice for 1 minute.
 8. Create a Hydrolysis Mixture containing 25 μg/mL rhAHCY and 2.5 μg/mL rhADA in Hydrolysis Buffer.
 9. Combine equal volumes of cooled reactions from step 7 and Hydrolysis Mixture.
 10. Incubate mixtures at 37 °C for one hour.
 11. Load 50 μL of each point of the standard curve into a plate. Include a curve blank containing 50 μL of Assay Buffer.
 12. Load 50 μL of each reaction mixture into a plate.
 13. Dilute ThioGlo to 100 μM in DMSO.
 14. Add 50 μL of 100 μM ThioGlo to each well.
 15. Incubate at room temperature for 5 minutes in the dark.
 16. Read the plate in endpoint mode at excitation and emission wavelengths of 380 nm and 445 nm, respectively.
 17. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted thiol produced* (pmol)}}{\text{Incubation time (min) x amount of enzyme (}\mu\text{g)}}$$

*Derived from the reduced glutathione standard curve using linear fitting and adjusted for Control.

Final Assay Conditions

Per Well:

- rhNNMT: 0.025 μg
- rhAHCY: 0.625 μg
- rhADA: 0.0625 μg
- S-adenosylmethionine: 0.025 mM
- Nicotinamide: 0.5 mM
- ThioGlo: 50 μM

PREPARATION AND STORAGE

Shipping The product is shipped with dry ice or equivalent. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

BACKGROUND

NNMT (Nicotinamide N-Methyltransferase) is a cytosolic enzyme that catalyzes the N-methylation of nicotinamide and other pyridines using S-adenosyl-L-methionine (AdoMet) as the methyl group donor (1, 2). The enzyme is highly expressed in liver (1) but is also detected in other organs. NNMT plays a significant role in nicotinamide metabolism (3) and in the detoxification of xenobiotics. The association with thyroid cancer and renal carcinoma makes NNMT useful as a tumor biomarker.

References:

1. Cantoni, G.L. *et al.* (1951) *J. Biol. Chem.* **189**:203.
2. Aksoy S. *et al.* (1994) *J. Biol. Chem.* **269**:14835.
3. D'Souza J. *et al.* (1980) *Xenobiotica* **10**:151.