R&D SYSTEMS
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Recombinant Human Glycine N-Methyltransferase/GNMT

Catalog Number: 6526-MT

DESCRIPTION

Source	*E. coli*-derived human Glycine N-Methyltransferase/GNMT protein Met1-Asp295 Accession # Q14749
N-terminal Sequence Analysis	Val2
Predicted Molecular Mass	33 kDa

SPECIFICATIONS

SDS-PAGE	31-35 kDa, reducing conditions
Activity	Measured by its ability to transfer a methyl group from S-adenosylmethionine to glycine. The specific activity is >190 pmol/min/µg.
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 NaH_2PO_4, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Methylation Buffer: 20 mM Tris, 2 mM $MgCl_2$, 1 mM EDTA, pH 9.0
- Hydrolysis Buffer: 100 mM HEPES, 2 mM $MgCl_2$, 1 mM EDTA, pH 7.0
- Recombinant Human Glycine N-Methyltransferase/GNMT (rhGNMT) (Catalog # 6526-MT)
- Recombinant Human Adenosylhomocysteinase/AHCY (rhAHCY) (Catalog # 6466-AH)
- Glutathione, reduced (Amresco, Catalog # 399), 250 mM stock in deionized water
- S-Adenosylmethionine (AdoMet) (Sigma, Catalog # A7007), 10 mM stock in 50% DMSO, 50% deionized water (v/v)
- Glycine (Sigma, Catalog # G7126), 1 M in deionized water
- Recombinant Human Adenosine Deaminase/ADA (rhADA) (Catalog # 7048-AD)
- ThioGlo® 3 Fluorescent Thiol Reagent (Covalent Associates, Inc., Catalog # T-003)
- DMSO (Sigma, Catalog # 154938)
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute AdoMet to 800 µM in Methylation Buffer.
2. Dilute Glycine to 20 mM in Methylation Buffer.
3. Form Substrate Mixture by combining equal volumes of 800 µM AdoMet and 20 mM Glycine.
4. Dilute rhGNMT to 0.8 µg/mL in Methylation Buffer.
5. Prepare methylation reaction by combining 50 µL of 0.8 µg/mL rhGNMT with 50 µL of Substrate Mixture. Also prepare a Substrate Blank containing Methylation Buffer in place of rhGNMT.
6. Incubate methylation reaction and substrate blank for 30 minutes at room temperature.
7. Stop methylation reaction by heating at 95-100 °C for 5 minutes. After stopping the reaction, cool on ice for 1 minute.
8. Prepare a standard curve by diluting the 250 mM reduced glutathione (250,000 pmol/µL) to 10 pmol/µL in Hydrolysis Buffer and performing six additional ½ serial dilutions. Include a standard curve blank consisting of Hydrolysis Buffer only.
9. Dilute rhAHCY to 10 ng/µL in Hydrolysis Buffer.
10. Dilute rhADA to 71.1 µg/mL in Hydrolysis Buffer.
11. Form Enzyme Mixture by combining equal volumes of 10 ng/µL rhAHCY and 71.1 µg/mL rhADA.
12. Prepare hydrolysis reaction by adding 100 µL of Enzyme Mixture to the stopped methylation reaction and blank.
13. Incubate the hydrolysis reaction, Substrate Blank, standard curve and standard curve blank for 1 hour at 37 °C.
14. Load 50 µL of hydrolysis reaction, Substrate Blank, and standard curve into wells of a black microplate.
15. Dilute ThioGlo® to 100 µM in DMSO.
16. Add 50 µL of 100 µM ThioGlo® to each well.
17. Incubate microplate at room temperature for 5 minutes in the dark.
18. Read the plate in endpoint mode at excitation and emission wavelengths of 380 nm and 445 nm, respectively.
19. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Thiol produced (pmol)}}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}}$$

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

Final Assay Conditions

Per Well:

- rhGNMT: 0.010 µg
- rhAHCY: 0.125 µg
- AdoMet: 100 µM
- Reduced glutathione curve: 500, 250, 125, 62.5, 31.25, 15.625, and 7.813 pmol
- rhADA: 0.889 µg
- ThioGlo®: 50 µM

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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

Glycine N-methyltransferase (GNMT) is a tetrameric cytosolic protein which catalyzes the transfer of a methyl group from S-adenosylmethionine (AdoMet) to glycine producing S-adenosylhomocysteine (AdoHcy) and sarcosine (1). GNMT plays a major role in maintaining normal AdoMet levels. GNMT is abundant in the liver where it is a major folate-binding protein. It binds 5-methyltetrahydrofolate pentaglutamate in vivo and in vitro, and the binding of the folate inhibits the activity of GNMT. A study of the rat enzyme showed differences in the kinetic parameters between recombinant GNMT and the protein isolated from liver (2). Based on structural information, the N-terminal part of the protein plays a major role in the transfer of the methyl group from AdoMet to glycine (3). It has been proposed that the difference in folate inhibitor binding between recombinant GNMT and the liver enzyme may be due to differential acetylation of the N-terminal valine (4). Hypermethioninemia is caused by defects in GNMT.

References:

1. Luka, Z. *et al.* (2009) J. Biol. Chem. **284**:22507.
2. Ogawa, H. *et al.* (1997) Biochem. J. **327**:407.
3. Takata, Y. *et al.* (2003) Biochemistry. **42**:8394.
4. Luka, Z. *et al.* (2008) Biochim. Biophys. Acta. **1784**:1342.

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