Recombinant Human N-Acetylglucosaminyltransferase I/MGAT1

Catalog Number: 8334-GT

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived Thr30-Asn445, with C-terminal 6-His tag Accession # P26572
N-terminal Sequence Analysis	Thr30
Predicted Molecular Mass	48 kDa

SPECIFICATIONS

SDS-PAGE	45-52 kDa, reducing conditions
Activity	Measured by its ability to transfer N-Acetyl-D-Glucosamine from UDP-GlcNAc to α1-3, α1-6-Mannotriose. The specific activity is >30 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	>90%, by SDS-PAGE under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
Formulation	Supplied as a 0.2 µm filtered solution in Tris and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Buffer A: 25 mM MES, 10 mM $MnCl_2$, 0.02% Brij-35, pH 6.5
- Buffer B: 100 mM Tris, 5 mM $CaCl_2$, pH 7.5
- Recombinant Human N-Acetylglucosaminyltransferase I/MGAT1 (rhMGAT1) (Catalog # 8334-GT)
- Donor Substrate: UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% ethanol
- Acceptor Substrate: α1-3, α1-6 Mannotriose (V-Labs, Catalog # M336), 20 mM stock in deionized water
- Glycosyltransferase Activity Kit (Catalog # EA001)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Buffer A for a 100 µM stock.
2. Prepare standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Buffer A. The standard curve has a range of 0.078 to 5 nmol per well.
3. Dilute rhMGAT1 to 20 µg/mL in Buffer A.
4. Create Substrate Mixture containing 0.4 mM UDP-GlcNAc and 2 mM α1-3, α1-6 Mannotriose in Buffer A.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Buffer A.
6. Load 25 µL of the 20 µg/mL rhMGAT1 into the plate. Include a control containing 25 µL of Buffer A.
7. Start the reaction by adding 25 µL of Substrate Mixture to the wells, excluding the standard curve.
8. Cover the plate with a plate sealer and incubate at 37 °C for 1 hour.
9. Dilute Coupling Phosphatase 1 to 2 µg/mL in Buffer B.
10. Add 50 µL of 2 µg/mL Coupling Phosphatase 1 to reaction wells and controls, excluding the standard curve. Also, add 50 µL of Buffer B to the wells containing the standard curve.
11. Mix and incubate for 10 minutes at room temperature.
12. Add 30 µL of the Malachite Green Reagent A to all wells.
13. Add 50 µL of deionized water to all wells.
14. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhMGAT1: 0.5 µg
- Coupling Phosphatase 1: 0.1 µg
- UDP-GlcNAc: 0.2 mM
- α1-3, α1-6 Mannotriose: 1 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with polar packs. 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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Mannosylglycoprotein N-acetyl-glucosaminyltransferase 1 (MGAT1), also known as GnT I, is a type II transmembrane Golgi enzyme that regulates the branching of N-glycans. By transferring a GlcNAc residue to the α3-linked mannose of the trimannosyl core of N-linked oligosaccharides, MGAT1 initiates the formation of complex and hybrid N-linked carbohydrates (1). Mice lacking MGAT1 activity die at mid-gestation, revealing an essential role for these carbohydrates (2). Branched N-glycans on cell surface proteins bind to galectins and allow the formation of a multivalent lattice thereby enhancing cell surface residency of growth factor receptors and focal adhesion proteins. Because of its key role in N-glycan synthesis, MGAT1 is a potential target for anti-cancer therapy (3). Enzymatic activity of the recombinant human MGAT1 was determined using a phosphatase coupled glycosyltransferase assay (4).

References:

1. Kumar R. *et al.* (1990) Proc. Natl. Acad. Sci. USA **87**:9948.
2. Ioffe, E. and Stanley, P. (1994) Proc. Natl. Acad. Sci. USA **91**:728.
3. Zavareh, R. *et al.* (2012) PLoS ONE **7**:e43721.
4. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

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