Recombinant Human MGAT4A His-tag

Catalog Number: 11496-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human MGAT4A protein Leu93-Asn535, with a C-terminal 6-His tag Accession # Q9UM21
N-terminal Sequence Analysis	Leu93
Predicted Molecular Mass	52 kDa

SPECIFICATIONS

SDS-PAGE	54-60 kDa, under reducing conditions.
Activity	Measured by its ability to transfer N-acetyl-α-D-glucosamine from UDP-N-Acetyl-α-D-glucosamine to glycan Cy5-Fuc labeled N2f. Able to convert >85% of the substrate glycan N2f, as measured under the described conditions.
Endotoxin Level	<0.10 EU per 1 µg of the protein by the LAL method.
Purity	>90%, 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 10 mM $MnCl_2$, pH 7.0
- Recombinant Human MGAT4A His-tag (rhMGAT4A) (Catalog # 11496-GT)
- Cy5-Fuc labeled N2f (Cy5-N2f) (Catalog # GL304)
- UDP-GlcNAc, 50 mM stock in 50% Ethanol
- 17% SDS-PAGE Gel
- Gel loading dye
- Gel Imager with Cy5 fluorescent dye detection capability

Assay

1. Dilute rhMGAT4A to 50 µg/mL with Assay Buffer.
2. Create a Reaction Mix containing 0.02 µM Cy5-N2f and 1 mM UDP-GlcNAc in Assay Buffer.
3. Combine 10 µL of rhMGAT4A and 10 µL of Reaction Mix. For a Control, combine 10 µL of Assay Buffer and 10 µL of Reaction Mix.
4. Incubate reaction and control at 37 °C for 60 minutes.
5. Add gel loading dye to each tube.
6. Load half the volume of each reaction and control onto a 17% SDS-PAGE gel. Let samples migrate at least 80% down the gel before stopping.
7. Acquire gel image and determine percent conversion of Cy5-N2f.

Final Assay Conditions

Per Reaction:

- rhMGAT4A: 0.5 µg
- Cy5-N2f: 0.2 pmol
- UDP-GlcNAc: 0.5 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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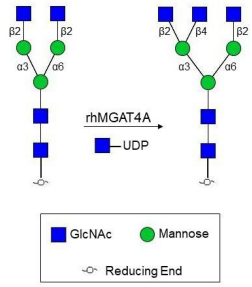
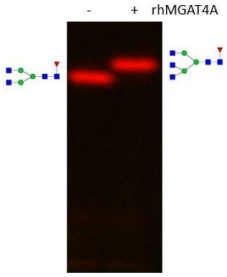
DATA

Enzyme Activity



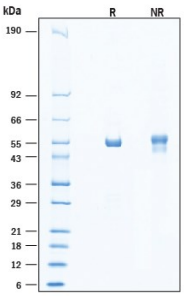
Recombinant Human MGAT4A His-tag Protein Enzyme Activity Diagram. Recombinant Human MGAT4A His-tag Protein (Catalog # 11496-GT) catalyzes a β1-4GlcNAc linkage to the α1-3Man arm of N-glycans.

Enzyme Activity



Recombinant Human MGAT4A His-tag Protein Enzyme Activity. Recombinant Human MGAT4A His-tag Protein (Catalog # 11496-GT) activity was measured by its ability to transfer N-acetyl-α-D-glucosamine from UDP-N-acetyl-α-D-glucosamine to glycan Cy5-Fuc labeled N2f (Catalog # GL304). Following treatment, a mobility shift was observed via SDS-PAGE gel.

SDS-PAGE



Recombinant Human MGAT4A His-tag Protein SDS-PAGE. 2 µg/lane of Recombinant Human MGAT4A His-tag Protein (Catalog # 11496-GT) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 54-60 kDa, under reducing conditions.

BACKGROUND

Recombinant human Alpha-1,3-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase A (MGAT4A), also known as GlcNAc-T IVa , is a metal-dependent, golgi single-pass type II membrane protein that contains an N-terminal transmembrane region, a catalytic domain, and a C-terminal carbohydrate binding module that regulates its catalytic activity (1, 2). MGAT4A is one of several human MGAT enzymes involved in the initiation and synthesis of N-glycans, with each enzyme having unique specificity and preferences (3). MGAT4 has two human isozymes that catalyze formation of the β1-4 GlcNAc branch in the α1-3 Mannose arm of N-glycans (4-6). The two isozymes have different expression profiles in tissues and cancer cell lines , with MGAT4A expression within gastrointestinal tissues such as the pancreas (2, 4, 5). Loss of MGAT4A in mouse models has been shown to induce type 2 diabetes (9,10). Expression and activity of MGAT4A is particularly important during differentiation and oncogenesis (7, 8) including within choriocarcinoma, invasive mole, and placental site trophoblastic tumors (11-13). The discovery of MGAT4A modulators may lead to new therapeutics for the treatment of these diseases (10). MGAT4A may also be useful as a tool for the glycoengineering of complex N-glycans. The activity of MGAT4A was demonstrated using a fluorescent gel shift assay.

References:

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