Recombinant Human MAN1A1 His-tag

Catalog Number: 10665-GH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human MAN1A1 protein Pro63-Glu653, with a C-terminal 6-His tag Accession # P33908.3
N-terminal Sequence Analysis	Pro63
Predicted Molecular Mass	67.7 kDa

SPECIFICATIONS

SDS-PAGE	63-65 kDa, under reducing conditions.
Activity	Measured by its ability to remove α-mannose from the high mannose glycan Man-9. A distinct band is observed in the rhMAN1A1 digested sample on SDS-PAGE gel, 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 $CaCl_2$. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Digestion Buffer: 50 mM MES, 15 mM $CaCl_2$, 1 mg/mL BSA, pH 6.0
- Labeling Buffer: 25 mM Tris, 150 mM NaCl, 10 mM $MnCl_2$, pH 7.5
- Recombinant Human MAN1A1 (rhMAN-1A1) (Catalog # 10665-GH)
- Oligomannose-9 (Man-9) (Dextra Laboratories, Catalog # MC1131), 0.1 mg/mL stock in deionized water
- Recombinant Human MGAT1 (rhMGAT1) (Catalog # 8334-GT)
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% ethanol, 50% deionized water
- Recombinant Human FUT8 (rhFUT8) (Catalog # 5768-GT)
- GDP-Cy5-Fucose (Catalog # ES301)
- 15% SDS-PAGE gel
- Reducing SDS-PAGE gel loading buffer
- Fluorescent imager

Assay

Digestion:

1. Dilute rhMAN1A1 to 20 µg/mL in Digestion buffer.
2. Dilute Man-9 to 20 µg/mL in Digestion Buffer.
3. Combine 5 µL of 20 µg/mL Man-9, 5 µL of 20 µg/mL rhMAN1A1 and 10 µL of Digestion Buffer. Include a Control containing 5 µL of 20 µg/mL Man-9 and 15 µL of Digestion Buffer.
4. Incubate at 37 °C for 2 hours.

Labeling:

1. Dilute rhMGAT1 to 100 µg/mL in Labeling Buffer.
2. Dilute UDP-GlcNAc to 1 mM in Labeling Buffer.
3. Dilute rhFUT8 to 100 µg/mL in Labeling Buffer.
4. Dilute GDP-Cy5-Fucose to 0.05 mM in Labeling Buffer.
5. Transfer 10 µL of each digestion to a new tube and add 5 µL of 100 µg/mL rhMGAT1, 5 µL of 1 mM UDP-GlcNAc, 5 µL of 100 µg/mL rhFUT8 and 5 µL of 0.05 mM GDP-Cy5-Fucose.
6. Incubate at 37 °C for 60 minutes.
7. Add 6 µL of Reducing SDS-PAGE gel loading buffer to each reaction.
8. Load 12 µL of each reaction onto a 15% SDS-PAGE gel and perform electrophoresis.
9. Analyze gel on a fluorescent imager.

Final Assay Conditions

Per Reaction:

- rhMAN1A1: 0.05 µg
- Man-9: 0.05 µg
- rhMGAT1: 0.5 µg
- UDP-GlcNAc: 5 nmol
- rhFUT8: 0.5 µg
- GDP-Cy5-Fucose: 0.25 nmol

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.

DATA

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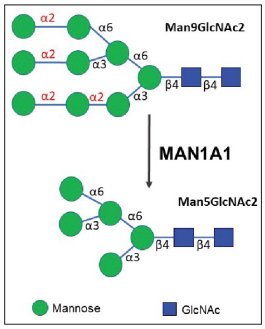
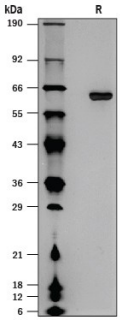
Enzyme Activity



Recombinant Human MAN1A1 His-tag Protein Enzyme Activity Diagram. MAN1A1 is involved in the removal of 4 distinct α-1,2-linked mannose residues from Man9GlcNAc2 to produce Man5GlcNAc2, an essential step of N-glycan maturation.

SDS-PAGE



Recombinant Human MAN1A1 His-tag Protein SDS-PAGE. 1 µg/lane of Recombinant Human MAN1A1 His-tag (Catalog # 10665-GT) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing a band at 63-65 kDa.

BACKGROUND

N-glycan maturation in Golgi apparatus starts with high-mannose glycan Man-9 that is capped with four 1,2-α-linked mannose residues at its non-reducing ends. During the process, these mannose residues are removed to generate Man-5 oligomannose glycan, a precursor for complex and hybrid N-glycans (1). Failure of removing these mannose residues will result in the display of high-mannose glycans on cell surface and extracellular matrix. Increased levels of high-mannose glycans on cell surface are usually associated with disease progress such as tumorigenesis and viral infection (2). The removal of 1,2-α-linked mannose residues are catalyzed by 4 α-mannosidases, including MAN1A1, MAN1B1, MAN1A2 and MAN1C1, that have overlapping substrate specificity and slight differences in enzyme activity (3). MAN1A1 is also a tumor-suppressor (4) and low levels of expression of MAN1A1 correlate with poor prognosis in breast cancer patients (5, 6).

References:

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3. Moremen, K.W. and Nairn, A.V. (2014) Handbook of Glycosyltransferases and Related Genes p1297.
4. Liu, T. *et al.* (2014) PLoS One **9**:e107941.
5. Milde-Langosch, K. *et al.* (2014) Breast Cancer Res. Treat. **145**:295.
6. Karen Legler, *et al.* (2018) Br. J. Cancer **118**:847.

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