

DESCRIPTION

Source Chinese Hamster Ovary cell line, CHO-derived human Glucosaminyl (N-acetyl) Transferase 2/GCNT2 protein
Asn26-Phe400, with a C-terminal 6-His
Accession # NP_001482.1

N-terminal Sequence Analysis Asn26

Predicted Molecular Mass 43.8 kDa

SPECIFICATIONS

SDS-PAGE 53-67 kDa, under reducing conditions.

Activity Measured by its ability to transfer GlcNAc from UDP-GlcNAc to Cy5-labeled Extended G2.

0.25 µg of Recombinant Human GCNT2 will convert >50% of its substrate to product, 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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, 1 mM DTT, pH 6.5
- Recombinant Human GCNT2 (rhGCNT2) (Catalog # 10847-GT)
- Cy5-labeled Extended G2 (Catalog # GL303)
- Recombinant Human B4GalT1 (rhB4GALT1) (Catalog # 3609-GT)
- UDP-Gal (Sigma, Catalog # U4500), 10 mM stock in Deionized Water
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% ethanol/50% Deionized Water
- 15% SDS-PAGE Gel
- Reducing SDS-PAGE gel loading buffer
- Fluorescent imager

- Assay**
1. Dilute rhGCNT2 to 25 µg/mL in Assay Buffer.
 2. Prepare a Master Mix containing 0.2 µM Cy5-labeled Extended G2, 1 mM UDP-GlcNAc, 1 mM UDP-Gal and 50 µg/mL rhB4GALT1 in Assay Buffer.
 3. Prepare reaction(s) by combining 10 µL of 25 µg/mL rhGCNT2 and 10 µL of Master Mix. Prepare a negative control by combining 10 µL of Assay Buffer and 10 µL of Master Mix.
 4. Incubate the reaction(s) and control at 37 °C for 90 minutes.
 5. Add 7 µL of reducing SDS-PAGE gel loading buffer to each reaction and control. Mix.
 6. Load 13.5 µL of each reaction and control per lane on a 15% SDS-PAGE gel. Run dye front down 80% of the length of the gel (minimum).
 7. Visualize the gel with a fluorescent imager.
 8. Calculate percent conversion of the substrate to product.

Final Assay Conditions

- Per Reaction:
- rhGCNT2: 0.25 µg
 - Cy5-labeled Extended G2: 2 pmol
 - UDP-Gal: 0.5 mM
 - UDP-GlcNAc: 0.5 mM
 - rhB4GALT1: 0.5 µg

PREPARATION AND STORAGE

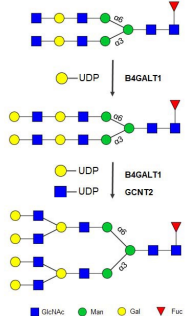
Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

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.

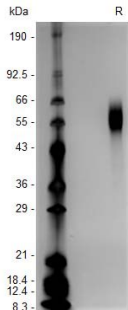
DATA

Enzyme Activity



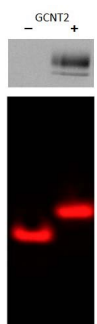
Recombinant Human GCNT2 His-tag Protein/GCNT2 Enzyme Activity Diagram. GCNT2 (Catalog # 10847-GT) recognizes poly-lactosamine structure and can tolerate core-6 fucose and its Cy5 derivatives.

SDS-PAGE



Recombinant Human GCNT2 His-tag Protein, CF SDS-PAGE
1 µg/lane of Recombinant Human GCNT2 His-tag (Catalog # 10847-GT) was resolved with SDS-PAGE under reducing (R) conditions and visualized by silver staining, showing bands at 53-63 kDa.

Gel Supershift Assay



Recombinant Human GCNT2 His-tag Protein, CF Fluorescent Gel Mobility Shift
Lane 1 contained substrate glycan extended G2 (Catalog # GL303). In the presence of rhGCNT2, the glycan was modified, and a mobility shift was observed.

BACKGROUND

N-acetyllactosaminide beta-1,6-N-acetylglucosaminyl-transferase (GCNT2) is a key branching enzyme that converts linear into branched poly-N-acetyllactosaminoglycans. It is responsible for the formation of the blood group I antigens during embryonic development and is closely associated with the development and maturation of erythroid cells (1, 2, 3). GCNT2 is a Golgi resident type II membrane protein with a typical domain structure of glycosyltransferases. GCNT2 has been found to be downregulated in melanomas, which leads to the loss of N-linked I-branched glycans and the synthesis of poly-N-acetyllactosamine (i-linear) glycans (4). In addition, GCNT2 has been found to play a role in breast cancer and lung cancer and knockdown of GCNT2 expression decreased cell migration and invasion *in vitro* (5). The activity of recombinant GCNT2 is demonstrated in an electrophoretic gel mobility shift assay using a fluorophore-labeled glycan as the substrate (6).

References:

1. Marti, F.A. *et al.* (1995) *Glycobiology* **5**:417.
2. Bierhuizen, M.F.A. *et al.* (1993) *Genes Dev.* **7**:468.
3. Inaba, N. *et al.* (2003) *blood* **101**:2870.
4. Sweeney, Jenna Geddes *et al.* (2018) *Nature communications* **9**:3368.
5. Haijun, Zhang *et al.* (2011) *Cancer Res.* **71**:4846.
6. Wu, Z.L. *et al* (2020) *Glycobiology* **30**:970.