Recombinant Human β-1,3-N-Acetylglucosaminyltransferase 6/B3GNT6

Catalog Number: 6505-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human Beta-1,3-N-Acetylglucosaminyltransferase 6/B3GNT6 protein Gln32-Ser384, with an N-terminal 6-His tag Accession # Q6ZMB0
N-terminal Sequence Analysis	His
Predicted Molecular Mass	40 kDa

SPECIFICATIONS

SDS-PAGE	43-55 kDa, reducing conditions
Activity	Measured by its ability to transfer GlcNAc from UDP-GlcNAc to 4-nitrophenyl-α-D-galactosaminide. The specific activity is >35 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	>95%, 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

- Assay Buffer: 25 mM Tris, 150 mM NaCl, 10 mM $MnCl_2$, 5 mM $CaCl_2$, 20% DMSO, pH 7.5
- Recombinant Human β-1,3-N-acetylglucosaminyltransferase 6/B3GNT6 (rhB3GNT6) (Catalog # 6505-GT)
- Coupling Enzyme: Recombinant Human CD39L3/ENTPD3 (rhCD39L3) (Catalog # 4400-EN)
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% EtOH (v/v)
- 4-Nitrophenyl N-acetyl-α-D-galactosaminide (4-NP-GalNAc) (Sigma, Catalog # N4264), 15 mM stock in DMSO
- Malachite Green Phosphate Detection Kit (Catalog # DY996)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute UDP-GlcNAc to 1.2 mM in Assay Buffer.
2. Dilute 4-NP-GalNAc to 3.6 mM in Assay Buffer.
3. Dilute rhCD39L3 to 6 µg/mL in Assay Buffer.
4. Prepare reaction mixture by combining equal volumes of 1.2 mM UDP-GlcNAc, 3.6 mM 4-NP-GalNAc, and 6 µg/mL rhCD39L3.
5. Dilute rhB3GNT6 to 12 µg/mL in Assay Buffer.
6. Dilute 1 M Phosphate Standard by adding 10 µL of the 1 M Phosphate Standard to 990 µL of deionized water for a 10 mM stock. Continue by adding 10 µL of the 10 mM Phosphate stock to 990 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
7. Continue standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5.0 nmol per well.
8. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
9. Load 25 µL of the 12 µg/mL rhB3GNT6 into the plate. Include a substrate blank containing 25 µL of Assay Buffer.
10. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
11. Cover the plate with parafilm or a plate sealer and incubate at 37 ºC for 60 minutes.
12. Add 30 µL of the Malachite Green Reagent A to all wells. Mix and incubate for 10 minutes at room temperature.
13. Add 100 µL of deionized water to all wells. Mix briefly.
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 Substrate.

Final Assay Conditions

Per Reaction:

- rhB3GNT6: 0.300 µg
- rhCD39L3: 50 ng
- UDP-GlcNAc: 0.2 mM
- 4-NP-GalNAc: 0.6 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

β-1,3-N-acetylglucosaminyltransferase 6 (B3GNT6) is also known as core 3 synthase due to its role in synthesis of the core 3 structure (GlcNAcβ1-3Gal-NAcα1-serine/threonine), an important precursor in the biosynthesis of mucin-type glycoproteins in digestive organs (1). Its expression is restricted to the stomach, colon and small intestine, where the core 3 structure is present. Down-regulation of the enzyme was found in gastric and colorectal carcinomas and it was suggested that it may be useful as a marker to distinguish between benign adenomas and premalignant lesions (2, 3). Prostate cancer cells transfected with core 3 synthase exhibited reduced migration and invasion compared with mock-transfected cells (4). When inoculated into nude mice, the transfected cells produced smaller tumors without metastasis in contrast to the robust tumor formation and metastasis observed in mock-transfected cells (4). Like other members of the β-1,3-N-acetylglucosaminyltransferase family, B3GNT6 is a Golgi-resident single-pass type II membrane protein.The activity of this enzyme has been measured with a phosphatase-coupled method (5).

References:

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3. Vavasseur, F. *et al.* (1995) Glycobiology **5**:351.
4. Lee, S.H. *et al.* (2009) J. Biol. Chem. **284**:17157.
5. Wu, Z.L. *et al.* (2010) Glycobiology doi: **10**.1093/glycob/cwq187.

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