Recombinant Human β-1,3-N-Acetylglucosaminyltransferase 4/ B3GNT4

Catalog Number: 7696-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Ala56-Arg378, with a C-terminal 6-His tag Accession # Q9C0J1
N-terminal Sequence Analysis	Ala56
Predicted Molecular Mass	37 kDa

SPECIFICATIONS

SDS-PAGE	40-55 kDa, reducing conditions
Activity	Measured by its ability to transfer N-acetylglucosamine from UDP-GlcNAc to β-lactose. The specific activity is >10 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 25 mM Tris, 10 mM $CaCl_2$, 10 mM $MnCl_2$ (supplied in kit), pH 7.5
- Recombinant Human β-1,3-N-Acetylglucosaminyltransferase 4/B3GNT4 (rhB3GNT4) (Catalog # 7696-GT)
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% ethanol
- β-Lactose (Sigma, Catalog # L3750), 250 mM stock in deionized water
- Glycosyltransferase Activity Kit (Catalog # EA001)
- 96-well Clear Plate (Costar, Catalog # 92592)
- 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 Assay Buffer for a 100 µM stock.
2. Prepare 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 nmol per well.
3. Prepare reaction mixture containing 80 mM β-Lactose, 2 mM UDP-GlcNAc, and 4 µg/mL Coupling Phosphatase I in Assay Buffer.
4. Dilute rhB3GNT4 to 12 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of the 12 µg/mL rhB3GNT4 into the plate. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of reaction mixture to the wells, excluding the standard curve and curve blank.
8. Seal the plate with parafilm or a plate sealer and incubate at 37 ºC for 2 hours.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. 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:

- rhB3GNT4: 0.3 µg
- Coupling Phosphatase I: 0.1 µg
- β-Lactose: 40 mM
- UDP-GlcNAc: 1.0 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

β1,3-Linked GlcNAc residues are present in the backbone of various biologically important glycans which are involved in many essential biological functions such as keratan sulfate synthesis in corneal tissue (1). The addition of such residues are catalyzed by a family of β1,3-N-acetylglucosaminyltransferases, that includes at least eight members (15). All of them are type II Golgi resident transmembrane proteins and have high homology to the β1,3-galactosyltransferase family. β1,3-N-acetylglucosaminyltransferase 4 or β3GNT4 is involved in polylactosamine synthesis and is mainly expressed in brain tissues such as whole brain, hippocampus, amygdala, cerebellum and caudate nucleus (6). The enzymatic activity of the recombinant protein was determined using a phosphatase coupled assay (7).

References:

1. Seko, A. and Yamashita, K. (2005) Glycobiology **15**:943.
2. Kataoka, K. and Huh, N.H. (2002) Biochem. Biophys. Res. Commun. **294**:843.
3. Iwai,T. *et al.* (2002) J. Biol. Chem. **277**:12802.
4. Togayachi, A. *et al.* (2001) J. Biol. Chem. **276**:22032.
5. Sasaki, K. *et al.* (1997) Proc. Natl. Acad. Sci. USA **94**:14294.
6. Shiraishi, N. *et al.* (2001) J. Biol. Chem. **276**:3498.
7. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

PRODUCT SPECIFIC NOTICES

Coomassie is a registered trademark of Imperial Chemical Industries Ltd.

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