Recombinant Human β-1,3-Glucuronyltransferase 1/B3GAT1

Catalog Number: 8560-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived His25-Ile334, with N-terminal 6-His tag Accession # Q9P2W7
N-terminal Sequence Analysis	His
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	50-59 kDa, reducing conditions
Activity	Measured by its ability to transfer GlcA from UDP-GlcA to lactose. The specific activity is >750 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.01 EU per 1 µg of the protein by the LAL method.
Purity	>85%, 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

- Glycosyltransferase Activity Kit (Catalog # EA001)
- Assay Buffer: 25 mM Tris, 150 mM NaCl, 5 mM $MnCl_2$, 5 mM $CaCl_2$, pH 7.0
- Recombinant Human β-1,3-Glucuronyltransferase 1/B3GAT1 (rhB3GAT1) (Catalog # 8560-GT)
- UDP-GlcA (Sigma, Catalog # U5625), 10 mM stock in deionized water.
- α-Lactose (Sigma, Catalog # L2643), 0.3 M stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- 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. This is the first point of the standard curve.
2. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare reaction mixture containing 1.25 mM UDP-GlcA, 60 mM α-Lactose, and 8 ng/µL Coupling Phosphatase 1 in Assay Buffer.
4. Dilute rhB3GAT1 to 2 ng/µL 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 2 ng/µL rhB3GAT1 into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of the reaction mixture to all wells, excluding the standard curve.
8. Seal plate and incubate at 37 °C for 20 minutes.
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 sealed plate 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:

- rhB3GAT1: 0.050 µg
- Coupling Phosphatase 1: 0.2 µg
- UDP-GlcA: 0.625 mM
- α-Lactose: 30 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.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

Recombinant Human β-1,3-Glucuronyltransferase 1/B3GAT1

Catalog Number: 8560-GT

BACKGROUND

B3GAT1 is a key enzyme involved in human natural killer1 (HNK1) epitope synthesis. It adds a glucuronic residue to the terminal lactosamine residue (Galβ14GlcNAc) of a glycoprotein or glycolipid, which can be further sulfated to become the HNK1 epitope, a unique trisaccharide structure, HSO_3-3GlcAβ1-3Galβ1-4GlcNAc (1, 2). The enzyme activity was found to be enhanced in the presence of sphingomyelin and phosphatidylinositol (3). The HNK1 carbohydrate epitope is characteristically expressed on a series of cell adhesion molecules in addition to some glycolipids in the extracellular matrix and on the cell surface in the nervous system, where it is involved in cell-cell and cell-substratum interaction and recognition during the development of the nervous system (4). Like most known glycosyltransferases, B3GAT1 is a type II Golgi-resident transmembrane protein with a short N-terminal cytoplasmic domain and a single pass transmembrane domain followed by an enzymatic domain in the lumen of Golgi apparatus. The enzyme activity was assayed using a phosphatase-coupled method (5).

References:

1. Terayama, K. *et al.* (1997) Proc. Natl. Acad. Sci. USA **94**:6093.
2. Shogo, O. *et al.* (1992) J. Biol. Chem. **267**: 22711.
3. Kakuda, S. *et al.* (2005) Glycobiology **2**:203.
4. Bollensen, E. and Schachner, M. (1987) Neurosci Lett. **82**:77.
5. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449