Recombinant Human β-1,3-Glucuronyltransferase 3/B3GAT3

Catalog Number: 6808-GT

DESCRIPTION

Source	*E. coli*-derived Glu72-Val335, with an N-terminal Met and 6-His tag Accession # O94766
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	30 kDa

SPECIFICATIONS

SDS-PAGE	30-35 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze UDP-GlcA. The specific activity is >1 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	>80%, 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

- Buffer A: 25 mM MES, 1 mM $MnCl_2$ (supplied in kit), pH 7.0
- Buffer B: 100 mM Tris, 5 mM $CaCl_2$, pH 7.5
- Recombinant Human β-1,3-Glucuronyltransferase 3/B3GAT3 (rhB3GAT3) (Catalog # 6808-GT)
- Substrate: UDP-GlcA (Sigma, Catalog # U5625), 10 mM stock in DMSO
- Glycosyltransferase Activity Kit (Catalog # EA001)
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute the 1 mM Phosphate standard stock by adding 40 µL to 360 µL of Buffer A for a 100 µM stock. This is the first point of the standard curve.
2. Prepare standard curve by performing six additional one-half serial dilutions of the 100 µM Phosphate stock in Buffer A. The standard curve has a range of 0.078 to 5 nmol per well.
3. Dilute Substrate to 1.25 mM in Buffer A.
4. Dilute rhB3GAT3 to 80 µg/mL in Buffer A.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Buffer A.
6. Load 25 µL of the 80 µg/mL rhB3GAT3 into the plate. Include 25 µL of Buffer A for a blank control.
7. Start the reaction by adding 25 µL of Substrate to the wells, excluding the standard curve.
8. Cover the plate with a plate sealer and incubate at 37 °C for 4 hours.
9. Dilute Coupling Phosphatase 1 to 2 µg/mL in Buffer B.
10. Add 50 µL of 2 µg/mL Coupling Phosphatase 1 to the reaction wells and blanks, excluding the standard curve. Add 50 µL of Buffer B to the standard curve.
11. Tap to mix and incubate for 10 minutes at room temperature.
12. Add 30 µL of the Malachite Green Reagent A to all wells.
13. Add 50 µL of deionized water to all wells.
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:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol)} \times (1000\ \text{pmol/nmol})}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Substrate Blank.

Final Assay Conditions

Per Reaction:

- rhB3GAT3: 2 µg
- Coupling Phosphatase 1: 100 ng
- Substrate: 0.625 mM

PREPARATION AND STORAGE

Shipping	The product is shipped with dry ice or equivalent. 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, -70 °C as supplied. ● 3 months, -70 °C under sterile conditions after opening.

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BACKGROUND

B3GAT3 is an essential enzyme involved in the synthesis of the linkage region of heparan sulfate and chondroitin sulfate proteoglycans (1). It transfers a glucuronic acid moiety from the uridine diphosphate-glucuronic acid (UDP-GlcA) to the trisaccharide Galβ1-3Galβ1-4Xyl covalently bound to a Ser residue at the glycosaminylglycan attachment site of proteoglycans. B3GAT3 knockout mice are embryonic lethal before the 8-cell stage due to failed cytokinesis, suggesting critical roles for chondroitin sulfate and heparan sulfate in embryonic cell division (2). Unlike the broad substrate specificity exhibited by B3GAT1, B3GAT3 shows strict specificity for Galβ1-3Galβ1 (3). The crystal structure shows the enzyme is an α/β protein with two subdomains that constitute the donor and acceptor binding sites with the active site lying in the cleft between the two subdomains (4). Like most known glycosyltransferases, B3GAT3 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. In the current assay, hydrolase activity against UDP-GlcA was measured using a phosphatase-coupled method (5).

References:

1. Kitagawa, H. *et al.* (1998) J. Biol. Chem. **273**:6615.
2. Isumikawa, T. *et al.* (1998) J. Biol. Chem. **285**:12190.
3. Fondeur-Gelinotte, M. *et al.* (2007) Glycobiology **17**:857.
4. Pedersen, L.C. *et al.* (2000) J. Biol. Chem. **275**:34580.
5. Wu, Z.L, *et al.* (2011) Glycobiology **21**:727.

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