Recombinant Human β-1,4-Glucuronyltransferase 1/B4GAT1 His-tag

Catalog Number: 6664-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human beta-1,4-Glucuronyltransferase 1/B4GAT1 protein His37-Cys415, with N-terminal 6-His tag Accession # O43505
Predicted Molecular Mass	43.8 kDa

SPECIFICATIONS

SDS-PAGE	42-53 kDa under reducing conditions
Activity	Measured by its ability to transfer GlcA from UDP-GlcA to Xylose. The specific actiivity is >55 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 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

- Glycosyltransferase Activity Kit (Catalog # EA001)
- Assay Buffer: 25 mM Tris, 10 mM $CaCl_2$, 10 mM $MnCl_2$ (supplied in kit), pH 7.5
- Recombinant Human β-1,4-Glucuronyltransferase 1/B4GAT1 His-tag (rhB4GAT1) (Catalog # 6664-GT)
- Donor Substrate: UDP-GlcA (Sigma, Catalog # U5625), 10 mM stock in deionized water
- Acceptor Substrate: Xylose (V-lab, Catalog # BX53), 100 mM 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. 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 0.8 mM UDP-GlcA, 40 mM Xylose, and 4 µg/mL Coupling Phosphatase I in Assay Buffer.
4. Dilute rhB4GAT1 to 20 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 20 µg/mL rhB4GAT1 into the plate. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of reaction mixture to all wells, excluding the standard curve and curve blank.
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 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:

- rhB4GAT1: 0.5 µg
- Coupling Phosphatase I: 0.1 µg
- Xylose: 20 mM
- UDP-GlcA: 0.4 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-1,4-Glucuronyltransferase 1/B4GAT1 His-tag

Catalog Number: 6664-GT

BACKGROUND

B4GAT1 was previously described in the literature as B3GNT1 (1). It is now characterized as a β1,4 glucuronyltransferase that is responsible for the synthesis of a glucuronyl-β1,4-xylosyl disaccharide found on α-dystroglycan (α- DG), a peripheral membrane protein that binds to several extracellular matrix components (2). Proper glycosylation of α-DG is critical to maintain structural integrity and force transmission between the cytoskeleton and the extracellular matrix for efficient signal transduction. Mutation of B4GAT1 will lead to failure of proper glycosylation of α-DG and loss of receptor binding of α-DG, therefore causes congenital muscular dystrophies (CMDs) (3). The enzymatic activity of recombinant human B4GAT1 was determined using a phosphatase-coupled assay (4) using xylose as acceptor substrate.

References:

1. Sasaki, K. *et al.* (1997) PNAS **94**:14294.
2. Willer, T. *et al.* (2014) eLife; **3**: e03941.
3. Barresi, R. and Campbell, K.P. (2006) J. Cell Sci. **119**:199.
4. 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