Recombinant Human B3GalT5 His-tag

Catalog Number: 10555-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human B3GalT5 protein Asn29-Val310, with a C-terminal 6-His tag Accession # Q9Y2C3.1
N-terminal Sequence Analysis	Asn29
Predicted Molecular Mass	34.7

SPECIFICATIONS

SDS-PAGE	40-45 kDa, under reducing conditions
Activity	Measured by its ability to transfer galactose from UDP-galactose to N-Acetyl-α-D-glucosamine. The specific activity is >200 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	>90%, 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

- Assay Buffer: 25 mM Tris, 150 mM NaCl, 10 mM $MgCl_2$, 10 mM $MnCl_2$, pH 7.5
- Glycosyltransferase Activity Kit (Catalog # EA001)
- Recombinant Human B3GalT5 (rhB3GalT5) (Catalog # 10555-GT)
- Donor Substrate: UDP-Galactose (Sigma, Catalog # U4500), 10 mM stock in deionized water
- Acceptor Substrate: GlcNAc (N-Acetyl-α-D-glucosamine) (Millipore, Catalog # 1079), 1 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 Activity 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. 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 nmoles per well.
3. Prepare reaction mixture containing 0.4 mM UDP-Galactose, 200 mM GlcNAc and 4 µg/mL Coupling Phosphatase 1 (supplied in kit) in Assay Buffer.
4. Dilute rhB3GalT5 to 8 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 8 µg/mL rhB3GalT5 into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of 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:

- rhB3GalT5: 0.2 µg
- Coupling Phosphatase 1: 0.1 µg
- UDP-Galactose: 0.2 mM
- GlcNAc: 100 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

B3GalT5 is a glycosyltransferase in the beta-1,3-galactosyltransferase (B3GalT) family that contains 5 members. It is synthesized as type II membrane-bound glycoproteins in the Golgi apparatus (1). The enzyme catalyzes the synthesis of type 1 lactosamine structure (Galβ1-3GlcNAc) in type 1 Lewis antigens (Le^a and Le^b). Le^a and Le^b are elevated in gastrointestinal and pancreatic cancers (2). Sialylated Le^a (Neu5Acα2-3Galβ1-3[Fucα1-4]GlcNAcβ), also known as CA19-9, is a tumor marker in the gastrointestinal tract (Stomach, colon, and pancrease) (3). Down-regulation of B3GalT5 will reduce the level of CA19-9 (4, 5). In contrast, beta-1,4-galactosyltransferases synthesize type 2 lactosamine structure (Galβ1-4GlcNAc) that is found in type 2 Lewis antigens (Le^x and Le^y). B3GalT5 is also known as SSEA-3 synthase and synthesizes the structure Galβ1-3GalNAc in stage-specific embryonic antigen-3 (SSEA-3) antigen (6). SSEA-3 plays a key role in identifying many types of mammalian cells with pluripotent and stem cell-like characteristics (7). It also catalyzes the transfer of Gal to GlcNAc-based acceptors with a preference for the Core-3 O-linked glycan GlcNAcβ1-3GalNAc structure (8). The activity of this enzyme has been measured with a phosphatase-coupled method (9).

References:

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