Recombinant Human GM2/GD2 Synthase/ B4GALNT1 His-tag

Catalog Number: 11777-BG

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human GM2/GD2 Synthase/ B4GALNT1 protein Ser26-Gln533, with an N-terminal 6-His tag Accession # Q00973.2
N-terminal Sequence Analysis	His
Predicted Molecular Mass	57 kDa

SPECIFICATIONS

SDS-PAGE	61-68 kDa, under reducing conditions.
Activity	Measured by its ability to hydrolyze UDP-GalNAc. The specific activity is >18 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<0.10 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)
- 10X Assay Buffer (supplied in kit): 250 mM Tris, 100 mM $CaCl_2$, pH 7.5
- $MnCl_2$ (supplied in kit): 100 mM
- Recombinant Human GM2/GD2 Synthase/B4GALNT1 (rhB4GALNT1) (Catalog # 11777-BG)
- Ethanol, Absolute
- GM3 Ganglioside, 1 mg/mL stock in 50%Ethanol/50% deionized water
- UDP-GalNAc, 10 mM stock in deionized water
- Clear 96-well Plate (Catalog # DY990)
- Plate Reader with Absorbance Read Capability

Assay

1. Prepare 1X Assay Buffer containing 10 mM $MnCl_2$ by combining 10X stocks and diluting 10-fold with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of 1X Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmoles per well.
4. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of 1X Assay Buffer.
5. Dilute rhB4GALNT1 to 20 µg/mL in 1X Assay Buffer.
6. Load 25 µL of 20 µg/mL rhB4GALNT1 into empty wells of the same plate as the curve. Include a Control containing 25 µL of 1X Assay Buffer.
7. Dilute Ethanol to 50% in deionized water.
8. Dilute prepared 50% Ethanol to 6.25% in 1X Assay Buffer.
9. Adding in order, prepare reaction mixture containing 0.1 mg/mL GM3 Ganglioside, 0.8 mM UDP-GalNAc, and 4 µg/mL Coupling Phosphatase 1 in prepared 6.25% Ethanol and mix gently after each addition.
10. Add 25 µL of the reaction mixture to all wells, excluding the standard curve.
11. Seal plate and incubate at 37 °C for 1 hour.
12. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
13. Add 100 µL of deionized water to all wells. Mix briefly.
14. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate for 20 minutes at room temperature.
15. Read plate at 620 nm (absorbance) in endpoint mode.
16. Calculate specific activity:

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

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

Final Assay Conditions

Per Reaction:

- rhB4GALNT1: 0.5 µg
- Coupling Phosphatase 1: 0.1 µg
- GM3 Ganglioside: 2.5 µg
- UDP-GalNAc: 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.

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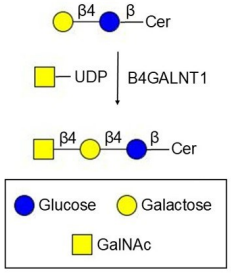
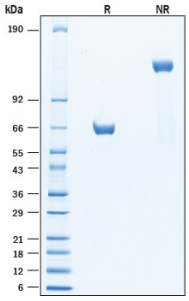
DATA

Enzyme Activity



Recombinant Human GM2/GD2 Synthase/ B4GALNT1 His-tag Enzyme Activity Diagram. Recombinant Human GM2/GD2 Synthase/ B4GALNT1 His-tag (Cat # 11777-BG) catalyzes the transfer of GalNAc onto ceramides (Cer) such as GM3 and GD3 by a beta-1,4 linkage, resulting in the formation of products such as GM2 and GD2.

SDS-PAGE



Recombinant Human GM2/GD2 Synthase/ B4GALNT1 His-tag SDS-PAGE. 2 µg/lane of Recombinant Human GM2/GD2 Synthase/ B4GALNT1 His-tag (Catalog # 11777-BG) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 61-68 kDa, under reducing conditions.

BACKGROUND

Beta-1,4 N-acetylgalactosaminyltransferase 1 (B4GALNT1), also known as GalNAc-T or GM2/GD2 synthase from the gene B4GALNT1, is a glycosylated, Mn-dependent type-II Golgi transmembrane homodimeric protein that belongs to the CAZy GT12 family of glycosyltransferases (GTs) (1-3). B4GALNT1 is a key enzyme of the ganglioside (sialic acid-containing glycosphingolipids) synthetic pathway, responsible for the synthesis of the complex gangliosides GM2 and GD2 from their respective precursors GM3 and GD3 (4); these products are the essential precursors of the major brain gangliosides. B4GALNT1 has a predicted domain structure consisting of a short 7 amino acid N-terminal cytoplasmic sequence followed by a transmembrane domain and a 56 kDa lumenal domain (3). Each monomer luminal domain of the B4GALNT1 homodimer possesses an N-terminal region that forms a non-catalytic domain with a section that wraps across the dimer and contribute loops and helices to both the upper and lower domains, while the C-terminal region forms much of the predicted catalytic domain including a conserved DxD motif required for UDP-binding and the manganese ion (3). In addition, there are hydrophobic residues present on two surface loops flanking the active site that insert into the lipid bilayer to allow the enzyme to process membrane-embedded substrates. The dimer is arranged in an antiparallel orientation with highly conserved disulfides including an inter-chain disulfide bond (3, 5). B4GALNT1 loss of function is caused by several mutations that disrupt the substrate binding site or dimerization site of the protein (3) resulting in a complex early-onset form of hereditary spastic paraplegia 26 (HSP26) (6). Overexpression or increased activity of B4GALNT1 is associated with several cancers and diseases including childhood neuroblastoma, hepatocellular carcinoma (HCC), Systemic Lupus Erythematosus (SLE) (3, 7-10). Upregulated catalytic products are present in GM2 gangliosidoses such as Tay-Sach's, Sandhoff, and AB variant, and can be antibody-mediated chemotherapeutic targets (11-13) or targeted for substrate reduction strategies (3, 14). Direct inhibition of B4GALNT1 may also represent an auxiliary therapeutic strategy along with immunotherapy in HCC where the enzyme plays a role in immunosuppression (15), as potential therapeutics in cancers where overexpression is driving pathogenesis, or where GM2 substrate reduction would be beneficial (3, 15).

References:

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