Recombinant Human ST6 Sialyltransferase 6/ST6GALNAC6

Catalog Number: 7425-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human ST6 Sialyltransferase 6/ST6GALNAC6 protein Asn65-Thr333, with a C-terminal 6-His tag Accession # Q969X2
N-terminal Sequence Analysis	Asn65
Predicted Molecular Mass	32 kDa

SPECIFICATIONS

SDS-PAGE	38-55 kDa, reducing conditions
Activity	Measured by its ability to transfer Neu5Ac from CMP-Neu5Ac to fetuin of fetal calf serum. The specific activity is >150 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	>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

- Assay Buffer: 25 mM MES, pH 6.0
- Recombinant Human Sialyltransferase 6/ST6GALNAC6 (rhST6GALNAC6) (Catalog # 7425-GT)
- CMP-Sialic Acid (Sigma, Catalog # C8271), 10 mM stock in deionized water
- Fetuin (Sigma, Catalog # F3385), 50 mg/mL stock in deionized water
- Sialyltransferase Activity Kit (Catalog # EA002)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM Phosphate Standard by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock.
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 nmoles per well.
3. Dilute CMP-Sialic Acid to 2.5 mM in Assay Buffer.
4. Dilute Coupling Phosphatase 2 to 20 µg/mL in Assay Buffer.
5. Prepare reaction mixture by combining 60 µL of 2.5 mM CMP-Sialic Acid, 60 µL of 20 µg/mL Coupling Phosphatase 2, 60 µL of 50 mg/mL Fetuin, and 120 µL of 100 mM $MnCl_2$ (supplied in kit). (Volume is sufficient for 12 wells.)
6. Dilute rhST6GALNAC to 5 µg/mL in Assay Buffer.
7. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
8. Load 25 µL of the 5 µg/mL rhST6GALNAC into the plate. Include a control containing 25 µL of Assay Buffer.
9. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
10. Cover the plate with a plate sealer and incubate at 37 ºC for 20 minutes.
11. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
12. Add 100 µL of deionized water to all wells. Mix briefly.
13. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
14. Read plate at 620 nm (absorbance) in endpoint mode.
15. 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

- rhST6GALNAC: 0.125 µg
- Coupling Phosphatase 2: 0.1 µg
- CMP-Sialic Acid: 250 µM
- Fetuin: 250 µg
- $MnCl_2$: 20 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

Gangliosides are acidic glycosphingolipids that contain one or more sialic acid residues (1). They are abundant in the nervous system, where they play crucial modulatory roles in cellular recognition, interaction, adhesion, and signal transduction, particularly during early developmental stages. The expression of gangliosides in the nervous system is developmentally regulated by various sialyltransferases (2). ST6GALNAC6 is widely expressed in the nervous tissues and many other mouse tissues such as colon, liver, and heart. It has known activity on gangliosides of GD1a, GT1b and GM1b (3). ST6GALNAC6 is also responsible for the biosynthesis of disialylgalactosylgloboside and disialyl Lewis a, representative tumor-associated antigens in pancreas and colon cancers (4, 5). The recombinant ST6GALNAC6 was active on fetuin from fetal calf serum when assayed using a phosphatase-coupled method (6) suggesting that ST6GALNAC6 is also active on N- or O-glycans.

References:

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4. Senda, M. *et al.*(2007) Biochem J. **402**:459.
5. Tsuchida, A. *et al.* (2003) J. Biol. Chem. **278**:22787.
6. Wu, Z.L. *et al.* (2011) Glycobiology **21**:727.

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