Recombinant Human ST8 α-2,8-Sialyltransferase 8A/ST8SIA1

Catalog Number: 6716-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human ST8 alpha-2,8-Sialyltransferase 8A/ST8SIA1 protein Tyr49-Ser356, with a C-terminal 6-His tag Accession # Q92185
N-terminal Sequence Analysis	Tyr49
Predicted Molecular Mass	36 kDa

SPECIFICATIONS

SDS-PAGE	40-60 kDa, reducing conditions
Activity	Measured by its ability to transfer Neu5Ac from CMP-Neu5Ac to fetuin of fetal calf serum. The specific activity is >15 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 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

- Sialyltransferase Activity Kit (Catalog # EA002)
- Assay Buffer: 50 mM MES, 5 mM $MgCl_2$, pH 6.5
- Recombinant Human α-2,8-Sialyltransferase 8A/ST8SIA1 (rhST8SIA1) (Catalog # 6716-GT)
- Donor Substrate: CMP-Neu5Ac (Sigma, Catalog # C8271), 10 mM stock in deionized water
- Acceptor Substrate: Fetuin (Sigma, Catalog # F3385), 50 mg/mL 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 Sialyltransferase 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. Complete the standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock using Assay Buffer. The standard curve has a range of 0.078 to 5 nmoles per well.
3. Dilute CMP-Neu5Ac to 2.5 mM in Assay Buffer.
4. Dilute Coupling Phosphatase 2 to 25 µg/mL in Assay Buffer.
5. Prepare reaction mixture by combining 50 µL of 2.5 mM CMP-Neu5Ac, 50 µL of 25 µg/mL Coupling Phosphatase 2, 62.5 µL of 50 mg/mL Fetuin, and 150 µL of Assay Buffer.
6. Dilute rhST8SIA1 to 40 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 40 µg/mL rhST8SIA1 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 parafilm or a plate sealer and incubate at 37 °C 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

Per Reaction:

- rhST8SIA1: 1.0 µg
- Coupling Phosphatase 2: 0.1 µg
- Fetuin: 250 µg
- CMP-Neu5Ac: 0.2 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 and are particularly prevalent on neuronal cells (1). Ganglioside GD3 is involved in cell adhesion and the growth of cultured malignant cells (2). ST8SIA1 is a sialyltransferase that catalyzes the transfer of sialic acid from CMP-sialic acid to GM3 (NeuNAcα2-3Galβ1-4Glc-Cer) to produce GD3 (NeuNAcα2-8NeuNAcα2-3Galβ1-4Glc-Cer) and GT3 (NeuNAcα2-8NeuNAcα2-8NeuNAcα2-3Galβ1-4Glc-Cer) in a successive manner (3); therefore the enzyme has both GD3 and GT3 synthase activity (4). ST8SIA1 is mainly expressed in adult and fetal brain, and its expression is enhanced in melanoma cell lines (3, 4, 5). Like most known glycosyltransferases, ST8SIA1 is predicted as a type II transmembrane protein with a short N-terminal cytoplasmic domain and a single-pass transmembrane domain followed by an enzymatic domain in the lumen of the Golgi apparatus. However, recently GD3 synthase activity was demonstrated at the surface of epithelial and melanoma cells, suggesting glycosphingolipid synthesis may occur at the cell membrane (6). Recombinant ST8SIA1 also showed activity on fetuin from fetal calf serum, when measured using a phosphatase-coupled method (7).

References:

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