Recombinant Human ST8 alpha-2,8-Sialyltransferase 6/ST8SIA6

Catalog Number: 9587-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Asp28-Ala398, with an N-terminal 6-His tag Accession # P61647
N-terminal Sequence Analysis	His
Predicted Molecular Mass	43 kDa

SPECIFICATIONS

SDS-PAGE	62-73 kDa, reducing conditions
Activity	Measured by its ability to transfer Neu5Ac from CMP-Neu5Ac to fetuin of fetal calf serum. The specific activity is >70 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

- Sialyltransferase Activity Kit (Catalog # EA002)
- Assay Buffer: 50 mM MES, 10 mM $MnCl_2$, pH 6.5
- Recombinant Human ST8 alpha-2,8-Sialyltransferase (rhST8SIA6) (Catalog # 9587-GT)
- CMP-Neu5Ac (CMP-sialic acid) (Sigma, Catalog # C8271), 10 mM stock in deionized water
- Fetuin (Sigma, Catalog # F3385), 50 mg/mL 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 nmol per well.
3. Prepare reaction mixture containing 0.5 mM CMP-Neu5Ac, 20 mg/mL Fetuin, and 4 µg/mL coupling phosphatase 2 (supplied in kit) in Assay Buffer.
4. Dilute rhST8SIA6 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 the 20 µg/mL rhST8SIA6 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 the wells, excluding the standard curve.
8. Incubate sealed plate 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:

- rhST8SIA6: 0.5 µg
- Coupling Phosphatase 2: 0.1 µg
- CMP-Neu5Ac: 250 µM
- Fetuin: 500 µg

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, -20 to -70 °C as supplied. ● 3 months, -20 to -70 °C under sterile conditions after opening.

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BACKGROUND

Polysialic acid (PSA), a glycan abundant on the neural cell adhesion molecule (NCAM) during embryonic development, negatively modulates the adhesive properties of NCAM (1). Following birth, PSA expression decreases promptly and becomes restricted to the hippocampus, hypothalamus, and olfactory bulb-areas of the brain that require continuous cell migration and synaptic plasticity (2). Expression of PSA in cancer cells has been suggested to increase tumor invasiveness and promote tumor growth (3). However; the mouse St8sia6 only has α-2,8 sialyltransferase activity toward both glycolipids and glycoproteins that has the NeuAc-α-2,3(6)-β-D-Gal sequence at the nonreducing ends of their carbohydrate groups, and forms NeuAc-α-2,8-NeuAc structures, but not oligosialic or polysialic acid structures (4). Like most glycosyltransferases, ST8SIA6 may be a Golgi-resident type II membrane protein. It is expressed in various tissues at low levels but is highly expressed in breast cancer and Dami megakaryocyte cell lines (5). The activity of this enzyme has been measured with a phosphatase-coupled method (6).

References:

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