Recombinant Human ST8 α-2,8-Sialyltransferase 8B/ST8SIA2

Catalog Number: 6590-GT

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human ST8 alpha-2,8-Sialyltransferase 8B/ST8SIA2 protein Asp24-Thr375, with an N-terminal 6-His tag Accession # Q92186
N-terminal Sequence Analysis	No sequence observed. Protein identity confirmed by detection of His-tag using Western analysis.
Predicted Molecular Mass	41 kDa

SPECIFICATIONS

SDS-PAGE	55-65 kDa, reducing conditions
Activity	Measured by its ability to transfer sialic acid from CMP-NeuAc to Recombinant Human NCAM-1/CD56 120 isoform (Catalog # 2408-NC). The specific activity is >85 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 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: 50 mM MES, 5 mM $MgCl_2$, pH 7.0
- Recombinant Human α-2,8-Sialyltransferase 8B/ST8SIA2 (rhST8SIA2) (Catalog # 6590-GT)
- Donor Substrate: CMP-Sialic Acid (Sigma, Catalog # C8271), 10 mM stock in deionized water
- Acceptor Substrate: Recombinant Human NCAM-1/CD56 (rhCD56) 120 isoform (Catalog # 2408-NC)
- 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 CMP-Sialic Acid to 3 mM in Assay Buffer.
2. Dilute Coupling Phosphatase 2 to 24 µg/mL in Assay Buffer.
3. Dilute rhCD56 to 240 µg/mL in Assay Buffer.
4. Prepare reaction mixture by combining 45 µL of 3 mM CMP-Sialic Acid, 45 µL of 24 µg/mL Coupling Phosphatase 2, and 180 µL of 240 µg/mL rhCD56.
5. Dilute rhST8SIA2 to 10 µg/mL in Assay Buffer.
6. 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. This is the first point of the standard curve.
7. 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 5.0 nmol per well.
8. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
9. Load 25 µL of the 10 µg/mL rhST8SIA2 into the plate. Include a Control containing 25 µL of Assay Buffer.
10. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
11. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 20 minutes.
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 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:

- rhST8SIA2: 0.250 µg
- Coupling Phosphatase 2: 100 ng
- rhCD56: 4 µg
- CMP-Sialic Acid: 250 µM

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

Polysialic acid (PSA), abundant on the neural cell adhesion molecule (NCAM) during embryonic development, acts as an anti-adhesive glycan to negatively modulate the adhesive properties of NCAM (1). PSA expression decreases promptly after birth, 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 to promote tumor growth (3). The temporal regulation of PSA is dependent on the expression of two polysialyltransferases, ST8SIA4 and ST8SIA2 (4, 5). ST8SIA2, also known as sialyltransferase X, is mainly expressed during embryonic development (4) and shows strict preference on NCAM (6). The high degree of substrate specificity is achieved through specific enzyme-substrate recognition at both the protein sequence and glycan structure levels (6, 7). Like most glycosyltransferases, ST8SIA2 is a Golgi-resident type II membrane protein. The activity of this enzyme has been measured with a phosphatase-coupled method (8).

References:

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