Recombinant Human ST3GAL6 Fc Chimera

Catalog Number: 10591-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human ST3GAL6 protein Asn40-Asp331, with N-termininal Fc Accession # Q9Y274.1
Predicted Molecular Mass	60.1 kDa

SPECIFICATIONS

SDS-PAGE	67-81 kDa, under reducing conditions.
Activity	Measured by its ability to transfer Neu5Ac from CMP-Neu5Ac to N-Acetyllactosamine. The specific activity is >50 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: 25 mM Tris, 150 mM NaCl, 5 mM $CaCl_2$, 10 mM $MnCl_2$, pH 7.5
- Sialyltransferase Activity Kit (Catalog # EA002)
- Recombinant Human ST3GAL6 (rhST3GAL6) (Catalog # 10591-GT)
- CMP-Sialic Acid (Calbiochem, Catalog # 233264), 2.5 mM stock in deionized water
- N-Acetyllactosamine (Dextra Laboratories, Catalog # GN204), 50 mM 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 nmol per well.
3. Prepare a reaction mixture containing 0.4 mM CMP-Sialic Acid, 8 mM N-Acetyllactosamine and 4 µg/mL Coupling Phosphatase 2 (supplied in kit) in Assay Buffer.
4. Dilute rhST3GAL6 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 20 µg/mL rhST3GAL6 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. Seal plate and incubate at 37 °C for 30 minutes.
9. Add 30 µL of the Malachite Green Reagent A (supplied in kit) to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells.
11. Add 30 µL of the Malachite Green Reagent B (supplied in kit) 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:

- rhST3GAL6: 0.5 µg
- Coupling Phosphatase 2: 0.1 µg
- CMP-Sialic Acid: 0.2 mM
- N-Acetyllactosamine: 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.

DATA

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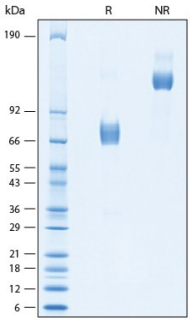
SDS-PAGE



Recombinant Human ST3GAL6 Fc Chimera Protein SDS-PAGE. 2 µg/lane of Recombinant Human ST3GAL6 Fc Chimera Protein (Catalog # 10591-GT) was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing bands at 67-81 kDa.

BACKGROUND

Sialyltransferases add sialic acid to glycoproteins or glycosphingolipids and play important roles in many biological processes including immune recognition, pathogen infection, and cell adhesion (1). ST3GAL6 is an α2,3-sialyltransferase and forms α2-3 linked sialic acid on Gal-β1,4-GlcNAc structure on glycoproteins and glycolipids (2). This enzyme has high specificity for some gangliosides and glycoproteins and may contribute to the formation of sialyl Lewis x (sLex), a carbohydrate important for cell-to-cell recognition and a blood group antigen (3). Study on various α2,3-sialyltransferases indicate that ST3GAL6 selectively sialylates EGFR therefore affects ERK and AKT signal transduction pathways and positively correlates to cell proliferation and colony formation, which is in sharp contrast to ST3GAL4 that selectively sialylates β1 integrin (4).

References:

1. Varki, A. (1999) Glycobiology **2**:25.
2. Okajima, T. *et al.* (1999) J Biol Chem. **274**:11479.
3. Glavey, S. *et al.* (2014) Blood **124**:1765.
4. Qi, F. *et al.* (2020) FASEB J. **34**:881.

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