Recombinant Human ST3GAL4 His-tag

Catalog Number: 10496-GT

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human ST3GAL4 protein Glu41-Phe333, with a C-terminal 6-His tag Accession # Q11206.1
Predicted Molecular Mass	34 kDa

SPECIFICATIONS

SDS-PAGE	39-41 kDa, under reducing conditions
Activity	Measured by its ability to transfer Neu5Ac from CMP-Neu5Ac to α-lactose. The specific activity is >100 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	>80%, 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 Tris, 5 mM $MnCl_2$, pH 7.0
- Sialyltransferase Activity Kit (Catalog # EA002)
- Recombinant Human ST3GAL4 (rhST3GAL4) (Catalog # 10496-GT)
- CMP-Sialic Acid (Sigma, Catalog # C8271), 10 mM stock in deionized water
- α-Lactose (Sigma, Catalog # L2643), 0.3 M 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. Prepare a reaction mixture containing 0.2 mM CMP-Sialic Acid, 36 mM α-Lactose and 2 µg/mL Coupling Phosphatase 2 (supplied in kit) in Assay Buffer.
4. Dilute rhST3GAL4 to 8 µg/mL in Assay Buffer. *Note: Perform single dilution step if possible to achieve concentration and avoid any acute mechanical stresses such as vortexing.
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 8 µg/mL rhST3GAL4 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 room temperature 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:

- rhST3GAL4: 0.2 µg
- Coupling Phosphatase 2: 0.05 µg
- CMP-Sialic Acid: 0.1 mM
- α-Lactose: 18 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, -20 to -70 °C as supplied. • 3 months, -20 to -70 °C under sterile conditions after opening.

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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). ST3GAL4 is an α2,3-sialyltransferase and forms α2-3 linked sialic acid on the Gal residue of the terminal Galβ1-4GlcNAc and Galβ1-3GalNAc structures found on glycoproteins and glycolipids (2, 3). ST3GAL4 is involved in the synthesis of sialyl Lewis x (sLe^x) glycan epitope that may serve as ligands for E- and P-selectins on activated endothelial cells in inflammatory responses (4). Down-regulation of ST3GAL4 mRNA may be one of the factors associated with the malignant progression of human renal cell carcinoma (5). ST3GAL4 is expressed as a type II membrane protein localized in the trans-Golgi apparatus but may also be proteolytically processed to form a soluble form. The activity of the recombinant human ST3GAL4 was determined using a phosphatase-coupled glycosyltransferase assay (6).

References:

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