**DESCRIPTION**

**Source**
Chinese Hamster Ovary cell line, CHO-derived
Leu25-Lys340, with C-terminal 6-His tag
Accession # Q9UNA3

**N-terminal Sequence Analysis**
Leu25

**Predicted Molecular Mass**
38 kDa

**SPECIFICATIONS**

**SDS-PAGE**
39-49 kDa, reducing conditions

**Activity**
Measured by its ability to transfer GlcNAc from UDP-GlcNAc to galactose.
The specific activity is >400 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**
- Glycosyltransferase Activity Kit (Catalog # EA001)
- 10X Assay Buffer (supplied in kit): 250 mM Tris, 100 mM CaCl₂, pH 7.5
- MnCl₂ (supplied in kit): 100 mM
- Recombinant Human α-1,4-N-Acetylglucosaminyltransferase 4/A4GNT (rhA4GNT) (Catalog # 8960-GT)
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% ethanol
- D-(+)-Galactose (Sigma, Catalog # G0625), 750 mM stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Prepare 1X Assay Buffer containing 10 mM MnCl₂ by combining 10X stocks and diluting 10-fold with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 μL of the 1 mM Phosphate Standard to 360 μL of 1X Assay Buffer for a 100 μM stock. This is the first point of the standard curve.
3. Complete the standard curve by performing six one-half serial dilutions of the 100 μM Phosphate stock using 1X Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
4. Prepare reaction mixture containing 5 mM UDP-GlcNAc, 200 mM D-(+)-Galactose, and 4 μg/mL Coupling Phosphatase 1 in 1X Assay Buffer.
5. Dilute rhA4GNT to 4 ng/μL in 1X Assay Buffer.
6. Load 50 μL of each dilution of the standard curve into a plate. Include a curve blank containing 50 μL of 1X Assay Buffer.
7. Load 25 μL of 4 ng/μL rhA4GNT into empty wells of the same plate as the curve. Include a Control containing 25 μL of 1X Assay Buffer.
8. Add 25 μL of the reaction mixture to all wells, excluding the standard curve.
9. Seal plate and incubate at 37°C for 20 minutes.
10. Add 30 μL of the Malachite Green Reagent A to all wells. Mix briefly.
11. Add 100 μL of deionized water to all wells. Mix briefly.
12. Add 30 μL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate for 20 minutes at room temperature.
13. Read plate at 620 nm (absorbance) in endpoint mode.
14. Calculate specific activity:

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\text{Specific Activity (pmol/min/μg)} = \frac{\text{Phosphate released}^* (\text{nmol}) \times (1000 \text{ pmol/nmol})}{\text{Incubation time (min)} \times \text{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:
- rhA4GNT: 0.1 μg
- Coupling Phosphatase 1: 0.1 μg
- UDP-GlcNAc: 2.5 mM
- D-(+)-Galactose: 100 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.
Alpha-1,4-N-acetylglucosaminyltransferase A4GNT is a type II Golgi resident membrane glycosyltransferase, but shows no significant sequence similarity with any other glycosyltransferases. It catalyzes the transfer of N-acetylgalactosamine (GlcNAc) to core 2 branched O-glycans and forms a unique glycan, GlcNAcα1→4Galβ1→R on mucin (1). Alpha-1,4-GlcNAc-capped mucin-type O-glycan inhibits cholesterol α-glucosyltransferase from Helicobacter pylori (Hp) and suppresses Hp growth (2). Hp infection is widespread and thought to be the causes of the development of gastric lesions including gastritis, intestinal metaplasia, and gastric carcinoma (3). The enzymatic activity of the recombinant protein was determined using a phosphatase-coupled assay (4).

References: