Recombinant Human Polypeptide GalNac Transferase 13/GALNT13

Catalog Number: 8906-GT

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived Ser29-Thr556, with a C-terminal 6-His tag Accession # Q8IUC8
N-terminal Sequence Analysis	Ser29
Predicted Molecular Mass	61 kDa

SPECIFICATIONS

SDS-PAGE	60-70 kDa, reducing conditions
Activity	Measured by its ability to transfer GalNAc from UDP-GalNAc to peptide EA2 from AnaSpec, Inc. The specific activity is >600 pmol/min/µg, as measured under the described specifications.
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 $CaCl_2$, pH 7.5
- $MnCl_2$ (supplied in kit): 100 mM
- Recombinant Human Polypeptide GalNAc Transferase 13/GALNT13 (rhGALNT13) (Catalog # 8906-GT)
- UDP-GalNAc (Sigma, Catalog # U5252), 10 mM stock in deionized water
- EA2 peptide (AnaSpec Inc., Catalog # 63841), 5 mM in 5 mM Tris, pH 7.0
- 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 $MnCl_2$ 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 1 mM UDP-GalNAc, 0.4 mM EA2 peptide, and 4 µg/mL Coupling Phosphatase 1 in 1X Assay Buffer.
5. Dilute rhGALNT13 to 4 µg/mL 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 µg/mL rhGALNT13 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:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol)} \times (1000\ \text{pmol/nmol})}{\text{Incubation time (min)} \times \text{amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhGALNT13: 0.1 µg
- Coupling Phosphatase 1: 0.1 µg
- UDP-GalNAc: 0.5 mM
- EA2 peptide: 0.2 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.

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BACKGROUND

O-glycosylation is a ubiquitous post-translational modification present in secreted and membrane-bound proteins. Polypeptide N-acetylgalactosaminyltransferases (GALNTs) catalyze the initial step for O-glycosylation by transferring GalNAc to Thr or Ser residues (GalNAc α1-O-Ser/Thr) in the Golgi compartment. Structurally, the GALNTs consist of an N-terminal catalytic domain tethered by a short linker to a C-terminal ricin-like lectin domain containing three potential carbohydrate-binding sites (1, 2). Twenty distinct GALNT isoforms have been detected in humans. These isoforms display both unique and overlapping substrate specificities (3, 4, 5) with no known universal consensus glycosylation sequence. Glycosylation of mucins results from the successive, often hierarchical, action of several specific GALNTs (6). GALNT13 is highly homologous to ubiquitously expressed GALNT1, but is restrictively expressed in the brain (7). In addition, GALNT13 is able to form trimeric Tn antigen, three consecutive GalNAc-Ser/Thr structures, on syndecans, which may further enhances cancer metastasis (7, 8). The enzymatic activity of recombinant human GALNT1 was determined using a phosphatase-coupled assay (9).

References:

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