R&D SYSTEMS
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Recombinant Human Polypeptide GalNac Transferase 1/GALNT1

Catalog Number: 7140-GT

DESCRIPTION

Source	Mouse myeloma cell line, NS0-derived human Polypeptide GalNac Transferase 1/GALNT1 protein Gly41-Phe559, with an N-terminal 6-His tag Accession # Q10472
N-terminal Sequence Analysis	Inconclusive results, intact N-terminus verified by anti-poly-His Western.
Predicted Molecular Mass	60 kDa

SPECIFICATIONS

SDS-PAGE	60-65 kDa, reducing conditions
Activity	Measured by its ability to transfer GalNAc from UDP-GalNAc to peptide EA2 from AnaSpec, Inc. The specific activity is >700 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 under reducing conditions and visualized by Colloidal Coomassie® Blue stain at 5 µg per lane.
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, 2.5 mM $MnCl_2$ (supplied in kit), 1 mM $CaCl_2$, pH 8.0
- Recombinant Human Polypeptide GalNac Transferase 1/GALNT1 (rhGALNT1) (Catalog # 7140-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
- Glycosyltransferase Activity Kit (Catalog # EA001)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock.
2. Prepare 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 nmol per well.
3. Dilute EA2 peptide to 1 mM in Assay Buffer.
4. Dilute Coupling Phosphatase I to 60 ng/µL in Assay Buffer.
5. Prepare reaction mixture by combining 32.5 uL of 10 mM UDP-GalNAc, 65 uL of 1 mM EA2 peptide, 43.3 uL of 60 ng/µL Coupling Phosphatase I, and 184.2 uL Assay Buffer (sufficient to test 12 wells).
6. Dilute rhGALNT1 to 1 ng/µL in Assay Buffer.
7. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
8. Load 25 µL of 1 ng/µL rhGALNT1 into the plate. Include a Control containing 25 µL of Assay Buffer.
9. Add 25 µL of reaction mixture to the wells, excluding the standard curve and curve blank.
10. Cover the plate with parafilm or a plate sealer and incubate at 37 °C for 20 minutes.
11. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
12. Add 100 µL of deionized water to all wells. Mix briefly.
13. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
14. Read plate at 620 nm (absorbance) in endpoint mode.
15. 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:

- rhGALNT1: 0.025 µg
- Coupling Phosphatase I: 0.2 µg
- EA2 peptide: 0.1 mM
- UDP-GalNAc: 0.5 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 of secreted and membrane-bound proteins. Polypeptide N-acetylgalactosaminyltransferases (GALNTs) calalyze the initial step for o-glycosylation: 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. Most of the isoforms display both unique and overlapping substrate specificities (3, 4) with no universal consensus glycosylation sequence. Glycosylation of mucins results from successive, often hierarchical, action of several specific GALNTs (5). GALNT1, in particular, is involved in the glycosylation of proteins essential for bone formation such as osteopontin and bone sialoprotein (6). Using a peptide library screening approach, GALNT1 was classified as an early transferase that has a preference for nonglycosylated or monoglycosylated substrates (5). The enzymatic activity of recombinant human GALNT1 was determined using a phosphatase-coupled assay (7).

References:

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PRODUCT SPECIFIC NOTICES

Coomassie is a registered trademark of Imperial Chemical Industries Ltd.

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