R&D SYSTEMS
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Recombinant Human C1GalT1

Catalog Number: 8659-GT

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human C1GalT1 protein Asp45-Pro363, with N-terminal 6-His tag (C1GalT1); Ile28-Asp318, with an N-terminal HA tag (C1GalT1C1) Accession # Q9NS00 (C1GalT1) & Q96EU7 (C1GalT1C1)
N-terminal Sequence Analysis	His (C1GalT1) & Tyr (C1GalT1C1)
Predicted Molecular Mass	38 kDa (C1GalT1) & 35 kDa (C1GalT1C1)

SPECIFICATIONS

SDS-PAGE	25-42 kDa, reducing conditions
Activity	Measured by its ability to transfer galactose from UDP-galactose to 4-nitrophenyl-α-D-galactosaminide. The specific activity is >2,750 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	>90%, 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)
- Assay Buffer: 25 mM Tris, 5 mM $CaCl_2$, 10 mM $MnCl_2$, pH 7.5
- Recombinant Human C1GalT1 (rhC1GalT1) (Catalog # 8659-GT)
- Uridine 5'-diphosphogalactose (UDP-Gal) (Sigma, Catalog # U4500), 10 mM stock in deionized water
- 4-Nitrophenyl N-acetyl-α-D-galactosaminide (4-NP-GalNAc) (Sigma, Catalog # N4264), 15 mM stock in DMSO
- 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 Glycosyltransferase 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 reaction mixture containing 1.2 mM UDP-Gal, 0.6 mM 4-NP-GalNAc, and 4 µg/mL Coupling Phosphatase 1 in Assay Buffer.
4. Dilute rhC1GALT1 to 0.6 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 0.6 µg/mL rhC1GALT1 into empty wells of the same plate as the curve. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of the reaction mixture to all wells, excluding the standard curve.
8. Seal plate and incubate at 37 °C for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate sealed plate 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 Well:

- rhC1GALT1: 0.015 µg
- Coupling Phosphatase 1: 0.1 µg
- UDP-Gal: 0.6 mM
- 4-NP-GalNAc: 0.3 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 (1). The synthesis of mucin-type O-glycans is initiated by the addition of GalNAc to threonine or serine residues on proteins by polypeptide N-acetylgalactosaminyltransferases (GALNTs) (2). The GalNAcα1-O-Ser/Thr structure is then extended by other glycosyltransferases to form eight types of core O-glycans (3). Core 1 β-3-galactosyltransferase (C1GalT1), in particular, synthesizes Gal-β1-3GalNAcα1-O-Ser/Thr, a precursor for all core 1 and core 2 based mucin-type O-glycans (4). These glycans play central roles in many processes, such as angiogenesis, thrombopoiesis, and kidney homeostasis (5). C1GalT1 forms a stable, non-covalent complex with Cosmc chaperone, C1GalT1C1, which is required for the full activity of C1GalT1 (6). Defective C1GalT1 causes a rare autoimmune disease called Tn syndrome (4) as well as susceptibility to IgA nephropathy (7). The recombinant C1GalT1 is co-purified with C1GalT1C1. The enzymatic activity of recombinant human C1GalT1 was determined using a phosphatase-coupled assay (8).

References:

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