# Recombinant Human GALNTL1

Catalog Number: 7850-GT

## DESCRIPTION

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| **Source** | Mouse myeloma cell line, NS0-derived<br>Asp27-Thr558 with C-terminal 6-His tag<br>Accession # Q8N428 |
| **N-terminal Sequence Analysis** | Asp27 |
| **Predicted Molecular Mass** | 61 kDa |

## SPECIFICATIONS

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| **SDS-PAGE** | 55-61 kDa, reducing conditions |
| **Activity** | Measured by its ability to transfer GalNAc from UDP-GalNAc to peptide EA2 from AnaSpec, Inc.<br>The specific activity is >90 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 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 (provided in kit): 25 mM Tris, 10 mM $MnCl_2$, 10 mM $CaCl_2$, pH 7.5
- Recombinant Human GALNTL1 (rhGALNTL1) (Catalog # 7850-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. Prepare reaction mixture by containing 1 mM UDP-GalNAc, 1 mM EA2 peptide, and 4 ng/µL Coupling Phosphatase I in Assay Buffer.
4. Dilute rhGALNTL1 to 10 ng/µL 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 the 1 ng/µL rhGALNTL1 into the plate. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of reaction mixture to the wells, excluding the standard curve and curve blank.
8. Cover the plate with parafilm or a plate sealer 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 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**

- rhGALNTL1: 0.25 µg
- Coupling Phosphatase I: 0.1 µg
- EA2 peptide: 0.5 mM
- UDP-GalNAc: 0.5 mM

## PREPARATION AND STORAGE

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| **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.**<br>• 6 months from date of receipt, -70 °C as supplied.<br>• 3 months, -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). GALNTL1 is active toward non-glycosylated peptides as well as some glycosylated peptides and is widely expressed in most tissues, especially high in heart, spinal cord and brain (7). Phylogenetically, GALNTL1 is closely related to GALNT2 and GALNT14 (8). The enzymatic activity of recombinant human GALNTL1 was determined using a phosphatase-coupled assay (9).

**References:**

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## PRODUCT SPECIFIC NOTICES





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