## DESCRIPTION

<table>
<thead>
<tr>
<th>Source</th>
<th>Chinese Hamster Ovary cell line, CHO-derived human B3GALNT2 protein Gly35-Arg500, with C-terminal 6x His tag Accession # Q8NCR0-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-terminal Sequence Analysis</td>
<td>Gly35</td>
</tr>
<tr>
<td>Predicted Molecular Mass</td>
<td>54 kDa</td>
</tr>
</tbody>
</table>

## SPECIFICATIONS

| SDS-PAGE | 55-63 kDa, reducing conditions |
| Activity | Measured by its ability to transfer GalNAc from UDP-GalNAc to benzyl-GlcNAc. The specific activity is >10 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)
- Assay Buffer: 50 mM HEPES, 10 mM MnCl₂ (supplied in kit), pH 7.5
- Recombinant Human B3GALNT2 (rhB3GALNT2) (Catalog # 1848-GT)
- Benzyl 2-Acetamido-2-deoxy-β-D-glucopyranoside (Benzyl-GlcNAc) (Sigma, Catalog # SC-221296), 25 mM stock in deionized water
- Uridine 5'-diphospho-N-acetylgalactosamine (UDP-GalNAc) (Sigma, Catalog # U5252), 10 mM stock in deionized water
- 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 0.4 mM UDP-GalNAc, 2 mM Benzyl-GlcNAc and 8 μg/mL Coupling Phosphatase 1 in Assay Buffer.
4. Dilute rhB3GALNT2 to 80 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 80 μg/mL rhB3GALNT2 into empty wells of the same plate as the curve. Include a Control containing 25 μL of Assay Buffer.
7. Add 25 μL of 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:

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

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

### Final Assay Conditions

Per Reaction:
- rhB3GALNT2: 2 μg
- Coupling Phosphatase 1: 0.2 μg
- UDP-GalNAc: 0.2 mM
- Benzyl-GlcNAc: 1 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.
B3GALNT2 was first identified in 2004 through a BLAST search (1). The expressed B3GALNT2 strictly transferred GalNAc to terminal GlcNAc residue and synthesized a unique carbohydrate structure GalNAc-β1,3-GlcNAc, which was not known in humans or other mammals previously. The related native structure was only found later in a study of congenital muscular dystrophies (CMD), a heterogeneous group of inherited diseases characterized by the onset of muscle weakness at birth or within 2 years of life. A common subgroup within the CMDs are the dystroglycanopathies, characterized by reduced functional glycosylation of α-dystroglycan (α-DG), a peripheral membrane protein that binds to several extracellular matrix components. Proper glycosylation of α-DG is critical to maintain structural integrity and force transmission between the cytoskeleton and the extracellular matrix for efficient signal transduction. Deleterious mutation in gene of B3GALNT2 was found in some of the patients of dystroglycanopathies (2). The complete structure of B3GALNT2 product was then found as an O-mannosyl trisaccharide GalNAc-β1,3-GlcNAc-β1,4-Man (3) with the mannose phosphorylated at the 6-position (4). The enzymatic activity of recombinant human B3GALNT2 was determined using a phosphatase-coupled assay (5) using GlcNAc β1-O-benzyl as acceptor substrate. Recombinant B3GALNT2 is also found to be highly active and specific towards O-GlcNAc and therefore is an ideal enzyme for the detection and labeling of O-GlcNAc (6). O-GlcNAc is a single GlcNAc residue attached to serine/threonine residues on nuclear and cytosolic proteins with regulatory roles from transcription, translation, cell signaling to cell cycle regulation (7).

References: