Recombinant Human O-GlcNAc Transferase/OGT

Catalog Number: 8446-GT

DESCRIPTION

Source	*E. coli*-derived Cys323-Glu1041, with C-terminal 6-His tag Accession # O15294
N-terminal Sequence Analysis	Cys323
Predicted Molecular Mass	81 kDa

SPECIFICATIONS

SDS-PAGE	70 kDa, reducing conditions
Activity	Measured by its ability to transfer GlcNAc from UDP-GclNAc to peptide OGT substrate from AnaSpec, Inc. The specific activity is >25 pmol/min/µg, as measured under the described conditions. See Activity Assay Protocol on www.RnDSystems.com.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>80%, 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, NaCl and TCEP. 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 CaCl2, pH 7.5
- MgCl2, 1 M stock in deionized water
- Recombinant Human O-GlcNAc Transferase/OGT (rhOGT) (Catalog # 8446-GT)
- OGT-Substrate (AnaSpec, Inc., Catalog # 63726), 5 mM stock in deionized water
- UDP-GlcNAc (Sigma, Catalog # U4375), 50 mM stock in 50% Ethanol/50% deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Prepare Assay Buffer by diluting 10X Assay Buffer to1X containing 10 mM $MgCl_2$ with deionized water.
2. Dilute 1 mM Phosphate Standard provided by the Glycosyltransferase Activity 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.
3. 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.
4. Prepare reaction mixture containing 0.8 mM UDP-GlcNAc, 0.4 mM OGT-Substrate, and 4 µg/mL Coupling Phosphatase 1 in Assay Buffer.
5. Dilute rhOGT to 40 µg/mL in Assay Buffer.
6. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
7. Load 25 µL of 40 µg/mL rhOGT into empty wells of the same plate as the curve. Include a Control containing 25 µL of 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 30 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:

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

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

Final Assay Conditions

Per Reaction:

- rhOGT: 1 µg
- Coupling Phosphatase 1: 0.1 µg
- OGT-Substrate: 0.2 mM
- UDP-GlcNAc: 0.4 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

The O-GlcNAc post-translational modification occurs by the addition of a single N-acetyl-glucosamine residue to serine/threonine residues of cellular proteins (1, 2). Unlike other types of glycosylation, the sugar residue is not elongated into complex oligosaccharides. In fact, O-GlcNAc shares many features with protein phosphorylation, a fundamental mechanism for intracellular communication. It is postulated that O-GlcNAc has a mutually exclusive relationship with phosphorylation (3, 4). While a variety of kinases and phosphatases are involved in phosphorylation, intracellular O-GlcNAc is introduced by a single O-GlcNAc transferase, OGT, and removed by a single glycosidase, OGA. O-GlcNAc is involved in many cellular processes, including stress responses, transcription, translation, cell signaling and cell cycle regulation and in many human diseases including diabetes, Alzheimer's disease and cancer (5, 6). By sensing levels of UDP-GlcNAc, OGT can respond to nutrient levels of all living systems via dynamically O-GlcNAcylating a wide range of nuclear and cytoplasmic proteins. Thus, strategies to modulate OGT activity may have therapeutic value for treating diabetic complications, cancer, and other diseases (7). In molecular structure, OGT comprises two distinct regions: an N-terminal region consisting of a series of tetratricopeptide repeat (TPR) units and a multidomain catalytic region (8). The enzyme activity of recombinant mouse OGT is measured using a phosphatase-coupled assay (9).

References:

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