Recombinant *B. thetaiotaomicron* O-GlcNAcase/OGA

Catalog Number: 6779-GH

DESCRIPTION

Source	*E. coli*-derived Gln22-Lys737, with an N-terminal Met and 6-His tag Accession # Q89ZI2
N-terminal Sequence Analysis	Met
Predicted Molecular Mass	83 kDa

SPECIFICATIONS

SDS-PAGE	66-76 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide (4-MU-GlcNAc) The specific activity is >3500 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	>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, NaCl, Brij and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM MES, 100 mM NaCl, pH 5.5
- Recombinant *B. thetaiotaomicron* O-GlcNAcase/OGA (r*Bt*OGA) (Catalog # 6779-GH)
- Substrate: 4-Methylumbelliferyl-N-acetyl-β-D-glucosaminide (Sigma, Catalog # M2133), 50 mM stock in DMSO
- F16 Black Maxisorp Plate (Nunc, Catalog # 475515)
- Fluorescent Plate Reader (Model: SpectraMax Gemini EM by Molecular Devices) or equivalent

Assay

1. Dilute r*Bt*OGA to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. Load into a plate 50 µL of 2 ng/µL r*Bt*OGA, and start the reaction by adding 50 µL of 2 mM Substrate. For Substrate Blanks, load 50 µL of Assay Buffer and 50 µL of 2 mM Substrate.
4. Read plate at excitation and emission wavelengths of 365 nm and 445 nm, respectively, in kinetic mode for 5 minutes.
5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted } V_{max}\text{* (RFU/min) x Conversion Factor** (pmol/RFU)}}{\text{amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Derived using calibration standard 4-Methylumbelliferone (4-MU) (Sigma, Catalog # M1381).

Final Assay Conditions

Per Well:

- r*Bt*OGA: 0.1 µg
- Substrate: 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.

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BACKGROUND

The addition of the monosaccharide β-N-acetyl-D-glucosamine to serine and threonine residues in proteins (O-GlcNAc glycosylation) is a dynamic, intracellular, post-translational modification that shares features with phosphorylation (1). Almost all major classes of intracellular proteins are modified with O-GlcNAc glycosylation. O-GlcNAc is known to regulate gene transcription, act as an energy sensor to desensitize insulin response, and coordinate phosphorylation to control protein activity (2, 3, 4, 5). In humans, O-GlcNAc is introduced by a single O-linked N-acetylglucosamine transferase, OGT, and removed by a single glycosidase, OGA. Both OGT and OGA are cytosolic. Enzymes with high sequence homology to human OGA have been found in human pathogens and symbionts (6, 7, 8), where these enzymes are proposed to metabolize O-GlcNAc in human proteins. OGA from the human gut symbiont *Bacteroides thetaiotaomicron* and its human counterpart are very similar in structure and function, and both enzymes operate via an unusual 'substrate-assisted' catalytic mechanism (8, 9). Recombinant *B. thetaiotaomicron* OGA can be used as an enzymatic tool to investigate O-GlcNAc glycosylation.

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