# Recombinant Human α-N-acetylglucosaminidase/NAGLU

Catalog Number: 7096-GH

## DESCRIPTION

| | |
|---|---|
| **Source** | Chinese Hamster Ovary cell line, CHO-derived human alpha-N-acetylglucosaminidase/NAGLU protein<br>Met1-Trp743, with a C-terminal 6-His tag<br>Accession # P54802 |
| **N-terminal Sequence Analysis** | Asp24 |
| **Predicted Molecular Mass** | 81 kDa |

## SPECIFICATIONS

| | |
|---|---|
| **SDS-PAGE** | 70-95 kDa, reducing conditions |
| **Activity** | Measured by its ability to hydrolyze 4-Nitrophenyl-N-acetyl-α-D-glucosaminide.<br>The specific activity is >900 pmol/min/µg, as measured under the decribed 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**

- Assay Buffer: 0.1 M Sodium Citrate, 0.25 M NaCl, pH 4.5
- Recombinant Human α-N-acetylglucosaminidase/NAGLU (rhNAGLU) (Catalog # 7096-GH)
- Substrate: 4-Nitrophenyl-N-acetyl-α-D-glucosaminide (Sigma, Catalog # N-8759), 5 mM stock in deionized water
- NaOH, 0.2 M stock in deionized water
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

**Assay**

1. Dilute rhNAGLU to 3 ng/µL in Assay Buffer.
2. Dilute Substrate to 3 mM in Assay Buffer.
3. In a plate combine 50 µL of rhNAGLU and 50 µL of 3 mM Substrate. Include a Substrate Blank containing 50 µL Assay Buffer and 50 µL of 3 mM Substrate.
4. Seal plate and incubate at 37 °C for 20 minutes.
5. Add 100 µL of 0.2 M NaOH to each well to stop the reaction and develop the color.
6. Read absorbance in endpoint mode at 402 nm.
7. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Abs* (OD) x well volume (L) x } 10^{12} \text{ pmol/mol}}{\text{Inc. time (min) x } \varepsilon^{**} \text{ } (M^{-1}cm^{-1}) \text{ x path corr.}^{***} \text{ (cm) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 17700 $M^{-1}cm^{-1}$

***Using the path correction 0.6 cm

**Final Assay Conditions**

Per Well:

- rhNAGLU: 0.150 µg
- Substrate: 750 µM

## 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

Human lysosomal α-N-acetylglucosaminidase is a hydrolase that catalyses the removal of terminal α-N-acetylglucosamine residues from heparan sulfate and heparin (1). Defects in this gene are the cause of mucopolysaccharidosis type IIIB (MPS-IIIB), also known as Sanfilippo syndrome B (2, 3, 4). Mucopolysaccharidosis types IIIA, C, and D are caused by mutations in other genes involved in the lysosomal degradation of heparan sulfate. Continuous lysosomal accumulation of heparan sulfate results in the clinical onset of disease, which is typified by severe central nervous system degeneration (5). Mucopolysaccharidosis type III differs from other mucopolysaccharidoses in that patients usually exhibit mild somatic changes with minimal skeletal abnormalities (6).

**References:**

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5. Beesley, C.E. *et al.* (2005) J. Inherit. Metab. Dis. **28**:759.
6. Yogalingam, G. and Hopwood, J.J. (2001) Hum. Mutat. **18**:264.



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