Recombinant Human α-N-acetylgalactosaminidase/NAGA

Catalog Number: 6717-GH

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived human alpha-N-acetylgalactosaminidase/NAGA protein Leu18-Gln411, with a C-terminal 6-His tag Accession # P17050
N-terminal Sequence Analysis	Leu18
Predicted Molecular Mass	46 kDa

SPECIFICATIONS

SDS-PAGE	50-60 kDa, reducing conditions
Activity	Measured by its ability to cleave α-N-acetylgalactosaminyl from 4-Nitrophenyl N-acetyl-α-D-galactosaminide. The specific activity is >1800 pmol/min/µg, as measured under the described condition.
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, NaCl, Brij and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M Sodium Citrate, 0.2 M NaCl, pH 4.0
- Recombinant Human α-N-acetylgalactosaminidase/NAGA (rhNAGA) (Catalog # 6717-GH)
- Substrate: 4-Nitrophenyl-N-acetyl-α-D-galactosaminide (Sigma, Catalog # N4264), 15 mM stock in DMSO
- 0.2 M Sodium Hydroxide
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhNAGA to 2 ng/µL in Assay Buffer.
2. Dilute Substrate to 2 mM in Assay Buffer.
3. Load 50 µL of 2 ng/µL rhNAGA and 50 µL of 2 mM Substrate into a clear 96-well plate. Also create a Substrate Blank by loading 50 µL of Assay Buffer and 50 µL of 2 mM Substrate.
4. Incubate plate at room temperature for 10 minutes.
5. Add 100 µL of 0.2 M NaOH to each well used in order to stop the reaction and develop the color.
6. Read absorbance in endpoint mode at 402 nm.
7. Calculate specific activity:

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

*Adjusted for Substrate Blank

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

***Using the path corr. 0.6 cm

†Based upon a 0.0002 L Volume

Final Assay Conditions

Per Reaction:

- rhNAGA: 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

NAGA is a lysosomal α-N-acetylgalactosaminidase that cleaves non-reducing α-N-acetylgalactosaminyl moieties from glycoconjugates (1). Mature NAGA has 394 amino acids and is trafficked to the lysosome via the mannose-6-phosphate receptor-mediated pathway (2). The enzyme is a retaining exoglycosidase, where both the substrate and product of the enzymatic reaction have the same anomeric configuration (3). Deficiency in NAGA results in increased urinary excretion and tissue accumulation of glycopeptides and oligosaccharides containing terminal α-N-acetylgalactosaminyl moieties (4), manifesting as Schindler's disease, an autosomal recessive disease with neuroaxonal dystrophy and other neurological symptoms (5). The enzyme can be used to remove α-N-acetylgalactosaminyl residues present on red blood cells thus converting blood type A to blood type O (6, 7, 8).

References:

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