Recombinant Human Glucosamine (N-acetyl)-6-Sulfatase/GNS

Catalog Number: 2484-SUC

Background

A member of the sulfatase family, GNS is required for the lysosomal degradation of the glycosaminoglycans (GAG) heparan sulfate and keratan sulfate (1, 2). It hydrolyzes the 6-sulfate group of the N-acetyl-D-glucosamine 6-sulfate units of the GAG. GNS deficiency results in mucopolysaccharidosis type IIID (MPS IIID or Sanfilippo D Syndrome), an inborn error leading to lysosomal accumulation of heparan sulfate. MPS IIID has profound mental deterioration, hyperactivity, and relatively mild somatic manifestations. The deduced amino acid sequence of human GNS consists of a signal peptide (residues 1 - 36) and a mature chain (residues 37 - 552) that may be further processed into N-terminal and C-terminal fragments (3). rhGNS corresponds to the single chain and has sulfatase activity described in Activity Assay Protocol.

References:

1. Parenti, G. *et al.* (1997) Curr. Opin. Genet. & Dev. **7**:386.
2. Neufeld, E.F. and J. Muenzer (2001) in *The Metabolic and Molecular Basis of Inherited Disease*, Scriver, C.R. *et al.* (eds.) pp. 3421 - 3452, New York, McGraw-Hill.
3. Robertson, D.A. *et al.* (1992) Biochem. J. **288**:539.

Description

Source	Chinese Hamster Ovary cell line, CHO-derived Val37 - Leu552 & Thr44 - Leu552, both with a C-terminal 10-His tag Accession # P15586
N-terminal Sequence Analysis	Val37 & Thr44
Predicted Molecular Mass	59 & 60 kDa

Specifications

SDS-PAGE	94 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS). The specific activity is > 250 pmoles/min/µg, as measured under the described conditions. See Activity Assay Protocol.
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 silver stain.
Formulation	Supplied as a 0.2 µm filtered solution in Tris, NaCl and Glycerol. See Certificate of Analysis for details.

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.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Sodium Acetate, 250 mM NaCl, pH 5.0
- Recombinant human GNS (R&D Systems, Catalog # 2484-SUC)
- Substrate: 4-Nitrocatechol Sulfate (PNCS) (Sigma, Catalog # N-7251), 100 mM stock in deionized water
- 0.2 M NaOH
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhGNS to 2 µg/mL in Assay Buffer.
2. Dilute PNCS substrate to 4 mM in Assay Buffer.
3. Combine 75 µL of 2 µg/mL rhGNS and 75 µL 4 mM PNCS. Include a Substrate Blank containing Assay Buffer in place of rhGNS.
4. Incubate at 37 °C for 2 hours.
5. Stop reactions and develop color by adding 150 µL of 0.2 M NaOH to each reaction.
6. Load into the wells of a clear 96-well plate 200 µL from each reaction.
7. Read at 510 nm (absorbance) in endpoint mode.
8. Calculate specific activity:

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

*Adjusted for Substrate Blank

**Derived using calibration standard *p*-Nitrocatechol (Sigma, Catalog # N-7126).

Final Assay Conditions Per Well

- rhGNS: 0.1 µg
- Substrate: 1 mM

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