Recombinant Human N-Acetylgalactosamine-6-Sulfatase/GALNS

Catalog Number: 8269-SU

DESCRIPTION

Source	*Spodoptera frugiperda*, *Sf* 21 (baculovirus)-derived human N-Acetylgalactosamine-6-Sulfatase/GALNS protein Ala27-His522, with an N-terminal 6-His tag Accession # P34059
N-terminal Sequence Analysis	His & Thr56
Predicted Molecular Mass	56 kDa

SPECIFICATIONS

SDS-PAGE	53-59 kDa, reducing conditions
Activity	Measured by its ability to hydrolyze the substrate 4-Nitrocatechol Sulfate (PNCS). The specific activity is >0.5 pmol/min/µg, as measured under the described conditions.
Endotoxin Level	<1.0 EU per 1 µg of the protein by the LAL method.
Purity	>90%, 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: 50 mM Sodium Actetate, 250 mM NaCl, pH 5.0
- Recombinant Human N-Acetylgalactosamine-6-Sulfatase/GALNS (rhGALNS) (Catalog # 8269-SU)
- Substrate: 4-Nitrocatechol Sulfate (PNCS) (Sigma, Catalog # N-7251), 100 mM stock in deionized water
- Sodium Hydroxide (NaOH) (Sigma, Catalog # 221465), 2 M stock in deionized water
- 96-well Clear Plate (Catalog # DY990)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute rhGALNS to 40 µg/mL in Assay Buffer.
2. Dilute PNCS to 4 mM in Assay Buffer.
3. Combine 200 µL of 40 µg/mL of rhGALNS and 200 µL of 4 mM of PNCS in microtubes. Include a Substrate Blank with 200 µL of Assay Buffer and 200 µL of 4 mM of PNCS.
4. Incubate at 37 °C overnight (16-20 hours).
5. Load 100 µL from each reaction and Substrate Blank vial into a plate in triplicate, minimally.
6. Stop reaction by adding 100 µL of 0.2 M NaOH to each well containing reactions and Substrate Blank.
7. Read plate at 510 nm (absorbance) in endpoint mode.
8. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Adjusted Abs* (OD) x Conversion Factor** (pmol/OD)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Adjusted for Substrate Blank

** Derived using calibration standard 4-Nitrocatechol (PNC) (Sigma, Catalog # N15553).

Final Assay Conditions

Per Well:

- rhGALNS: 2 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.

BACKGROUND

GALNS is a lysosomal sulfatase that hydrolyzes the 6-sulfate groups of the N-acetyl-D-galactosamine 6-sulfate units of chondroitin sulfate and of the D-galactose 6-sulfate units of keratan sulfate (1). The conversion to 3-oxoalanine (also known as C-formylglycine, FGly) of a cysteine residue is critical for catalytic activity of the enzyme (2). Deficiencies of this enzyme lead to Morquio A syndrome or mucopolysaccharidosis 4A (MPS4A), a lysosomal storage disorder characterized by intracellular accumulation of keratan sulfate and chondroitin-6-sulfate (3). Key clinical features of this disease include short stature, skeletal dysplasia, dental anomalies, and corneal clouding (4). Current therapeutic method for lysosomal storage diseases is enzyme replacement therapy (5).

References:

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3. Rivera-Colon, Y. *et al.* (2013) J. Mol. Biol. **423**:736.
4. Fukuda, S. *et al.* (1992) J. Clin. Invest. **90**:1049.
5. Ohashi, T. (2012) Pediatr. Endocrinol. Rev. 10 Suppl **1**:26.

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