Recombinant Human N-Deacetylase/N-Sulfotransferase 2/NDST2

Catalog Number: 7689-ST

DESCRIPTION

Source	Human embryonic kidney cell, HEK293-derived Ala81-Gly883, with C-terminal addition of HPGGGSGGGSGGGSHHHHHH Accession # P52849
N-terminal Sequence Analysis	Ala81
Predicted Molecular Mass	94 kDa

SPECIFICATIONS

SDS-PAGE	90-100 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to heparan sulfate. The specific activity is >1,250 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	>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 and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM Tris, 15 mM $MgCl_2$, pH 7.5 (supplied in kit)
- Recombinant Human N-Deacetylase/N-Sulfotransferase 2/NDST2 (rhNDST2) (Catalog # 7689-ST)
- PAPS (3'-Phosphoadenosine-5'-phosphosulfate) (Catalog # ES019)
- Heparan sulfate (Celsus Labs, Catalog # HO-3105), 50 mg/mL stock in deionized water
- Universal Sulfotransferase Activity Kit (Catalog # EA003)
- 96-well Clear Plate (Costar, Catalog # 92592)
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM Phosphate Standard provided by the Universal Sulfotransferase Kit by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock.
2. Prepare standard curve by performing six one-half serial dilutions of the 100 µM Phosphate stock in Assay Buffer. The standard curve has a range of 0.078 to 5 nmol per well.
3. Prepare reaction mixture containing 0.8 mM PAPS, 8 mg/mL heparan sulfate, and 20 µg/mL Coupling Phosphatase 3 in Assay Buffer.
4. Dilute rhNDST2 to 1.6 µg/mL in Assay Buffer.
5. Load 50 µL of each dilution of the standard curve into a plate. Include a curve blank containing 50 µL of Assay Buffer.
6. Load 25 µL of the 1.6 µg/mL rhNDST2 into the plate. Include a Control containing 25 µL of Assay Buffer.
7. Add 25 µL of reaction mixture to the wells, excluding the standard curve.
8. Cover the plate with a plate sealer and incubate at 37 °C for 20 minutes.
9. Add 30 µL of the Malachite Green Reagent A to all wells. Mix briefly.
10. Add 100 µL of deionized water to all wells. Mix briefly.
11. Add 30 µL of the Malachite Green Reagent B to all wells. Mix and incubate for 20 minutes at room temperature.
12. Read plate at 620 nm (absorbance) in endpoint mode.
13. Calculate specific activity:

$$\text{Specific Activity (pmol/min/µg)} = \frac{\text{Phosphate released* (nmol) x (1000 pmol/nmol)}}{\text{Incubation time (min) x amount of enzyme (µg)}}$$

*Derived from the phosphate standard curve using linear or 4-parameter fitting and adjusted for Control.

Final Assay Conditions

Per Reaction:

- rhNDST2: 0.04 µg
- Coupling Phosphatase 3: 0.5 µg
- PAPS: 0.4 mM
- Heparan sulfate: 200 µg

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

Heparan sulfate is a highly sulfated polysaccharide that can be found on the cell surface and within extracellular matrix. It is typically covalently attached to the protein core of proteoglycans, such as syndecans and glypicans (1, 2). Heparin, on the other hand, is a highly sulfated version of heparan sulfate and is predominantly found in mast cells. Both heparin and heparan sulfate contain disaccharide repeats of uronic acid and N-acetylglucosamine and are modified by the same sulfotransferases (1, 2). The uronic acid residues can be sulfated at the 2-O position by heparan sulfate 2-O sulfotransferase (HS2ST). The N-acetylglucosamine residues can be sulfated at the N, 3-O, and 6-O positions by N-deacetylase/N-sulfotransferases (NDSTs), heparan sulfate 3-O sulfotransferases and heparan sulfate 6-O sulfotransferases, respectively. All these enzymes are Golgi resident proteins. NDST-mediated modifications are believed to occur before modifications by other sulfotransferases (3). There are four NDSTs in the human genome and all are dual enzymes with both deacetylase and sulfotransferase domains (4, 5). While NDST1 is mainly responsible for heparan sulfate synthesis, NDST2 is mainly responsible for the synthesis of heparin in mast cells (5). Disrupting the NDST2 gene causes production failure of fully sulfated heparin (6, 7).

References:

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