Recombinant Mouse Carbohydrate Sulfotransferase 3/CHST3

Catalog Number: 5356-ST

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Glu39-Thr472, with an N-terminal 6-His tag Accession # NP_058083
N-terminal Sequence Analysis	His
Predicted Molecular Mass	50 kDa

SPECIFICATIONS

SDS-PAGE	60-80 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to chondroitin sulfate. The specific activity is >350 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 silver stain.
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, 125 mM NaCl, 50 mM $MgCl_2$, pH 7.5
- Recombinant Mouse Carbohydrate Sulfotransferase 3/CHST3 (rmCHST3) (Catalog # 5356-ST)
- Donor Substrate: Adenosine 3'-phosphate 5'-phosphosulfate (PAPS) (Catalog # ES019)
- Acceptor Substrate: Chondroitin Sulfate (Sigma, Catalog # C6737), 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 Phosphate Standard by adding 40 µL of the 1 mM Phosphate Standard to 360 µL of Assay Buffer for a 100 µM stock. This is the first point of the standard curve.
2. Continue 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 5.0 nmol per well.
3. Prepare a reaction mixture containing 0.571 mM PAPS, 14.3 mg/mL chondroitin sulfate, and 14.3 µg/mL Coupling Phosphatase 3.
4. Dilute rmCHST3 to 4.17 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 15 µL of the 4.17 µg/mL rmCHST3 into the plate. Include a Control containing 15 µL of Assay Buffer.
7. Add 35 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:

- rmCHST3: 0.0625 µg
- Coupling Phosphatase 3: 0.500 µg
- PAPS: 0.4 mM
- Chondrointin Sulfate: 500 µg

PREPARATION AND STORAGE

Shipping

The product is shipped with dry ice or equivalent. 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, -70 °C as supplied.
- 3 months, -70 °C under sterile conditions after opening.

bio-techne®
Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 Canada TEL 855 668 8722 China TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449

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BACKGROUND

The mouse CHST family is comprised of 13 enzymes. All members of this family are Golgi-localized type II membrane proteins. Only the luminal and enzymatic domain is expressed in each of our recombinant CHST proteins. These enzymes transfer sulfate (i.e., sulfonate) onto the 6-O or 4-O positions of GalNAc, Gal and GlcNAc residues on glycoproteins, proteoglycans and glycolipids (1). This sulfation often creates specific epitopes that can be recognized by extracellular matrix proteins, cell surface receptors and viruses (2). CHST3, also known as chondroitin 6-O-sulfotransferase, transfers sulfate to position 6 of GalNAc residues on chondroitin sulfate (3). Chondroitin sulfate constitutes the predominant proteoglycan present in cartilage and is distributed on the surfaces of many cells and extracellular matrices. Loss of CHST3 function in human results in severe chondrodysplasia (4). CHST3 can also sulfate Gal residues of keratan sulfate and Gal residues in sialyl N-acetyllactosamine (sialyl LacNAc) oligosaccharides (5). Mouse CHST3 shares 85% amino acid sequence identity to the human ortholog.

References:

1. Hemmerich, S. and Rosen, S. (2000) Glycobiology **10**:849.
2. Bowman, K. G. and Bertozzi, C. R. (1999) Chem. Biol. **5**:447.
3. Uchimura, K. *et al.* (2002) J. Biol. Chem. **277**:1443.
4. Thiele, H. *et al.* (2004) Proc. Natl. Acad. Sci. U. S. A. **101**:10155.
5. Yusa, A. *et al.* (2006) J. Biol. Chem. **281**: 20393.

Global bio-techne.com info@bio-techne.com techsupport@bio-techne.com TEL +1 612 379 2956
USA TEL 800 343 7475 **Canada** TEL 855 668 8722 **China** TEL +86 (21) 52380373
Europe | Middle East | Africa TEL +44 (0)1235 529449