Recombinant Mouse Carbohydrate Sulfotransferase 4/CHST4

Catalog Number: 5547-ST

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Arg30-Gly388 with an N-terminal 6-His tag Accession # Q9R1I1
N-terminal Sequence Analysis	His
Predicted Molecular Mass	42 kDa

SPECIFICATIONS

SDS-PAGE	40-60 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to N-acetyl-D-glucosamine. The specific activity is >35 pmol/min/µg, 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, NaCl and Glycerol. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 0.1 M Tris, 30 mM $MgCl_2$, pH 7.5
- Recombinant Mouse Carbohydrate Sulfotransferase 4/CHST4 (rmCHST4) (Catalog # 5547-ST)
- Donor Substrate: 3′-Phosphoadenosine-5′-phosphosulfate/PAPS (PAPS) (Catalog # ES019)
- Acceptor Substrate: N-acetyl-α-D-glucosamine (GlcNAc) (Calbiochem, Catalog #1079), 1 M stock in deionized water
- Universal Sulfotransferase Activity Kit (Catalog # EA003)
- 96-well Clear Plate
- Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

Assay

1. Dilute 1 mM 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 reaction mixture by containing 0.286 mM PAPS, 0.643 M GlcNAc, 0.0143 mg/mL Coupling Phosphatase 3.
4. Dilute rmCHST4 to 40 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 40 µg/mL rmCHST4 into the plate. Include a Substrate Blank 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:

- rmCHST4: 0.6 µg
- Coupling Phosphatase 3: 0.5 µg
- PAPS: 0.2 mM
- GlcNAc: 450 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

The mouse CHST family is comprised of 13 enzymes. All members of this family are Golgi-localized type II membrane proteins (1). Only the luminal and enzymatic domain is expressed in each of the 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 (2). This sulfation often creates specific epitopes that can be recognized by extracellular matrix proteins, cell surface receptors and viruses (3). Human CHST4, also known as high endothelial cell N-acetylglucosamine 6-O-sulfotransferase (HEC-GlcNAc6ST) or L-selectin ligand sulfotransferase (LSST), catalyzes the transfer of sulfate to position 6 of non-reducing GlcNAc residues within mucin-associated glycans that ultimately serve as L-selectin ligands (4). It has a catalytic preference for core 2-branched mucin-type O-glycans, but also has activity toward core 3 type of O-glycans (5). Mouse CHST4 shares 72% amino acid sequence identity with the human ortholog. The enzyme activity was measured using a phosphatase coupled method (6).

References:

1. deGraffenried, D. and Bertozzi, C.R. (2003) J. Biol. Chem. **278**:40282.
2. Hemmerich, S. and Rosen, S. (2000) Glycobiology **10**:849.
3. Bowman, K. G. and Bertozzi, C. R. (1999) Chem. Biol. **5**:447.
4. Bistrup, A. *et al.* (1999) J. Cell Biol. **145**:899.
5. Uchimura, K. *et al.* (2002) J. Biol. Chem. **277**: 3979.
6. Prather,B. *et al.* (2012) Anal. Biochem. **423**:86.

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