Recombinant Human Carbohydrate Sulfotransferase 2/CHST2

Catalog Number: 5107-ST

DESCRIPTION

Source	Chinese Hamster Ovary cell line, CHO-derived Tyr76-Leu530, with an N-terminal 5-His tag Accession # Q9Y4C5
N-terminal Sequence Analysis	His
Predicted Molecular Mass	50 kDa

SPECIFICATIONS

SDS-PAGE	50-60 kDa, reducing conditions
Activity	Measured by its ability to transfer sulfate from PAPS to N-acetyl-D-glucosamine. The specific activity is >125 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 (provided in kit): 50 mM Tris, 15 mM $MgCl_2$, pH 7.5
- Recombinant Human Carbohydrate Sulfotransferase 2/CHST2 (rhCHST2) (Catalog # 5107-ST)
- 3'-Phosphoadenosine-5'-phosphosulfate/PAPS (Catalog # ES019)
- 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 (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 0.4 mM PAPS, 0.1 M GlcNAc, and 20 µg/mL Coupling Phosphatase 3 in Assay Buffer.
4. Dilute rhCHST2 to 16 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 16 µg/mL rhCHST2 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:

- rhCHST2: 0.4 µg
- Coupling Phosphatase 3: 0.5 µg
- PAPS: 0.2 mM
- GlcNAc: 50 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 CHST family is comprised of 14 genes in both human and mouse. 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). Human CHST2, also known as N-acetylglucosamine-6-O-sulfotransferase 1 (GlcNAc6ST-1) and Gal/GalNAc/GlcNAc 6-O-sulfotransferase (GST-2), was previously shown to act on non-reducing GlcNAc residues (3). The enzyme is known to be involved in biosynthesis of L-selectin ligand sialyl 6-sulfo Lewis X (4) and therefore plays a role in lymphocyte homing (5).The enzymatic activity of the recombinant human CHST2 was measured using a phosphatase-coupled assay (6).

References:

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