SANTA CRUZ BIOTECHNOLOGY, INC.

CHST1 siRNA (m): sc-62111

BACKGROUND

Sulfotransferase enzymes catalyze the sulfate conjugation of many hormones, neurotransmitters, drugs, and xenobiotic compounds. These enzymes differ in their tissue distributions and substrate specificities, although the gene structure (number and length of exons) is similar among family members. Carbohydrate sulfotransferase 1 (CHST1), also referred to as KSGal6ST or KSST, is a keratan sulfate sulfotransferase. It is predominantly expressed in brain and skeletal muscle and localizes to the *trans*-Golgi network. CHST1 is responsible for mediating the sulfation of keratan in the cornea, which is important in maintaining corneal transparency. In particular, CHST1 catalyzes the transfer of sulfate groups from 3'-phosphoadenosine 5'-phosphosulfate to position six of internal or terminal galactose residues (preferentially the residues adjacent to sulfated GlcNAc) on growing keratan sulfate chains. CHST1 also contributes to the generation of L-selectin ligands. Mutations in the CHST1 gene may play a role in macular corneal dystrophy (MCD).

REFERENCES

1. Fukuta, M., et al. 1998. Molecular cloning and characterization of human keratan sulfate Gal-6-sulfotransferase. J. Biol. Chem. 272: 32321-32328.
2. Tu, L., et al. 1999. L-selectin ligands expressed by human leukocytes are HECA-452 antibody-defined carbohydrate epitopes preferentially displayed by P-selectin glycoprotein ligand-1. J. Immunol. 163: 5070-5078.
3. Li, X., et al. 1999. CHST1 and CHST2 sulfotransferases expressed by human vascular endothelial cells: cDNA cloning, expression, and chromosomal localization. Genomics 55: 345-347.
4. Torii, T., et al. 2000. Sulfation of sialyl N-acetyllactosamine oligosaccharides and fetuin oligosaccharides by keratan sulfate Gal-6-sulfotransferase. Glycobiology 10: 203-211.
5. Li, X., et al. 2001. CHST1 and CHST2 sulfotransferase expression by vascular endothelial cells regulates shear-resistant leukocyte rolling via L-selectin. J. Leukoc. Biol. 69: 565-574.
6. Uchimura, K., et al. 2002. Functional analysis of the chondroitin 6-sulfotransferase gene in relation to lymphocyte subpopulations, brain development, and oversulfated chondroitin sulfates. J. Biol. Chem. 277: 1443-1450.

CHROMOSOMAL LOCATION

Genetic locus: Chst1 (mouse) mapping to 2 E1.

PRODUCT

CHST1 siRNA (m) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see CHST1 shRNA Plasmid (m): sc-62111-SH and CHST1 shRNA (m) Lentiviral Particles: sc-62111-V as alternate gene silencing products.

For independent verification of CHST1 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-62111A, sc-62111B and sc-62111C.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNAses and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

CHST1 siRNA (m) is recommended for the inhibition of CHST1 expression in mouse cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10 µM in 66 µl. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

GENE EXPRESSION MONITORING

CHST1 (A-2): sc-365021 is recommended as a control antibody for monitoring of CHST1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgGκ BP-FITC: sc-516140 or m-IgGκ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor CHST1 gene expression knockdown using RT-PCR Primer: CHST1 (m)-PR: sc-62111-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.