

## HS6ST1 siRNA (h): sc-94375

### BACKGROUND

Heparan sulfate structures, which are responsible for executing multiple biologic activities, are generated and regulated by heparan sulfate (HS) biosynthetic enzymes. HS6ST1 (heparan sulfate 6-O-sulfotransferase 1), also known as HS6ST, is a 411 amino acid single-pass type II membrane protein that exists as multiple alternatively spliced isoforms and belongs to the sulfotransferase 6 family. Expressed in fetal brain, HS6ST1 functions as a 6-O-sulfation enzyme that specifically catalyzes the transfer of sulfate from 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the N-sulfoglucosamine residue (GlcNS) HS. HS6ST1 is subject to post-translational N-glycosylation and is encoded by a functional gene on human chromosome 2 and a pseudogene on human chromosome 1 (known as LOC728969).

### REFERENCES

1. Habuchi, H., et al. 1998. Molecular characterization and expression of heparan-sulfate 6-sulfotransferase. Complete cDNA cloning in human and partial cloning in Chinese hamster ovary cells. *J. Biol. Chem.* 273: 9208-9213.
2. Habuchi, H., et al. 2000. The occurrence of three isoforms of heparan sulfate 6-O-sulfotransferase having different specificities for hexuronic acid adjacent to the targeted N-sulfoglucosamine. *J. Biol. Chem.* 275: 2859-2868.
3. Habuchi, H., et al. 2003. Biosynthesis of heparan sulphate with diverse structures and functions: two alternatively spliced forms of human heparan sulphate 6-O-sulphotransferase-2 having different expression patterns and properties. *Biochem. J.* 371: 131-142.
4. Edavettal, S.C., et al. 2004. A conformational change in heparan sulfate 3-O-sulfotransferase-1 is induced by binding to heparan sulfate. *Biochemistry* 43: 4680-4688.
5. Online Mendelian Inheritance in Man, OMIM™. 2005. Johns Hopkins University, Baltimore, MD. MIM Number: 604846. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
6. Backen, A.C., et al. 2007. Heparan sulphate synthetic and editing enzymes in ovarian cancer. *Br. J. Cancer* 96: 1544-1548.

### CHROMOSOMAL LOCATION

Genetic locus: HS6ST1 (human) mapping to 2q14.3.

### PRODUCT

HS6ST1 siRNA (h) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HS6ST1 shRNA Plasmid (h): sc-94375-SH and HS6ST1 shRNA (h) Lentiviral Particles: sc-94375-V as alternate gene silencing products.

For independent verification of HS6ST1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-94375A, sc-94375B and sc-94375C.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

HS6ST1 siRNA (h) is recommended for the inhibition of HS6ST1 expression in human 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  $\mu$ M in 66  $\mu$ l. 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

HS6ST1 (C-9): sc-398231 is recommended as a control antibody for monitoring of HS6ST1 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 HS6ST1 gene expression knockdown using RT-PCR Primer: HS6ST1 (h)-PR: sc-94375-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.