SANTA CRUZ BIOTECHNOLOGY, INC.

GlcAT-I siRNA (h): sc-96989

BACKGROUND

GlcAT-I (glucuronosyltransferase-I), also known as β-1,3-glucuronyltransferase 3 (B3GAT3), is a 335 amino acid single-pass type II membrane protein belonging to the glycosyltransferase 43 family. By using manganese as a cofactor, GlcAT-I catalyzes the formation of the glycosaminoglycan-protein linkage by way of a glucuronyl transfer reaction that is present in the final step of the biosynthesis of the linkage region of proteoglycans. Present as a disulfide-linked homodimer, GlcAT-I shows strict specificity for Gal-β-1,3-Gal-β-1,4-Xyl. Ubiquitously expressed, GlcAT-I is N-glycosylated and is localized to the Golgi apparatus membrane.

REFERENCES

1. Kitagawa, H., et al. 1998. Molecular cloning and expression of glucuronyltransferase I involved in the biosynthesis of the glycosaminoglycan-protein linkage region of proteoglycans. J. Biol. Chem. 273: 6615-6618.
2. Tone, Y., et al. 1999. Characterization of recombinant human glucuronyltransferase I involved in the biosynthesis of the glycosaminoglycan-protein linkage region of proteoglycans. FEBS Lett. 459: 415-420.
3. Ouzzine, M., et al. 2000. Structure/function of the human Ga1-β-1,3-glucuronosyltransferase. Dimerization and functional activity are mediated by two crucial cysteine residues. J. Biol. Chem. 275: 28254-28260.
4. Pedersen, L.C., et al. 2000. Heparan/chondroitin sulfate biosynthesis. Structure and mechanism of human glucuronyltransferase I. J. Biol. Chem. 275: 34580-34585.
5. Gulberti, S., et al. 2003. The functional glycosyltransferase signature sequence of the human β-1,3-glucuronosyltransferase is a XDD motif. J. Biol. Chem. 278: 32219-32226.
6. Venkatesan, N., et al. 2004. Stimulation of proteoglycan synthesis by glucuronosyltransferase-I gene delivery: a strategy to promote cartilage repair. Proc. Natl. Acad. Sci. USA 101: 18087-18092.

CHROMOSOMAL LOCATION

Genetic locus: B3GAT3 (human) mapping to 11q12.3.

PRODUCT

GlcAT-I 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GlcAT-I shRNA Plasmid (h): sc-96989-SH and GlcAT-I shRNA (h) Lentiviral Particles: sc-96989-V as alternate gene silencing products.

For independent verification of GlcAT-I (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-96989A, sc-96989B and sc-96989C.

PROTOCOLS

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

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

GlcAT-I siRNA (h) is recommended for the inhibition of GlcAT-I 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 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

GlcAT-I (D-7): sc-390475 is recommended as a control antibody for monitoring of GlcAT-I 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GlcAT-I gene expression knockdown using RT-PCR Primer: GlcAT-I (h)-PR: sc-96989-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.