SANTA CRUZ BIOTECHNOLOGY, INC.

COL9A1 siRNA (h): sc-45635

BACKGROUND

The Collagen Type IX protein (also known as Collagen α1 Type IX) is encoded by the COL9A1 gene which possesses two promoter regions and codes for both a long chain Collagen Type IX protein expressed in the cartilage, and a shorter Collagen Type IX protein expressed in the cornea and vitreous. Collagen Type IX forms a heterotrimer with Collagen α2 Type IX and Collagen α3 Type IX. When it is expressed in hyaline cartilage, Collagen Type IX posseses a large N-terminal globular domain (NC4). The COL9A1 gene is also expressed in the human inner ear, and disruption of this gene in mice results in hearing loss, indicating the role of Collagen Type IX in hearing. Mutations in the COL9A1 gene are associated with multiple epiphyseal dysplasia (MED), a chondrodysplasia, in humans. Collagen Type IX is often co-localized with Collagen Type II, and may play a role in the interaction of fibrils between Collagen Type II and Collagen Type IX.

REFERENCES

1. McCormick, D., et al. 1987. Structure of the glycosaminoglycan domain in the Collagen Type IX-proteoglycan. Proc. Natl. Acad. Sci. USA 84: 4044-4048.
2. Muragaki, Y., et al. 1990. The complete primary structure of two distinct forms of human α 1 (IX) Collagen chains. Eur. J. Biochem. 192: 703-708.
3. Warman, M.L., et al. 1993. Physical and linkage mapping of the human and murine genes for the α 1 chain of Collagen Type IX (COL9A1). Genomics 17: 694-698.
4. Fassler, R., et al. 1994. Mice lacking α 1 (IX) Collagen develop noninflammatory degenerative joint disease. Proc. Natl. Acad. Sci. USA 91: 5070-5074.
5. Czarny-Ratajczak, M., et al. 2001. A mutation in COL9A1 causes multiple epiphyseal dysplasia: further evidence for locus heterogeneity. Am. J. Hum. Genet. 69: 969-980.
6. Zhang, P., et al. 2003. Regulation of human COL9A1 gene expression. Activation of the proximal promoter region by Sox-9. J. Biol. Chem. 278: 117-123.
7. Asamura, K., et al. 2005. Collagen Type IX is crucial for normal hearing. Neuroscience 132: 493-500.

CHROMOSOMAL LOCATION

Genetic locus: COL9A1 (human) mapping to 6q13.

PRODUCT

COL9A1 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 COL9A1 shRNA Plasmid (h): sc-45635-SH and COL9A1 shRNA (h) Lentiviral Particles: sc-45635-V as alternate gene silencing products.

For independent verification of COL9A1 (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-45635A, sc-45635B and sc-45635C.

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

COL9A1 siRNA (h) is recommended for the inhibition of COL9A1 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

COL9A1 (H-7): sc-376969 is recommended as a control antibody for monitoring of COL9A1 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 COL9A1 gene expression knockdown using RT-PCR Primer: COL9A1 (h)-PR: sc-45635-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.