SANTA CRUZ BIOTECHNOLOGY, INC.

COL8A2 siRNA (h): sc-72951

BACKGROUND

COL8A2 (collagen α-2(VIII) chain), also known as endothelial collagen, is a 703 amino acid secreted protein. COL8A2 is a major component of the Descemet membrane (basement membrane) of corneal endothelial cells. COL8A2 can form homodimers as well as heterodimers with COL8A1. Defects in COL8A2 are a cause for posterior polymorphous corneal dystrophy (PPCD) and Fuchs endothelial corneal dystrophy (FECD), both being disorders with visual impairment occurring in adulthood. COL8A2 is also the cause of posterior polymorphous corneal dystrophy 2 (PPCD2), a rare familial disorder that occurs from birth onwards.

REFERENCES

1. Muragaki, Y., et al. 1991. The α 2(VIII) collagen gene. A novel member of the short chain collagen family located on the human chromosome 1. J. Biol. Chem. 266: 7721-7727.
2. Biswas, S., et al. 2001. Missense mutations in COL8A2, the gene encoding the α2 chain of type VIII collagen, cause two forms of corneal endothelial dystrophy. Hum. Mol. Genet. 10: 2415-2423.
3. Gottsch, J.D., et al. 2005. Inheritance of a novel COL8A2 mutation defines a distinct early-onset subtype of fuchs corneal dystrophy. Invest. Ophthalmol. Vis. Sci. 46: 1934-1939.
4. Adiguzel, E., et al. 2006. Migration and growth are attenuated in vascular smooth muscle cells with type VIII collagen-null alleles. Arterioscler. Thromb. Vasc. Biol. 26: 56-61.
5. Turner, N.J., et al. 2006. α2(VIII) collagen substrata enhance endothelial cell retention under acute shear stress flow via an α2β1 integrin-dependent mechanism: an *in vitro* and *in vivo* study. Circulation 114: 820-829.

CHROMOSOMAL LOCATION

Genetic locus: COL8A2 (human) mapping to 1p34.3.

PRODUCT

COL8A2 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 COL8A2 shRNA Plasmid (h): sc-72951-SH and COL8A2 shRNA (h) Lentiviral Particles: sc-72951-V as alternate gene silencing products.

For independent verification of COL8A2 (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-72951A, sc-72951B and sc-72951C.

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

COL8A2 siRNA (h) is recommended for the inhibition of COL8A2 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

COL8A2 (1F4): sc-293350 is recommended as a control antibody for monitoring of COL8A2 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 COL8A2 gene expression knockdown using RT-PCR Primer: COL8A2 (h)-PR: sc-72951-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.