SANTA CRUZ BIOTECHNOLOGY, INC.

COL4A6 siRNA (m): sc-142470

BACKGROUND

Collagens are fibrous, extracellular matrix proteins with high tensile strength and are the major components of connective tissue, such as tendons and cartilage. Collagens contain triple helix domains and frequently show lateral self-association in order to form complex connective tissues. Several collagens play a role in cell adhesion, which is important for maintaining normal tissue architecture and function. COL4A6 (collagen, type IV, α 6) is a 1,691 amino acid secreted protein belonging to the type IV collagen family. Type IV collagen, a major component of the basement membrane (BM), is composed of six genetically distinct α(IV) chains, α1(IV) to α6(IV). Defects in the gene encoding COL4A6 is believed is the cause of X-linked Alport syndrome (AS), which is characterized by macroscopic hematuria, cataracts and leiomyomatosis.

REFERENCES

1. Zheng, K., et al. 1999. Absence of the α6(IV) chain of collagen type IV in Alport syndrome is related to a failure at the protein assembly level and does not result in diffuse leiomyomatosis. Am. J. Pathol. 154: 1883-1891.
2. Segal, Y., et al. 2001. Regulation of the paired type IV collagen genes COL4A5 and COL4A6. Role of the proximal promoter region. J. Biol. Chem. 276: 11791-11797.
3. Mothes, H., et al. 2002. Alport syndrome associated with diffuse leiomyomatosis: COL4A5-COL4A6 deletion associated with a mild form of Alport nephropathy. Nephrol. Dial. Transplant. 17: 70-74.
4. Anker, M.C., et al. 2003. Alport syndrome with diffuse leiomyomatosis. Am. J. Med. Genet. A 119A: 381-385.
5. Thielen, B.K., et al. 2003. Deletion mapping in Alport syndrome and Alport syndrome-diffuse leiomyomatosis reveals potential mechanisms of visceral smooth muscle overgrowth. Hum. Mutat. 22: 419.
6. Sund, M., et al. 2005. Bifunctional promoter of type IV collagen COL4A5 and COL4A6 genes regulates the expression of α5 and α6 chains in a distinct cell-specific fashion. Biochem. J. 387: 755-761.

CHROMOSOMAL LOCATION

Genetic locus: Col4a6 (mouse) mapping to X F1.

PRODUCT

COL4A6 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 COL4A6 shRNA Plasmid (m): sc-142470-SH and COL4A6 shRNA (m) Lentiviral Particles: sc-142470-V as alternate gene silencing products.

For independent verification of COL4A6 (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-142470A, sc-142470B and sc-142470C.

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

COL4A6 siRNA (m) is recommended for the inhibition of COL4A6 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

COL4A6 (G-2): sc-398655 is recommended as a control antibody for monitoring of COL4A6 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 COL4A6 gene expression knockdown using RT-PCR Primer: COL4A6 (m)-PR: sc-142470-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.