

COL6A3 siRNA (h): sc-94560

BACKGROUND

Collagens (COLs) are fibrous, extracellular matrix proteins with high tensile strength that function as the major components of connective tissue, such as tendons and cartilage. All COL proteins contain a triple helix domain and frequently show lateral self-association in order to form complex connective tissues. There are several types of COL proteins, including fibril-forming interstitial COLs (types I, II, III and V), basement membrane COLs (type IV) and beaded filament COLs (type VI). COL6A3 (collagen α -3(VI) chain), is a 3,176 amino acid secreted protein that contains one fibronectin type-III domain, one MPII inhibitor domain and 12 VWFA domains and functions as the third (and largest) of 3 α chains of the type VI COL protein complex. Existing as a trimer with two other type VI α proteins, COL6A3 acts as a cell-binding protein that plays an important role in the organization of matrix components. Defects in the gene encoding COL6A3 are the cause of Bethlem myopathy (BM), a rare autosomal proximal myopathy, and Ullrich congenital muscular dystrophy (UCMD), an autosomal recessive congenital myopathy. Multiple isoforms of COL6A3 exist due to alternative splicing events.

REFERENCES

- Weil, D., et al. 1988. Cloning and chromosomal localization of human genes encoding the three chains of type VI collagen. *Am. J. Hum. Genet.* 42: 435-445.
- Demir, E., et al. 2002. Mutations in COL6A3 cause severe and mild phenotypes of Ullrich congenital muscular dystrophy. *Am. J. Hum. Genet.* 70: 1446-1458.
- Mercuri, E., et al. 2002. Collagen VI involvement in Ullrich syndrome: a clinical, genetic, and immunohistochemical study. *Neurology* 58: 1354-1359.
- Baker, N.L., et al. 2005. Dominant collagen VI mutations are a common cause of Ullrich congenital muscular dystrophy. *Hum. Mol. Genet.* 14: 279-293.
- Lampe, A.K. and Bushby, K.M. 2005. Collagen VI related muscle disorders. *J. Med. Genet.* 42: 673-685.
- Lamande, S.R., et al. 2006. The C5 domain of the collagen VI α 3(VI) chain is critical for extracellular microfibril formation and is present in the extracellular matrix of cultured cells. *J. Biol. Chem.* 281: 16607-16614.

CHROMOSOMAL LOCATION

Genetic locus: COL6A3 (human) mapping to 2q37.3.

PRODUCT

COL6A3 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see COL6A3 shRNA Plasmid (h): sc-94560-SH and COL6A3 shRNA (h) Lentiviral Particles: sc-94560-V as alternate gene silencing products.

For independent verification of COL6A3 (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-94560A, sc-94560B and sc-94560C.

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

APPLICATIONS

COL6A3 siRNA (h) is recommended for the inhibition of COL6A3 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 μ M in 66 μ 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

COL6A3 (A-5): sc-515335 is recommended as a control antibody for monitoring of COL6A3 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 COL6A3 gene expression knockdown using RT-PCR Primer: COL6A3 (h)-PR: sc-94560-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Li, F., et al. 2012. Identification of NME5 as a contributor to innate resistance to gemcitabine in pancreatic cancer cells. *FEBS J.* 279: 1261-1273.

RESEARCH USE

For research use only, not for use in diagnostic procedures.