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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Fibulin-5 siRNA (h): sc-43121

BACKGROUND

Fibulin proteins contribute to normal development of elastic fiber systems in various types of organs that require elasticity, such as vasculature, lung and skin. Fibulin-5 (EVEC, UP50, DANCE) is an integrin-binding extracellular matrix protein that mediates endothelial cell adhesion. Fibulin-5 is also a calcium-dependent elastin-binding protein that scaffolds cells to elastic fibers, thereby preventing elastinopathy in the skin, lung, and vasculature. The Arg-Gly-Asp (RGD) motif in Fibulin-5 interacts with cell surface integrins α_V/β_3 , α_V/β_5 and $\alpha 9\beta 1$, serves as an anchorage for elastic fibers to cells, and promotes organization of elastic fibers. The human Fibulin-5 gene maps to chromosome 14q32.12 and encodes a 488 amino acid protein.

REFERENCES

1. Kowal, R.C., et al. 1999. Assignment of Fibulin-5 (FBLN5) to human chromosome 14q31 by *in situ* hybridization and radiation hybrid mapping. *Cytogenet. Cell Genet.* 87: 2-3.
2. Yanagisawa, H., et al. 2002. Fibulin-5 is an elastin-binding protein essential for elastic fibre development *in vivo*. *Nature* 415: 168-171.
3. Nakamura, T., et al. 2002. Fibulin-5/DANCE is essential for elasto-genesis *in vivo*. *Nature* 415: 171-175.
4. Midwood, K.S. and Schwarzbauer, J.E. 2002. Elastic fibers: building bridges between cells and their matrix. *Curr. Biol.* 12: R279-R281.
5. Schiemann, W.P., et al. 2002. Context-specific effects of Fibulin-5 (DANCE/EVEC) on cell proliferation, motility and invasion. Fibulin-5 is induced by transforming growth factor- β and affects protein kinase cascades. *J. Biol. Chem.* 277: 27367-27377.

CHROMOSOMAL LOCATION

Genetic locus: FBLN5 (human) mapping to 14q32.12.

PRODUCT

Fibulin-5 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 Fibulin-5 shRNA Plasmid (h): sc-43121-SH and Fibulin-5 shRNA (h) Lentiviral Particles: sc-43121-V as alternate gene silencing products.

For independent verification of Fibulin-5 (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-43121A, sc-43121B and sc-43121C.

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

Fibulin-5 siRNA (h) is recommended for the inhibition of Fibulin-5 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

Fibulin-5 (3F8D5,7F8D5): sc-517203 is recommended as a control antibody for monitoring of Fibulin-5 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 Fibulin-5 gene expression knockdown using RT-PCR Primer: Fibulin-5 (h)-PR: sc-43121-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.

SELECT PRODUCT CITATIONS

1. Choudhury, R., et al. 2009. Differential regulation of elastic fiber formation by Fibulin-4 and -5. *J. Biol. Chem.* 284: 24553-24567.
2. Xiao, W., et al. 2013. Nogo-B promotes the epithelial-mesenchymal transition in HeLa cervical cancer cells via Fibulin-5. *Oncol. Rep.* 29: 109-116.

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.