SANTA CRUZ BIOTECHNOLOGY, INC.

Villin siRNA (h): sc-29521

BACKGROUND

Caldesmon, Filamin 1, Nebulin and Villin are differentially expressed and regulated Actin binding proteins. Both muscular (CDh) and non-muscular (CDl) forms of Caldesmon have been identified and each has been shown to bind to Actin as well as to calmodulin and myosin. CDh is expressed predominantly on thin filaments in smooth muscle, whereas CDl is widely expressed in non-muscle tissues and cells. Filamin 1, which is ubiquitously expressed and exists as a homodimer, functions to crosslink Actin to filaments. Nebulin is a large filamentous protein specific to muscle tissue that may function as a ruler for filament length. Several isoforms of Nebulin are produced by alternative exon usage. Villin is Ca^{2+}-regulated and is the major structural component of the brush border of absorptive cells.

REFERENCES

1. Weihing, R.R. 1988. Actin-binding and dimerization domains of HeLa cell Filamin. Biochemistry 27: 1865-1869.
2. Marston, S., Pinter, K. and Bennett, P. 1992. Caldesmon binds to smooth muscle Myosin and Myosin rod and crosslink thick filaments to Actin filaments. J. Muscle Res. Cell. Motil. 13: 206-218.
3. Maunoury, R., Robine, S., Pringault, E., Leonard, N., Gaillard, J.A. and Louvard, D. 1992. Developmental regulation of Villin gene expression in the epithelial cell lineages of mouse digestive and urogenital tracts. Development 115: 717-728.
4. Labeit, S. and Kolmerer, B. 1995. The complete primary structure of human Nebulin and its correlation to muscle structure. J. Mol. Biol. 248: 308-315.
5. Huber, P.A., El-Mezgueldi, M., Grabarek, Z., Slatter, D.A., Levine, B.A. and Marston, S.B. 1996. Multiple-sited interaction of caldesmon with Ca^{2+}-Calmodulin. Biochem. J. 316: 413-420.
6. Zhang, J.Q., Luo, G., Herrera, A.H., Paterson, B. and Horowits, R. 1996. cDNA cloning of mouse Nebulin. Evidence that the Nebulin-coding sequence is highly conserved among vertebrates. Eur. J. Biochem. 239: 835-841.

CHROMOSOMAL LOCATION

Genetic locus: VIL1 (human) mapping to 2q35.

PRODUCT

Villin 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 Villin shRNA Plasmid (h): sc-29521-SH and Villin shRNA (h) Lentiviral Particles: sc-29521-V as alternate gene silencing products.

For independent verification of Villin (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-29521A, sc-29521B and sc-29521C.

RESEARCH USE

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

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

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

Villin (1D2C3): sc-58897 is recommended as a control antibody for monitoring of Villin 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 Villin gene expression knockdown using RT-PCR Primer: Villin (h)-PR: sc-29521-PR (20 µl, 436 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.