SANTA CRUZ BIOTECHNOLOGY, INC.

MYL9 siRNA (h): sc-35939

BACKGROUND

Myosin is a highly conserved, ubiquitously expressed protein that interacts with Actin to generate the force for cellular movements. Conventional Myosins are hexameric proteins consisting of two heavy chain subunits, a pair of non-phosphorylatable light chain subunits and a pair of phosphorylatable light chain subunits. Three general classes of myosin have been cloned: smooth muscle Myosins, striated muscle Myosins and non-muscle myosins. Myosin light chain 9, which is encoded by MYL9, is one of the numerous regulatory Myosin light chains. Regulatory Myosin light chains, also known as MLCs, regulate contraction in smooth muscle and non-muscle cells via phosphorylation by Myosin light chain kinase (MLCK). Phosphorylation of regulatory Myosin light chains is catalyzed by MLCK in the presence of calcium and calmodulin and it increases the Actin-activated Myosin ATPase activity, thereby regulating the contractile activity. Myosin light chain is also located in striated skeletal muscle, where its function remains undefined.

REFERENCES

1. Kumar, C.C., et al. 1989. Characterization and differential expression of human vascular smooth muscle Myosin light chain 2 isoform in non-muscle cells. Biochemistry 28: 4027-4035.
2. Kolodney, M.S., et al. 1999. Ca^{2+}-independent Myosin II phosphorylation and contraction in chicken embryo fibroblasts. J. Physiol. 515: 87-92.
3. Sward, K., et al. 2000. Inhibition of Rho-associated kinase blocks agonist-induced Ca^{2+} sensitization of Myosin phosphorylation and force in guinea-pig ileum. J. Physiol. 522: 33-49.
4. Numata, T., et al. 2001. Functional role of the C-terminal domain of smooth muscle Myosin light chain kinase on the phosphorylation of smooth muscle Myosin. J. Biochem. 129: 437-444.
5. Nobe, H., et al. 2003. Rho kinase mediates serum-induced contraction in fibroblast fibers independent of Myosin LC20 phosphorylation. Am. J. Physiol. Cell Physiol. 284: 599-606.
6. Szczesna-Cordary, D., et al. 2005. The E22K mutation of Myosin RLC that causes familial hypertrophic cardiomyopathy increases calcium sensitivity of force and ATPase in transgenic mice. J. Cell Sci. 118: 3675-3683.

CHROMOSOMAL LOCATION

Genetic locus: MYL9 (human) mapping to 20q11.23.

PRODUCT

MYL9 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 MYL9 shRNA Plasmid (h): sc-35939-SH and MYL9 shRNA (h) Lentiviral Particles: sc-35939-V as alternate gene silencing products.

For independent verification of MYL9 (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-35939A, sc-35939B and sc-35939C.

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

MYL9 siRNA (h) is recommended for the inhibition of MYL9 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

MYL9/MYL12A/B (E-4): sc-28329 is recommended as a control antibody for monitoring of MYL9 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 MYL9 gene expression knockdown using RT-PCR Primer: MYL9 (h)-PR: sc-35939-PR (20 µl, 479 bp). 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.

PROTOCOLS

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