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Myosin Ib siRNA (h): sc-44600

BACKGROUND

Actin is a highly conserved protein that is expressed in all eukaryotic cells. Actin filaments can form both stable and labile structures and are crucial components of microvilli and the contractile apparatus of muscle cells. Troponin facilitates interaction between Actin and Myosin by binding to Ca^{2+} . Troponin is made up of at least two subunits, which are divergent in cardiac muscle, fast skeletal muscle and slow skeletal muscle. Myosin is a hexamer of two heavy chains (MHC) and four light chains (MLC) that interacts with Actin to generate the force for diverse cellular movements, including cytokinesis, phagocytosis and muscle contraction. Myosin Ib (MYO1B), also designated Myosin I α or MYH-1c, is a motor protein that is involved in cell migration, neurite outgrowth and vesicular transport. In multivesicular endosomes, Myosin Ib has been implicated in protein cargo traffic control.

REFERENCES

1. Marion, S., et al. 2005. Signalization and cytoskeleton activity through Myosin Ib during the early steps of phagocytosis in *Entamoeba histolytica*: a proteomic approach. *Cell. Microbiol.* 7: 1504-1518.
2. Salas-Cortes, L., et al. 2005. Myosin Ib modulates the morphology and the protein transport within multi-vesicular sorting endosomes. *J. Cell Sci.* 118: 4823-4832.
3. de Lanerolle, P., et al. 2005. Actin and Myosin I in the nucleus: what next? *Nat. Struct. Mol. Biol.* 12: 742-746.
4. Takeda, T., et al. 2005. Role of fission yeast Myosin I in organization of sterol-rich membrane domains. *Curr. Biol.* 15: 1331-1336.
5. Clark, R., et al. 2005. Loop 1 of transducer region in mammalian class I Myosin, Myosin Ib, modulates Actin affinity, ATPase activity and nucleotide access. *J. Biol. Chem.* 280: 30935-30942.

CHROMOSOMAL LOCATION

Genetic locus: MYO1B (human) mapping to 2q32.3.

PRODUCT

Myosin Ib 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 Myosin Ib shRNA Plasmid (h): sc-44600-SH and Myosin Ib shRNA (h) Lentiviral Particles: sc-44600-V as alternate gene silencing products.

For independent verification of Myosin Ib (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-44600A, sc-44600B and sc-44600C.

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.

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

Myosin Ib siRNA (h) is recommended for the inhibition of Myosin Ib 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

Myosin Ib (F-8): sc-393053 is recommended as a control antibody for monitoring of Myosin Ib 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 Myosin Ib gene expression knockdown using RT-PCR Primer: Myosin Ib (h)-PR: sc-44600-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.