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ACTA2 siRNA (m): sc-43591

BACKGROUND

All eukaryotic cells express Actin, which often constitutes as much as 50% of total cellular protein. Actin filaments can form both stable and labile structures and are crucial components of microvilli and the contractile apparatus of muscle cells. While lower eukaryotes, such as yeast, have only one Actin gene, higher eukaryotes have several isoforms of Actin encoded by a family of genes. At least six types of Actin are present in mammalian tissues and fall into three classes, designated α -Actin, β -Actin and γ -Actin. ACTA2 (Actin, α 2, smooth muscle, aorta) is a 377 amino acid protein that localizes to the cytoplasm and the cytoskeleton and is involved in cell motility and structural integrity. Defects in the gene encoding ACTA2 are the cause of aortic aneurysm familial thoracic type 6 (AAT6), a permanent dilation of the aorta that results in a loss of smooth muscle cells and a fragmentation of elastic fibers.

REFERENCES

1. Doolittle, R.F. 1995. The origins and evolution of eukaryotic proteins. *Philos. Trans. R. Soc. Lond., B, Biol. Sci.* 349: 235-240.
2. Maccioni, R.B., et al. 1995. Role of microtubule-associated proteins in the control of microtubule assembly. *Physiol. Rev.* 75: 835-864.
3. Schutt, C.E., et al. 1995. A discourse on modeling F-Actin. *J. Struct. Biol.* 115: 186-198.
4. Barkalow, K., et al. 1995. Actin cytoskeleton. Setting the pace of cell movement. *Curr. Biol.* 5: 1000-1002.
5. Nobes, C.D., et al. 1995. Rho, Rac, and Cdc42 GTPases regulate the assembly of multimolecular focal complexes associated with Actin stress fibers, lamellipodia and filopodia. *Cell* 81: 53-62.

CHROMOSOMAL LOCATION

Genetic locus: Acta2 (mouse) mapping to 19 C3.

PRODUCT

ACTA2 siRNA (m) 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 ACTA2 shRNA Plasmid (m): sc-43591-SH and ACTA2 shRNA (m) Lentiviral Particles: sc-43591-V as alternate gene silencing products.

For independent verification of ACTA2 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-43591A, sc-43591B and sc-43591C.

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

ACTA2 siRNA (m) is recommended for the inhibition of ACTA2 expression in mouse 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

α -Actin (1A4): sc-32251 is recommended as a control antibody for monitoring of ACTA2 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 ACTA2 gene expression knockdown using RT-PCR Primer: ACTA2 (m)-PR: sc-43591-PR (20 μ l, 446 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.