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ACTA2 shRNA (m) Lentiviral Particles: sc-43591-V

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 C1.

PRODUCT

ACTA2 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ACTA2 siRNA (m): sc-43591 and ACTA2 shRNA Plasmid (m): sc-43591-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

ACTA2 shRNA (m) Lentiviral Particles is recommended for the inhibition of α -Actin expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

α -Actin (1A4): sc-32251 is recommended as a control antibody for monitoring of α -Actin 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor α -Actin 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 $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.