

ACTA1 siRNA (h): sc-108076

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 encoded by a family of genes. At least six types of Actin are present in mammalian tissues and fall into three classes. α Actin expression is limited to various types of muscle, whereas β and γ are the principle constituents of filaments in other tissues. Members of the small GTPase family regulate the organization of the Actin cytoskeleton. Rho controls the assembly of Actin stress fibers and focal adhesion, Rac regulates Actin filament accumulation at the plasma membrane and Cdc42 stimulates formation of filopodia.

REFERENCES

1. Koy, A., et al. 2007. Nemaline myopathy with exclusively intranuclear rods and a novel mutation in ACTA1 (Q139H). *Neuropediatrics* 38: 282-286.
2. North, K.N., et al. 2008. Skeletal muscle α -Actin diseases. *Adv. Exp. Med. Biol.* 642: 15-27.
3. Laing, N.G., et al. 2009. Mutations and polymorphisms of the skeletal muscle α -Actin gene (ACTA1). *Hum. Mutat.* 30: 1267-1277.
4. Feng, J.J., et al. 2009. Genotype-phenotype correlations in ACTA1 mutations that cause congenital myopathies. *Neuromuscul. Disord.* 19: 6-16.
5. Garcia-Angarita, N., et al. 2009. Severe nemaline myopathy associated with consecutive mutations E74D and H75Y on a single ACTA1 allele. *Neuromuscul. Disord.* 19: 481-484.
6. Stenzel, W., et al. 2010. Fetal akinesia caused by a novel Actin filament aggregate myopathy skeletal muscle Actin gene (ACTA1) mutation. *Neuromuscul. Disord.* 20: 531-533.
7. Stern-Straeter, J., et al. 2011. Characterization of human myoblast differentiation for tissue-engineering purposes by quantitative gene expression analysis. *J. Tissue Eng. Regen. Med.* 5: e197-e206.

CHROMOSOMAL LOCATION

Genetic locus: ACTA1 (human) mapping to 1q42.13.

PRODUCT

ACTA1 siRNA (h) is a target-specific 19-25 nt siRNA 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 ACTA1 shRNA Plasmid (h): sc-108076-SH and ACTA1 shRNA (h) Lentiviral Particles: sc-108076-V as alternate gene silencing products.

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

ACTA1 siRNA (h) is recommended for the inhibition of ACTA1 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

ACTA1 (733H2P): sc-517660 is recommended as a control antibody for monitoring of ACTA1 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 ACTA1 gene expression knockdown using RT-PCR Primer: ACTA1 (h)-PR: sc-108076-PR (20 μ l). 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.