SANTA CRUZ BIOTECHNOLOGY, INC.

ACTA1 siRNA (m): sc-140840

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. ACTA1 (Actin, α skeletal muscle) is 377 amino acid protein belonging to the Actin family. Localizing to cytoplasm and ctyoskeleton, ACTA1 is ubiquitously expressed in eukaryotic cells. ACTA1 is essential for muscle contraction; mutations to this gene result in severe congenital-onset disease, with varying degrees of severity. Nemaline myopathy type 3 (NEM3), a muscular disorder resulting in muscle weakness and abnormal thread or rod-like structures in muscle fibers, is also associated with defects to ACTA1.

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.

CHROMOSOMAL LOCATION

Genetic locus: Acta1 (mouse) mapping to 8 E2.

PRODUCT

ACTA1 siRNA (m) is a pool of 2 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 ACTA1 shRNA Plasmid (m): sc-140840-SH and ACTA1 shRNA (m) Lentiviral Particles: sc-140840-V as alternate gene silencing products.

For independent verification of ACTA1 (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-140840A and sc-140840B.

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 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

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

Smooth Muscle Actin (B4): sc-53142 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-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 ACTA1 gene expression knockdown using RT-PCR Primer: ACTA1 (m)-PR: sc-140840-PR (20 µl, 593 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.