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Troponin T-SS siRNA (h): sc-43251

BACKGROUND

Actin is a highly conserved protein that is expressed in all eukaryotic cells and interacts with Myosin to generate the force for diverse cellular movements, including cytokinesis, phagocytosis and muscle contraction. Troponin facilitates the interaction between Actin and Myosin by binding to calcium. Troponin is made up of at least two subunits, which are divergent in cardiac muscle, fast skeletal muscle and slow skeletal muscle. Structures of skeletal muscle Troponin are composed of Troponin C (the sensor), Troponin I (the regulator) and three Troponin T (the link to the muscle thin filament) proteins, one of which functions as a Tropomyosin-binding protein and is known as Troponin T-SS (Troponin T-Slow Skeletal). Defects in the gene encoding Troponin T-SS are the cause of nemaline myopathy type 5 (NEM5), a form of nemaline myopathy characterized by mild contractures of the shoulders and hips, tremors and respiratory problems that often lead to death. Troponin T-SS is expressed as three isoforms due to alternative splicing events.

REFERENCES

1. Stefancsik, R., et al. 1998. Identification and mutagenesis of a highly conserved domain in Troponin T responsible for troponin I binding: potential role for coiled coil interaction. *Proc. Natl. Acad. Sci. USA* 95: 957-962.
2. Mukherjea, P., et al. 1999. Altered regulatory function of two familial hypertrophic cardiomyopathy Troponin T mutants. *Biochemistry* 38: 13296-13301.
3. Barton, P.J., et al. 1999. Close physical linkage of human troponin genes: organization, sequence, and expression of the locus encoding cardiac troponin I and slow skeletal Troponin T. *Genomics* 57: 102-109.
4. Yuasa, K., et al. 1999. A novel interaction of cGMP-dependent protein kinase I with Troponin T. *J. Biol. Chem.* 274: 37429-37434.
5. Johnston, J.J., et al. 2000. A novel nemaline myopathy in the Amish caused by a mutation in Troponin T1. *Am. J. Hum. Genet.* 67: 814-821.

CHROMOSOMAL LOCATION

Genetic locus: TNNT1 (human) mapping to 19q13.42.

PRODUCT

Troponin T-SS 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 Troponin T-SS shRNA Plasmid (h): sc-43251-SH and Troponin T-SS shRNA (h) Lentiviral Particles: sc-43251-V as alternate gene silencing products.

For independent verification of Troponin T-SS (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-43251A, sc-43251B and sc-43251C.

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

Troponin T-SS siRNA (h) is recommended for the inhibition of Troponin T-SS 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

Troponin T-SS (A-9): sc-365014 is recommended as a control antibody for monitoring of Troponin T-SS 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 Troponin T-SS gene expression knockdown using RT-PCR Primer: Troponin T-SS (h)-PR: sc-43251-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.