SANTA CRUZ BIOTECHNOLOGY, INC.

Troponin C fast skeletal siRNA (m): sc-36737

BACKGROUND

Actin is a highly conserved protein that is expressed in all eukaryotic cells. Actin filaments can form both stable and labile structures and are crucial components of microvilli and the contractile apparatus of muscle cells. Myosin is a hexamer of two heavy chains (MHC) and four light chains (MLC) that interacts with Actin 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 Troponin T (the link to the muscle thin filament). Troponin C is dumbbell-shaped and has a hydrophobic pocket that increases the contractile force of muscle fibers. Troponin C has two isoforms: fast and slow. Fast Troponin C has two calcium binding sites while slow/cardiac Troponin C has a single calcium binding site.

REFERENCES

1. Parmacek, M.S., et al. 1989. Structure and expression of the murine slow/cardiac Troponin C gene. J. Biol. Chem. 264: 13217-13225.
2. Koppe, R.I., et al. 1989. cDNA clone and expression analysis of rodent fast and slow skeletal muscle Troponin I mRNAs. J. Biol. Chem. 264: 14327-14333.
3. Ausoni, S., et al. 1994. Structure and regulation of the mouse cardiac Troponin I gene. J. Biol. Chem. 269: 339-346.
4. Potter, J.D., et al. 1995. A direct regulatory role for Troponin T and a dual role for troponin C in the Ca^{2+} regulation of muscle contraction. J. Biol. Chem. 270: 2557-2562.
5. Barkalow, K., et al. 1995. Actin cytoskeleton. Setting the pace of cell movement. Curr. Biol. 5: 1000-1002.
6. Baker, J.P., et al. 1998. Myosins: matching functions with motors. Curr. Opin. Cell Biol. 10: 80-86.

CHROMOSOMAL LOCATION

Genetic locus: Tnnc2 (mouse) mapping to 2 H3.

PRODUCT

Troponin C fast skeletal 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Troponin C fast skeletal shRNA Plasmid (m): sc-36737-SH and Troponin C fast skeletal shRNA (m) Lentiviral Particles: sc-36737-V as alternate gene silencing products.

For independent verification of Troponin C fast skeletal (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-36737A, sc-36737B and sc-36737C.

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

Troponin C fast skeletal siRNA (m) is recommended for the inhibition of Troponin C fast skeletal 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

Troponin C fast skeletal (E-7): sc-48347 is recommended as a control antibody for monitoring of Troponin C fast skeletal 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 Troponin C fast skeletal gene expression knockdown using RT-PCR Primer: Troponin C fast skeletal (m)-PR: sc-36737-PR (20 µl). Annealing temperature for the primers should be 55-60S° C and the extension temperature should be 68-72° C.

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.