SANTA CRUZ BIOTECHNOLOGY, INC.

Titin siRNA (m): sc-154286

BACKGROUND

Titin, also known as connectin, is a large protein involved in the temporal and spatial control of the assembly of the highly ordered sarcomeres (contractile units) of striated muscle. In addition to sarcomere assembly Titin also functions to maintain the structural integrity of the contracting myofibrils within the muscle as well as organizing the machinery for condensation of chromosomes in dividing cells. Titin is a giant protein composed of 27,000 amino acids and contains an autoregulated serine kinase catalytic domain as well as a calcium/calmodulin binding region that are involved in its activation. Activated Titin phosphorylates the muscle protein Telethonin, a sarcomeric protein abundant in heart and skeletal muscle, implicating Titin activity in the reorganization of the cytoskeleton during myofibrillogenesis.

REFERENCES

1. Trinick, J. 1996. Titin as a scaffold and spring. Cytoskeleton. Curr. Biol. 6: 258-260.
2. Valle, G., et al. 1997. Telethonin, a novel sarcomeric protein of heart and skeletal muscle. FEBS Lett. 415: 163-168.
3. Means, A.R. 1998. The clash in Titin. Nature 395: 846-847.
4. Mayans, O., et al. 1998. Structural basis for activation of the Titin kinase domain during myofibrillogenesis. Nature 395: 863-869.
5. Gregorio, C.C., et al. 1999. Muscle assembly: a titanic achievement? Curr. Opin. Cell Biol. 11: 18-25.
6. Trinick, J. et al. 1999. Titin: a molecular control freak. Trends Cell Biol. 9: 377-380.
7. Niederlander, N., et al. 2004. Regulation of the Actin-Myosin interaction by Titin. Eur. J. Biochem. 271: 4572-4581.
8. Fukuda, N., et al. 2005. Phosphorylation of Titin modulates passive stiffness of cardiac muscle in a Titin isoform-dependent manner. J. Gen. Physiol. 125: 257-271.
9. Harris, B.N., et al. 2005. Calcium transients regulate Titin organization during myofibrillogenesis. Cell Motil. Cytoskeleton 60: 129-139.

CHROMOSOMAL LOCATION

Genetic locus: Ttn (mouse) mapping to 2 C3.

PRODUCT

Titin 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 Titin shRNA Plasmid (m): sc-154286-SH and Titin shRNA (m) Lentiviral Particles: sc-154286-V as alternate gene silencing products.

For independent verification of Titin (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-154286A, sc-154286B and sc-154286C.

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

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

Titin (E-2): sc-271946 is recommended as a control antibody for monitoring of Titin 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 Titin gene expression knockdown using RT-PCR Primer: Titin (m)-PR: sc-154286-PR (20 µl). 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.

PROTOCOLS

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