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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 



Titin shRNA (h) Lentiviral Particles: sc-43463-V

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

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2. Valle, G., et al. 1997. Telethonin, a novel sarcomeric protein of heart and skeletal muscle. *FEBS Lett.* 415: 163-168.
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3. Mayans, O., et al. 1998. Structural basis for activation of the Titin kinase domain during myofibrillogenesis. *Nature* 395: 863-869. Published erratum appears in *Nature*. 1999. 397: 719.
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.

CHROMOSOMAL LOCATION

Genetic locus: TTN (human) mapping to 2q31.2.

STORAGE

Store lentiviral particles at -80°C . Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4°C for up to one week. Avoid repeated freeze thaw cycles.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PRODUCT

Titin shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μl frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Titin siRNA (h): sc-43463 and Titin shRNA Plasmid (h): sc-43463-SH as alternate gene silencing products.

APPLICATIONS

Titin shRNA (h) Lentiviral Particles is recommended for the inhibition of Titin expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μl frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Titin gene expression knockdown using RT-PCR Primer: Titin (h)-PR: sc-43463-PR (20 μl , 460 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

PROTOCOLS

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