

Obscurin siRNA (h): sc-75987

BACKGROUND

Belonging to the protein kinase superfamily, Obscurin is a 7,968 amino acid protein that is specifically expressed in skeletal and cardiac muscle. Containing a GTPase nucleotide exchange factor (GEF) domain, Obscurin is localized in the sarcomere near the M-band and Z-disk where it participates in the G-protein regulated pathways that control the formation of new myofibrils. Obscurin contains 55 immunoglobulin (Ig)-like domains, through which it interacts with proteins such as Titin and calmodulin. During cardiac hypertrophy, the gene encoding Obscurin is upregulated to produce additional contractile units. Knockdown of Obscurin mRNA results in the disruption of M-bands and A-bands and reduction of Myosin and myomesin levels, suggesting that Obscurin is required for regular sarcomere structure. There are six isoforms of Obscurin that are produced as a result of alternative splicing events.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608616. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
2. Kontogianni-Konstantopoulos, A., et al. 2003. Obscurin is a ligand for small Ankyrin-1 in skeletal muscle. *Mol. Biol. Cell* 14: 1138-1148.
3. Kontogianni-Konstantopoulos, A., et al. 2004. Obscurin regulates the organization of Myosin into A bands. *Am. J. Physiol., Cell Physiol.* 287: C209-C217.
4. Kontogianni-Konstantopoulos, A., et al. 2006. *De novo* myofibrillogenesis in C2C12 cells: evidence for the independent assembly of M bands and Z disks. *Am. J. Physiol., Cell Physiol.* 290: C626-C637.
5. Raeker, M.O., et al. 2006. Obscurin is required for the lateral alignment of striated myofibrils in zebrafish. *Dev. Dyn.* 235: 2018-2029.
6. Armani, A., et al. 2006. Molecular interactions with Obscurin are involved in the localization of muscle-specific small Ankyrin-1 isoforms to subcompartments of the sarcoplasmic reticulum. *Exp. Cell Res.* 312: 3546-3558.

CHROMOSOMAL LOCATION

Genetic locus: OBSCN (human) mapping to 1q42.13.

PRODUCT

Obscurin 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 Obscurin shRNA Plasmid (h): sc-75987-SH and Obscurin shRNA (h) Lentiviral Particles: sc-75987-V as alternate gene silencing products.

For independent verification of Obscurin (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-75987A, sc-75987B and sc-75987C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

Obscurin siRNA (h) is recommended for the inhibition of Obscurin 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

Obscurin (5C20): sc-517125 is recommended as a control antibody for monitoring of Obscurin 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 Obscurin gene expression knockdown using RT-PCR Primer: Obscurin (h)-PR: sc-75987-PR (20 μ l, 577 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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