

# myotubularin siRNA (h): sc-44356

## BACKGROUND

X-linked recessive myotubular myopathy is a congenital muscular disease characterized by severe hypotonia and generalized muscle weakness that, in most cases, leads to early postnatal death. The gene responsible for myotubular myopathy MTM1 encodes a dual specificity phosphatase, named myotubularin, which is highly conserved through evolution. The gene for MTM1 is localized to a 300 kb critical region on human Xq28 between IDS and GRBRA3. Human MTM1, a 603 amino-acid protein, is mutated in myotubular myopathy. The largely related protein hMTMR2 is found mutated in a recessive form of Charcot-Marie-Tooth neuropathy. Myotubularin is primarily a lipid phosphatase that acts on phosphatidylinositol 3-monophosphate and is involved in the regulation of the phosphatidylinositol 3-kinase (PI 3-kinase) pathway and membrane trafficking. Wild-type myotubularin can directly dephosphorylate PI3P and PI4P in vitro. Thus, it decreases PI3P levels by down-regulating PI3K activity and by facilitating the degradation of PI3P.

## REFERENCES

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2. de Gouyon, B., et al. 1996. Comparative mapping on the mouse X chromosome defines a myotubular myopathy equivalent region. *Mamm. Genome* 7: 575-579.
3. Laporte, J., et al. 1997. Mutations in the MTM1 gene implicated in X-linked myotubular myopathy. *Hum. Mol. Genet.* 6: 1505-1511.
4. Buj-Bello, A., et al. 1999. Identification of novel mutations in the MTM1 gene causing severe and mild forms of X-linked myotubular myopathy. *Hum. Mutat.* 14: 320-325.
5. Hane, B.G., et al. 1999. Germline mosaicism in X-linked myotubular myopathy. *Clin. Genet.* 56: 77-81.
6. Blondeau, F., et al. 2000. Myotubularin, a phosphatase deficient in myotubular myopathy, acts on phosphatidylinositol 3-kinase and phosphatidylinositol 3-phosphate pathway. *Hum. Mol. Genet.* 9: 2223-2229.

## CHROMOSOMAL LOCATION

Genetic locus: MTM1 (human) mapping to Xq28.

## PRODUCT

myotubularin 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see myotubularin shRNA Plasmid (h): sc-44356-SH and myotubularin shRNA (h) Lentiviral Particles: sc-44356-V as alternate gene silencing products.

For independent verification of myotubularin (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-44356A, sc-44356B and sc-44356C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

myotubularin siRNA (h) is recommended for the inhibition of myotubularin 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  $\mu$ M in 66  $\mu$ 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

myotubularin (F-1): sc-377309 is recommended as a control antibody for monitoring of myotubularin 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor myotubularin gene expression knockdown using RT-PCR Primer: myotubularin (h)-PR: sc-44356-PR (20  $\mu$ 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.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.