

MTMR5 siRNA (h): sc-61092

BACKGROUND

Myotubularin and the myotubularin-related proteins (MTMR1-9) belong to a highly conserved family of eukaryotic phosphatases. They are protein tyrosine phosphatases that utilize inositol phospholipids, rather than phosphoproteins, as substrates. MTMR family members hydrolyze both phosphatidylinositol 3-phosphate (PtdIns3P) and PtdIns(3,5)P₂. MTMR2 interacts with MTMR5, an inactive family member that increases the enzymatic activity of MTMR2 and dictates its subcellular localization. Mutations in MTMR2 cause autosomal recessive Charcot-Marie-Tooth type 4B1 (CMT4B1), which is characterized by reduced nerve conduction velocities, focally folded myelin sheaths and demyelination. MTMR3 and MTMR4 can either interact with each other or self associate. MTMR6 regulates the activity of the calcium-activated potassium channel 3.1. MTMR9 regulates the activity of MTMR7 and MTMR8.

REFERENCES

1. Laporte, J., et al. 1997. Mutations in the MTM1 gene implicated in X-linked myotubular myopathy. *Hum. Mol. Genet.* 6: 1505-1511.
2. Laporte, J., et al. 1998. Mutations in the MTM1 gene implicated in X-linked myotubular myopathy. ENMC international consortium on myotubular myopathy. European Neuro-Muscular Center. *Hum. Mol. Genet.* 6: 1505-1511.
3. 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.
4. Kim, S.A., et al. 2003. Regulation of myotubularin-related (MTMR)2 phosphatidylinositol phosphatase by MTMR5, a catalytically inactive phosphatase. *Proc. Natl. Acad. Sci. USA* 100: 4492-4497.
5. Mochizuki, Y. and Majerus, P.W. 2003. Characterization of myotubularin-related protein 7 and its binding partner, myotubularin-related protein 9. *Proc. Natl. Acad. Sci. USA* 100: 9768-9773.
6. Srivastava, S., et al. 2005. Phosphatidylinositol 3-phosphate indirectly activates KCa3.1 via 14 amino acids in the carboxy terminus of KCa3.1. *Mol. Biol. Cell* 17: 146-154.

CHROMOSOMAL LOCATION

Genetic locus: SBF1 (human) mapping to 22q13.33.

PRODUCT

MTMR5 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 MTMR5 shRNA Plasmid (h): sc-61092-SH and MTMR5 shRNA (h) Lentiviral Particles: sc-61092-V as alternate gene silencing products.

For independent verification of MTMR5 (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-61092A, sc-61092B and sc-61092C.

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

MTMR5 siRNA (h) is recommended for the inhibition of MTMR5 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

MTMR5 (B-9): sc-393488 is recommended as a control antibody for monitoring of MTMR5 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 MTMR5 gene expression knockdown using RT-PCR Primer: MTMR5 (h)-PR: sc-61092-PR (20 μ 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 for detailed protocols and support products.