SANTA CRUZ BIOTECHNOLOGY, INC.

POMT2 siRNA (m): sc-61382

BACKGROUND

O-mannosylation is an essential protein modification in eukaryotes that is initiated by an evolutionarily conserved family of protein O-mannosyltransferases. POMT2 encodes an integral membrane protein which localizes to the endoplasmic reticulum (ER) and shares significant sequence similarity with a family of protein O-mannosyltransferases of *S. cerevisiae*. The deduced 750 amino acid protein has a seven transmembrane helical structure with a central hydrophilic domain surrounded by five N-terminal and two C-terminal transmembrane regions. Like other known members of its family, POMT2 lacks a characteristic ER-targeting or -retention signal and contains five N-glycosylation sites. POMT2 shares 36% sequence identity with human POMT1 and RNA dot blot analysis reveals highest expression of mouse POMT2 in testis.

REFERENCES

1. Willer, T., et a. 2002. Characterization of POMT2, a novel member of the PMT protein O-mannosyltransferase family specifically localized to the acrosome of mammalian spermatids. Glycobiology 12: 771-783.
2. Akasaka-Manya, K., et al. 2004. Mutations of the POMT1 gene found in patients with Walker-Warburg syndrome lead to a defect of protein O-mannosylation. Biochem. Biophys. Res. Commun. 325: 75-79.
3. Ichimiya, T., et al. 2004. The twisted abdomen phenotype of *Drosophila* POMT1 and POMT2 mutants coincides with their heterophilic protein O-mannosyltransferase activity. J. Biol. Chem. 279: 42638-42647.
4. Manya, H., et al. 2004. Demonstration of mammalian protein O-mannosyltransferase activity: coexpression of POMT1 and POMT2 required for enzymatic activity. Proc. Natl. Acad. Sci. USA 101: 500-505.
5. van Reeuwijk, J., et al. 2005. POMT2 mutations cause α-dystroglycan hypo-glycosylation and Walker-Warburg syndrome. J. Med. Genet. 42: 907-912.
6. Manya, H., et al. 2006. Molecular cloning and characterization of rat POMT1 and POMT2. Glycobiology 16: 863-873.
7. Mercuri, E., et al. 2006. POMT2 mutation in a patient with "MEB-like" phenotype. Neuromuscul. Disord. 16: 446-448.

CHROMOSOMAL LOCATION

Genetic locus: Pomt2 (mouse) mapping to 12 D2.

PRODUCT

POMT2 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 POMT2 shRNA Plasmid (m): sc-61382-SH and POMT2 shRNA (m) Lentiviral Particles: sc-61382-V as alternate gene silencing products.

For independent verification of POMT2 (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-61382A, sc-61382B and sc-61382C.

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

POMT2 siRNA (m) is recommended for the inhibition of POMT2 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

POMT2 (G-3): sc-393487 is recommended as a control antibody for monitoring of POMT2 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 POMT2 gene expression knockdown using RT-PCR Primer: POMT2 (m)-PR: sc-61382-PR (20 µl, 572 bp). 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.