SANTA CRUZ BIOTECHNOLOGY, INC.

POMT1 siRNA (m): sc-61380

BACKGROUND

O-mannosylation is an essential protein modification in eukaryotes that is initiated by an evolutionarily conserved family of protein O-mannosyltransferases. The POMT1 (protein O-mannosyltransferase 1) protein consists of 725 amino acids. POMT1 contains 7 to 12 presumed transmembrane regions and a C-terminal ER membrane retention signal; RT-PCR reveals several mRNA splice variants. RNA dot blot analysis indicates ubiquitous expression of POMT1, with maximum levels in testis and high levels in fetal brain and pituitary tissues. Walker-Warburg syndrome (WWS), a severe, recessive, congenital muscular dystrophy associated with defects in neuronal migration that produce complex brain and eye abnormalities, is caused by mutations in the POMT1 gene.

REFERENCES

1. 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.
2. 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.
3. Willer, T., et al. 2004. Targeted disruption of the Walker-Warburg syndrome gene POMT1 in mouse results in embryonic lethality. Proc. Natl. Acad. Sci. USA 101: 14126-14131.
4. Yamamoto, T., et al. 2004. Expression and localization of fukutin, POMGnT1, and POMT1 in the central nervous system: consideration for functions of fukutin. Med. Electron Microsc. 37: 200-207.
5. Balci, B., et al. 2005. An autosomal recessive limb girdle muscular dystrophy (LGMD2) with mild mental retardation is allelic to Walker-Warburg syndrome (WWS) caused by a mutation in the POMT1 gene. Neuromuscul. Disord. 15: 271-275.
6. Currier, S.C., et al. 2005. Mutations in POMT1 are found in a minority of patients with Walker-Warburg syndrome. Am. J. Med. Genet. A 133A: 53-57.
7. D'Amico, A., et al. 2006. Expanding the clinical spectrum of POMT1 phenotype. Neurology 66: 1564-1567.

CHROMOSOMAL LOCATION

Genetic locus: Pomt1 (mouse) mapping to 2 B.

PRODUCT

POMT1 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 POMT1 shRNA Plasmid (m): sc-61380-SH and POMT1 shRNA (m) Lentiviral Particles: sc-61380-V as alternate gene silencing products.

For independent verification of POMT1 (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-61380A, sc-61380B and sc-61380C.

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

POMT1 siRNA (m) is recommended for the inhibition of POMT1 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

POMT1 (G-10): sc-390451 is recommended as a control antibody for monitoring of POMT1 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 POMT1 gene expression knockdown using RT-PCR Primer: POMT1 (m)-PR: sc-61380-PR (20 µl, 535 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.