

POMGnT1 siRNA (h): sc-40865

BACKGROUND

The product of the POMGnT1 gene, protein O-mannose β -1, 2-N-acetylglucosaminyltransferase, participates in O-mannosyl glycan synthesis. POMGnT1 is an N(in)/C(out) (type II) membrane protein localized in the medial-Golgi that initiates the conversion of high mannose N-glycans to complex N-glycans. Specifically, POMGnT1 is a glycosylation enzyme that participates in the synthesis of O-mannosyl glycan, a laminin-binding ligand of α -dystroglycan that is rarely synthesized in mammals. Mutations in the POMGnT1 gene cause muscle-eye-brain disease (MEB), an autosomal recessive disorder characterized by congenital muscular dystrophy, ocular abnormalities and lissencephaly. Altered glycosylation of α -dystroglycan may play a critical role in the pathomechanism of MEB as well as Walker-Warburg syndrome (WWS), characterized by the absence of glycosylation of α -dystroglycan. The human POMGnT1 gene maps to chromosome 1p34.1 and encodes a 660-amino acid type II transmembrane protein.

REFERENCES

- Burke, J., et al. 1992. The transmembrane and flanking sequences of β -1, 2-N-acetylglucosaminyltransferase I specify medial-Golgi localization. *J. Biol. Chem.* 267: 24433-24440.
- Yoshida, A., et al. 2001. Muscular dystrophy and neuronal migration disorder caused by mutations in a glycosyltransferase, POMGnT1. *Dev. Cell* 1: 717-724.
- Kano, H., et al. 2002. Deficiency of α -dystroglycan in muscle-eye-brain disease. *Biochem. Biophys. Res. Commun.* 291: 1283-1286.
- Michele, D.E., et al. 2002. Post-translational disruption of dystroglycan-ligand interactions in congenital muscular dystrophies. *Nature* 418: 417-422.
- Beltran-Valero De Bernabe, D., et al. 2002. Mutations in the O-mannosyltransferase gene POMT1 give rise to the severe neuronal migration disorder Walker-Warburg syndrome. *Am. J. Hum. Genet.* 71: 1033-1043.
- Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 606822. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: POMGNT1 (human) mapping to 1p34.1.

PRODUCT

POMGnT1 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 POMGnT1 shRNA Plasmid (h): sc-40865-SH and POMGnT1 shRNA (h) Lentiviral Particles: sc-40865-V as alternate gene silencing products.

For independent verification of POMGnT1 (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-40865A, sc-40865B and sc-40865C.

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

POMGnT1 siRNA (h) is recommended for the inhibition of POMGnT1 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

POMGnT1 (JD.23): sc-130459 is recommended as a control antibody for monitoring of POMGnT1 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 POMGnT1 gene expression knockdown using RT-PCR Primer: POMGnT1 (h)-PR: sc-40865-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.