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SANTA CRUZ BIOTECHNOLOGY, INC.

DPM1 siRNA (m): sc-41510

BACKGROUND

Biosynthesis of glycosylphosphatidylinositol and N-glycan pre-cursor is dependent upon a mannosyl donor, dolichol phosphate-mannose (DPM). DPM synthase, a transmembrane protein, is associated with membranes of the rough endoplasmic reticulum and catalyzes mannosyl transfer from GDP-mannose hydrophobic long-chain acceptor dolichyl-phosphate. DPM synthase in various organisms are grouped into two types. One type is a single-component enzyme, represented by *Saccharomyces cerevisiae,* and the other is a multicomponent enzyme which is represented by human DPM synthase and consists of three subunits: DPM1, DPM2 and DPM3. DPM1 is not sufficient for DPM synthesis, which requires the 84 amino acid DPM2 protein for localization to the ER and stable expression of DPM1. The third subunit, DPM3, comprises 92 amino acids, and it is associated with DPM1 via its C-terminal domain and with DPM2 via its N-terminal region. The stability of DPM1 is directly dependent upon DPM3, which is stablized by DPM2. DPM synthase activity is associated with an ER phosphoprotein. In addition, a mitochondrial DPM synthase exists, which is located on the cytosolic face of the outer membrane of mitochondria.

REFERENCES

1. Gasnier, F., et al. 1992. Mitochondrial dolichyl-phosphate mannose synthase. Purification and immunogold localization by electron microscopy. Eur. J. Biochem. 206: 853-858.
2. Forsee, W.T., et al. 1997. Characterization of recombinant yeast dolichyl mannosyl phophate synthase and site-directed mutagenesis of its cysteine residues. Eur. J. Biochem. 244: 935-938.
3. Maeda, Y., et al. 1998. DPM2 regulates biosynthesis of dolichol phosphate-mannose in mammalian cells: correct subcellular localization and stablization of DPM1, and binding of dolichol phosphate. EMBO J. 17: 4920-4929.
4. Tomita, S., et al. 1998. A homologue of *Saccharomyces cerevisiae* DPM1p is not sufficient for synthesis of dolichol-phosphate-mannose in mammalian cells. J. Biol. Chem. 273: 9249-9254.
5. Banerjee, D.K., et al. 1999. Mannosylphosphodolichol synthase activity is associated with a 32 kDa phosphoprotein. Biosci. Rep. 19: 169-177.
6. Maeda, Y., et al. 2000. Human dolichol-phosphate-mannose synthase consists of three subunits, DPM1, DPM2 and DPM3. EMBO J. 19: 2475-2482.

CHROMOSOMAL LOCATION

Genetic locus: Dpm1 (mouse) mapping to 2 H3.

PRODUCT

DPM1 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 DPM1 shRNA Plasmid (m): sc-41510-SH and DPM1 shRNA (m) Lentiviral Particles: sc-41510-V as alternate gene silencing products.

For independent verification of DPM1 (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-41510A, sc-41510B and sc-41510C.

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

DPM1 siRNA (m) is recommended for the inhibition of DPM1 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

DPM1 (A-5): sc-515721 is recommended as a control antibody for monitoring of DPM1 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 DPM1 gene expression knockdown using RT-PCR Primer: DPM1 (m)-PR: sc-41510-PR (20 µl). 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.