

## PIG-N siRNA (m): sc-152254

### BACKGROUND

Several cell surface proteins are attached to the membrane through their C-terminal domain and a glycosylphosphatidylinositol (GPI) moiety. Phosphatidylinositol-glycans (PIGs) are multi-pass transmembrane proteins that localize to the endoplasmic reticulum. PIGs are crucial for the synthesis of N-acetylglucosaminyl-phosphatidylinositol, a very early intermediate in GPI-anchor biosynthesis. PIGs play a role in the recognition of either the GPI attachment signal or the lipid portion of GPI. PIG-N (phosphatidylinositol-glycan biosynthesis class N protein), also known as GPI ethanolamine phosphate transferase 1 and MCD4 homolog, is a 931 amino acid enzyme of the endoplasmic reticulum that transfers ethanolamine phosphate to the first  $\alpha$ -1,4-linked mannose of the glycosylphosphatidylinositol precursor of GPI-anchor. The gene encoding PIG-N is localized near a region of human chromosome 18 that may be implicated in chronic recurrent multifocal osteomyelitis.

### REFERENCES

1. Yeh, E.T., et al. 1994. Biosynthesis and processing of the glycosylphosphatidylinositol anchor in mammalian cells. *Semin. Immunol.* 6: 73-80.
2. Hong, Y., et al. 1999. PIG-N, a mammalian homologue of yeast Mcd4p, is involved in transferring phosphoethanolamine to the first mannose of the glycosylphosphatidylinositol. *J. Biol. Chem.* 274: 35099-35106.
3. Gaynor, E.C., et al. 1999. MCD4 encodes a conserved endoplasmic reticulum membrane protein essential for glycosylphosphatidylinositol anchor synthesis in yeast. *Mol. Biol. Cell* 10: 627-648.
4. Barz, W.P. and Walter, P. 1999. Two endoplasmic reticulum (ER) membrane proteins that facilitate ER-to-Golgi transport of glycosylphosphatidylinositol-anchored proteins. *Mol. Biol. Cell* 10: 1043-1059.
5. Yada, T., et al. 2001. Its8, a fission yeast homolog of Mcd4 and PIG-N, is involved in GPI anchor synthesis and shares an essential function with calcineurin in cytokinesis. *J. Biol. Chem.* 276: 13579-13586.

### CHROMOSOMAL LOCATION

Genetic locus: Pign (mouse) mapping to 1 E2.1.

### PRODUCT

PIG-N 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIG-N shRNA Plasmid (m): sc-152254-SH and PIG-N shRNA (m) Lentiviral Particles: sc-152254-V as alternate gene silencing products.

For independent verification of PIG-N (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-152254A, sc-152254B and sc-152254C.

### PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.

### 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

### APPLICATIONS

PIG-N siRNA (m) is recommended for the inhibition of PIG-N 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  $\mu$ M in 66  $\mu$ 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.

### RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PIG-N gene expression knockdown using RT-PCR Primer: PIG-N (m)-PR: sc-152254-PR (20  $\mu$ 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.