# PIG-F siRNA (m): sc-72327



The Power to Question

# **BACKGROUND**

Phosphatidylinositol-glycans (PIGs) are multi-pass transmembrane proteins that localize to the endoplasmic reticulum. PIGs exhibit various functions but all are crucial for the biosynthesis of the glycosylphosphatidylinositol (GPI)-anchor. Some PIG proteins are components of the GPI transamidase complex and play a role in the recognition of either the GPI attachment signal or the lipid portion of GPI. Other PIGs belong to the glycosyltransferase complex and function in the transfer of N-acetylglucosamine (GlcNAc) to phosphatidylinositol (PI). A variety of other PIGs play distinct roles in GPI synthesis. PIG-F functions as an auxiliary subunit of ethanolamine phosphate (EtNP) transferases. It associates with PIG-O and is required for its expression and stability. Together these two PIGs function as an EtNP transferase and catalyze the transfer of EtNP to the third mannose (Man-3) of GPI. A mutation in the gene encoding PIG-F may result in a block of EtNP addition to Man-3 and lead to an absence of GPI-anchored proteins.

# **REFERENCES**

- Inoue, N., et al. 1993. Cloning of a human gene, PIG-F, a component of glycosylphosphatidylinositol anchor biosynthesis, by a novel expression cloning strategy. J. Biol. Chem. 268: 6882-6885.
- Taron, C.H., et al. 2000. Glycosylphosphatidylinositol biosynthesis defects in Gpi11p- and Gpi13p-deficient yeast suggest a branched pathway and implicate gpi13p in phosphoethanolamine transfer to the third mannose. Mol. Biol. Cell 11: 1611-1630.
- Hong, Y., et al. 2000. Requirement of PIG-F and PIG-O for transferring phosphoethanolamine to the third mannose in glycosylphosphatidylinositol. J. Biol. Chem. 275: 20911-20919.
- Delorenzi, M., et al. 2002. Genes for glycosylphosphatidylinositol toxin biosynthesis in *Plasmodium falciparum*. Infect. Immun. 70: 4510-4522.
- Murakami, Y., et al. 2003. PIG-W is critical for inositol acylation but not for flipping of glycosylphosphatidylinositol-anchor. Mol. Biol. Cell 14: 4285-4295.
- Shishioh, N., et al. 2005. GPI7 is the second partner of PIG-F and involved in modification of glycosylphosphatidylinositol. J. Biol. Chem. 280: 9728-9734.
- 7. Bowman, S.M., et al. 2006. Mutational analysis of the glycosylphosphatidylinositol (GPI) anchor pathway demonstrates that GPI-anchored proteins are required for cell wall biogenesis and normal hyphal growth in *Neurospora crassa*. Eukaryotic Cell 5: 587-600.
- Pittet, M. and Conzelmann, A. 2007. Biosynthesis and function of GPI proteins in the yeast *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1771: 405-420.
- 9. Orlean, P. and Menon, A.K. 2007. Thematic review series: lipid posttranslational modifications. GPI anchoring of protein in yeast and mammalian cells, or: how we learned to stop worrying and love glycophospholipids. J. Lipid Res. 48: 993-1011.

# CHROMOSOMAL LOCATION

Genetic locus: Pigf (mouse) mapping to 17 E4.

#### **PRODUCT**

PIG-F 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  $\mu\text{M}$  solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIG-F shRNA Plasmid (m): sc-72327-SH and PIG-F shRNA (m) Lentiviral Particles: sc-72327-V as alternate gene silencing products.

For independent verification of PIG-F (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-72327A, sc-72327B and sc-72327C.

# 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-F siRNA (m) is recommended for the inhibition of PIG-F 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.

# **RT-PCR REAGENTS**

Semi-quantitative RT-PCR may be performed to monitor PIG-F gene expression knockdown using RT-PCR Primer: PIG-F (m)-PR: sc-72327-PR (20  $\mu$ I). 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.