SANTA CRUZ BIOTECHNOLOGY, INC.

PIG-L siRNA (h): sc-93912



BACKGROUND

Phosphatidylinositol-glycans (PIGs) are transmembrane proteins that localize to endoplasmic reticulum (ER). PIGs are crucial for the synthesis of N-acetylglucosaminyl-phosphatidylinositol (GlcNAc-PI), a very early intermediate in glycosylphosphatidylinositol (GPI)-anchor biosynthesis. 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. PIG-L (phosphatidylinositol glycan anchor biosynthesis, class L), also known as N-acetylglucosaminyl-phosphatidylinositol de-N-acetylase, is a 252 amino acid ER single-pass membrane protein. Encoded by a gene that maps to human chromosome 17p11.2, PIG-L shares 77% identity with rat PIG-L, and both are orthologs of Saccharomyces cerevisiae Gpi12, which results in a lethal phenotype when disrupted. Enhanced by metal ions, in particular Mn²⁺ and Ni²⁺, PIG-L catalyzes the second step of GPI biosynthesis, which is the de-N-acetylation of GlcNAc-PI.

REFERENCES

- 1. Kinoshita, T., et al. 1997. GPI-anchor synthesis in mammalian cells: genes, their products, and a deficiency. J. Biochem. 122: 251-257.
- Nakamura, N., et al. 1997. Expression cloning of PIG-L, a candidate N-acetylglucosaminyl-phosphatidylinositol deacetylase. J. Biol. Chem. 272: 15834-15840.
- Watanabe, R., et al. 1998. The first step of glycosylphosphatidylinositol biosynthesis is mediated by a complex of PIG-A, PIG-H, PIG-C and GPI1. EMBO J. 17: 877-885.
- Watanabe, R., et al. 1999. Mammalian PIG-L and its yeast homologue Gpi12p are N-acetylglucosaminylphosphatidylinositol de-N-acetylases essential in glycosylphosphatidylinositol biosynthesis. Biochem. J. 339: 185-192.
- Ferguson, M.A., et al. 1999. The GPI biosynthetic pathway as a therapeutic target for African sleeping sickness. Biochim. Biophys. Acta 1455: 327-340.
- 6. Gordon, V.M., et al. 1999. Clostridium septicum α toxin uses glycosylphosphatidylinositol-anchored protein receptors. J. Biol. Chem. 274: 27274-27280.
- Abrami, L., et al. 2001. Cross-talk between caveolae and glycosylphosphatidylinositol-rich domains. J. Biol. Chem. 276: 30729-30736.
- 8. Chang, T., et al. 2002. Cloning of *Trypanosoma brucei* and *Leishmania major* genes encoding the GlcNAc-phosphatidylinositol de-N-acetylase of glycosylphosphatidylinositol biosynthesis that is essential to the African sleeping sickness parasite. J. Biol. Chem. 277: 50176-50182.
- McCarthy, A.A., et al. 2004. Crystal structure of MshB from *Mycobacterium tuberculosis*, a deacetylase involved in mycothiol biosynthesis. J. Mol. Biol. 335: 1131-1141.

CHROMOSOMAL LOCATION

Genetic locus: PIGL (human) mapping to 17p11.2.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PRODUCT

PIG-L 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 PIG-L shRNA Plasmid (h): sc-93912-SH and PIG-L shRNA (h) Lentiviral Particles: sc-93912-V as alternate gene silencing products.

For independent verification of PIG-L (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-93912A, sc-93912B and sc-93912C.

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

PIG-L siRNA (h) is recommended for the inhibition of PIG-L 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PIG-L gene expression knockdown using RT-PCR Primer: PIG-L (h)-PR: sc-93912-PR (20 μ I). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.