SANTA CRUZ BIOTECHNOLOGY, INC.

PIG-W siRNA (h): sc-93698

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 including mannosylation of the GPI-anchor. PIG-W (Phosphatidylinositol-glycan biosynthesis class W protein) is a 504 amino acid multi-pass membrane protein that functions in the third step of GPI biosynthesis and acylates the inositol ring of phosphatidylinositol.

REFERENCES

1. Stevens, V.L. and Raetz, C.R. 1991. Defective glycosyl phosphatidylinositol biosynthesis in extracts of three Thy-1 negative lymphoma cell mutants. J. Biol. Chem. 266: 10039-10042.
2. Ryals, P.E., Pak, Y. and Thompson, G.A. 1991. Phosphatidylinositol-linked glycans and phosphatidylinositol-anchored proteins of *Tetrahymena* mimbres. J. Biol. Chem. 266: 15048-15053.
3. Scallon, B.J., Fung, W.J., Tsang, T.C., Li, S., Kado-Fong, H., Huang, K.S. and Kochan, J.P. 1991. Primary structure and functional activity of a phosphatidylinositol-glycan-specific phospholipase D. Science 252: 446-448.
4. Ueda, E., Nishimura, J., Kitani, T., Nasu, K., Kageyama, T., Kim, Y.U., Takeda, J. and Kinoshita, T. 1992. Deficient surface expression of glycosylphosphatidylinositol-anchored proteins in B cell lines established from patients with paroxysmal nocturnal hemoglobinuria. Int. Immunol. 4: 1263-1271.
5. Güther, M.L. and Ferguson, M.A. 1993. The microanalysis of glycosylphosphatidylinositol glycans. Methods Mol. Biol. 14: 99-117.
6. Watanabe, R., Inoue, N., Westfall, B., Taron, C.H., Orlean, P., Takeda, J. and Kinoshita, T. 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.
7. Murakami, Y., Siripanyapinyo, U., Hong, Y., Kang, J.Y., Ishihara, S., Nakakuma, H., Maeda, Y. and Kinoshita, T. 2003. PIG-W is critical for inositol acylation but not for flipping of glycosylphosphatidylinositol-anchor. Mol. Biol. Cell 14: 4285-4295.
8. Online Mendelian Inheritance in Man, OMIM™. 2006. Johns Hopkins University, Baltimore, MD. MIM Number: 610275. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
9. Rodríguez, A.E., Couto, A., Echaide, I., Schnittger, L. and Florin-Christensen, M. 2010. *Babesia bovis* contains an abundant parasite-specific protein-free glycerophosphatidylinositol and the genes predicted for its assembly. Vet. Parasitol. 167: 227-235.

CHROMOSOMAL LOCATION

Genetic locus: PIGW (human) mapping to 17q12.

PRODUCT

PIG-W 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIG-W shRNA Plasmid (h): sc-93698-SH and PIG-W shRNA (h) Lentiviral Particles: sc-93698-V as alternate gene silencing products.

For independent verification of PIG-W (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-93698A, sc-93698B and sc-93698C.

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

PIG-W siRNA (h) is recommended for the inhibition of PIG-W 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 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-W gene expression knockdown using RT-PCR Primer: PIG-W (h)-PR: sc-93698-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.