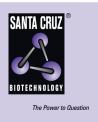
SANTA CRUZ BIOTECHNOLOGY, INC.

PIG-O siRNA (m): sc-152255



BACKGROUND

Phosphatidylinositol-glycans (PIGs) are transmembrane proteins that localize to endoplasmic reticulum. PIGs exhibit a variety of functions, but all are crucial for 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 (GPI-N-acetylglucosaminyltransferase or GPI-GnT) and function in the transfer of N-acetylglucosamine (GlcNAc) to phosphatidylinositol (PI). An array of PIGs play distinct roles in GPI synthesis. PIG-0 (phosphatidylinositol glycan anchor biosynthesis, class O), also known as GPI ethanolamine phosphate transferase 3, is a 1,089 amino acid protein that exists as two alternatively spliced isoforms. Associating with PIG-F for stabilization, PIG-O functions as an ethanolamine phosphate (EtNP) transferase and catalyzes the transfer of EtNP to the GPI third mannose, which links the GPI-anchor to the protein C-terminus by an amide bond.

REFERENCES

- Kawagoe, K., Kitamura, D., Okabe, M., Taniuchi, I., Ikawa, M., Watanabe, T., Kinoshita, T. and Takeda, J. 1996. Glycosylphosphatidylinositol-anchor-deficient mice: implications for clonal dominance of mutant cells in paroxysmal nocturnal hemoglobinuria. Blood 87: 3600-3606.
- Kinoshita, T. and Inoue, N. 2000. Dissecting and manipulating the pathway for glycosylphos-phatidylinositol-anchor biosynthesis. Curr. Opin. Chem. Biol. 4: 632-638.
- Hong, Y., Maeda, Y., Watanabe, R., Inoue, N., Ohishi, K. and Kinoshita, T. 2000. Requirement of PIG-F and PIG-O for transferring phosphoethanolamine to the third mannose in glycosylphosphatidylinositol. J. Biol. Chem. 275: 20911-20919.
- Maeda, Y., Watanabe, R., Harris, C.L., Hong, Y., Ohishi, K., Kinoshita, K. and Kinoshita, T. 2001. PIG-M transfers the first mannose to glycosylphosphatidylinositol on the lumenal side of the ER. EMBO J. 20: 250-261.
- Eisenhaber, B., Maurer-Stroh, S., Novatchkova, M., Schneider, G. and Eisenhaber, F. 2003. Enzymes and auxiliary factors for GPI lipid anchor biosynthesis and post-translational transfer to proteins. Bioessays 25: 367-385.
- Kang, J.Y., Hong, Y., Ashida, H., Shishioh, N., Murakami, Y., Morita, Y.S., Maeda, Y. and Kinoshita, T. 2005. PIG-V involved in transferring the second mannose in glycosylphosphatidylinositol. J. Biol. Chem. 280: 9489-9497.
- Shishioh, N., Hong, Y., Ohishi, K., Ashida, H., Maeda, Y. and Kinoshita, T. 2005. GPI7 is the second partner of PIG-F and involved in modification of glycosylphosphatidylinositol. J. Biol. Chem. 280: 9728-9734.
- Kinoshita, T., Fujita, M. and Maeda, Y. 2008. Biosynthesis, remodelling and functions of mammalian GPI-anchored proteins: recent progress. J. Biochem. 144: 287-294.

PROTOCOLS

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

CHROMOSOMAL LOCATION

Genetic locus: Pigo (mouse) mapping to 4 A5.

PRODUCT

PIG-O 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIG-O shRNA Plasmid (m): sc-152255-SH and PIG-O shRNA (m) Lentiviral Particles: sc-152255-V as alternate gene silencing products.

For independent verification of PIG-0 (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-152255A, sc-152255B and sc-152255C.

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-0 siRNA (m) is recommended for the inhibition of PIG-0 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 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-O gene expression knockdown using RT-PCR Primer: PIG-O (m)-PR: sc-152255-PR (20 μ 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.