

PIG-A siRNA (h): sc-61349

BACKGROUND

Phosphatidylinositol-glycans (PIGs) are multi-pass transmembrane proteins that localize to the endoplasmic reticulum. PIGs are crucial for the synthesis of N-acetylglucosaminyl-phosphatidylinositol (GlcNAc-PI), a very early intermediate in glycosylphosphatidylinositol (GPI)-anchor biosynthesis. The 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. Phosphatidylinositol glycan class A (PIG-A), an endoplasmic reticulum (ER) transmembrane protein, contains a large cytoplasmic domain, which displays homology to the bacterial GlcNAc transferase RfaK, and a small luminal domain, which plays a role in targeting the PIG-A protein to the rough ER. PIG-A associates with PIG-C, PIG-H, PIG-P and PIG-Q, as well as DPM2, and interacts directly with PIG-Y. Defects in PIG-A cause paroxysmal nocturnal hemoglobinuria (PNH), an acquired genetic hematologic disorder.

REFERENCES

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- Takeda, J., et al. 1993. Deficiency of the GPI anchor caused by a somatic mutation of the PIG-A gene in paroxysmal nocturnal hemoglobinuria. *Cell* 73: 703-711.
- Watanabe, R., et al. 1996. PIG-A and PIG-H, which participate in glycosylphosphatidylinositol anchor form a protein complex in the endoplasmic reticulum. *J. Biol. Chem.* 271: 26868-26875.
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- Hu, R., et al. 2005. PIG-A mutations in normal hematopoiesis. *Blood* 105: 3848-3854.
- Hanaoka, N., et al. 2006. Immunoselection by natural killer cells of PIG-A mutant cells missing stress-inducible ULBP. *Blood* 107: 1184-1191.

CHROMOSOMAL LOCATION

Genetic locus: PIGA (human) mapping to Xp22.2.

PRODUCT

PIG-A 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-A shRNA Plasmid (h): sc-61349-SH and PIG-A shRNA (h) Lentiviral Particles: sc-61349-V as alternate gene silencing products.

For independent verification of PIG-A (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-61349A, sc-61349B and sc-61349C.

PROTOCOLS

See our web site at 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 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-A siRNA (h) is recommended for the inhibition of PIG-A 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.

GENE EXPRESSION MONITORING

PIG-A (H-6): sc-374194 is recommended as a control antibody for monitoring of PIG-A gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG κ BP-HRP: sc-516102 or m-IgG κ BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker[™] Molecular Weight Standards: sc-2035, UltraCruz[®] Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG κ BP-FITC: sc-516140 or m-IgG κ BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz[®] Mounting Medium: sc-24941 or UltraCruz[®] Hard-set Mounting Medium: sc-359850.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PIG-A gene expression knockdown using RT-PCR Primer: PIG-A (h)-PR: sc-61349-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.