SANTA CRUZ BIOTECHNOLOGY, INC.

PIG-S siRNA (h): sc-62808

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 (GPIT) 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-S is a component of GPIT, a multisubunit membrane-bound complex that recognizes the C-terminal signal sequences on proproteins, cleaves them and replaces them with specific GPI lipids. PIG-S is required for the generation of the carbonyl intermediate.

REFERENCES

1. Ohishi, K., et al. 2001. PIG-S and PIG-T, essential for GPI anchor attachment to proteins, form a complex with GAA1 and GPI8. EMBO J. 20: 4088-4098.
2. Hong, Y., et al. 2003. Human PIG-U and yeast Cdc91p are the fifth subunit of GPI transamidase that attaches GPI-anchors to proteins. Mol. Biol. Cell 14: 1780-1789.
3. Nagamune, K., et al. 2003. GPI transamidase of *Trypanosoma brucei* has two previously uncharacterized (trypanosomatid transamidase 1 and 2) and three common subunits. Proc. Natl. Acad. Sci. USA 100: 10682-10687.
4. Vainauskas, S. and Menon, A.K. 2004. A conserved proline in the last transmembrane segment of Gaa1 is required for glycosylphosphatidylinositol (GPI) recognition by GPI transamidase. J. Biol. Chem. 279: 6540-6545.
5. Zhu, Y., et al. 2005. Gpi17p does not stably interact with other subunits of glycosylphosphatidylinositol transamidase in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta 1735: 79-88.
6. Vainauskas, S. and Menon, A.K. 2006. Ethanolamine phosphate linked to the first mannose residue of glycosylphosphatidylinositol (GPI) lipids is a major feature of the GPI structure that is recognized by human GPI transamidase. J. Biol. Chem. 281: 38358-38364.
7. Hong, Y., et al. 2006. TbGPI16 is an essential component of GPI transamidase in *Trypanosoma brucei*. FEBS Lett. 580: 603-606.

CHROMOSOMAL LOCATION

Genetic locus: PIGS (human) mapping to 17q11.2.

PRODUCT

PIG-S 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-S shRNA Plasmid (h): sc-62808-SH and PIG-S shRNA (h) Lentiviral Particles: sc-62808-V as alternate gene silencing products.

For independent verification of PIG-S (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-62808A, sc-62808B and sc-62808C.

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

GENE EXPRESSION MONITORING

PIG-S (G-10): sc-373701 is recommended as a control antibody for monitoring of PIG-S 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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-S gene expression knockdown using RT-PCR Primer: PIG-S (h)-PR: sc-62808-PR (20 µl, 453 bp). 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.