

PIG-T siRNA (h): sc-62810

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-T 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-T is disulfide-linked to PIG-K and functions to stabilize the complex and promote GPIT activity. Overexpression of PIG-T is associated with breast cancer.

REFERENCES

1. 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.
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. Ohishi, K., et al. 2003. Two subunits of glycosylphosphatidylinositol transamidase, GPI8 and PIG-T, form a functionally important intermolecular disulfide bridge. *J. Biol. Chem.* 278: 13959-13967.
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. Wu, G., et al. 2006. Overexpression of glycosylphosphatidylinositol (GPI) transamidase subunits phosphatidylinositol glycan class T and/or GPI anchor attachment 1 induces tumorigenesis and contributes to invasion in human breast cancer. *Cancer Res.* 66: 9829-9836.

CHROMOSOMAL LOCATION

Genetic locus: PIGT (human) mapping to 20q13.12.

PRODUCT

PIG-T 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-T shRNA Plasmid (h): sc-62810-SH and PIG-T shRNA (h) Lentiviral Particles: sc-62810-V as alternate gene silencing products.

For independent verification of PIG-T (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-62810A, sc-62810B and sc-62810C.

RESEARCH USE

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

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-T siRNA (h) is recommended for the inhibition of PIG-T 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-T gene expression knockdown using RT-PCR Primer: PIG-T (h)-PR: sc-62810-PR (20 μ l, 574 bp). 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.