

# PIG-K siRNA (h): sc-62804

## BACKGROUND

PPHosphatidylinositol-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-K (also called hGPI8) is the catalytic subunit of GPIT, a multi-subunit membrane-bound complex that recognizes the C-terminal signal sequences on proproteins, cleaves them and replaces them with specific GPI lipids. PIG-K functions as a cysteine protease and contains a Cis-His catalytic dyad. It is responsible for cleaving the signal sequence on proteins and forming the amide bond between GPI and the protein.

## REFERENCES

1. Kodukula, K., et al. 1995. Processing of nascent proteins to glycosylphosphatidylinositol-anchored forms in cell-free systems. *Methods Enzymol.* 250: 536-547.
2. Ramalingam, S., et al. 1996. COOH-terminal processing of nascent polypeptides by the glycosylphosphatidylinositol transamidase in the presence of hydrazine is governed by the same parameters as glycosylphosphatidylinositol addition. *Proc. Natl. Acad. Sci. USA* 93: 7528-7533.
3. Benghezal, M., et al. 1997. Yeast Gpi8p is essential for GPI anchor attachment on to proteins. *EMBO J.* 15: 6575-6583.
4. Meyer, U., et al. 2002. The glycosylphosphatidylinositol (GPI) signal sequence of human placental alkaline phosphatase is not recognized by human Gpi8p in the context of the yeast GPI anchoring machinery. *Mol. Microbiol.* 46: 745-748.
5. Nagamune, K., et al. 2003. GPI transamidase of *Trypanosoma brucei* has two previously uncharacterized (trypanosomatid transamidase 1 and three common subunits. *Proc. Natl. Acad. Sci. USA* 100: 10682-10687.
6. Eisenhaber, B., et al. 2003. Enzymes and auxiliary factors for GPI lipid anchor biosynthesis and post-translational transfer to proteins. *Bioessays* 25: 367-385.

## CHROMOSOMAL LOCATION

Genetic locus: PIGK (human) mapping to 1p31.1.

## PRODUCT

PIG-K 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIG-K shRNA Plasmid (h): sc-62804-SH and PIG-K shRNA (h) Lentiviral Particles: sc-62804-V as alternate gene silencing products.

For independent verification of PIG-K (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-62804A, sc-62804B and sc-62804C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

PIG-K siRNA (h) is recommended for the inhibition of PIG-K 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  $\mu$ M in 66  $\mu$ 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-K (G-6): sc-398611 is recommended as a control antibody for monitoring of PIG-K 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 $\lambda$  BP-HRP: sc-516132 or m-IgG $\lambda$  BP-HRP (Cruz Marker): sc-516132-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\lambda$  BP-FITC: sc-516185 or m-IgG $\lambda$  BP-PE: sc-516186 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PIG-K gene expression knockdown using RT-PCR Primer: PIG-K (h)-PR: sc-62804-PR (20  $\mu$ l, 445 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](http://www.scbt.com) for detailed protocols and support products.