SANTA CRUZ BIOTECHNOLOGY, INC.

PIG-K siRNA (m): sc-62805

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-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 proproteins 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 .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 (mouse) mapping to 3 H3.

PRODUCT

PIG-K 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PIG-K shRNA Plasmid (m): sc-62805-SH and PIG-K shRNA (m) Lentiviral Particles: sc-62805-V as alternate gene silencing products.

For independent verification of PIG-K (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-62805A, sc-62805B and sc-62805C.

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-K siRNA (m) is recommended for the inhibition of PIG-K 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 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-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-IgGλ BP-HRP: sc-516132 or m-IgGλ BP-HRP (Cruz Marker): sc-516132-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-IgGλ BP-FITC: sc-516185 or m-IgGλ BP-PE: sc-516186 (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-K gene expression knockdown using RT-PCR Primer: PIG-K (m)-PR: sc-62805-PR (20 µl). 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.