

PGAP1 siRNA (h): sc-94514

BACKGROUND

PGAP1 (post-GPI attachment to proteins 1), also known as Bst1 or GPI inositol-deacylase, is a 922 amino acid multi-pass membrane protein that belongs to the GPI inositol-deacylase family and exists as four alternatively spliced isoforms. Encoded by a gene that maps to human chromosome 2q33.1, PGAP1 localizes to Endoplasmic reticulum membrane and is involved in inositol deacylation of glycosylphosphatidylinositol- (GPI) anchored proteins. GPI inositol deacylation may be vital for streamlined transport of GPI-anchored proteins from Endoplasmic reticulum to Golgi apparatus, as well as inducing mature GPI that are capable of protein attachment. Human and rat PGAP1 share 90% amino acid sequence identity and both contain an identical lipase consensus motif with a putative catalytic serine. PGAP1 is also linked to otocephaly and male infertility.

REFERENCES

1. Tanaka, S., Maeda, Y., Tashima, Y. and Kinoshita, T. 2004. Inositol deacylation of glycosylphosphatidylinositol-anchored proteins is mediated by mammalian PGAP1 and yeast Bst1p. *J. Biol. Chem.* 279: 14256-14263.
2. Maeda, Y., Ashida, H. and Kinoshita, T. 2006. CHO glycosylation mutants: GPI anchor. *Meth. Enzymol.* 416: 182-205.
3. Tashima, Y., Taguchi, R., Murata, C., Ashida, H., Kinoshita, T. and Maeda, Y. 2006. PGAP2 is essential for correct processing and stable expression of GPI-anchored proteins. *Mol. Biol. Cell* 17: 1410-1420.
4. Ueda, Y., Yamaguchi, R., Ikawa, M., Okabe, M., Morii, E., Maeda, Y. and Kinoshita, T. 2007. PGAP1 knock-out mice show otocephaly and male infertility. *J. Biol. Chem.* 282: 30373-30380.
5. Maeda, Y., Tashima, Y., Houjou, T., Fujita, M., Yoko-o, T., Jigami, Y., Taguchi, R. and Kinoshita, T. 2007. Fatty acid remodeling of GPI-anchored proteins is required for their raft association. *Mol. Biol. Cell* 18: 1497-1506.
6. Kinoshita, T., Fujita, M. and Maeda, Y. 2008. Biosynthesis, remodelling and functions of mammalian GPI-anchored proteins: recent progress. *J. Biochem.* 144: 287-294.
7. Urquhart, J., Black, G.C. and Clayton-Smith, J. 2009. 4.5 Mb microdeletion in chromosome band 2q33.1 associated with learning disability and cleft palate. *Eur. J. Med. Genet.* 52: 454-457.
8. Fujita, M. and Kinoshita, T. 2010. Structural remodeling of GPI anchors during biosynthesis and after attachment to proteins. *FEBS Lett.* 584: 1670-1677.
9. Kamnasaran, D., Morin, F. and Gekas, J. 2010. Prenatal diagnosis and molecular genetic studies on a new case of agnathia-otocephaly. *Fetal Pediatr. Pathol.* 29: 207-211.

CHROMOSOMAL LOCATION

Genetic locus: PGAP1 (human) mapping to 2q33.1.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

PRODUCT

PGAP1 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 PGAP1 shRNA Plasmid (h): sc-94514-SH and PGAP1 shRNA (h) Lentiviral Particles: sc-94514-V as alternate gene silencing products.

For independent verification of PGAP1 (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-94514A, sc-94514B and sc-94514C.

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

PGAP1 siRNA (h) is recommended for the inhibition of PGAP1 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 PGAP1 gene expression knockdown using RT-PCR Primer: PGAP1 (h)-PR: sc-94514-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.