SANTA CRUZ BIOTECHNOLOGY, INC.

PIS1 siRNA (r): sc-270262

BACKGROUND

PIS1, also known as CDIPT (CDP-diacylglycerol—inositol 3-phosphatidyltransferase) or PIS (phosphatidylinositol synthase), is a 213 amino acid multi-pass membrane protein that belongs to the CDP-alcohol phosphatidyltransferase class-I family and exists as two alternatively spliced isoforms. Localizing to Golgi apparatus and the cytoplasmic side of endoplasmic reticulum, PIS1 is widely expressed, with highest expression in adult liver and skeletal muscle. PIS1 catalyzes the biosynthesis of phosphatidylinositol (PtdIns), as well as the PtdIns-inositol exchange reaction, which is due to the reverse reaction of PtdIns synthase and is CMP-dependent. PIS1 may also reduce excessive cellular PtdIns. Highly conserved in yeast and mammals, PIS1 is encoded by a gene that maps to human chromosome 16p11.2.

REFERENCES

1. Takenawa, T., et al. 1982. Effect of unsaturated fatty acids and Ca^{2+} on phosphatidylinositol synthesis and breakdown. J. Biochem. 91: 793-799.
2. Anderson, M.S., et al. 1996. Carbon source regulation of PIS1 gene expression in *Saccharomyces cerevisiae* involves the MCM1 gene and the two-component regulatory gene, SLN1. J. Biol. Chem. 271: 26596-26601.
3. Fujita, H., et al. 1997. PIS1, a negative regulator of the action of auxin transport inhibitors in *Arabidopsis thaliana*. Plant J. 12: 583-595.
4. Gardocki, M.E., et al. 2003. Expression of the yeast PIS1 gene requires multiple regulatory elements including a Rox1p binding site. J. Biol. Chem. 278: 38646-38652.
5. Gardocki, M.E., et al. 2005. Genomic analysis of PIS1 gene expression. Eukaryotic Cell 4: 604-614.
6. Han, S.H., et al. 2005. Regulation of the PIS1-encoded phosphatidylinositol synthase in *Saccharomyces cerevisiae* by zinc. J. Biol. Chem. 280: 29017-29024.
7. Rohatgi, N., et al. 2006. Novel molecular targets of smokeless tobacco (khaini) in cell culture from oral hyperplasia. Toxicology 224: 1-13.

CHROMOSOMAL LOCATION

Genetic locus: Cdipt (rat) mapping to 1q36.

PRODUCT

PIS1 siRNA (r) 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 PIS1 shRNA Plasmid (r): sc-270262-SH and PIS1 shRNA (r) Lentiviral Particles: sc-270262-V as alternate gene silencing products.

For independent verification of PIS1 (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270262A, sc-270262B and sc-270262C.

PROTOCOLS

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

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

PIS1 siRNA (r) is recommended for the inhibition of PIS1 expression in rat 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

PIS1 (C-2): sc-514255 is recommended as a control antibody for monitoring of PIS1 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 PIS1 gene expression knockdown using RT-PCR Primer: PIS1 (r)-PR: sc-270262-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.