SANTA CRUZ BIOTECHNOLOGY, INC.

PNPase siRNA (m): sc-61372

BACKGROUND

Mitochondrial polyribonucleotide nucleotidyltransferase, also designated 3'-5' RNA exonuclease, OLD35, PNPase or PNPT1, is an evolutionaly conserved protein in which the mouse protein shares 90% identity with the human version. PNPase (polyribonucleotide nucleotidyltransferase 1) participates in mRNA degradation and hydrolyzes single-stranded ribonucleotides in the 3' to 5' direction. PNPase forms homotrimers and is upregulated in response to interferon-β induction. The N-terminus of PNPase contains a putative mitochondrial targeting sequence; mutation analysis confirms that N-terminal sequences of PNPase target the protein to the mitochondria. Endogenous PNPase also co-localizes with a mitochondrial marker protein in HeLa cells.

REFERENCES

1. Bermúdez-Cruz, R.M., et al. 2005. Conserved domains in polynucleotide phosphorylase among eubacteria. Biochimie 87: 737-745.
2. Bollenbach, T.J., et al. 2005. RNR1, a 3'-5' exoribonuclease belonging to the RNR superfamily, catalyzes 3' maturation of chloroplast ribosomal RNAs in *Arabidopsis thaliana*. Nucleic Acids Res. 33: 2751-2563.
3. Oussenko, I.A., et al. 2005. Participation of 3'-to-5' exoribonucleases in the turnover of *Bacillus subtilis* mRNA. J. Bacteriol. 187: 2758-2767.
4. Sarkar, D., et al. 2005. Defining the domains of human polynucleotide phosphorylase (hPNPase^{OLD-35}) mediating cellular senescence. Mol. Cell. Biol. 25: 7333-7343.
5. Gewartowski, K., et al. 2006. Upregulation of human PNPase mRNA by β-interferon has no effect on protein level in melanoma cell lines. Acta Biochim. Pol. 53: 179-188.
6. Chen, H.W., et al. 2007. Human polynucleotide phosphorylase: location matters. Trends Cell Biol. 17: 600-608.
7. Portnoy, V., et al. 2008. Analysis of the human polynucleotide phosphorylase (PNPase) reveals differences in RNA binding and response to phosphate compared to its bacterial and chloroplast counterparts. RNA 14: 297-309.
8. Slomovic, S. and Schuster, G. 2008. Stable PNPase RNAi silencing: its effect on the processing and adenylation of human mitochondrial RNA. RNA 14: 310-323.

CHROMOSOMAL LOCATION

Genetic locus: Pnpt1 (mouse) mapping to 11 A3.3.

PRODUCT

PNPase 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 PNPase shRNA Plasmid (m): sc-61372-SH and PNPase shRNA (m) Lentiviral Particles: sc-61372-V as alternate gene silencing products.

For independent verification of PNPase (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-61372A, sc-61372B and sc-61372C.

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

PNPase siRNA (m) is recommended for the inhibition of PNPase 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

PNPase (D-1): sc-271479 is recommended as a control antibody for monitoring of PNPase 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).

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PNPase gene expression knockdown using RT-PCR Primer: PNPase (m)-PR: sc-61372-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.