



PNK siRNA (m): sc-45370

BACKGROUND

Bifunctional polynucleotide phosphatase/kinase (PNK), also referred to as polynucleotide kinase 3'-phosphatase, is a member of the DNA 3' phosphatase family. PNK is the protein expressed by the gene PNKP and contains a 3'-phosphatase domain with similarity to L-2-haloacid dehalogenases and a reported ATP binding site. PNK is a nuclear protein that is involved in DNA repair following damage caused by radiation or oxidation. The protein catalyzes the phosphorylation of DNA at the hydroxy termini but can also dephosphorylate its 3'-phosphate termini. The highest levels of expression of PNK occur in testis, pancreas, spleen, kidney and heart.

REFERENCES

1. Jilani, A., et al. 1999. Molecular cloning of the human gene, PNKP, encoding a polynucleotide kinase 3'-phosphatase and evidence for its role in repair of DNA strand breaks caused by oxidative damage. *J. Biol. Chem.* 274: 24176-24186.
2. Karimi-Busheri, F., et al. 1999. Molecular characterization of a human DNA kinase. *J. Biol. Chem.* 274: 24187-24194.
3. Fanta, M., et al. 2001. Production, characterization, and epitope mapping of monoclonal antibodies against human polydeoxyribonucleotide kinase. *Hybridoma* 20: 237-242.
4. Meijer, M., et al. 2002. PNK1, a DNA kinase/phosphatase required for normal response to DNA damage by gamma-radiation or camptothecin in *Schizosaccharomyces pombe*. *J. Biol. Chem.* 277: 4050-4055.
5. Plo, I., et al. 2003. Association of XRCC1 and tyrosyl DNA phosphodiesterase (Tdp1) for the repair of topoisomerase I-mediated DNA lesions. *DNA Repair* 2: 1087-1100.
6. Zhu, H., et al. 2004. Characterization of polynucleotide kinase/phosphatase enzymes from *Mycobacteriophages* omega and Cjw1 and vibriophage KVP40. *J. Biol. Chem.* 279: 26358-26369.
7. Martins, A., et al. 2005. An end-healing enzyme from *Clostridium thermocellum* with 5' kinase, 2', 3' phosphatase and adenylyltransferase activities. *RNA* 11: 1271-1280.
8. Bernstein, N.K., et al. 2005. The molecular architecture of the mammalian DNA repair enzyme, polynucleotide kinase. *Mol. Cell* 17: 657-670.

CHROMOSOMAL LOCATION

Genetic locus: Pnkp (mouse) mapping to 7 B4.

PRODUCT

PNK 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PNK shRNA Plasmid (m): sc-45370-SH and PNK shRNA (m) Lentiviral Particles: sc-45370-V as alternate gene silencing products.

For independent verification of PNK (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-45370A, sc-45370B and sc-45370C.

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

PNK siRNA (m) is recommended for the inhibition of PNK 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 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.

GENE EXPRESSION MONITORING

PNK (F-3): sc-271505 is recommended as a control antibody for monitoring of PNK 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 PNK gene expression knockdown using RT-PCR Primer: PNK (m)-PR: sc-45370-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.

PROTOCOLS

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