SANTA CRUZ BIOTECHNOLOGY, INC.

PNUTS siRNA (h): sc-61377

BACKGROUND

Eukaryotic protein phosphorylation and dephosphorylation on serine and threonine residues regulates numerous cell functions, including division, homeostasis and apoptosis. A group of proteins that play a major role in this process are the serine/threonine protein phosphatases. Protein phosphatase (PP) holoenzyme is a trimeric complex that contains a regulatory subunit, a variable subunit and a catalytic subunit. Families of PP catalytic subunits include PP1, PP2A, PP2B, PP2C, PPX and PP5. Regulatory subunits include nuclear inhibitor of PP1 (NIPP1), PP1 nuclear targeting subunit (PNUTS), PP2A-A, PP2A-B, PP2A-B56, PP2A-C, PP2B-B and PR48. PNUTS, also designated CAT53 or FB19, is encoded by the gene PPP1R10. PNUTS acts as an inhibitor for the phosphatase activity of PP1α and PP1γ. It is a nuclear protein primarily detected in nucleoplasmic bodies and within nucleoli. PNUTS expression levels are highest in brain, heart, lung, placenta, liver, kidney, pancreas and skeletal muscle.

REFERENCES

1. Kreivi, J.P., et al. 1997. Purification and characterisation of p99, a nuclear modulator of protein phosphatase 1 activity. FEBS Lett. 420: 57-62.
2. Totaro, A., et al. 1998. Cloning of a new gene (FB19) within HLA class I region. Biochem. Biophys. Res. Commun. 250: 555-557.
3. Kim, Y.M., et al. 2003. PNUTS, a protein phosphatase 1 (PP1) nuclear targeting subunit. Characterization of its PP1- and RNA-binding domains and regulation by phosphorylation. J. Biol. Chem. 278: 13819-13828.
4. Lesage, B., et al. 2004. Interactor-mediated nuclear translocation and retention of protein phosphatase-1. J. Biol. Chem. 279: 55978-55984.
5. Tran, H.T., et al. 2004. Proteomic characterization of protein phosphatase complexes of the mammalian nucleus. Mol. Cell. Proteomics 3: 257-265.
6. Landsverk, H.B., et al. 2005. PNUTS enhances *in vitro* chromosome decondensation in a PP1-dependent manner. Biochem. J. 390: 709-717.

CHROMOSOMAL LOCATION

Genetic locus: PPP1R10 (human) mapping to 6p21.33.

PRODUCT

PNUTS 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see PNUTS shRNA Plasmid (h): sc-61377-SH and PNUTS shRNA (h) Lentiviral Particles: sc-61377-V as alternate gene silencing products.

For independent verification of PNUTS (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-61377A, sc-61377B and sc-61377C.

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

PNUTS siRNA (h) is recommended for the inhibition of PNUTS 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 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

PNUTS (F-8): sc-271681 is recommended as a control antibody for monitoring of PNUTS 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 PNUTS gene expression knockdown using RT-PCR Primer: PNUTS (h)-PR: sc-61377-PR (20 µl, 598 bp). 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.