

PHACTR3 siRNA (h): sc-76114

BACKGROUND

In eukaryotes, the phosphorylation and dephosphorylation of proteins on serine and threonine residues is an essential means of regulating a broad range of cellular functions, including division, homeostasis and apoptosis. A group of proteins that are intimately involved in this process are the protein phosphatases. PHACTR3 (phosphatase and actin regulator 3), also known as scapinin (scaffold-associated PP1-inhibiting protein), is a 559 amino acid nuclear matrix protein that inhibits protein phosphatase 1 (PP1) activity by binding actin and PP1 α , the catalytic subunit. PHACTR3 is highly expressed in brain, and expressed at lower levels in placenta, thymus and spleen. Mutations in the gene encoding PHACTR3 are found in non-small cell lung carcinoma and these mutations are associated with worse prognosis. There are three isoforms of PHACTR3 that are expressed as a result of alternative splicing events. PHACTR3 is upregulated in several tumor types, with isoform 3 being the major form in GOTO, HL-60 and U937 cell lines.

REFERENCES

1. Sagara, J., et al. 2003. Scapinin, a putative protein phosphatase-1 regulatory subunit associated with the nuclear nonchromatin structure. *J. Biol. Chem.* 278: 45611-45619.
2. Allen, P.B., et al. 2004. Phactrs 1-4: A family of protein phosphatase 1 and actin regulatory proteins. *Proc. Natl. Acad. Sci. USA* 101: 7187-7192.
3. Online Mendelian Inheritance in Man, OMIM™. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 608725. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Worch, S., et al. 2006. Genomic organization and expression pattern of scapinin (PHACTR3) in mouse and human. *Cytogenet. Genome Res.* 115: 23-29.
5. Tang, L.Y., et al. 2007. Quantitative phosphoproteome profiling of Wnt3a-mediated signaling network: indicating the involvement of ribonucleoside-diphosphate reductase M2 subunit phosphorylation at residue serine 20 in canonical Wnt signal transduction. *Mol. Cell. Proteomics* 6: 1952-1967.

CHROMOSOMAL LOCATION

Genetic locus: PHACTR3 (human) mapping to 20q13.32.

PRODUCT

PHACTR3 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 PHACTR3 shRNA Plasmid (h): sc-76114-SH and PHACTR3 shRNA (h) Lentiviral Particles: sc-76114-V as alternate gene silencing products.

For independent verification of PHACTR3 (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-76114A, sc-76114B and sc-76114C.

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 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

PHACTR3 siRNA (h) is recommended for the inhibition of PHACTR3 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.

GENE EXPRESSION MONITORING

PHACTR3 (H-3): sc-390843 is recommended as a control antibody for monitoring of PHACTR3 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 PHACTR3 gene expression knockdown using RT-PCR Primer: PHACTR3 (h)-PR: sc-76114-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.