SANTA CRUZ BIOTECHNOLOGY, INC.

NCK1 siRNA (h): sc-40967

BACKGROUND

The NCK family of SH2/SH3 adaptor proteins consists of two members, NCK1 (NCKα) and NCK2 (NCKβ), which couple tyrosine kinase signaling, including the EGF and PDGF receptor-pathways, to downstream signaling proteins. Specifically, overexpression of Nck1 in NIH/3T3 cells decreases DNA synthesis stimulated by EGF. Furthermore, the SH2 domain of NCK2 inhibits EGF- and PDGF-induced DNA synthesis. The SH3 domain of NCK binds a proline-rich domain on PAK, a known actin cytoskeleton regulator. The NCK protein thus mediates the interaction between PAK and RAC. The NCK2 protein binds human PDGFR-β (Tyr 1009); overexpression of Nck2 inhibits PDGF-induced membrane ruffling and lamellipod formation. Various growth factor receptors, cell surface antigens and adhesion molecules phosphorylate mammalian NCK1 and NCK2. The human NCK1 and NCK2 genes map to chromosomes 3q22.3 and 2q12, respectively.

REFERENCES

1. Park, D., et al. 1992. Phosphorylation of NCK in response to a variety of receptors, phorbol myristate acetate, and cyclic AMP. Mol. Cell. Biol. 12: 5816-5823.
2. Huebner, K., et al. 1994. Chromosome locations of genes encoding human signal transduction adapter proteins, Nck (NCK), Shc (SHC1), and Grb2 (GRB2). Genomics 22: 281-287.
3. Chen, M., et al. 1998. Identification of NCK family genes, chromosomal localization, expression, and signaling specificity. J. Biol. Chem. 273: 25171-25178.
4. Chen, M., et al. 2000. NCKβ adapter regulates Actin polymerization in NIH/3T3 fibroblasts in response to platelet-derived growth factor BB. Mol. Cell. Biol. 20: 7867-7880.
5. Buday, L., et al. 2002. The NCK family of adapter proteins. Regulators of Actin cytoskeleton. Cell. Signal. 14: 723-731.
6. Kiosses, W.B., et al. 2002. A dominant-negative p65 PAK peptide inhibits angiogenesis. Circ. Res. 90: 697-702.
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CHROMOSOMAL LOCATION

Genetic locus: NCK1 (human) mapping to 3q22.3.

PRODUCT

NCK1 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 NCK1 shRNA Plasmid (h): sc-40967-SH and NCK1 shRNA (h) Lentiviral Particles: sc-40967-V as alternate gene silencing products.

For independent verification of NCK1 (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-40967A, sc-40967B and sc-40967C.

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

NCK1 siRNA (h) is recommended for the inhibition of NCK1 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

NCK1 (20B.1H9): sc-20026 is recommended as a control antibody for monitoring of NCK1 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 NCK1 gene expression knockdown using RT-PCR Primer: NCK1 (h)-PR: sc-40967-PR (20 µl, 489 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.

PROTOCOLS

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