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# NCK1/2 shRNA (h) Lentiviral Particles: sc-43959-V

## BACKGROUND

The NCK family of SH2/SH3 adaptor proteins consists of two members, NCK1 (NCK $\alpha$ ) and NCK2 (NCK $\beta$ ), 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- $\beta$  (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 2q12.2, 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. NCK $\beta$  adapter regulates Actin polymerization in NIH 3T3 fibroblasts in response to platelet-derived growth factor BB. *Mol. Cell. Biol.* 20: 7867-7880.

## CHROMOSOMAL LOCATION

Genetic locus: NCK1 (human) mapping to 3q22.3, NCK2 (human) mapping to 2q12.2.

## PRODUCT

NCK1/2 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 4 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200  $\mu$ l frozen stock containing  $1.0 \times 10^6$  infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see NCK1/2 siRNA (h): sc-43959 and NCK1/2 shRNA Plasmid (h): sc-43959-SH as alternate gene silencing products.

## STORAGE

Store lentiviral particles at  $-80^\circ$  C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at  $4^\circ$  C for up to one week. Avoid repeated freeze thaw cycles.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) or our catalog for detailed protocols and support products.

## APPLICATIONS

NCK1/2 shRNA (h) Lentiviral Particles is recommended for the inhibition of NCK1 and NCK2 expression in human cells.

## SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200  $\mu$ l frozen viral stock containing  $1.0 \times 10^6$  infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

## GENE EXPRESSION MONITORING

NCK1/2 (G-3): sc-365802 is recommended as a control antibody for monitoring of NCK1/2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NCK1/2 gene expression knockdown using RT-PCR Primer: NCK1/2 (h)-PR: sc-43959-PR (20  $\mu$ l). Annealing temperature for the primers should be  $55-60^\circ$  C and the extension temperature should be  $68-72^\circ$  C.

## BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

## RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.