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Nek3 shRNA (h) Lentiviral Particles: sc-43550-V



The Power to Ouestion

BACKGROUND

NIMA was originally shown in *Aspergillus nidulans* to be necessary for entry into mitosis. NIMA-related mammalian proteins have since been identified as Nek1, Nek2, Nek3 and Nek4 (also designated STK2 or NRK2). High expression of Nek1 is seen in male and female germ cell lines of mouse. Nek2 is the closest known mammalian relative to NIMA. Like NIMA, Nek2 expression peaks at the $\rm G_2$ to M phase transition. Nek3 is a predominantly cytoplasmic enzyme that was detectable in all organs studied. Levels of Nek3 seem to remain unchanged throughout the cell cycle, but appear to be elevated in $\rm G_0$ -arrested, quiescent fibroblasts. In developing testicular germ cells, differential patterns of expression were seen for Nek1, Nek2 and Nek4, indicating possible overlapping, but non-identical functions.

REFERENCES

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- Schultz, S.J., et al. 1994. Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of Aspergillus nidulans. Cell Growth Differ. 5: 625-635.
- Rhee, K., et al. 1997. The NIMA-related kinase 2, Nek2, is expressed in specific stages of the meiotic cell cycle and associates with meiotic chromosomes. Development 124: 2167-2177.
- Fry, A.M., et al. 1997. Charcterization of mammalian DNA-related kinases. Meth. Enzymol. 283: 270-282.
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CHROMOSOMAL LOCATION

Genetic locus: NEK3 (human) mapping to 13q14.3.

PRODUCT

Nek3 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0 x 10⁶ 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 Nek3 siRNA (h): sc-43550 and Nek3 shRNA Plasmid (h): sc-43550-SH as alternate gene silencing products.

STORAGE

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

APPLICATIONS

Nek3 shRNA (h) Lentiviral Particles is recommended for the inhibition of Nek3 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0 x 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

Nek3 (E-10): sc-390872 is recommended as a control antibody for monitoring of Nek3 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) Immunofluo-rescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Nek3 gene expression knockdown using RT-PCR Primer: Nek3 (h)-PR: sc-43550-PR (20 μ l, 501 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

PROTOCOLS

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