

Nek3 siRNA (m): sc-149905

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 G₂ 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 G₀-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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2. Letwin, K., et al. 1992. A mammalian dual specificity protein kinase, Nek1, is related to the NIMA cell cycle regulator and highly expressed in meiotic germ cells. *EMBO J.* 11: 3521-3531.
3. 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.
4. Rhee, K. and Wolgemuth, D.J. 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.
5. Fry, A.M. and Nigg, E.A. 1997. Characterization of mammalian DNA-related kinases. *Methods Enzymol.* 283: 270-282.
6. Tanaka, K. and Nigg, E.A. 1999. Cloning and characterization of the murine Nek3 protein kinase, a novel member of the NIMA family of putative cell cycle regulators. *J. Biol. Chem.* 274: 13491-13497.
7. Chen, A., et al. 1999. NIMA-related kinases: isolation and characterization of murine Nek3 and Nek4 cDNAs, and chromosomal localization of Nek1, Nek2 and Nek3. *Gene* 234: 127-137.

CHROMOSOMAL LOCATION

Genetic locus: Nek3 (mouse) mapping to 8 A2.

PRODUCT

Nek3 siRNA (m) is a pool of 2 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 Nek3 shRNA Plasmid (m): sc-149905-SH and Nek3 shRNA (m) Lentiviral Particles: sc-149905-V as alternate gene silencing products.

For independent verification of Nek3 (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-149905A and sc-149905B.

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

Nek3 siRNA (m) is recommended for the inhibition of Nek3 expression in mouse 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

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 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 Nek3 gene expression knockdown using RT-PCR Primer: Nek3 (m)-PR: sc-149905-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.

PROTOCOLS

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