SANTA CRUZ BIOTECHNOLOGY, INC.

Nek6 siRNA (m): sc-61173

BACKGROUND

NIMA was originally shown in *Aspergillus nidulans* to be necessary for entry into mitosis. NIMA-related mammalian proteins have since been identified as Nek1-4 and Nek 6-9. High expression of Nek1 is seen in male and female germ cell lines of mice. Nek2 is the closest known mammalian relative to NIMA. Like NIMA, Nek2 expression peaks at the G_2 to M phase transition. Nek3, Nek6, Nek7 and Nek9 also regulate mitosis. Nek1 and Nek8 have been linked with polycystic kidney disease, and Nek4 expression is present in most primary carcinomas. Nek6 localizes to the cytoplasm and is expressed ubiquitously, with highest expression observed in the heart and skeletal muscle. It is activated during M phase and is required for chromosome segregation at the metaphase-anaphase transition and, consequently, mitotic progression.

REFERENCES

1. Osmani, S.A., Pu, R.T. and Morris, N.R. 1988. Mitotic induction and maintenance by overexpression of a G_2-specific gene that encodes a potential protein kinase. Cell 53: 237-244.
2. Letwin, K., Mizzen, L., Motro, B., Ben-David, Y., Bernstein, A. and Pawson, T. 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., Fry, A.M., Sutterlin, C., Ried, T. and Nigg, E.A. 1994. Cell cycle-dependent expression of Nek2, a novel human protein kinase related to the NIMA mitotic regulator of *Aspergillus nidulans*. Cell Growth Diff. 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. Charcterization 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.

CHROMOSOMAL LOCATION

Genetic locus: Nek6 (mouse) mapping to 2 B.

PRODUCT

Nek6 siRNA (m) 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 Nek6 shRNA Plasmid (m): sc-61173-SH and Nek6 shRNA (m) Lentiviral Particles: sc-61173-V as alternate gene silencing products.

For independent verification of Nek6 (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-61173A, sc-61173B and sc-61173C.

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

Nek6 siRNA (m) is recommended for the inhibition of Nek6 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 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

Nek6 (D-7): sc-374491 is recommended as a control antibody for monitoring of Nek6 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 Nek6 gene expression knockdown using RT-PCR Primer: Nek6 (m)-PR: sc-61173-PR (20 µl). 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.