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SANTA CRUZ BIOTECHNOLOGY, INC.

Nek5 siRNA (m): sc-149906

BACKGROUND

The phosphorylation and dephosphorylation of proteins on serine and threonine residues is an essential means of regulating a broad range of cellular functions in eukaryotes, including cell division, homeostasis and apoptosis. A group of proteins that are intimately involved in this process are the serine/threonine (Ser/Thr) protein kinases. Nek5 (NimA-related protein kinase 5) is a 708 amino acid protein that is related to NIMA, a protein that was originally discovered in Aspergillus nidulans and is necessary for entry into mitosis. One of several members of the Set/Thr protein kinase super family, Nek5 contains one protein kinase domain through which it catalyzes the ATP-dependent phosphorylation of target proteins. Like NIMA, Nek5 may be involved in mitotic regulation and cell cycle control.

REFERENCES

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4. Lu, K.P., et al. 1995. The NIMA kinase: a mitotic regulator in *Aspergillus nidulans* and vertebrate cells. Prog. Cell Cycle Res. 1: 187-205.
5. Li, J.J., et al. 2006. Mitotic kinases: the key to duplication, segregation, and cytokinesis errors, chromosomal instability, and oncogenesis. Pharmacol. Ther. 111: 974-984.
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8. Salaun, P., et al. 2008. Cdk1, Plks, Auroras, and Neks: the mitotic bodyguards. Adv. Exp. Med. Biol. 617: 41-56.

CHROMOSOMAL LOCATION

Genetic locus: Nek5 (mouse) mapping to 8 A2.

PRODUCT

Nek5 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 Nek5 shRNA Plasmid (m): sc-149906-SH and Nek5 shRNA (m) Lentiviral Particles: sc-149906-V as alternate gene silencing products.

For independent verification of Nek5 (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-149906A, sc-149906B and sc-149906C.

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

Nek5 siRNA (m) is recommended for the inhibition of Nek5 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

Nek5 (OC-65): sc-130492 is recommended as a control antibody for monitoring of Nek5 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 Nek5 gene expression knockdown using RT-PCR Primer: Nek5 (m)-PR: sc-149906-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.