SANTA CRUZ BIOTECHNOLOGY, INC.

Nek9 siRNA (h): sc-61178

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. Nek9 [NIMA (never in mitosis gene a)-related kinase 9], also known as serine/threonine-protein kinase Nek9, NERCC, NERCC1, MGC16714, MGC138306 or DKFZp434D0935, is a 979 amino acid protein that localizes to cytoplasm and nucleus. Highly expressed in liver, heart, kidney and testis, Nek9 is also expressed at lower levels in fibroblasts and smooth muscle cells. Nek9 regulates G_1/S transition and S phase progression by influencing spindle dynamics and chromosome separation. Nek9 phosphorylates different histones (Histone H3 on serine and threonine), myelin basic protein, β-casein (serine) and BICD2. Nek9 interacts with Ran GTPase, Nek6, Nek7, BICD2, SSRP1 and SUPT16H/FACT complex.

REFERENCES

1. Roig, J., et al. 2002. Nercc1, a mammalian NIMA-family kinase, binds the Ran GTPase and regulates mitotic progression. Genes Dev. 16: 1640-1658.
2. Belham, C., et al. 2003. A mitotic cascade of NIMA family kinases. Nercc1/Nek9 activates the Nek6 and Nek7 kinases. J. Biol. Chem. 278: 34897-34909.
3. O'Connell, M.J., et al. 2003. Never say never. The NIMA-related protein kinases in mitotic control. Trends Cell Biol. 13: 221-228.
4. Tan, B.C., et al. 2004. Nek9, a novel FACT-associated protein, modulates interphase progression. J. Biol. Chem. 279: 9321-9330.
5. Quarmby, L.M., et al. 2005. Caught Nek-ing: cilia and centrioles. J. Cell Sci. 118: 5161-5169.
6. Roig, J., et al. 2005. Active Nercc1 protein kinase concentrates at centrosomes early in mitosis and is necessary for proper spindle assembly. Mol. Biol. Cell 16: 4827-4840.

CHROMOSOMAL LOCATION

Genetic locus: NEK9 (human) mapping to 14q24.3.

PRODUCT

Nek9 siRNA (h) 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 Nek9 shRNA Plasmid (h): sc-61178-SH and Nek9 shRNA (h) Lentiviral Particles: sc-61178-V as alternate gene silencing products.

For independent verification of Nek9 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-61178A, sc-61178B and sc-61178C.

PROTOCOLS

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

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

Nek9 siRNA (h) is recommended for the inhibition of Nek9 expression in human 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

Nek9 (39-7): sc-100401 is recommended as a control antibody for monitoring of Nek9 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 Nek9 gene expression knockdown using RT-PCR Primer: Nek9 (h)-PR: sc-61178-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Phadke, M., et al. 2018. Dabrafenib inhibits the growth of BRAF-WT cancers through CDK16 and Nek9 inhibition. Mol. Oncol. 12: 74-88.

RESEARCH USE

For research use only, not for use in diagnostic procedures.