SANTA CRUZ BIOTECHNOLOGY, INC.

Ninein siRNA (h): sc-61195

BACKGROUND

Ninein is a centrosomal protein necessary for the positioning and anchorage of the microtubule minus-end in epithelial cells. The protein is presumably a centrosome maturation factor and may play a role in microtubule nucleation. Overexpression of Ninein does not alter nucleation or elongation of microtubules, but rather suppresses their release. Ninein associates with GSK3B (GSK3-β) via its C-terminal domain, and also interacts with C14orf166; the latter is thought to prevent phosphorylation of Ninein by GSK-3β. Ninein is a component of the core centrosome, where it is arranged in a tubular conformation with its open and closed ends contained within the centrosome. It demonstrates ubiquitous expression and shows predominant expression in heart and skeletal muscle tissues. The coiled-coil region from Asn 1611 to Pro 1693 is necessary for targeting Ninein to the centrosome.

REFERENCES

1. Hong, Y.R., et al. 2000. Cloning and characterization of a novel human Ninein protein that interacts with the glycogen synthase kinase 3β. Biochim. Biophys. Acta 1492: 513-516.
2. Nagase, T., et al. 2000. Prediction of the coding sequences of unidentified human genes. XVIII. The complete sequences of 100 new cDNA clones from brain which code for large proteins *in vitro*. DNA Res. 7: 273-281.
3. Hong, Y.R., et al. 2001. Genomic organization and molecular characterization of the human Ninein gene. Biochem. Biophys. Res. Commun. 279: 989-995.
4. Ou, Y.Y., et al. 2002. CEP110 and Ninein are located in a specific domain of the centrosome associated with centrosome maturation. J. Cell Sci. 115: 1825-1835.
5. Chen, C.H., et al. 2003. Molecular characterization of human Ninein protein: two distinct subdomains required for centrosomal targeting and regulating signals in cell cycle. Biochem. Biophys. Res. Commun. 308: 975-983.
6. Baird, D.H., et al. 2004. Distribution of the microtubule-related protein Ninein in developing neurons. Neuropharmacology 47: 677-683.
7. Stillwell, E.E., et al. 2004. Human Ninein is a centrosomal autoantigen recognized by CREST patient sera and plays a regulatory role in microtubule nucleation. Cell Cycle 3: 923-930.

CHROMOSOMAL LOCATION

Genetic locus: NIN (human) mapping to 14q22.1.

PRODUCT

Ninein 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 Ninein shRNA Plasmid (h): sc-61195-SH and Ninein shRNA (h) Lentiviral Particles: sc-61195-V as alternate gene silencing products.

For independent verification of Ninein (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-61195A, sc-61195B and sc-61195C.

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

Ninein siRNA (h) is recommended for the inhibition of Ninein 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

Ninein (F-5): sc-376420 is recommended as a control antibody for monitoring of Ninein 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 Ninein gene expression knockdown using RT-PCR Primer: Ninein (h)-PR: sc-61195-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. Jay, J., et al. 2015. JAK2 tyrosine kinase phosphorylates and is negatively regulated by centrosomal protein Ninein. Mol. Cell. Biol. 35: 111-131.

RESEARCH USE

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