SANTA CRUZ BIOTECHNOLOGY, INC.

NEDD1 siRNA (h): sc-72378

BACKGROUND

NEDD1 (neural precursor cell expressed, developmentally down-regulated 1), also known as GCP-WD, is a homolog of the *Drosophila* protein known as Dgp71WD. It is a ubiquitously expressed, evolutionarily conserved protein and contains eight WD40 repeats and a coiled coil domain at the C-terminus. NEDD1 is a subunit of the γ-tubulin ring complex (γTuRC) and plays an important role in mitosis. During mitosis NEDD1 is phosphorylated and functions in forming the association of γ-tubulin with the spindle. The state of phosphorylation of NEDD1 is also important for determining its cellular localization. NEDD1 is responsible for targeting γTuRC to the centrosome and spindle and is therefore required for centrosomal and chromatin-mediated microtubule nucleation. The inhibition of NEDD1 results in the loss of γTuRC from the centrosome and a sequential loss of microtubule nucleation. Due to its critical role in mitosis, NEDD1 may be a potential target for anticancer therapies.

REFERENCES

1. Kumar, S., et al. 1994. Induction of apoptosis by the mouse NEDD2 gene, which encodes a protein similar to the product of the *Caenorhabditis elegans* cell death gene Ced-3 and the mammalian IL-1 β-converting enzyme. Genes Dev. 8: 1613-1626.
2. Kumar, S., et al. 1994. Molecular cloning and biological activity of a novel developmentally regulated gene encoding a protein with β-transducin-like structure. J. Biol. Chem. 269: 11318-11326.
3. Takai, S., et al. 1995. Assignment of the developmentally regulated gene NEDD1 to human chromosome 12q22 by fluorescence *in situ* hybridization. Hum. Genet. 95: 96-98.
4. Koul, S., et al. 2004. Characteristic promoter hypermethylation signatures in male germ cell tumors. Mol. Cancer 1: 8.
5. Haren, L., et al. 2006. NEDD1-dependent recruitment of the γ-tubulin ring complex to the centrosome is necessary for centriole duplication and spindle assembly. J. Cell Biol. 172: 505-515.

CHROMOSOMAL LOCATION

Genetic locus: NEDD1 (human) mapping to 12q23.1.

PRODUCT

NEDD1 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 NEDD1 shRNA Plasmid (h): sc-72378-SH and NEDD1 shRNA (h) Lentiviral Particles: sc-72378-V as alternate gene silencing products.

For independent verification of NEDD1 (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-72378A, sc-72378B and sc-72378C.

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

NEDD1 siRNA (h) is recommended for the inhibition of NEDD1 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

NEDD1 (H-3): sc-398733 is recommended as a control antibody for monitoring of NEDD1 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 NEDD1 gene expression knockdown using RT-PCR Primer: NEDD1 (h)-PR: sc-72378-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.