SANTA CRUZ BIOTECHNOLOGY, INC.

NEDD4-L siRNA (h): sc-75894

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

NEDD4-L (neural precursor cell expressed, developmentally downregulated 4-like), also known as RSP5, NEDD4-2 or NEDL3, is a 975 amino acid protein that localizes to the cytoplasm and contains four WW domains, one HECT domain and one C2 domain. Expressed ubiquitously with highest expression in pancreas, prostate and kidney, NEDD4-L functions as an E3 ubiquitin-protein ligase that, characteristic of E3 ligase proteins, accepts ubiquitin (in the form of a thioester) from an E2 ubiquitin-conjugating enzyme and transfers that ubiquitin residue to substrates targeted for degradation. Through its ability to ubiquitinate and induce the proteasome-dependent degradation of proteins such as Smad2 and TGFβ RII, NEDD4-L is thought to inhibit the TGFβ signaling pathway, thereby regulating the signaling pathways that control cell growth and differentiation. NEDD4-L is expressed as eight isoforms due to alternative splicing events.

REFERENCES

1. Chen, H., et al. 2001. NEDD4-L on human chromosome 18q21 has multiple forms of transcripts and is a homologue of the mouse Nedd4-2 gene. Eur. J. Hum. Genet. 9: 922-930.
2. Harvey, K.F., et al. 2002. N4WBP5, a potential target for ubiquitination by the Nedd4 family of proteins, is a novel Golgi-associated protein. J. Biol. Chem. 277: 9307-9317.
3. Qi, H., et al. 2003. Androgens differentially regulate the expression of NEDD4-L transcripts in LNCaP human prostate cancer cells. Mol. Cell. Endocrinol. 210: 51-62.

CHROMOSOMAL LOCATION

Genetic locus: NEDD4L (human) mapping to 18q21.31.

PRODUCT

NEDD4-L 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 NEDD4-L shRNA Plasmid (h): sc-75894-SH and NEDD4-L shRNA (h) Lentiviral Particles: sc-75894-V as alternate gene silencing products.

For independent verification of NEDD4-L (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-75894A, sc-75894B and sc-75894C.

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

NEDD4-L siRNA (h) is recommended for the inhibition of NEDD4-L 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

NEDD4-L (C-8): sc-514954 is recommended as a control antibody for monitoring of NEDD4-L 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 NEDD4-L gene expression knockdown using RT-PCR Primer: NEDD4-L (h)-PR: sc-75894-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. Heise, C.J., et al. 2010. Serum and glucocorticoid-induced kinase (SGK) 1 and the epithelial sodium channel are regulated by multiple with no lysine (WNK) family members. J. Biol. Chem. 285: 25161-25167.
2. Kang, Y., et al. 2015. Regulation of the human ether-a-go-go-related gene (hERG) potassium channel by Nedd4 family interacting proteins (Ndfips). Biochem. J. 472: 71-82.
3. Chen, Y., et al. 2021. NEDD4L-induced ubiquitination mediating UBE2T degradation inhibits progression of lung adenocarcinoma via PI3K-Akt signaling. Cancer Cell Int. 21: 631.

RESEARCH USE

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