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# NEDD4-L siRNA (m): sc-149898

## BACKGROUND

NEDD4-L (neural precursor cell expressed, developmentally down-regulated 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 TGF $\beta$  RII, NEDD4-L is thought to inhibit the TGF $\beta$  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. NEDD4L 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 NEDD4L transcripts in LNCaP human prostate cancer cells. *Mol. Cell. Endocrinol.* 210: 51-62.
4. van Bemmelen, M.X., et al. 2004. Cardiac voltage-gated sodium channel Nav1.5 is regulated by Nedd4-2 mediated ubiquitination. *Circ. Res.* 95: 284-291.
5. Hryciw, D.H., et al. 2004. Nedd4-2 functionally interacts with CIC-5: involvement in constitutive albumin endocytosis in proximal tubule cells. *J. Biol. Chem.* 279: 54996-55007.
6. Ichimura, T., et al. 2005. 14-3-3 proteins modulate the expression of epithelial Na<sup>+</sup> channels by phosphorylation-dependent interaction with Nedd4-2 ubiquitin ligase. *J. Biol. Chem.* 280: 13187-13194.

## CHROMOSOMAL LOCATION

Genetic locus: Nedd4l (mouse) mapping to 18 E1.

## PRODUCT

NEDD4-L 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NEDD4-L shRNA Plasmid (m): sc-149898-SH and NEDD4-L shRNA (m) Lentiviral Particles: sc-149898-V as alternate gene silencing products.

For independent verification of NEDD4-L (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-149898A, sc-149898B and sc-149898C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

NEDD4-L siRNA (m) is recommended for the inhibition of NEDD4-L 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  $\mu$ M in 66  $\mu$ l. 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-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>TM</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> 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 (m)-PR: sc-149898-PR (20  $\mu$ l). 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](http://www.scbt.com) for detailed protocols and support products.