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SZABO-SCANDIC Handels GmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

NEDL1 siRNA (m): sc-149899

BACKGROUND

NEDL1 (NEDD4-like ubiquitin-protein ligase 1), also known as HECW1 (HECT, C2 and WW domain containing E3 ubiquitin protein ligase 1), is a 1,606 amino acid cytoplasmic protein predominantly expressed in neurons of adult and fetal brain. NEDL1 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. NEDL1 mediates ubiquitination and subsequent degradation of Dvl-1 and targets mutant SOD-1. NEDL1 forms cytotoxic aggregates with Dvl, TRAP-γ and mutant SOD1 that lead to motor neuron death in FALS (familial amyotrophic lateral sclerosis). Individuals affected by FALS (also known as Lou Gehrig's disease) experience muscle weakness and atrophy throughout the body. FALS is caused by the degeneration of upper and lower motor neurons resulting in loss of signal to muscles.

REFERENCES

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2. Al-Chalabi, A. and Leigh, P.N. 2000. Recent advances in amyotrophic lateral sclerosis. *Curr. Opin. Neurol.* 13: 397-405.
3. Miyazaki, K., et al. 2004. NEDL1, a novel ubiquitin-protein isopeptide ligase for dishevelled-1, targets mutant superoxide dismutase-1. *J. Biol. Chem.* 279: 11327-11335.
4. Sang, Q., et al. 2006. NEDD4-WW domain-binding protein 5 (NDFIP1) is associated with neuronal survival after acute cortical brain injury. *J. Neurosci.* 26: 7234-7244.
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6. Phukan, J., et al. 2007. Cognitive impairment in amyotrophic lateral sclerosis. *Lancet Neurol.* 6: 994-1003.
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CHROMOSOMAL LOCATION

Genetic locus: Hecw1 (mouse) mapping to 13 A1.

PRODUCT

NEDL1 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NEDL1 shRNA Plasmid (m): sc-149899-SH and NEDL1 shRNA (m) Lentiviral Particles: sc-149899-V as alternate gene silencing products.

For independent verification of NEDL1 (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-149899A, sc-149899B and sc-149899C.

RESEARCH USE

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

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

NEDL1 siRNA (m) is recommended for the inhibition of NEDL1 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 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NEDL1 gene expression knockdown using RT-PCR Primer: NEDL1 (m)-PR: sc-149899-PR (20 μl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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