

NEDL2 siRNA (h): sc-95007

BACKGROUND

NEDL2 (NEDD4-like E3 ubiquitin-protein ligase 2), also known as HECW2 (HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2), is a 1,572 amino acid cytoplasmic protein that is involved in protein modification and ubiquitination and exists as two alternatively spliced isoforms. NEDL2 acts as an E3 ubiquitin-protein ligase that enhances p73 transcription activation, ubiquitination and stabilization, and is highly expressed in adult heart, lung and brain. Containing one C2 domain, two WW domains and a single HECT (E6AP-type E3 ubiquitin-protein ligase) domain, NEDL2 is encoded by a gene that maps to human chromosome 2, which consists of 237 million bases, encodes over 1,400 genes and makes up approximately 8% of the human genome. A number of genetic diseases are linked to genes on chromosome 2 including Harlequin ichthyosis, sitosterolemia and Alström syndrome.

REFERENCES

- Patel, S.B., et al. 1998. Mapping a gene involved in regulating dietary cholesterol absorption. The sitosterolemia locus is found at chromosome 2p21. *J. Clin. Invest.* 102: 1041-1044.
- Shulenin, S., et al. 2001. An ATP-binding cassette gene (ABCG5) from the ABCG (white) gene subfamily maps to human chromosome 2p21 in the region of the sitosterolemia locus. *Cytogenet. Cell Genet.* 92: 204-208.
- Hearn, T., et al. 2002. Mutation of ALMS1, a large gene with a tandem repeat encoding 47 amino acids, causes Alström syndrome. *Nat. Genet.* 31: 79-83.
- Miyazaki, K., et al. 2003. A novel HECT-type E3 ubiquitin ligase, NEDL2, stabilizes p73 and enhances its transcriptional activity. *Biochem. Biophys. Res. Commun.* 308: 106-113.
- Kelsell, D.P., et al. 2005. Mutations in ABCA12 underlie the severe congenital skin disease harlequin ichthyosis. *Am. J. Hum. Genet.* 76: 794-803.
- Oberst, A., et al. 2005. Regulation of the p73 protein stability and degradation. *Biochem. Biophys. Res. Commun.* 331: 707-712.

CHROMOSOMAL LOCATION

Genetic locus: HECW2 (human) mapping to 2q32.3.

PRODUCT

NEDL2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NEDL2 shRNA Plasmid (h): sc-95007-SH and NEDL2 shRNA (h) Lentiviral Particles: sc-95007-V as alternate gene silencing products.

For independent verification of NEDL2 (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-95007A, sc-95007B and sc-95007C.

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

APPLICATIONS

NEDL2 siRNA (h) is recommended for the inhibition of NEDL2 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 μ M in 66 μ 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NEDL2 gene expression knockdown using RT-PCR Primer: NEDL2 (h)-PR: sc-95007-PR (20 μ 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.