

NLE1 siRNA (h): sc-62691

BACKGROUND

The notch signaling pathway is an evolutionary conserved system that is involved in intracellular communication. Notch receptors play an important role in development and cell-fate decisions. Notchless is a loss-of-function mutant allele that encodes for protein Nle1 (notchless homolog 1). Nle1 is a 485 amino acid WD40-repeat protein that binds to the cytoplasmic domain of Notch, regulating its signaling activity in *Drosophila melanogaster* and in mice. Deletion of the Nle1 gene in mice during the early stages of development results in embryonic death, while gene deletion in the late stages of development leads to activation of a caspase-3-dependent apoptotic pathway. In plants, Nle1 is crucial for normal cellular growth and development. Underexpression during shoot proliferation causes pleiotropic defects such as delayed flowering and abnormal organ maturation. It may also play a role in 60S ribosomal subunit biogenesis in yeast. Nle1 contains eight WD40 domains and produces one isoform due to alternative splicing.

REFERENCES

1. Royet, J., Bouwmeester, T. and Cohen, S.M. 1998. Notchless encodes a novel WD40-repeat-containing protein that modulates Notch signaling activity. *EMBO J.* 17: 7351-7360.
2. Nal, B., Mohr, E., Silva, M.I., Tagett, R., Navarro, C., Carroll, P., Depetris, D., Verthuy, C., Jordan, B.R. and Ferrier, P. 2002. Wdr12, a mouse gene encoding a novel WD-repeat protein with a notchless-like amino-terminal domain. *Genomics* 79: 77-86.
3. Cormier, S., Le Bras, S., Souilhol, C., Vandormael-Pournin, S., Durand, B., Babinet, C., Baldacci, P. and Cohen-Tannoudji, M. 2006. The murine ortholog of notchless, a direct regulator of the notch pathway in *Drosophila melanogaster*, is essential for survival of inner cell mass cells. *Mol. Cell Biol.* 26: 3541-3549.
4. Chantha, S.C., Emerald, B.S. and Matton, D.P. 2006. Characterization of the plant Notchless homolog, a WD repeat protein involved in seed development. *Plant Mol. Biol.* 62: 897-912.
5. Chantha, S.C. and Matton, D.P. 2007. Underexpression of the plant NOTCHLESS gene, encoding a WD-repeat protein, causes pleiotropic phenotype during plant development. *Planta* 225: 1107-1120.

CHROMOSOMAL LOCATION

Genetic locus: NLE1 (human) mapping to 17q12.

PRODUCT

NLE1 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 NLE1 shRNA Plasmid (h): sc-62691-SH and NLE1 shRNA (h) Lentiviral Particles: sc-62691-V as alternate gene silencing products.

For independent verification of NLE1 (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-62691A, sc-62691B and sc-62691C.

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

NLE1 siRNA (h) is recommended for the inhibition of NLE1 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.

GENE EXPRESSION MONITORING

NLE1 (G-5): sc-377142 is recommended as a control antibody for monitoring of NLE1 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 κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 NLE1 gene expression knockdown using RT-PCR Primer: NLE1 (h)-PR: sc-62691-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.

PROTOCOLS

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