

Nup98 siRNA (h): sc-43535

BACKGROUND

Nuclear pore complexes (NPCs) are the channels for the bi-directional movement of macromolecules between the nucleus and cytoplasm, and contain more than 100 different subunits. Many of them belong to a family called nucleoporins, which are characterized by the presence of O-linked N-acetylglucosamine moieties and a distinctive pentapeptide repeat (XFXFG). The Nup98 gene encodes precursor proteins that generate two nucleoplasmically oriented nucleoporins, Nup98 and Nup96. The O-linked glycoprotein, Nup98 is a component of the nuclear pore complex. Nup98 is essential for gastrulation, a developmental stage that is associated with rapid cell proliferation, but dispensable for basal cell growth. Nup98 plays a role in RNA export from the nucleus and it appears to be an essential component of multiple RNA export pathways. Nup98 is a member of the GLFG nucleoporin family. The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for Nup98 and class I homeoprotein HoxA9. Nup98-HoxA9 fusion protein may promote leukemogenesis through inhibiting of HoxA9-mediated terminal differentiation and/or aberrant nucleocytoplasmic transport.

REFERENCES

1. Radu, A., et al. 1995. The peptide repeat domain of nucleoporin Nup98 functions as a docking site in transport across the nuclear pore complex. *Cell* 81: 215-222.
2. Borrow, J., et al. 1996. The t(7;11)(p15;p15) translocation in acute myeloid leukaemia fuses the genes for nucleoporin Nup98 and class I homeoprotein HoxA9. *Nat. Genet.* 12: 159-167.

CHROMOSOMAL LOCATION

Genetic locus: NUP98 (human) mapping to 11p15.4.

PRODUCT

Nup98 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 Nup98 shRNA Plasmid (h): sc-43535-SH and Nup98 shRNA (h) Lentiviral Particles: sc-43535-V as alternate gene silencing products.

For independent verification of Nup98 (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-43535A, sc-43535B and sc-43535C.

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

Nup98 siRNA (h) is recommended for the inhibition of Nup98 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

Nup98 (C-7): sc-74553 is recommended as a control antibody for monitoring of Nup98 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 Nup98 gene expression knockdown using RT-PCR Primer: Nup98 (h)-PR: sc-43535-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Funasaka, T., et al. 2011. RNA export factor RAE1 contributes to Nup98-HOXA9-mediated leukemogenesis. *Cell Cycle* 10: 1456-1467.
2. Funasaka, T., et al. 2013. Nucleoporin Nup98 mediates galectin-3 nuclear-cytoplasmic trafficking. *Biochem. Biophys. Res. Commun.* 434: 155-161.

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.