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Nup98 shRNA (h) Lentiviral Particles: sc-43535-V

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.
3. Powers, M.A., et al. 1997. The vertebrate GLFG nucleoporin, Nup98, is an essential component of multiple RNA export pathways. *J. Cell Biol.* 136: 241-250.
4. Fontoura, B.M., et al. 2001. The nucleoporin Nup98 associates with the intranuclear filamentous protein network of TPR. *Proc. Natl. Acad. Sci. USA* 98: 3208-3213.

CHROMOSOMAL LOCATION

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

PRODUCT

Nup98 shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Nup98 siRNA (h): sc-43535 and Nup98 shRNA Plasmid (h): sc-43535-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Nup98 shRNA (h) Lentiviral Particles is recommended for the inhibition of Nup98 expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 $55-60^{\circ}$ C and the extension temperature should be $68-72^{\circ}$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

PROTOCOLS

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