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SZABO-SCANDIC HandelsgmbH

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) 

Nogo shRNA (h) Lentiviral Particles: sc-43974-V

BACKGROUND

CNS white matter is selectively inhibitory for axonal out-growth. Nogo (also designated NI250 and Reticulon 4-A) is an oligodendrocyte-specific member of the Reticulon family and is a component of CNS white matter that inhibits axon outgrowth, induces collapse of growth cones of chick dorsal root ganglion cells, and inhibits the spreading of 3T3 fibroblasts. Other members of the reticulon protein family do not inhibit axon extension and are thought to have a role in ER function. Nogo is expressed by oligodendrocytes but not by Schwann cells, and associates primarily with the endoplasmic reticulum. Nogo exists in three different splice forms, Nogo-A, -B and -C.

REFERENCES

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- van de Velde, H.J., et al. 1994. NSP-encoded reticulons, neuroendocrine proteins of a novel gene family associated with membranes of the endoplasmic reticulum. *J. Cell Sci.* 107: 2403-2416.
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CHROMOSOMAL LOCATION

Genetic locus: RTN4 (human) mapping to 2p16.1.

PRODUCT

Nogo 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 Nogo siRNA (h): sc-43974 and Nogo shRNA Plasmid (h): sc-43974-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

Nogo shRNA (h) Lentiviral Particles is recommended for the inhibition of Nogo 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

Nogo (C-4): sc-271878 is recommended as a control antibody for monitoring of Nogo 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 IgM-HRP: sc-2064 (dilution range: 1:500-1:5,000), TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgM-FITC: sc-2082 (dilution range: 1:100-1:400) or goat anti-mouse IgM-TR: sc-2983 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Nogo gene expression knockdown using RT-PCR Primer: Nogo (h)-PR: sc-43974-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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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