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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 siRNA (h): sc-43974

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

- Schwab, M.E., et al. 1985. Dissociated neurons regenerate into sciatic but not optic nerve explants in culture irrespective of neurotrophic factors. *J. Neurosci.* 5: 2415-2423.
- Schwab, M.E., et al. 1988. Oligodendrocytes and CNS myelin are nonpermissive substrates for neurite growth and fibroblast spreading *in vitro*. *J. Neurosci.* 8: 2381-2393.
- Caroni, P., et al. 1988. Two membrane protein fractions from rat central myelin with inhibitory properties for neurite growth and fibroblast spreading. *J. Cell Biol.* 106: 1281-1288.
- 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.
- Spillmann, A.A., et al. 1998. Identification and characterization of a bovine neurite growth inhibitor (bNI-220). *J. Biol. Chem.* 273: 19283-19293.

CHROMOSOMAL LOCATION

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

PRODUCT

Nogo 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 Nogo shRNA Plasmid (h): sc-43974-SH and Nogo shRNA (h) Lentiviral Particles: sc-43974-V as alternate gene silencing products.

For independent verification of Nogo (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-43974A, sc-43974B and sc-43974C.

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

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

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

SELECT PRODUCT CITATIONS

- Lee, J.E., et al. 2012. Nongenomic STAT5-dependent effects on Golgi apparatus and endoplasmic reticulum structure and function. *Am. J. Physiol., Cell Physiol.* 302: C804-C820.
- Rodríguez-Feo, J.A., et al. 2015. A new role for reticulon-4B/NOGO-B in the intestinal epithelial barrier function and inflammatory bowel disease. *Am. J. Physiol. Gastrointest. Liver Physiol.* 308: G981-G993.

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.