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neurocan siRNA (h): sc-41901

BACKGROUND

The lecticans are a family of chondroitin proteoglycans, including aggrecan, versican, neurocan and brevican, that contain a C-type lectin domain. Neurocan is a glycoprotein synthesized primarily by neurons, and its expression levels are highest during embryonic brain development and during the early postnatal period. Neurocan is a component of the extracellular matrix in the central nervous system that can bind to various other CNS matrix components, such as heparin, Tenascin-R, HB-GAM and NCAM, suggesting that it plays a role in axon guidance and neurite growth. Neurocan is a 1,257 amino acid, precursor protein in embryonic and neonatal rats that is proteolytically processed in the adult CNS into an N-terminal fragment, which localizes to the cytoplasm of glial cells. Neurocan expression (including the re-expression of the neonatal isoform) increases significantly in adults in the glial scar resulting from cortical injury.

REFERENCES

1. Retzler, C., et al. 1996. Structural and electron microscopic analysis of neurocan and recombinant neurocan fragments. *J. Biol. Chem.* 271: 17107-17113.
2. Milev, P., et al. 1998. High affinity binding and overlapping localization of neurocan and phosphacan/protein-tyrosine phosphatase- ζ/β with Tenascin-R, amphoterin, and the heparin-binding growth associated molecule. *J. Biol. Chem.* 273: 6998-7005.
3. Milev, P., et al. 1998. Differential regulation of expression of hyaluronan-binding proteoglycans in developing brain: aggrecan, versican, neurocan, and brevican. *Biochem. Biophys. Res. Commun.* 247: 207-212.
4. Matsui, F., et al. 1998. Occurrence of a N-terminal proteolytic fragment of neurocan, not a C-terminal half, in perineuronal net in the adult rat cerebrum. *Brain Res.* 790: 45-51.
5. McKeon, R., et al. 1999. The chondroitin sulfate proteoglycans neurocan and phosphacan are expressed by reactive astrocytes in the chronic CNS glial scar. *J. Neurosci.* 19: 10778-10788.
6. Rauch, U., et al. 2001. Neurocan: a brain chondroitin sulfate proteoglycan. *Cell. Mol. Life Sci.* 58: 1842-1856.

CHROMOSOMAL LOCATION

Genetic locus: NCAN (human) mapping to 19p13.11.

PRODUCT

neurocan 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 neurocan shRNA Plasmid (h): sc-41901-SH and neurocan shRNA (h) Lentiviral Particles: sc-41901-V as alternate gene silencing products.

For independent verification of neurocan (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-41901A, sc-41901B and sc-41901C.

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

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

neurocan (C-9): sc-515237 is recommended as a control antibody for monitoring of neurocan 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 neurocan gene expression knockdown using RT-PCR Primer: neurocan (h)-PR: sc-41901-PR (20 μ l, 558 bp). 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.