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netrin-4 siRNA (h): sc-44504

BACKGROUND

Netrin proteins are a family of laminin-related secreted proteins that provide guidance signals for axonal growth and cell migration during development. Netrin signaling is dependent on the concentration of calcium outside the cell and the level of PKA activity. In axonal cells, a reduction in PKA activity converts the responsiveness of the axons to the netrin proteins, as the cells are repelled, rather than attracted, by the netrin gradient. Netrin-4 is related to the Laminin β chains, and is therefore also designated β -netrin. It is present in the basement membranes of the vasculature, lateral olfactory tract, kidney and ovary. In humans, the gene encoding for the netrin-4 protein is localized to chromosome 12q22. High levels of netrin-4 mRNA have also been detected in many cells and tissues, including cerebral cortex, hippocampus, amygdaloid nuclei and Purkinje cells. Netrin-4 is important in neural, kidney and vascular development.

REFERENCES

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- Van Raay, T.J., et al. 1997. The NTN2L gene encoding a novel human netrin maps to the autosomal dominant polycystic kidney disease region on chromosome 16p13.3. *Genomics* 41: 279-282.
- Wang, H., et al. 1999. Netrin-3, a mouse homolog of human NTN2L, is highly expressed in sensory ganglia and shows differential binding to netrin receptors. *J. Neurosci.* 19: 4938-4947.
- Koch, M., et al. 2000. A novel member of the netrin family, β -netrin, shares homology with the β chain of Laminin: identification, expression, and functional characterization. *J. Cell Biol.* 151: 221-234.
- Liu, Y., et al. 2004. Novel role for netrins in regulating epithelial behavior during lung branching morphogenesis. *Curr. Biol.* 14: 897-905.
- Zhang, C., et al. 2004. Identification of a novel alternative splicing form of human netrin-4 and analyzing the expression patterns in adult rat brain. *Brain Res. Mol. Brain Res.* 130: 68-80.

CHROMOSOMAL LOCATION

Genetic locus: NTN4 (human) mapping to 12q22.

PRODUCT

netrin-4 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 netrin-4 shRNA Plasmid (h): sc-44504-SH and netrin-4 shRNA (h) Lentiviral Particles: sc-44504-V as alternate gene silencing products.

For independent verification of netrin-4 (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-44504A, sc-44504B and sc-44504C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

netrin-4 siRNA (h) is recommended for the inhibition of netrin-4 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

netrin-4 (A-7): sc-365280 is recommended as a control antibody for monitoring of netrin-4 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 netrin-4 gene expression knockdown using RT-PCR Primer: netrin-4 (h)-PR: sc-44504-PR (20 μ l, 449 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.