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# netrin-1 siRNA (h): sc-42044

## BACKGROUND

Netrin proteins are a family of Laminin-related secreted proteins that provide guidance signals for axonal growth and cell migration during development. Netrin-1, which is the mammalian homolog of UNC-6 from *C. elegans*, is largely expressed in the developing nervous system and in mesodermal tissues. Netrin-1 is expressed by the floor plate as either a cell-associated protein or in a diffusible form, and it binds to several surface receptor components, including deleted in colorectal cancer (DCC) and neogenin. During embryonic development, netrin-1 diffuses through the neuronal epithelium, where it forms a chemoattractant gradient that directs axonal migration to the ventral midline of the spinal cord. Netrin-2 and the corresponding mouse homolog netrin-3 are expressed primarily in the lower two-thirds of the spinal cord, and, like netrin-1, they can either attract or repel commissural axons at a distance. 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.

## REFERENCES

- Kennedy, T.E., et al. 1994. Netrins are diffusible chemotropic factors for commissural axons in the embryonic spinal cord. *Cell* 78: 425-435.
- Colamarino, S.A., et al. 1995. The axonal chemoattractant netrin-1 is also a chemorepellent for trochlear motor axons. *Cell* 81: 621-629.
- Livesey, F.J., et al. 1997. Netrin and netrin receptor expression in the embryonic mammalian nervous system suggests roles in retinal, striatal, nigral, and cerebellar development. *Mol. Cell. Neurosci.* 8: 417-429.

## CHROMOSOMAL LOCATION

Genetic locus: NTN1 (human) mapping to 17p13.1.

## PRODUCT

netrin-1 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see netrin-1 shRNA Plasmid (h): sc-42044-SH and netrin-1 shRNA (h) Lentiviral Particles: sc-42044-V as alternate gene silencing products.

For independent verification of netrin-1 (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-42044A, sc-42044B and sc-42044C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

netrin-1 siRNA (h) is recommended for the inhibition of netrin-1 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  $\mu$ M in 66  $\mu$ 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-1 (5H8): sc-293197 is recommended as a control antibody for monitoring of netrin-1 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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-1 gene expression knockdown using RT-PCR Primer: netrin-1 (h)-PR: sc-42044-PR (20  $\mu$ l, 428 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

- Fitamant, J., et al. 2008. Netrin-1 expression confers a selective advantage for tumor cell survival in metastatic breast cancer. *Proc. Natl. Acad. Sci. USA* 105: 4850-4855.
- Delloye-Bourgeois, C., et al. 2009. Interference with netrin-1 and tumor cell death in non-small cell lung cancer. *J. Natl. Cancer Inst.* 101: 237-247.
- Delloye-Bourgeois, C., et al. 2009. Netrin-1 acts as a survival factor for aggressive neuroblastoma. *J. Exp. Med.* 206: 833-847.

## RESEARCH USE

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

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.