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netrin-4 shRNA (h) Lentiviral Particles: sc-44504-V

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-q23. 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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- 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.
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CHROMOSOMAL LOCATION

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

PRODUCT

netrin-4 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 netrin-4 siRNA (h): sc-44504 and netrin-4 shRNA Plasmid (h): sc-44504-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

netrin-4 shRNA (h) Lentiviral Particles is recommended for the inhibition of netrin-4 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

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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker[™] compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker[™] Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz[™] Mounting Medium: sc-24941.

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 $55-60^\circ\text{C}$ 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.

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