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NGF shRNA (h) Lentiviral Particles: sc-43970-V

BACKGROUND

Neurotrophins function to regulate naturally occurring cell death of neurons during development. The prototype neurotrophin is nerve growth factor (NGF), originally discovered in the 1950s as a soluble peptide promoting the survival of, and neurite outgrowth from, sympathetic ganglia. Three additional structurally homologous neurotrophic factors have been identified. These include brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4 (NT-4) (also designated NT-5). These various neurotrophins stimulate the *in vitro* survival of distinct, but partially overlapping, populations of neurons. The cell surface receptors through which neurotrophins mediate their activity have been identified. For instance, the Trk A receptor is the preferential receptor for NGF, but also binds NT-3 and NT-4. The Trk B receptor binds both BDNF and NT-4 equally well, and binds NT-3 to a lesser extent, while the Trk C receptor only binds NT-3.

REFERENCES

1. Oppenheim, R.W. 1991. Cell death during development of the nervous system. *Annu. Rev. Neurosci.* 14: 453-501.
2. Thoenen, H. 1991. The changing scene of neurotrophic factors. *Trends Neurosci.* 14: 165-170.
3. Chao, M.V. 1992. Neurotrophin receptors: a window into neuronal differentiation. *Neuron* 9: 583-593.
4. Korsching, S. 1993. The neurotrophic factor concept: a reexamination. *J. Neurosci.* 13: 2739-2748.
5. Ip, N.Y., et al. 1993. Similarities and differences in the way neurotrophins interact with the Trk receptors in neuronal and nonneuronal cells. *Neuron* 10: 137-149.

CHROMOSOMAL LOCATION

Genetic locus: NGF (human) mapping to 1p13.2.

PRODUCT

NGF 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 NGF siRNA (h): sc-43970 and NGF shRNA Plasmid (h): sc-43970-SH as alternate gene silencing products.

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.

APPLICATIONS

NGF shRNA (h) Lentiviral Particles is recommended for the inhibition of mature NGF 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

NGF (E-12): sc-365944 is recommended as a control antibody for monitoring of NGF 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 mature NGF gene expression knockdown using RT-PCR Primer: NGF (h)-PR: sc-43970-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

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.

PROTOCOLS

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