

CNTFR α siRNA (m): sc-35077

BACKGROUND

Ciliary neurotrophic factor, or CNTF, is a neurotrophic cytokine that promotes the survival and differentiation of a number of cell types including sensory, sympathetic and motor neurons. CNTF, LIF and IL-6 belong to a family of cytokines that share structural homology and signal through identical receptor components. The CNTF receptor (CNTFR) is comprised of CNTFR α , a CNTFR-specific chain, and a heterodimer of the gp130 chain common to the IL-6 and LIF receptor and the LIFR β chain. The CNTFR complex has been shown to augment DNA synthesis through the activation of transcription factors Stat1 and Stat3. CNTF has been implicated as a protein involved in the pathogenesis of amyotrophic lateral sclerosis, or ALS. However, unlike mice lacking CNTF, mice containing a homozygous null mutation in the gene encoding the CNTFR α chain die perinatally and display severe motor neuron deficits. This data suggests the existence of a second CNTFR ligand that plays a critical role in development of the neonatal nervous system.

REFERENCES

1. He, C., et al. 1995. Preparation and a structure-function analysis of human ciliary neurotrophic factor. *Neurosci. Res.* 23: 327-333.
2. Saggio, I., et al. 1995. CNTF variants with increased biological potency and receptor selectivity define a functional site of receptor interaction. *EMBO J.* 14: 3045-3054.
3. De Serio, A., et al. 1995. *In vitro* binding of ciliary neurotrophic factor to its receptors: evidence for the formation of an IL-6-type hexameric complex. *J. Mol. Biol.* 254: 795-800.
4. Orrell, R.W., et al. 1995. Investigation of a null mutation of the CNTF gene in familial amyotrophic lateral sclerosis. *J. Neurol. Sci.* 132: 126-128.

CHROMOSOMAL LOCATION

Genetic locus: *Cntfr* (mouse) mapping to 4 A5.

PRODUCT

CNTFR α siRNA (m) 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 CNTFR α shRNA Plasmid (m): sc-35077-SH and CNTFR α shRNA (m) Lentiviral Particles: sc-35077-V as alternate gene silencing products.

For independent verification of CNTFR α (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35077A, sc-35077B and sc-35077C.

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.

STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20 $^{\circ}$ C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20 $^{\circ}$ 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

CNTFR α shRNA (m) Lentiviral Particles is recommended for the inhibition of CNTFR α expression in mouse 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

CNTFR α (AN-B2): sc-9993 is recommended as a control antibody for monitoring of CNTFR α 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 CNTFR α gene expression knockdown using RT-PCR Primer: CNTFR α (m)-PR: sc-35077-PR (20 μ l). Annealing temperature for the primers should be 55-60 $^{\circ}$ C and the extension temperature should be 68-72 $^{\circ}$ C.