

GFR α -3 siRNA (h): sc-41972

BACKGROUND

Glial cell line-derived neurotrophic factor (GDNF) and the related neurotrophic factor neurturin (NTN) are potent survival factors for central and peripheral neurons. GDNF is a glycosylated, disulfide-bonded homodimer that is distantly related to the TGF β superfamily of growth factors. Three receptors for these factors, GFR α -1 (also designated GDNFR- α , RETL1 or TrnR-1), GFR α -2 (also designated GDNFR- β , RETL2, NTNR- α or TrnR-2) and GFR α -3 have been identified. The receptors do not contain transmembrane domains and are attached to the cell membrane by glycosyl-phosphoinositol linkage. Both GFR α -1 and GFR α -2 have been shown to mediate the GDNF-dependent and NTN-dependent phosphorylation and activation of the tyrosine kinase Ret. GFR α -3 is expressed only during development.

REFERENCES

1. Lin, L.F., et al. 1993. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. *Science* 260: 1130-1132.
2. Jing, S., et al. 1996. GDNF-induced activation of the Ret protein tyrosine kinase is mediated by GDNFR- α , a novel receptor for GDNF. *Cell* 85: 1113-1124.
3. Treanor, J.J., et al. 1996. Characterization of a multi-component receptor for GDNF. *Nature* 382: 80-83.
4. Kotzbauer, P.T., et al. 1996. Neurturin, a relative of glial-cell-line-derived neurotrophic factor. *Nature* 384: 467-470.
5. Baloh, R.H., et al. 1997. TrnR2, a novel receptor that mediates neurturin and GDNF signaling through Ret. *Neuron* 18: 793-802.
6. Naveilhan, P., et al. 1998. Expression and regulation of GFR α -3, a glial cell line-derived neurotrophic factor family receptor. *Proc. Natl. Acad. Sci. USA* 95: 1295-1300.

CHROMOSOMAL LOCATION

Genetic locus: GFRA3 (human) mapping to 5q31.2.

PRODUCT

GFR α -3 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 GFR α -3 shRNA Plasmid (h): sc-41972-SH and GFR α -3 shRNA (h) Lentiviral Particles: sc-41972-V as alternate gene silencing products.

For independent verification of GFR α -3 (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-41972A, sc-41972B and sc-41972C.

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

GFR α -3 siRNA (h) is recommended for the inhibition of GFR α -3 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

GFR α -3 (C-3): sc-398618 is recommended as a control antibody for monitoring of GFR α -3 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 GFR α -3 gene expression knockdown using RT-PCR Primer: GFR α -3 (h)-PR: sc-41972-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.