

# GFR $\alpha$ -3 siRNA (m): sc-41973

## 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 $\beta$  superfamily of growth factors. Three receptors for these factors, GFR $\alpha$ -1 (also designated GDNFR- $\alpha$ , RETL1 or TrnR-1), GFR $\alpha$ -2 (also designated GDNFR- $\beta$ , RETL2, NTN- $\alpha$  or TrnR-2) and GFR $\alpha$ -3 have been identified. The receptors do not contain transmembrane domains and are attached to the cell membrane by glycosyl-phosphoinositol linkage. Both GFR $\alpha$ -1 and GFR $\alpha$ -2 have been shown to mediate the GDNF-dependent and NTN-dependent phosphorylation and activation of the tyrosine kinase Ret. GFR $\alpha$ -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- $\alpha$ , 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 $\alpha$ -3, a glial cell line-derived neurotrophic factor family receptor. *Proc. Natl. Acad. Sci. USA* 95: 1295-1300.

## CHROMOSOMAL LOCATION

Genetic locus: Gfra3 (mouse) mapping to 18 B1.

## PRODUCT

GFR $\alpha$ -3 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see GFR $\alpha$ -3 shRNA Plasmid (m): sc-41973-SH and GFR $\alpha$ -3 shRNA (m) Lentiviral Particles: sc-41973-V as alternate gene silencing products.

For independent verification of GFR $\alpha$ -3 (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-41973A, sc-41973B and sc-41973C.

## 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.

## 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  $\mu$ 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

GFR $\alpha$ -3 siRNA (m) is recommended for the inhibition of GFR $\alpha$ -3 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  $\mu$ 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

GFR $\alpha$ -3 (C-3): sc-398618 is recommended as a control antibody for monitoring of GFR $\alpha$ -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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> 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<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor GFR $\alpha$ -3 gene expression knockdown using RT-PCR Primer: GFR $\alpha$ -3 (m)-PR: sc-41973-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60 $^{\circ}$  C and the extension temperature should be 68-72 $^{\circ}$  C.