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# Neuronatin siRNA (h): sc-43966

## BACKGROUND

The paternally imprinted Neuronatin gene (NNAT) is initially expressed in rhombomeres and the pituitary gland and is later expressed more widely, but much less abundantly, in the central and peripheral nervous systems. The human NNAT gene maps to chromosome 20q11.23 and contains an imprinting region associated with morphological abnormalities and early neonatal lethality. Specifically, hypermethylation of the NNAT gene occurs in both myeloid and lymphoid acute pediatric leukemias and may inhibit NNAT expression. The Neuronatin protein consists of two isoforms,  $\alpha$  and  $\beta$ , which are the products of alternative splicing. The  $\alpha$  form of the Neuronatin gene is encoded by three exons, whereas the  $\beta$  form is missing the second exon. Neuronatin mRNA expression is abundant in undifferentiated PC-12 cells. Treatment of these cells with nerve growth factor (NGF), which contributes to neuronal differentiation, downregulates Neuronatin mRNA expression. NNAT-1.9 PC-12 cells exhibit an increase in nigericin, rotenone and valinomycin sensitivity; NNAT transfection restores wild-type PC-12 resistance. These results suggest a potential protective role for Neuronatin against toxic insult during development.

## REFERENCES

- Joseph, R., et al. 1996. Neuronatin mRNA in PC-12 cells: downregulation by nerve growth factor. *Brain Res.* 738: 32-38.
- Kikyo, N., et al. 1997. Genetic and functional analysis of Neuronatin in mice with maternal or paternal duplication of distal Chr 2. *Dev. Biol.* 190: 66-77.
- Evans, H.K., et al. 2001. The Neuronatin gene resides in a "micro-imprinted" domain on human chromosome 20q11.2. *Genomics* 77: 99-104.
- Zheng, S., et al. 2002. The fetal and neonatal brain protein Neuronatin protects PC-12 cells against certain types of toxic insult. *Brain Res. Dev. Brain Res.* 136: 101-110.
- Kuerbitz, S.J., et al. 2002. Hypermethylation of the imprinted NNAT locus occurs frequently in pediatric acute leukemia. *Carcinogenesis* 23: 559-564.

## CHROMOSOMAL LOCATION

Genetic locus: NNAT (human) mapping to 20q11.23.

## PRODUCT

Neuronatin 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Neuronatin shRNA Plasmid (h): sc-43966-SH and Neuronatin shRNA (h) Lentiviral Particles: sc-43966-V as alternate gene silencing products.

For independent verification of Neuronatin (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-43966A, sc-43966B and sc-43966C.

## 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° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° 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

Neuronatin siRNA (h) is recommended for the inhibition of Neuronatin 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  $\mu$ 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.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Neuronatin gene expression knockdown using RT-PCR Primer: Neuronatin (h)-PR: sc-43966-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.