SANTA CRUZ BIOTECHNOLOGY, INC.

NF-M siRNA (h): sc-36050

BACKGROUND

Neurofilament-M (NF-M), for neurofilament medium polypeptide, a member of the intermediate filament family, is a major component of neuronal cytoskeletons. Neurofilaments are dynamic structures; they contain phosphorylation sites for a large number of protein kinases, including protein kinase A, protein kinase C, cyclin-dependent kinase 5, extracellular signal regulated kinase, glycogen synthase kinase-3, and stress-activated protein kinase γ. In addition to their role in the control of axon caliber, neurofilaments may affect other cytoskeletal elements, such as microtubules and actin filaments. Changes in neurofilament phosphorylation or metabolism are frequently observed in neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Alzheimer's disease.

REFERENCES

1. Levy, E., et al. 1987. Structure and evolutionary origin of the gene encoding NF-M, the middle-molecular-mass neurofilament protein. Eur. J. Biochem. 166: 71-77.
2. Angelides, K.J., et al. 1989. Assembly and exchange of intermediate filament proteins of neurons: neurofilaments are dynamic structures. J. Cell Biol. 108: 1495-1506.
3. Sihag, R.K., et al. 1989. *In vivo* phosphorylation of distinct domains of the 70 kilodalton neurofilament subunit involves different protein kinases. J. Biol. Chem. 264: 457 -464.
4. Hisanaga, S., et al. 1990. Effects of phosphorylation of the neurofilament L protein on filamentous structures. Cell Regul. 1: 237-248.
5. Gonda, Y., et al. 1990. Involvement of protein kinase C in the regulation of assembly-disassembly of neurofilaments *in vitro*. Biochem. Biophys. Res. Commun. 167: 1316 -1325.
6. Nakamura, Y., et al. 1997. Abnormal distribution of neurofilament L in neurons with Alzheimer's disease. Neurosci. Lett. 225: 201-204.
7. Nakamura, Y., et al. 1999. Casein kinase II is responsible for phosphorylation of NF-L at Ser-473. FEBS Lett. 455: 83-86.
8. Strong, M.J. 1999. Neurofilament metabolism in sporadic amyotrophic lateral sclerosis. J. Neurol. Sci. 169: 170-177.

CHROMOSOMAL LOCATION

Genetic locus: NEFM (human) mapping to 8p21.2.

PRODUCT

NF-M 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NF-M shRNA Plasmid (h): sc-36050-SH and NF-M shRNA (h) Lentiviral Particles: sc-36050-V as alternate gene silencing products.

For independent verification of NF-M (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-36050A, sc-36050B and sc-36050C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NF-M siRNA (h) is recommended for the inhibition of NF-M 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 µM in 66 µl. 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

NF-M (E-9): sc-398532 is recommended as a control antibody for monitoring of NF-M 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 NF-M gene expression knockdown using RT-PCR Primer: NF-M (h)-PR: sc-36050-PR (20 µl, 527 bp). 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.

PROTOCOLS

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