

NMNAT-3 siRNA (h): sc-62695

BACKGROUND

NMNAT proteins are essential cofactors involved in the fundamental processes of cell metabolism. They belong to the eukaryotic NMN adenylyltransferase family. NMNATs participate in the synthesis of NAD⁺ by catalyzing the condensation of nicotinamide mononucleotide and ATP. The presence of magnesium and other divalent cations increases their enzymatic activity. The interaction of NMNATs with nuclear proteins is likely to be modulated by phosphorylation. NMNAT proteins contain at least three potential phosphorylation sites and may act as substrates for nuclear kinases. NMNAT-3 (nicotinamide mononucleotide adenylyltransferase-3), also designated PNAT3, is a 252 amino acid protein that localizes to the mitochondria. Highly expressed in the spleen and lungs, NMNAT-3 is able to form homotetramers. Two isoforms exist due to alternative splicing events.

REFERENCES

1. Sestini, S., et al. 2000. Enzyme activities leading to NAD synthesis in human lymphocytes. *Arch. Biochem. Biophys.* 379: 277-282.
2. Raffaelli, N., et al. 2002. Identification of a novel human nicotinamide mononucleotide adenylyltransferase. *Biochem. Biophys. Res. Commun.* 297: 835-840.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2004. Johns Hopkins University, Baltimore, MD. MIM Number: 608702. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Berger, F., et al. 2005. Subcellular compartmentation and differential catalytic properties of the three human nicotinamide mononucleotide adenylyltransferase isoforms. *J. Biol. Chem.* 280: 36334-36341.
5. Mulligan, M.K., et al. 2006. Toward understanding the genetics of alcohol drinking through transcriptome meta-analysis. *Proc. Natl. Acad. Sci. USA* 103: 6368-6373.

CHROMOSOMAL LOCATION

Genetic locus: NMNAT3 (human) mapping to 3q23.

PRODUCT

NMNAT-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 NMNAT-3 shRNA Plasmid (h): sc-62695-SH and NMNAT-3 shRNA (h) Lentiviral Particles: sc-62695-V as alternate gene silencing products.

For independent verification of NMNAT-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-62695A, sc-62695B and sc-62695C.

PROTOCOLS

See our web site at 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 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

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

NMNAT-3 (D-10): sc-390433 is recommended as a control antibody for monitoring of NMNAT-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 NMNAT-3 gene expression knockdown using RT-PCR Primer: NMNAT-3 (h)-PR: sc-62695-PR (20 μ l, 544 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.