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NMNAT-1 siRNA (h): sc-45502

BACKGROUND

Nicotinamide adenine dinucleotide (NMNAT) is an essential cofactor involved in fundamental processes in cell metabolism. NMNAT plays a key role in NAD⁺ biosynthesis, catalysing the condensation of nicotinamide mononucleotide and ATP, and yielding NAD⁺ and pyrophosphate. NMNAT appears to be a substrate of nuclear kinases and contains at least three potential phosphorylation sites. The interaction of NMNAT with nuclear proteins is likely to be modulated by phosphorylation. NMNAT is widely expressed with highest levels in skeletal muscle, heart, liver and kidney.

REFERENCES

1. D'Angelo, I., et al. 2000. Structure of nicotinamide mononucleotide adenylyltransferase: a key enzyme in NAD⁺ biosynthesis. *Structure* 8: 993-1004.
2. Schweiger, M., et al. 2001. Characterization of recombinant human nicotinamide mononucleotide adenylyl transferase (NMNAT), a nuclear enzyme essential for NAD synthesis. *FEBS Lett.* 492: 95-100.
3. Mack, T.G., et al. 2001. Wallerian degeneration of injured axons and synapses is delayed by a Ube4b/NMNAT chimeric gene. *Nat. Neurosci.* 4: 1199-1206.
4. Gillingwater, T.H., et al. 2002. Age-dependent synapse withdrawal at axotomized neuromuscular junctions in Wld(s) mutant and Ube4b/NMNAT transgenic mice. *J. Physiol.* 543: 739-755.
5. Werner, E., et al. 2002. Crystallization and preliminary X-ray analysis of human nicotinamide mononucleotide adenylyltransferase (NMNAT). *Acta Crystallogr. D Biol. Crystallogr.* 58: 140-142.
6. SWISS-PROT/TrEMBL (Q9HAN9). World Wide Web URL: <http://www.expasy.ch/sprot/sprot-top.html>.

CHROMOSOMAL LOCATION

Genetic locus: NMNAT1 (human) mapping to 1p36.22.

PRODUCT

NMNAT-1 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-1 shRNA Plasmid (h): sc-45502-SH and NMNAT-1 shRNA (h) Lentiviral Particles: sc-45502-V as alternate gene silencing products.

For independent verification of NMNAT-1 (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-45502A, sc-45502B and sc-45502C.

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° 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-1 siRNA (h) is recommended for the inhibition of NMNAT-1 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-1 (B-7): sc-271557 is recommended as a control antibody for monitoring of NMNAT-1 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-1 gene expression knockdown using RT-PCR Primer: NMNAT-1 (h)-PR: sc-45502-PR (20 μ l, 596 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.