SANTA CRUZ BIOTECHNOLOGY, INC.

NMNAT-2 siRNA (m): sc-62694

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The Power to Question

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-2 (nicotinamide mononucleotide adenylyltransferase-2) is a 307 amino acid protein that is highly expressed in the brain, especially in the cerebrum, cerebellum, occipital lobe, frontal lobe, temporal lobe and putamen. It is also detected at lower levels in the heart and skeletal muscle. Two isoforms exist due to alternate splicing.

REFERENCES

1. Gillingwater, T.H., et al. 2002. Age-dependent synapse withdrawal at axotomised neuromuscular junctions in Wld^s mutant and Ube4b/ NMNAT transgenic mice. J. Physiol. 543: 739-755.
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: 608701. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Berger, F., et al. 2005. Subcellular compart-mentation and differential catalytic properties of the three human nicotin-amide mononucleotide adenylyltransferase isoforms. J. Biol. Chem. 280: 36334-36341.
5. Berger, F., et al. 2007. Regulation of poly(ADP-ribose) polymerase 1 activity by the phosphorylation state of the nuclear NAD biosynthetic enzyme NMN adenylyltransferase-1. Proc. Natl. Acad. Sci. USA 104: 3765-3770.

CHROMOSOMAL LOCATION

Genetic locus: Nmnat2 (mouse) mapping to 1 G3.

PRODUCT

NMNAT-2 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NMNAT-2 shRNA Plasmid (m): sc-62694-SH and NMNAT-2 shRNA (m) Lentiviral Particles: sc-62694-V as alternate gene silencing products.

For independent verification of NMNAT-2 (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-62694A, sc-62694B and sc-62694C.

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 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

NMNAT-2 siRNA (m) is recommended for the inhibition of NMNAT-2 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 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

NMNAT-2 (B-10): sc-515206 is recommended as a control antibody for monitoring of NMNAT-2 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 NMNAT-2 gene expression knockdown using RT-PCR Primer: NMNAT-2 (m)-PR: sc-62694-PR (20 µl). 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.