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SANTA CRUZ BIOTECHNOLOGY, INC.

NADSYN1 siRNA (m): sc-149797

BACKGROUND

NAD (nicotinamide adenine dinucleotide) is a cofactor that participates in a wide variety of functions, including metabolic redox reactions, cell signaling events and post-translational protein modifications. The synthesis of NAD within the cell is dependent upon a number of enzymes, called NAD synthetases, that work in concert to catalyze the reactions that form NAD. NADSYN1 (NAD synthetase 1) is a 706 amino acid protein that contains one CN (carbon-nitrogen) hydrolase domain and is a member of the NAD synthetase family. Expressed at high levels in testis, kidney, liver and small intestine, NADSYN1 catalyzes the ATP-dependent conversion of deamido-NAD^+ to free NAD^+. NADSYN1 exists as a homohexamer that uses both ammonia and glutamate as amide donors. NADSYN1 is present in human promyelocytic leukemia and glioma cell lines, suggesting a possible role in tumor formation.

REFERENCES

1. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 608285. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
2. Hara, N., et al. 2003. Molecular identification of human glutamine- and ammonia-dependent NAD synthetases. Carbon-nitrogen hydrolase domain confers glutamine dependency. J. Biol. Chem. 278: 10914-10921.
3. Jauch, R., et al. 2005. Structures of *Escherichia coli* NAD synthetase with substrates and products reveal mechanistic rearrangements. J. Biol. Chem. 280: 15131-15140.
4. Bellinzoni, M., et al. 2005. Glutamine amidotransferase activity of NAD^+ synthetase from *Mycobacterium tuberculosis* depends on an amino-terminal nitrilase domain. Res. Microbiol. 156: 173-177.
5. Wojcik, M., et al. 2006. Glutamine-dependent NAD^+ synthetase. How a two-domain, three-substrate enzyme avoids waste. J. Biol. Chem. 281: 33395-33402.

CHROMOSOMAL LOCATION

Genetic locus: Nadsyn1 (mouse) mapping to 7 F5.

PRODUCT

NADSYN1 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 NADSYN1 shRNA Plasmid (m): sc-149797-SH and NADSYN1 shRNA (m) Lentiviral Particles: sc-149797-V as alternate gene silencing products.

For independent verification of NADSYN1 (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-149797A, sc-149797B and sc-149797C.

PROTOCOLS

See our web site at www.scbt.com or our catalog 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

NADSYN1 siRNA (m) is recommended for the inhibition of NADSYN1 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

NADSYN1 (S-15): sc-168703 is recommended as a control antibody for monitoring of NADSYN1 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 (secondary) reagents are recommended: 1) Western Blotting: use donkey anti-goat IgG-HRP: sc-2020 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible donkey anti-goat IgG-HRP: sc-2033 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use donkey anti-goat IgG-FITC: sc-2024 (dilution range: 1:100-1:400) or donkey anti-goat IgG-TR: sc-2783 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NADSYN1 gene expression knockdown using RT-PCR Primer: NADSYN1 (m)-PR: sc-149797-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.