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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

linkedin.com/company/szaboscandic

SANTA CRUZ BIOTECHNOLOGY, INC.

NMNAT-1 shRNA (m) Lentiviral Particles: sc-45503-V

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 Fold Des. 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 axotomised 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.

CHROMOSOMAL LOCATION

Genetic locus: Nmnat1 (mouse) mapping to 4 E2.

PRODUCT

NMNAT-1 shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see NMNAT-1 siRNA (m): sc-45503 and NMNAT-1 shRNA Plasmid (m): sc-45503-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

NMNAT-1 shRNA (m) Lentiviral Particles is recommended for the inhibition of NMNAT-1 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

NMNAT-1 (H-109): sc-98249 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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 goat anti-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor NMNAT-1 gene expression knockdown using RT-PCR Primer: NMNAT-1 (m)-PR: sc-45503-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.