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NNT siRNA (m): sc-150013

BACKGROUND

The process of cellular respiration is carried out by integral inner mitochondrial membrane proteins that facilitate the harnessing of energy released by the oxidation of NADH. NNT (nicotinamide nucleotide transhydrogenase), also known as mitochondrial NAD(P) transhydrogenase or pyridine nucleotide transhydrogenase, is a 1,086 amino acid multi-pass mitochondrial inner membrane protein. NNT is a homodimer with an N-terminal section belonging to the AlaDH/PNT family and a C-terminal section belonging to the PNT β subunit family. NNT catalyzes the transfer of a hydride ion from NADH to NADP⁺ and functions as a mitochondrial inner membrane proton pump. Using the energy of the proton gradient created by the electron transport chain, NNT produces high concentrations of NADPH, which is used in free radical detoxification and biosynthesis.

REFERENCES

1. Forsmark-Andrée, P., et al. 1996. Oxidative modification of nicotinamide nucleotide transhydrogenase in submitochondrial particles: effect of endogenous ubiquinol. *Arch. Biochem. Biophys.* 336: 113-120.
2. Arkblad, E.L., et al. 1996. The cDNA sequence of proton-pumping nicotinamide nucleotide transhydrogenase from man and mouse. *Biochim. Biophys. Acta* 1273: 203-205.
3. Zieger, B. and Ware, J. 1997. Cloning and deduced amino acid sequence of human nicotinamide nucleotide transhydrogenase. *DNA Seq.* 7: 369-373.
4. White, S.A., et al. 2000. The high-resolution structure of the NAD(P)⁺-binding component (dIII) of proton-translocating transhydrogenase from human heart mitochondria. *Structure* 8: 1-12.

CHROMOSOMAL LOCATION

Genetic locus: Nnt (mouse) mapping to 13 D2.3.

PRODUCT

NNT 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NNT shRNA Plasmid (m): sc-150013-SH and NNT shRNA (m) Lentiviral Particles: sc-150013-V as alternate gene silencing products.

For independent verification of NNT (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-150013A, sc-150013B and sc-150013C.

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

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

NNT (B-3): sc-390236 is recommended as a control antibody for monitoring of NNT 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 NNT gene expression knockdown using RT-PCR Primer: NNT (m)-PR: sc-150013-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Zhao, H., et al. 2019. Single-cell transcriptomics of human oocytes: environment-driven metabolic competition and compensatory mechanisms during oocyte maturation. *Antioxid. Redox Signal.* 30: 542-559.

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.