

NNMT siRNA (h): sc-61213

BACKGROUND

Nicotinamide N-methyltransferase (NNMT) catalyzes the N-methylation of nicotinamide and other pyridines. NNMT activity in the human liver has a bimodal frequency distribution, indicating that its enzyme activity may be modulated through a genetic polymorphism, which could have functional implications for individual differences in drug and xenobiotic toxicity. The gene that encodes human NNMT is approximately 16.5 kb in length, consists of three exons and two introns and maps to 11q23.2. NNMT isolated from the human liver was determined to be 969 nucleotides in length, with a 792 nucleotide open reading frame that encodes a 264 amino acid protein. The NNMT gene is presumed to be a significant genetic determinant of plasma homocysteine levels in Spanish families, since it encodes an enzyme involved in homocysteine synthesis.

REFERENCES

1. Yan, L., et al. 1998. Mouse nicotinamide N-methyltransferase gene: molecular cloning, structural characterization, and chromosomal localization. *DNA Cell Biol.* 17: 659-667.
2. Yan, L., et al. 1999. Human nicotinamide N-methyltransferase pharmacogenetics: gene sequence analysis and promoter characterization. *Pharmacogenetics* 9: 307-316.
3. Parsons, R.B., et al. 2002. Expression of nicotinamide N-methyltransferase (E.C. 2.1.1.1) in the Parkinsonian brain. *J. Neuropathol. Exp. Neurol.* 61: 111-124.
4. Parsons, R.B., et al. 2003. High expression of nicotinamide N-methyltransferase in patients with idiopathic Parkinson's disease. *Neurosci. Lett.* 342: 13-16.

CHROMOSOMAL LOCATION

Genetic locus: NNMT (human) mapping to 11q23.2.

PRODUCT

NNMT 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 NNMT shRNA Plasmid (h): sc-61213-SH and NNMT shRNA (h) Lentiviral Particles: sc-61213-V as alternate gene silencing products.

For independent verification of NNMT (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-61213A, sc-61213B and sc-61213C.

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

NNMT siRNA (h) is recommended for the inhibition of NNMT 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

NNMT (G-4): sc-376048 is recommended as a control antibody for monitoring of NNMT 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 NNMT gene expression knockdown using RT-PCR Primer: NNMT (h)-PR: sc-61213-PR (20 μ l, 458 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Xie, X., et al. 2014. Nicotinamide N-methyltransferase enhances the capacity of tumorigenesis associated with the promotion of cell cycle progression in human colorectal cancer cells. *Arch. Biochem. Biophys.* 564: 52-66.
2. Zhang, J., et al. 2014. Down-regulation of nicotinamide N-methyltransferase induces apoptosis in human breast cancer cells via the mitochondria-mediated pathway. *PLoS ONE* 9: e89202.
3. Xie, X., et al. 2016. Nicotinamide N-methyltransferase enhances resistance to 5-fluorouracil in colorectal cancer cells through inhibition of the ASK1-p38 MAPK pathway. *Oncotarget* 7: 45837-45848.

RESEARCH USE

For research use only, not for use in diagnostic procedures.