

PNMTase siRNA (h): sc-106772

BACKGROUND

Phenylethanolamine N-methyltransferase (PNMT/PNMTase) catalyzes the synthesis of epinephrine from norepinephrine, the last step of catecholamine biosynthesis. Human PNMT, a 282 amino acid polypeptide, shares significant homology with tyrosine hydroxylase. Expression of PNMT is regulated by hormonal and neural stimuli because its promoter contains sequences responding to cholinergic and depolarization stimuli. Cortisol and Egr-1 enhance PNMT expression, which controls the adrenaline production in adrenaline-secreting pheochromocytomas. Protein kinase A also up-regulates PNMT expression, whereas protein kinase C causes down-regulation and pituitary adenylate cyclase-activating polypeptide lowers PNMT activity. PNMT is expressed in a tissue-specific manner based on an alternative splicing mechanism, termed intron retention, to produce two splice variants. This mechanism is sensitive to regulation by glucocorticoids in the brain. The spliced, intronless mRNA is down-regulated postnatally, while the intron-retained mRNA is constitutively expressed through adulthood. At all stages of development, only the intron-less message is expressed in adrenals. PNMT gene is associated with increased susceptibility to the sporadic form of early-onset Alzheimer disease.

REFERENCES

1. Baetge, E.E., et al. 1986. Complete nucleotide and deduced amino acid sequence of bovine phenylethanolamine N-methyltransferase: partial amino acid homology with rat tyrosine hydroxylase. *Proc. Natl. Acad. Sci. USA* 83: 5454-5458.
2. Kaneda, N., et al. 1988. Molecular cloning of cDNA and chromosomal assignment of the gene for human phenylethanolamine N-methyltransferase, the enzyme for epinephrine biosynthesis. *J. Biol. Chem.* 263: 7672-7677.
3. Lee, Y.S., et al. 1999. Neural regulation of phenylethanolamine N-methyltransferase (PNMT) gene expression in bovine chromaffin cells differs from other catecholamine enzyme genes. *J. Mol. Neurosci.* 12: 53-68.
4. Unsworth, B.R., et al. 1999. Tissue-specific alternative mRNA splicing of phenylethanolamine N-methyltransferase (PNMT) during development by intron retention. *Int. J. Dev. Neurosci.* 17: 45-55.

CHROMOSOMAL LOCATION

Genetic locus: PNMT (human) mapping to 17q12.

PRODUCT

PNMTase siRNA (h) is a pool of 2 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 PNMTase shRNA Plasmid (h): sc-106772-SH and PNMTase shRNA (h) Lentiviral Particles: sc-106772-V as alternate gene silencing products.

For independent verification of PNMTase (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-106772A and sc-106772B.

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

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

PNMTase (C-7): sc-393995 is recommended as a control antibody for monitoring of PNMTase 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 MarkerTM 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 PNMTase gene expression knockdown using RT-PCR Primer: PNMTase (h)-PR: sc-106772-PR (20 μ l). 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.

PROTOCOLS

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