

NAPRT siRNA (h): sc-77596

BACKGROUND

NAPRT (nicotinate phosphoribosyltransferase), also known as FHA-HIT-interacting protein or nicotinate phosphoribosyltransferase domain-containing protein 1, is a 538 amino acid member of the NAPRTase protein family. Localized to the cytoplasm, NAPRT is involved in the biosynthesis of the cofactor NAD⁺. NAPRT catalyzes the conversion of nicotinic acid (NA) to NA mononucleotide (NaMN). This conversion is essential to increase cellular NAD levels, which prevents oxidative stress of the cells. NAPRT is expressed as three isoforms produced by alternative splicing events. The gene that encodes NAPRT maps to human chromosome 8, which makes up nearly 146 million bases and encodes about 800 genes. Translocation of portions of chromosome 8 with amplifications of the c-Myc gene are found in some leukemias and lymphomas, and are typically associated with a poor prognosis. Portions of chromosome 8 have been linked to schizophrenia and bipolar disorder.

REFERENCES

1. Wildenauer, D.B. and Schwab, S.G. 1999. Chromosomes 8 and 10 workshop. *Am. J. Med. Genet.* 88: 239-243.
2. Magni, G., et al. 2004. Enzymology of NAD⁺ homeostasis in man. *Cell. Mol. Life Sci.* 61: 19-34.
3. McQueen, M.B., et al. 2005. Combined analysis from eleven linkage studies of bipolar disorder provides strong evidence of susceptibility loci on chromosomes 6q and 8q. *Am. J. Hum. Genet.* 77: 582-595.
4. Nusbaum, C., et al. 2006. DNA sequence and analysis of human chromosome 8. *Nature* 439: 331-335.
5. Hara, N., et al. 2007. Elevation of cellular NAD levels by nicotinic acid and involvement of nicotinic acid phosphoribosyltransferase in human cells. *J. Biol. Chem.* 282: 24574-24582.

CHROMOSOMAL LOCATION

Genetic locus: NAPRT1 (human) mapping to 8q24.3.

PRODUCT

NAPRT 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 NAPRT shRNA Plasmid (h): sc-77596-SH and NAPRT shRNA (h) Lentiviral Particles: sc-77596-V as alternate gene silencing products.

For independent verification of NAPRT (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-77596A, sc-77596B and sc-77596C.

PROTOCOLS

See our web site at www.scbt.com 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 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

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

NAPRT (B-8): sc-398404 is recommended as a control antibody for monitoring of NAPRT 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 NAPRT gene expression knockdown using RT-PCR Primer: NAPRT (h)-PR: sc-77596-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.