SANTA CRUZ BIOTECHNOLOGY, INC.

NADK siRNA (h): sc-88094

BACKGROUND

NADK (NAD kinase) is a 446 amino acid protein that belongs to the NAD kinase family. Expressed at high levels in placenta and at moderate levels in colon, kidney, brain, heart, liver, spleen, lung, testis and stomach, NADK functions to catalyze the transfer of a phosphate group from ATP to NAD^+, thereby generating $NADP^+$. Once formed, $NADP^+$ can be reduced to NADPH, which can subsequently act as an eletron donor in biosynthetic reactions. Through its ability to catalyze the formation of $NADP^+$, NADK is able to control the concentration of NADPH within the cell. NADK uses divalent metal cations (such as zinc and manganese) as cofactors and exhibits the highest rate of enzymatic activity at a pH of 7.5.

REFERENCES

1. Lerner, F., et al. 2001. Structural and functional characterization of human NAD kinase. Biochem. Biophys. Res. Commun. 288: 69-74.
2. Online Mendelian Inheritance in Man, OMIM™. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 611616. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
3. Turner, W.L., et al. 2004. Cloning and characterization of two NAD kinases from *Arabidopsis*. identification of a calmodulin binding isoform. Plant Physiol. 135: 1243-1255.
4. Grose, J.H., et al. 2006. Evidence that feedback inhibition of NAD kinase controls responses to oxidative stress. Proc. Natl. Acad. Sci. USA 103: 7601-7606.
5. Pollak, N., et al. 2007. NAD kinase levels control the NADPH concentration in human cells. J. Biol. Chem. 282: 33562-33571.
6. Singh, R., et al. 2007. Oxidative stress evokes a metabolic adaptation that favors increased NADPH synthesis and decreased NADH production in *Pseudomonas fluorescens*. J. Bacteriol. 189: 6665-6675.

CHROMOSOMAL LOCATION

Genetic locus: NADK (human) mapping to 1p36.33.

PRODUCT

NADK 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NADK shRNA Plasmid (h): sc-88094-SH and NADK shRNA (h) Lentiviral Particles: sc-88094-V as alternate gene silencing products.

For independent verification of NADK (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-88094A, sc-88094B and sc-88094C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

NADK siRNA (h) is recommended for the inhibition of NADK 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 µM in 66 µl. 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

NADK (J-07): sc-100347 is recommended as a control antibody for monitoring of NADK 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 NADK gene expression knockdown using RT-PCR Primer: NADK (h)-PR: sc-88094-PR (20 µl). 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.