

pyridoxal kinase siRNA (h): sc-61423

BACKGROUND

Pyridoxal kinase belongs to the pyridoxine kinase family and phosphorylates vitamin B6, a step necessary for the conversion of vitamin B6 to pyridoxal 5' phosphate (PLP), the active form of vitamin B6. PLP acts as a coenzyme and functions to maintain homeostasis. Pyridoxal kinase is a 312-amino acid cytoplasmic protein that may act as a homodimer and is expressed ubiquitously. There are three known isoforms of pyridoxal kinase, and isoform 3 expression is observed in adult testis and spermatozoa. The optimum pH for pyridoxal kinase is between 5.5 and 6.0. PDXK, the gene that encodes the pyridoxal kinase protein, maps to chromosome 21q22.3 and may be a candidate gene for autoimmune polyglandular disease type 1, a genetic disorder that has been mapped to the same region on chromosome 21.

REFERENCES

1. Hanna, M.C., et al. 1997. Human pyridoxal kinase. cDNA cloning, expression, and modulation by ligands of the benzodiazepine receptor. *J. Biol. Chem.* 272: 10756-10760.
2. Lee, H.S., et al. 2000. Human pyridoxal kinase: overexpression and properties of the recombinant enzyme. *Mol. Cells* 10: 452-459.
3. Online Mendelian Inheritance in Man, OMIM[™]. 2002. Johns Hopkins University, Baltimore, MD. MIM Number: 179020. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
4. Fang, X., et al. 2004. Expression of a novel pyridoxal kinase mRNA splice variant, PKH-T, in human testis. *Asian J. Androl.* 6: 83-91.
5. Bach, S., et al. 2005. Roscovitine targets, protein kinases and pyridoxal kinase. *J. Biol. Chem.* 280: 31208-31219.
6. Tang, L., et al. 2005. Crystal structure of pyridoxal kinase in complex with roscovitine and derivatives. *J. Biol. Chem.* 280: 31220-31229.
7. Hwang, I.K., et al. 2005. Age-dependent changes of pyridoxal phosphate synthesizing enzymes immunoreactivities and activities in the gerbil hippocampal CA1 region. *Mech. Ageing Dev.* 126: 1322-1330.
8. Klugmann, M., et al. 2006. A novel role of circadian transcription factor DBP in hippocampal plasticity. *Mol. Cell. Neurosci.* 31: 303-314.

CHROMOSOMAL LOCATION

Genetic locus: PDXK (human) mapping to 21q22.3.

PRODUCT

pyridoxal kinase 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 pyridoxal kinase shRNA Plasmid (h): sc-61423-SH and pyridoxal kinase shRNA (h) Lentiviral Particles: sc-61423-V as alternate gene silencing products.

For independent verification of pyridoxal kinase (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-61423A, sc-61423B and sc-61423C.

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

pyridoxal kinase siRNA (h) is recommended for the inhibition of pyridoxal kinase 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

pyridoxal kinase (E-2): sc-365173 is recommended as a control antibody for monitoring of pyridoxal kinase 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 pyridoxal kinase gene expression knockdown using RT-PCR Primer: pyridoxal kinase (h)-PR: sc-61423-PR (20 μ l, 567 bp). 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.