

DGUOK siRNA (h): sc-77139

BACKGROUND

DGUOK (deoxyguanosine kinase), also known as DGK, is a 277 amino acid protein that localizes to mitochondria and exists as multiple alternatively spliced isoforms. Functioning as a homodimer and highly expressed in a variety of tissues, including liver, muscle and brain, DGUOK uses ATP to catalyze the conversion of deoxyguanosine to dGMP. Via its catalytic activity, DGUOK is essential for the phosphorylation of purine deoxyribonucleosides in the mitochondrial matrix and is an important antiviral and chemotherapeutic tool. Defects in the gene encoding DGUOK are the cause of hepatocerebral mitochondrial DNA depletion syndrome (MDS), a group of disorders that result in reduced mtDNA (mitochondrial DNA) copy number and are characterized by liver failure and neurologic abnormalities.

REFERENCES

1. Johansson, M., et al. 1996. Cloning and expression of human deoxyguanosine kinase cDNA. *Proc. Natl. Acad. Sci. USA* 93: 7258-7262.
2. Mandel, H., et al. 2001. The deoxyguanosine kinase gene is mutated in individuals with depleted hepatocerebral mitochondrial DNA. *Nat. Genet.* 29: 337-341.
3. Taanman, J.W., et al. 2002. A novel mutation in the deoxyguanosine kinase gene causing depletion of mitochondrial DNA. *Ann. Neurol.* 52: 237-239.
4. Salviati, L., et al. 2002. Mitochondrial DNA depletion and dGK gene mutations. *Ann. Neurol.* 52: 311-317.
5. Taanman, J.W., et al. 2003. Mitochondrial DNA depletion can be prevented by dGMP and dAMP supplementation in a resting culture of deoxyguanosine kinase-deficient fibroblasts. *Hum. Mol. Genet.* 12: 1839-1845.
6. Mancuso, M., et al. 2005. New DGK gene mutations in the hepatocerebral form of mitochondrial DNA depletion syndrome. *Arch. Neurol.* 62: 745-747.
7. Mousson de Camaret, B., et al. 2007. Kinetic properties of mutant deoxyguanosine kinase in a case of reversible hepatic mtDNA depletion. *Biochem. J.* 402: 377-385.
8. Online Mendelian Inheritance in Man, OMIM™. 2007. Johns Hopkins University, Baltimore, MD. MIM Number: 601465. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>

CHROMOSOMAL LOCATION

Genetic locus: DGUOK (human) mapping to 2p13.1.

PRODUCT

DGUOK 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 DGUOK shRNA Plasmid (h): sc-77139-SH and DGUOK shRNA (h) Lentiviral Particles: sc-77139-V as alternate gene silencing products.

For independent verification of DGUOK (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-77139A and sc-77139B.

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

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

DGUOK (H-3): sc-376267 is recommended as a control antibody for monitoring of DGUOK 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 DGUOK gene expression knockdown using RT-PCR Primer: DGUOK (h)-PR: sc-77139-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.