

sMtCK siRNA (h): sc-38969

BACKGROUND

Creatine kinases (CK) are a large family of isoenzymes that regulate levels of ATP in subcellular compartments, where they provide ATP at sites of fluctuating energy demand by the transfer of phosphates between creatine and adenine nucleotides. CKs provide the energy of phosphate hydrolysis necessary to drive the normal function of many cellular systems including muscle, electrocytes, retina photoreceptor cells, brain cells, kidney, salt glands, myometrium, placenta, pancreas, thymus, thyroid, intestinal epithelial cells, endothelial cells, cartilage and bone cells, macrophages, blood platelets, tumor and cancer cells. Human cytoplasmic CK-Brain (CK-B, BCK) is a 381 amino acid, brain tissue specific isoform of CK. Human cytoplasmic CK-Muscle (CK-M, MCK) is a muscle tissue specific isoform of CK. Human cytoplasmic CK-Mitochondrial (MtCK, Mi-CK) is a 416 amino acid mitochondrial specific isoform of CK. Cytosolic CKs are important in the energetic regulation of Ca²⁺-pumps and in the maintenance of Ca²⁺-homeostasis.

REFERENCES

1. Mariman, E.C., et al. 1987. Structure and expression of the human creatine kinase B gene. *Genomics* 1: 126-137.
2. Nigro, J.M., et al. 1987. cDNA cloning and mapping of the human creatine kinase M gene to 19q13. *Am. J. Hum. Genet.* 40: 115-125.
3. Mariman, E.C., et al. 1989. Complete nucleotide sequence of the human creatine kinase B gene. *Nucleic Acids Res.* 17: 6385.
4. Haas, R.C., et al. 1989. Isolation and characterization of the gene and cDNA encoding human mitochondrial creatine kinase. *J. Biol. Chem.* 264: 2890-2897.
5. Wallimann, T., et al. 1994. Creatine kinase in non-muscle tissues and cells. *Mol. Cell. Biochem.* 133-134: 193-220.
6. Wallimann, T., et al. 1998. Some new aspects of creatine kinase (CK): compartmentation, structure, function and regulation for cellular and mitochondrial bioenergetics and physiology. *Biofactors* 8: 229-234.

CHROMOSOMAL LOCATION

Genetic locus: CKMT2 (human) mapping to 5q14.1.

PRODUCT

sMtCK 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 sMtCK shRNA Plasmid (h): sc-38969-SH and sMtCK shRNA (h) Lentiviral Particles: sc-38969-V as alternate gene silencing products.

For independent verification of sMtCK (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-38969A, sc-38969B and sc-38969C.

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

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

sMtCK (C-6): sc-390489 is recommended as a control antibody for monitoring of sMtCK 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 sMtCK gene expression knockdown using RT-PCR Primer: sMtCK (h)-PR: sc-38969-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.