



creatine kinase-M siRNA (r): sc-270230

BACKGROUND

Creatine kinases (CKs) 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. Creatine kinases 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, and tumor and cancer cells. Human cytoplasmic creatine kinase-B, also designated CK-B and BCK, is a 381 amino acid, brain tissue-specific isoform of creatine kinase. Human cytoplasmic creatine kinase-M (CK-M, MCK) is a muscle tissue-specific isoform of creatine kinase. Human cytoplasmic creatine kinase-Mi (Mi-CK, MtCK) is a 416 amino acid mitochondrial-specific isoform of creatine kinase. Cytosolic creatine kinases are important in the energetic regulation of Ca^{2+} -pumps and in the maintenance of Ca^{2+} -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. 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.
4. Mariman, E.C., et al. 1989. Complete nucleotide sequence of the human creatine kinase B gene. *Nucleic Acids Res.* 17: 6385.
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: Ckm (rat) mapping to 1q21.

PRODUCT

creatine kinase-M siRNA (r) 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see creatine kinase-M shRNA Plasmid (r): sc-270230-SH and creatine kinase-M shRNA (r) Lentiviral Particles: sc-270230-V as alternate gene silencing products.

For independent verification of creatine kinase-M (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270230A and sc-270230B.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

creatine kinase-M siRNA (r) is recommended for the inhibition of creatine kinase-M expression in rat 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

creatine kinase-M (G-9): sc-365046 is recommended as a control antibody for monitoring of creatine kinase-M 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 creatine kinase-M gene expression knockdown using RT-PCR Primer: creatine kinase-M (r)-PR: sc-270230-PR (20 μl). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.