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creatine kinase-M shRNA (h2) Lentiviral Particles: sc-43700-V

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 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. 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.

CHROMOSOMAL LOCATION

Genetic locus: CKM (human) mapping to 19q13.32.

PRODUCT

creatine kinase-M shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see creatine kinase-M siRNA (h2): sc-43700 and creatine kinase-M shRNA Plasmid (h2): sc-43700-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

creatine kinase-M shRNA (h2) Lentiviral Particles is recommended for the inhibition of creatine kinase-M expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

creatine kinase-M (G-9): sc-365046 is recommended as a control antibody for monitoring of 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

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 (h2)-PR: sc-43700-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.