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mHMGCS shRNA (h) Lentiviral Particles: sc-44503-V

BACKGROUND

HMG-CoA synthase exists as both a mitochondrial (mHMGCS) and cytoplasmic (cHMGCS) enzyme that condenses acetyl-CoA with acetoacetyl-CoA to form HMG-CoA. The HMG-CoA produced by cHMGCS is transformed into mevalonate by HMG-CoA reductase, which starts isoprenoid biosynthesis. End products of the isoprenoid pathway include cholesterol, ubiquinone, dolichol, isopentenyl adenosine and farnesyl groups. mHMGCS, together with HMG-CoA lyase, is responsible for ketone body biosynthesis. mHMGCS is expressed in liver and kidney. Fasting, cAMP and fatty acids increase the level of transcription of mHMGCS, while feeding and Insulin repress it. A regulatory element within the mHMGCS promoter confers transcriptional regulation by PPAR, RXR, COUP-TF and HNF-4.

REFERENCES

1. Apte, J., et al. 1990. Rat mitochondrial and cytosolic 3-hydroxy-3-methylglutaryl-CoA synthases are encoded by two different genes. *Proc. Natl. Acad. Sci. USA* 87: 3874-3878.
2. Russ, A.P., et al. 1992. Amplification and direct sequencing of a cDNA encoding human cytosolic 3-hydroxy-3-methylglutaryl-coenzyme A synthase. *Biochim. Biophys. Acta* 1132: 329-331.
3. Mascaro, C., et al. 1995. Molecular cloning and tissue expression of human mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase. *Arch. Biochem. Biophys.* 317: 385-390.
4. Hegardt, F.G., et al. 1998. Transcriptional regulation of mitochondrial HMG-CoA synthase in the control of ketogenesis. *Biochimie* 80: 803-806.
5. Rodriguez, J.C., et al. 1998. The hepatocyte nuclear factor 4 (HNF-4) represses the mitochondrial HMG-CoA synthase gene. *Biochem. Biophys. Res. Commun.* 242: 692-696.

CHROMOSOMAL LOCATION

Genetic locus: HMGCS2 (human) mapping to 1p12.

PRODUCT

mHMGCS shRNA (h) 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 μ l frozen stock containing 1.0×10^6 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 mHMGCS siRNA (h): sc-44503 and mHMGCS shRNA Plasmid (h): sc-44503-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

mHMGCS shRNA (h) Lentiviral Particles is recommended for the inhibition of mHMGCS expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 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

mHMGCS (G-11): sc-376092 is recommended as a control antibody for monitoring of mHMGCS 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 mHMGCS gene expression knockdown using RT-PCR Primer: mHMGCS (h)-PR: sc-44503-PR (20 μ l). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ 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.