SANTA CRUZ BIOTECHNOLOGY, INC.

mHMGCS siRNA (m): sc-44502

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. Ayte, 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.
6. Hegardt, F.G., et al. 1999. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase: a control enzyme in ketogenesis. Biochem. J. 338: 569-582.
7. Mascaro, C., et al. 2000. Sterol regulatory element binding protein-mediated effect of fluvastatin on cytosolic 3-hydroxy-3-methylglutaryl-coenzyme A synthase transcription. Arch. Biochem. Biophys. 374: 286-292.

CHROMOSOMAL LOCATION

Genetic locus: Hmgcs2 (mouse) mapping to 3 F2.2.

PRODUCT

mHMGCS siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see mHMGCS shRNA Plasmid (m): sc-44502-SH and mHMGCS shRNA (m) Lentiviral Particles: sc-44502-V as alternate gene silencing products.

For independent verification of mHMGCS (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-44502A, sc-44502B and sc-44502C.

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

mHMGCS siRNA (m) is recommended for the inhibition of mHMGCS expression in mouse 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

mHMGCS (B-8): sc-393256 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 reagents are recommended: 1) Western Blotting: use m-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 mHMGCS gene expression knockdown using RT-PCR Primer: mHMGCS (m)-PR: sc-44502-PR (20 µl). 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.