

MCAD siRNA (h): sc-60996

BACKGROUND

Acyl-CoA dehydrogenase is a family of enzymes that localize to the mitochondrion and target acyl chain lengths of 4 to 16 by use of the mitochondrial fatty acid β -oxidation pathway. In mammalian tissue, many straight-chain acyl-CoA dehydrogenases possess different substrate specificities. In rare cases, irregularities in medium-chain acyl-CoA dehydrogenase can cause fasting hypoglycemia, hepatic dysfunction and encephalopathy, often resulting in death in infancy. MCAD, also designated acyl-CoA dehydrogenase, medium-chain (ACADM) and MCADH, is a homotetramer. The MCAD gene encodes a 421 amino acid protein with characteristics of mitochondrial protein transit peptides. The protein shows 88% sequence identity with MCAD of pig origin. Medium-chain acyl-CoA dehydrogenase catalyzes the initial reaction in the β -oxidation of C4 to C12 straight-chain acyl-CoAs.

REFERENCES

1. Matsubara, Y., et al. 1986. Molecular cloning of cDNAs encoding rat and human medium-chain acyl-CoA dehydrogenase and assignment of the gene to human chromosome 1. *Proc. Natl. Acad. Sci. USA* 83: 6543-6547.
2. Kelly, D.P., et al. 1987. Nucleotide sequence of medium-chain acyl-CoA dehydrogenase mRNA and its expression in enzyme-deficient human tissue. *Proc. Natl. Acad. Sci. USA* 84: 4068-4072.
3. O'Reilly, L., et al. 2004. The Y42H mutation in medium-chain acyl-CoA dehydrogenase, which is prevalent in babies identified by MS/MS-based newborn screening, is temperature sensitive. *Eur. J. Biochem.* 271: 4053-4063.
4. Lee, P.J., et al. 2005. L-carnitine and exercise tolerance in medium-chain acyl-coenzyme A dehydrogenase (MCAD) deficiency: a pilot study. *J. Inher. Metab. Dis.* 28: 141-152.
5. Blois, B., et al. 2005. Newborns with C8-acylcarnitine level over the 90th centile have an increased frequency of the common MCAD 985A>G mutation. *J. Inher. Metab. Dis.* 28: 551-556.
6. Corydon, T.J., et al. 2005. Downregulation of HSP 60 expression by RNAi impairs folding of medium-chain acyl-CoA dehydrogenase wildtype and disease-associated proteins. *Mol. Genet. Metab.* 85: 260-270.

CHROMOSOMAL LOCATION

Genetic locus: ACADM (human) mapping to 1p31.1.

PRODUCT

MCAD 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 MCAD shRNA Plasmid (h): sc-60996-SH and MCAD shRNA (h) Lentiviral Particles: sc-60996-V as alternate gene silencing products.

For independent verification of MCAD (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-60996A, sc-60996B and sc-60996C.

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

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

MCAD (E-8): sc-271931 is recommended as a control antibody for monitoring of MCAD 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 MCAD gene expression knockdown using RT-PCR Primer: MCAD (h)-PR: sc-60996-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.

PROTOCOLS

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