SANTA CRUZ BIOTECHNOLOGY, INC.

CPTI-M siRNA (m): sc-40383

BACKGROUND

The mitochondrial β-oxidation of long-chain fatty acids is initiated by the sequential action of carnitine palmitoyltransferase (CPT) I (outer membrane and detergent labile) and II (inner membrane and detergent stable), together with carnitine carrier. CPTI catalyzes the first reaction in the transport of long-chain fatty acids from the cytoplasm to the mitochondrion, a rate-limiting step in β-oxidation. Two types of CPTI are known, the liver (CPTIA) and muscle (CPTIB) isoforms. The muscle type protein is specially expressed in heart and skeletal muscle. Membrane-bound CPTI, but not CPTII, is inhibited reversibly by malonyl-coenzyme A (CoA). Unlike CPTII, CPTI requires membrane integrity for catalytic function. In addition, glutamic acid 3 and histidine 5 are necessary for malonyl CoA inhibition and binding to liver CPTI, but not for catalytic activity.

REFERENCES

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2. McGarry, J.D., et al. 1989. Regulation of ketogenesis and the renaissance of carnitine palmitoyltransferase. Diabetes Metab. Rev. 5: 271-284.
3. Woeltje, K.F., et al. 1990. Inter-tissue and inter-species characteristics of the mitochondrial carnitine palmitoyltransferase enzyme system. J. Biol. Chem. 265: 10714-10719.
4. Britton, C.H., et al. 1995. Human liver mitochondrial carnitine palmitoyltransferase I: characterization of its cDNA and chromosomal localization and partial analysis of the gene. Proc. Natl. Acad. Sci. USA 92: 1984-1988.
5. Yamazaki, N., et al. 1996. Isolation and characterization of cDNA and genomic clones encoding human muscle type carnitine palmitoyltransferase I. Biochim. Biophys. Acta 1307: 157-161.
6. Zhu, H., et al. 1997. Functional studies of yeast-expressed human heart muscle carnitine palmitoyltransferase I. Arch. Biochem. Biophys. 347: 53-61.
7. Yamazaki, N., et al. 1997. Structural features of the gene encoding human muscle type carnitine palmitoyltransferase I. FEBS Lett. 409: 401-406.
8. Woldegiorgis, G., et al. 2000. Functional characterization of mammalian mitochondrial carnitine palmitoyltransferases I and II expressed in the yeast Pichia pastoris. J. Nutr. 130: 310-314.

CHROMOSOMAL LOCATION

Genetic locus: Cpt1b (mouse) mapping to 15 E3.

PRODUCT

CPTI-M 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 CPTI-M shRNA Plasmid (m): sc-40383-SH and CPTI-M shRNA (m) Lentiviral Particles: sc-40383-V as alternate gene silencing products.

For independent verification of CPTI-M (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-40383A, sc-40383B and sc-40383C.

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

CPTI-M siRNA (m) is recommended for the inhibition of CPTI-M 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

CPTI (E-7): sc-393070 is recommended as a control antibody for monitoring of CPTI-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-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 CPTI-M gene expression knockdown using RT-PCR Primer: CPTI-M (m)-PR: sc-40383-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.