SANTA CRUZ BIOTECHNOLOGY, INC.

CROT siRNA (m): sc-142579

BACKGROUND

CROT (carnitine O-octanoyltransferase), also called COT (carnitine octanoyltransferase), is a member of the carnitine/choline acetyltransferase protein family, which also includes CAT, CPTI, CPTI-M and CPTII. Carnitine/choline acetyltransferase family members are essential for the β-oxidation of fatty acids. CROT localizes to peroxisomes and is highly expressed in liver, kidney and proximal intestinal epithelium. CROT plays a role in lipid metabolism, catalyzing the reversible conversion of acyl-CoAs to their corresponding carnitine esters—a crucial step in facilitating the transport of fatty acids out of peroxisomes to mitochondria, where they can be further degraded. With a preference for straight and branched medium-chain acyl-CoAs (C6-C10 chain length), CROT plays an important role in energy metabolism in eukaryotes. In addition, CROT activity can be inhibited by malonyl-CoA.

REFERENCES

1. Ferdinandusse, S., et al. 1999. Molecular cloning and expression of human carnitine octanoyltransferase: evidence for its role in the peroxisomal β-oxidation of branched-chain fatty acids. Biochem. Biophys. Res. Commun. 263: 213-218.
2. van der Leij, F.R., et al. 2000. Genomics of the human carnitine acyltransferase genes. Mol. Genet. Metab. 71: 139-153.
3. Jong-Yeon, K., et al. 2002. Long- and medium-chain fatty acid oxidation is increased in exercise-trained human skeletal muscle. Metab. Clin. Exp. 51: 460-464.
4. Jogl, G., et al. 2004. Structure and function of carnitine acyltransferases. Ann. N.Y. Acad. Sci. 1033: 17-29.
5. Cordente, A.G., et al. 2004. Redesign of carnitine acetyltransferase specificity by protein engineering. J. Biol. Chem. 279: 33899-33908.
6. Cordente, A.G., et al. 2006. Mutagenesis of specific amino acids converts carnitine acetyltransferase into carnitine palmitoyltransferase. Biochemistry 45: 6133-6141.
7. Alfirevic, A., et al. 2007. Tacrine-induced liver damage: an analysis of 19 candidate genes. Pharmacogenet. Genomics 17: 1091-1100.
8. Wanders, R.J., et al. 2007. Peroxisomes, Refsum's disease and the α- and ω-oxidation of phytanic acid. Biochem. Soc. Trans. 35: 865-869.

CHROMOSOMAL LOCATION

Genetic locus: Crot (mouse) mapping to 5 A1.

PRODUCT

CROT 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 CROT shRNA Plasmid (m): sc-142579-SH and CROT shRNA (m) Lentiviral Particles: sc-142579-V as alternate gene silencing products.

For independent verification of CROT (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-142579A, sc-142579B and sc-142579C.

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

CROT siRNA (m) is recommended for the inhibition of CROT 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

CROT (H-1): sc-365976 is recommended as a control antibody for monitoring of CROT 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 CROT gene expression knockdown using RT-PCR Primer: CROT (m)-PR: sc-142579-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.