

# ACOT2 siRNA (h): sc-72435

## BACKGROUND

Acyl-CoA thioesterases (ACOTs) are a group of enzymes that catalyze the hydrolysis of acyl-CoA to form Coenzyme A (CoA) and a free fatty acid. Through their catalytic activity, ACOTs are able to regulate the level of fatty acids and acyl-CoAs within the cell. ACOT1 (acyl-CoA thioesterase 1, also known as CTE1) and ACOT2 (acyl-CoA thioesterase 2, also known as PTE2) are members of the ACOT family and exhibit different cellular localization, with ACOT1 existing as a monomer in the cytoplasm and ACOT2 localized primarily to mitochondria. Characteristic of most ACOT proteins, ACOT1 and ACOT2 catalyze the conversion of Palmitoyl-CoA and water to free CoA and palmitate, a reaction that is important for the regulation of intercellular fatty acid levels. ACOT2 is expressed as multiple alternatively spliced isoforms and, like ACOT1, is encoded by a gene which maps to human chromosome 14q24.3.

## REFERENCES

- Jones, J.M. and Gould, S.J. 2000. Identification of PTE2, a human peroxisomal long-chain acyl-CoA thioesterase. *Biochem. Biophys. Res. Commun.* 275: 233-240.
- Ishizuka, M., et al. 2004. Overexpression of human acyl-CoA thioesterase upregulates peroxisome biogenesis. *Exp. Cell Res.* 297: 127-141.
- Westin, M.A., et al. 2004. Molecular cloning and characterization of two mouse peroxisome proliferator-activated receptor  $\alpha$  (PPAR $\alpha$ )-regulated peroxisomal acyl-CoA thioesterases. *J. Biol. Chem.* 279: 21841-21848.
- Hunt, M.C., et al. 2005. A revised nomenclature for mammalian acyl-CoA thioesterases/hydrolases. *J. Lipid Res.* 46: 2029-2032.
- Hunt, M.C., et al. 2006. Analysis of the mouse and human acyl-CoA thioesterase (ACOT) gene clusters shows that convergent, functional evolution results in a reduced number of human peroxisomal ACOTs. *FASEB J.* 20: 1855-1864.
- Online Mendelian Inheritance in Man, OMIM<sup>™</sup>. 2006. Johns Hopkins University, Baltimore, MD. MIM Number: 6099726. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
- Rudolph, M.C., et al. 2007. Lipid synthesis in lactation: diet and the fatty acid switch. *J. Mammary Gland Biol. Neoplasia* 12: 269-281.

## CHROMOSOMAL LOCATION

Genetic locus: ACOT2 (human) mapping to 14q24.3.

## PRODUCT

ACOT2 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ACOT2 shRNA Plasmid (h): sc-72435-SH and ACOT2 shRNA (h) Lentiviral Particles: sc-72435-V as alternate gene silencing products.

For independent verification of ACOT2 (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-72435A, sc-72435B and sc-72435C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

ACOT2 siRNA (h) is recommended for the inhibition of ACOT2 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  $\mu$ M in 66  $\mu$ 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

ACOT1/2 (F-2): sc-373917 is recommended as a control antibody for monitoring of ACOT2 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ACOT2 gene expression knockdown using RT-PCR Primer: ACOT2 (h)-PR: sc-72435-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.