

ACAT-2 siRNA (m): sc-61909

BACKGROUND

ACAT-1 (acetyl-coenzyme A acetyltransferase 1), also known as acetoacetyl coenzyme A thiolase or mitochondrial acetoacetyl-CoA thiolase, is an enzyme involved in the formation and degradation of ketone bodies and is necessary for the proper metabolic processing of isoleucine. ACAT-2 (acetyl-CoA acetyltransferase 2), also known as acetyl-CoA transferase-like protein or cytosolic acetoacetyl-CoA thiolase, is a 397 amino acid protein that belongs to the thiolase family and exists as a homotetramer. Both acetoacetyl-CoA specific thiolases, ACAT-1 and ACAT-2, catalyze the formation of acetoacetyl-CoA from two acetyl-CoA molecules. These enzymes are also capable of the reverse reaction, the cleavage of acetoacetyl-CoA into two acetyl-CoA molecules.

REFERENCES

1. Groot, C.J., et al. 1977. A patient with severe neurologic symptoms and acetoacetyl-CoA thiolase deficiency. *Pediatr. Res.* 11: 1112-1116.
2. Willison, K., et al. 1987. The human homologue of the mouse t-complex gene, TCP1, is located on chromosome 6 but is not near the HLA region. *EMBO J.* 6: 1967-1974.
3. Igual, J.C., et al. 1992. Phylogenetic analysis of the thiolase family. Implications for the evolutionary origin of peroxisomes. *J. Mol. Evol.* 35: 147-155.
4. Ashworth, A. 1993. Two acetyl-CoA acetyltransferase genes located in the t-complex region of mouse chromosome 17 partially overlap the *Tcp-1* and *Tcp-1x* genes. *Genomics* 18: 195-198.
5. Song, X.Q., et al. 1994. Molecular cloning and nucleotide sequence of complementary DNA for human hepatic cytosolic acetoacetyl-coenzyme A thiolase. *Biochem. Biophys. Res. Commun.* 201: 478-485.

CHROMOSOMAL LOCATION

Genetic locus: *Acat2* (mouse) mapping to 17 A1.

PRODUCT

ACAT-2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ACAT-2 shRNA Plasmid (m): sc-61909-SH and ACAT-2 shRNA (m) Lentiviral Particles: sc-61909-V as alternate gene silencing products.

For independent verification of ACAT-2 (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-61909A, sc-61909B and sc-61909C.

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.

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

ACAT-2 siRNA (m) is recommended for the inhibition of ACAT-2 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 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

ACAT-2 (4A5): sc-293307 is recommended as a control antibody for monitoring of ACAT-2 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 ACAT-2 gene expression knockdown using RT-PCR Primer: ACAT-2 (m)-PR: sc-61909-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.