

ACAT-2 siRNA (h): sc-61908

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

- Groot, C.J., et al. 1977. A patient with severe neurologic symptoms and acetoacetyl-CoA thiolase deficiency. *Pediatr. Res.* 11: 1112-1116.
- 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.
- 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.
- 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.
- 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.
- Masuno, M., et al. 1996. Assignment of the human cytosolic acetoacetyl-coenzyme A thiolase (ACAT2) gene to chromosome 6q25.3-q26. *Genomics* 36: 217-218.

CHROMOSOMAL LOCATION

Genetic locus: ACAT2 (human) mapping to 6q25.3.

PRODUCT

ACAT-2 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 ACAT-2 shRNA Plasmid (h): sc-61908-SH and ACAT-2 shRNA (h) Lentiviral Particles: sc-61908-V as alternate gene silencing products.

For independent verification of ACAT-2 (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-61908A, sc-61908B and sc-61908C.

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 (h) is recommended for the inhibition of ACAT-2 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

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 (h)-PR: sc-61908-PR (20 μ l, 434 bp). 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.