SANTA CRUZ BIOTECHNOLOGY, INC.

ACAT-1 siRNA (h): sc-96390

BACKGROUND

ACAT-1 (acetyl-coenzyme A acetyltransferase 1) is a mitochondrial enzyme involved in the formation and degradation of ketone bodies and is necessary for the proper metabolic processing of isoleucine. Rare defects in the gene encoding ACAT-1 lead to β-ketothiolase deficiency, which is characterized by ketoacidotic attacks. ACAT-2 (acetyl-coenzyme A acetyltransferase 2) is considered a cytosolic protein and is crucial for cholesterol synthesis. Specifically, 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. Thompson, S.L. and Krisans, S.K. 1990. Rat liver peroxisomes catalyze the initial step in cholesterol synthesis. The condensation of acetyl-CoA units into acetoacetyl-CoA. J. Biol. Chem. 265: 5731-5735.
2. 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.
3. Masuno, M., et al. 1996. Assignment of the human cytosolic acetoacetyl-coenzyme A thiolase (ACAT-2) gene to chromosome 6q25.3-q26. Genomics 36: 217-218.

CHROMOSOMAL LOCATION

Genetic locus: ACAT1 (human) mapping to 11q22.3.

PRODUCT

ACAT-1 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ACAT-1 shRNA Plasmid (h): sc-96390-SH and ACAT-1 shRNA (h) Lentiviral Particles: sc-96390-V as alternate gene silencing products.

For independent verification of ACAT-1 (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-96390A, sc-96390B and sc-96390C.

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

ACAT-1 siRNA (h) is recommended for the inhibition of ACAT-1 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 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

ACAT-1 (AT15E5): sc-517387 is recommended as a control antibody for monitoring of ACAT-1 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 ACAT-1 gene expression knockdown using RT-PCR Primer: ACAT-1 (h)-PR: sc-96390-PR (20 µl, 420 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Huttunen, H.J., et al. 2007. Knockdown of ACAT-1 reduces amyloidogenic processing of APP. FEBS Lett. 581: 1688-1692.
2. Bemlih, S., et al. 2010. Acyl-coenzyme A: cholesterol acyltransferase inhibitor Avasimibe affect survival and proliferation of glioma tumor cell lines. Cancer Biol. Ther. 9: 1025-1032.
3. Zhang, J., et al. 2018. Importance of TFEB acetylation in control of its transcriptional activity and lysosomal function in response to histone deacetylase inhibitors. Autophagy 14: 1043-1059.
4. Zhao, H., et al. 2019. Single-cell transcriptomics of human oocytes: environment-driven metabolic competition and compensatory mechanisms during oocyte maturation. Antioxid. Redox Signal. 30: 542-559.
5. Sun, X., et al. 2022. Histone deacetylase inhibitors inhibit cervical cancer growth through Parkin acetylation-mediated mitophagy. Acta Pharm. Sin. B 12: 838-852.

RESEARCH USE

For research use only, not for use in diagnostic procedures.