

# ACAD-8 siRNA (h): sc-61932

## BACKGROUND

ACAD-8 (acyl-CoA dehydrogenase family member 8), also known as isobutyryl-CoA dehydrogenase (IBD) or activator-recruited cofactor 42 kDa component (ARC42), consists of an N-terminal  $\alpha$ -helical domain, a  $\beta$ -sheet domain and another  $\alpha$ -helical domain at the C-terminal. The ACAD family of enzymes are involved in the catabolism of fatty acids and amino acids. They provide a major source of energy for the heart and skeletal muscle. ACAD-8 is a mitochondrial flavoprotein involved in valine degradation. It is responsible for converting isobutyryl-CoA to methacrylyl-CoA. ACAD-8 localizes to the mitochondrial matrix and exists as a homotetramer. Deficiency of ACAD-8 results in carnitine deficiency, dilated cardiomyopathy and formula feeding intolerance. The excretion of isobutyryl-glycine in urine is a sign of an ACAD-8 related defect.

## REFERENCES

1. Roe, C.R., et al. 1999. Isolated isobutyryl-CoA dehydrogenase deficiency: an unrecognized defect in human valine metabolism. *Mol. Genet. Metab.* 65: 264-271.
2. Näär, A.M., et al. 1999. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* 398: 828-832.
3. Nguyen, T.V., et al. 2002. Identification of isobutyryl-CoA dehydrogenase and its deficiency in humans. *Mol. Genet. Metab.* 77: 68-79.
4. Zhang, J., et al. 2002. Cloning and functional characterization of ACAD-9, a novel member of human acyl-CoA dehydrogenase family. *Biochem. Biophys. Res. Commun.* 297: 1033-1042.
5. Sass, J.O., et al. 2004. Isobutyryl-CoA dehydrogenase deficiency: isobutyrylglycinuria and ACAD8 gene mutations in two infants. *J. Inher. Metab. Dis.* 27: 741-745.
6. Battaile, K.P., et al. 2004. Structures of isobutyryl-CoA dehydrogenase and enzyme-product complex: comparison with isovaleryl- and short-chain acyl-CoA dehydrogenases. *J. Biol. Chem.* 279: 16526-16534.

## CHROMOSOMAL LOCATION

Genetic locus: ACAD8 (human) mapping to 11q25.

## PRODUCT

ACAD-8 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 ACAD-8 shRNA Plasmid (h): sc-61932-SH and ACAD-8 shRNA (h) Lentiviral Particles: sc-61932-V as alternate gene silencing products.

For independent verification of ACAD-8 (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-61932A, sc-61932B and sc-61932C.

## RESEARCH USE

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

## 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

ACAD-8 siRNA (h) is recommended for the inhibition of ACAD-8 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

ACAD-8 (D-5): sc-390038 is recommended as a control antibody for monitoring of ACAD-8 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™ Molecular Weight Standards: sc-2035, UltraCruz® 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® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ACAD-8 gene expression knockdown using RT-PCR Primer: ACAD-8 (h)-PR: sc-61932-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.