SANTA CRUZ BIOTECHNOLOGY, INC.

EHHADH siRNA (h): sc-78261

BACKGROUND

Peroxisomes play an important role in the oxidation of fatty acids via β-oxidation, which is carried out by two distinct pathways; the L-hydroxy-specific classical β-oxidation for very long straight-chain fatty acids and the D-hydroxy-specific β-oxidation for branched-chain fatty acids. A defect in either pathway can result in elevated serum levels of fatty-acids, leading to severe mental retardation and early death. As an L-hydroxy-specific enzyme, EHHADH (enoyl-CoA-hydratase:3-hydroxyacyl-CoA dehydrogenase), also known as Peroxisomal L-bifunctional enzyme, is a 723 amino acid protein has an essential tripeptide sequence on its carboxyl-terminus that is required for peroxisomal transport. EHHADH-null mice only exhibit a blunted peroxisome proliferative response when challenged with a peroxisome proliferator. Since there were no observed changes in lipid metabolism, this evidence suggests that enoyl-CoAs were diverted to the D-hydroxy-specific β-oxidation system for metabolism.

REFERENCES

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2. Hoefler, G., et al. 1994. cDNA cloning of the human peroxisomal enoyl-CoA hydratase: 3-hydroxyacyl-CoA dehydrogenase bifunctional enzyme and localization to chromosome 3q26.3-3q28: a free left Alu Arm is inserted in the 3' noncoding region. Genomics 19: 60-67.
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CHROMOSOMAL LOCATION

Genetic locus: EHHADH (human) mapping to 3q27.2.

PRODUCT

EHHADH 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 EHHADH shRNA Plasmid (h): sc-78261-SH and EHHADH shRNA (h) Lentiviral Particles: sc-78261-V as alternate gene silencing products.

For independent verification of EHHADH (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-78261A, sc-78261B and sc-78261C.

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

EHHADH siRNA (h) is recommended for the inhibition of EHHADH 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

EHHADH (D-2): sc-393123 is recommended as a control antibody for monitoring of EHHADH 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 EHHADH gene expression knockdown using RT-PCR Primer: EHHADH (h)-PR: sc-78261-PR (20 µl). 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 for detailed protocols and support products.