SANTA CRUZ BIOTECHNOLOGY, INC.

HADHB siRNA (m): sc-62436

BACKGROUND

HADHB (trifunctional enzyme subunit β (mitochondrial), Acetyl-CoA acyltransferase) is a 474 amino acid protein encoded by the human gene HADHB. HADHB belong to the thiolase family, which are ubiquitous enzymes that catalyze the reversible thiolytic cleavage of 3-ketoacyl-CoA into acyl-CoA and acetyl-CoA, a 2-step reaction involving a covalent intermediate formed with a catalytic cysteine. HADHB is found in the mitochondrion as an octamer of 4 α (HADHA) and 4 β (HADHB) subunits. Defects in HADHB are a cause of trifunctional protein deficiency (TFP deficiency). The clinical manifestations are very variable and include hypoglycemia, cardiomyopathy and sudden death. Phenotypes with mainly hepatic and neuromyopathic involvement can also be distinguished. Biochemically, TFP deficiency is defined by the loss of all three enzyme activities of the TFP complex.

REFERENCES

1. Ushikubo, S., et al. 1996. Molecular characterization of mitochondrial trifunctional protein deficiency: formation of the enzyme complex is important for stabilization of both α- and β-subunits. Am. J. Hum. Genet. 58: 979-988.
2. Aoyama, T., et al. 1998. Fluorescence *in situ* hybridization mapping of the α and β subunits (HADHA and HADHB) of human mitochondrial fatty acid β-oxidation multienzyme complex to 2p23 and their evolution. Cytogenet. Cell Genet. 79: 221-224.
3. Gevaert, K., et al. 2003. Exploring proteomes and analyzing protein processing by mass spectrometric identification of sorted N-terminal peptides. Nat. Biotechnol. 21: 566-569.
4. Spiekerkoetter, U., et al. 2003. Molecular and phenotypic heterogeneity in mitochondrial trifunctional protein deficiency due to β-subunit mutations. Hum. Mutat. 21: 598-607.
5. Adams, D.J., et al. 2003. HADHB, HuR, and CP1 bind to the distal 3'-untranslated region of human renin mRNA and differentially modulate renin expression. J. Biol. Chem. 278: 44894-44903.

CHROMOSOMAL LOCATION

Genetic locus: Hadhb (mouse) mapping to 5 B1.

PRODUCT

HADHB 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HADHB shRNA Plasmid (m): sc-62436-SH and HADHB shRNA (m) Lentiviral Particles: sc-62436-V as alternate gene silencing products.

For independent verification of HADHB (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-62436A, sc-62436B and sc-62436C.

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 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

HADHB siRNA (m) is recommended for the inhibition of HADHB 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 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

HADHB (E-1): sc-271495 is recommended as a control antibody for monitoring of HADHB 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 HADHB gene expression knockdown using RT-PCR Primer: HADHB (m)-PR: sc-62436-PR (20 µl). 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 for detailed protocols and support products.