

HADHA siRNA (m): sc-75221

BACKGROUND

HADHA (trifunctional enzyme subunit alpha, mitochondrial), also known as TP- α , is the 763 amino acid α subunit of the mitochondrial trifunctional protein, which catalyzes the last three steps of mitochondrial β -oxidation of long chain fatty acids. This mitochondrial complex is composed of four α (HADHA) and four β (HADHB) subunits, and the α subunit (HADHA) is responsible for catalyzing the 3-hydroxyacyl-CoA dehydrogenase and enoyl-CoA hydratase activities. Mutations in the HADHA gene can lead to long-chain 3-hydroxyacyl-coenzyme A dehydrogenase (LCHAD) deficiency or mitochondrial trifunctional protein deficiency. LCHAD deficiency is characterized by a deficiency of the dehydrogenase activity with normal hydratase activity and moderately decreased thiolase activity. In mitochondrial trifunctional protein deficiency, all three activities of the protein, dehydrogenase, hydratase, and thiolase, are deficient.

REFERENCES

1. Aoyama, T., et al. 1997. 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.
2. Spiekerkoetter, U., et al. 2002. Uniparental disomy of chromosome 2 resulting in lethal trifunctional protein deficiency due to homozygous α -subunit mutations. *Hum. Mutat.* 20: 447-451.
3. Das, A.M., et al. 2006. Isolated mitochondrial long-chain ketoacyl-CoA thiolase deficiency resulting from mutations in the HADHB gene. *Clin. Chem.* 52: 530-534.

CHROMOSOMAL LOCATION

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

PRODUCT

HADHA 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see HADHA shRNA Plasmid (m): sc-75221-SH and HADHA shRNA (m) Lentiviral Particles: sc-75221-V as alternate gene silencing products.

For independent verification of HADHA (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-75221A, sc-75221B and sc-75221C.

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

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

HADHA (E-8): sc-374497 is recommended as a control antibody for monitoring of HADHA 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 HADHA gene expression knockdown using RT-PCR Primer: HADHA (m)-PR: sc-75221-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. 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.

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.