

NDUFA5 siRNA (h): sc-89625

BACKGROUND

NDUFA5 (NADH-ubiquinone oxidoreductase a subunit 5), also designated Complex I-13kD-B, is one of 45 subunits comprising complex I of the oxidative phosphorylation electron transport chain. The multi-subunit NADH:ubiquinone oxidoreductase (complex I) is the first enzyme complex in the electron transport chain of the mitochondria. Complex I deficiency is the most common respiratory chain defect, resulting in various combinations of cardiac, hepatic, and renal disorders. Through use of chaotropic agents, complex I can be separated into three different fractions: a flavoprotein fraction, a hydrophobic protein (HP) fraction and an iron-sulfur protein (IP) fraction. NDUFA5 is a 116 amino acid protein that is ubiquitously expressed with highest levels in heart, skeletal muscle and brain.

REFERENCES

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2. Duncan, A.M., et al. 1992. Localization of the human 75-kDa Fe-S protein of NADH-coenzyme Q reductase gene (NDUFS1) to 2q33→q34. *Cytogenet. Cell Genet.* 60: 212-213.
3. Tensing, K., et al. 1999. Genomic organization of the human complex I 13-kDa subunit gene NDUFA5. *Cytogenet. Cell Genet.* 84: 125-127.
4. Stojanovski, D., et al. 2004. Levels of human Fis1 at the mitochondrial outer membrane regulate mitochondrial morphology. *J. Cell Sci.* 117: 1201-1210.
5. Karahan, O.I., et al. 2005. Ultrasound evaluation of peritoneal catheter tunnel in catheter related infections in CAPD. *Int. Urol. Nephrol.* 37: 363-366.
6. Martin, M.A., et al. 2005. Leigh syndrome associated with mitochondrial complex I deficiency due to a novel mutation in the NDUFS1 gene. *Arch. Neurol.* 62: 659-661.
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CHROMOSOMAL LOCATION

Genetic locus: NDUFA5 (human) mapping to 7q31.32.

PRODUCT

NDUFA5 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NDUFA5 shRNA Plasmid (h): sc-89625-SH and NDUFA5 shRNA (h) Lentiviral Particles: sc-89625-V as alternate gene silencing products.

For independent verification of NDUFA5 (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-89625A, sc-89625B and sc-89625C.

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

NDUFA5 siRNA (h) is recommended for the inhibition of NDUFA5 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 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

NDUFA5 (A-3): sc-393273 is recommended as a control antibody for monitoring of NDUFA5 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 NDUFA5 gene expression knockdown using RT-PCR Primer: NDUFA5 (h)-PR: sc-89625-PR (20 μ l). 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.