

NDUFS1 siRNA (m): sc-61165

BACKGROUND

The multisubunit NADH:ubiquinone oxidoreductase (complex I) is the first enzyme complex in the electron transport chain of mitochondria. Through use of chaotropic agents, complex I can be separated into three different fractions: a flavoprotein fraction, an iron-sulfur protein (IP) fraction and a hydrophobic protein (HP) fraction. The IP fraction contains NDUFS1-7. NDUFS1, a 75 kDa protein, is the largest subunit of complex I and is thought to be the first of the Fe-S proteins to accept electrons from an NADH-flavoprotein reductase within the complex. NDUFS1 may even form part of the active site crevice where NADH is oxidized. NDUFS1 is also a critical caspase substrate in mitochondria, and caspase cleavage of NDUFS1 is required for several mitochondrial changes associated with apoptosis.

REFERENCES

1. Chow, W., et al. 1991. Determination of the cDNA sequence for the human mitochondrial 75 kDa Fe-S protein of NADH-coenzyme Q reductase. *Eur. J. Biochem.* 201: 547-50.
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. Bénit, P., et al. 2001. in mitochondrial complex I deficiency. *Am. J. Hum. Genet.* 68: 1344-1352.
4. Karahan, O.I., et al. 2005. Ultrasound evaluation of peritoneal catheter tunnel in catheter related infections in CAPD. *Int. Urol. Nephrol.* 37: 363-366.
5. 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.
6. Smeitink, J.A., et al. 2005. Cell biological consequences of mitochondrial NADH: ubiquino deficiency. *Curr. Neurovasc. Res.* 1: 29-40.

CHROMOSOMAL LOCATION

Genetic locus: *Ndufs1* (mouse) mapping to 1 C2.

PRODUCT

NDUFS1 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 NDUFS1 shRNA Plasmid (m): sc-61165-SH and NDUFS1 shRNA (m) Lentiviral Particles: sc-61165-V as alternate gene silencing products.

For independent verification of NDUFS1 (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-61165A, sc-61165B and sc-61165C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

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

NDUFS1 siRNA (m) is recommended for the inhibition of NDUFS1 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

NDUFS1 (E-8): sc-271510 is recommended as a control antibody for monitoring of NDUFS1 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 NDUFS1 gene expression knockdown using RT-PCR Primer: NDUFS1 (m)-PR: sc-61165-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.