



COX5a siRNA (h): sc-62150

BACKGROUND

The cytochrome c oxidase (COX) family of proteins function as the final electron donor in the respiratory chain to drive a proton gradient across the inner mitochondrial membrane, ultimately resulting in the production of water. The mammalian COX apoenzyme is a dimer, with each monomer consisting of 13 subunits, some of which are mitochondrial and some of which are nuclear. Found in the inner mitochondrial membrane, COX5a, one of two subunits of the COX5 protein, is the heme A-containing chain of the oxidase family that converts one molecule of oxygen and four molecules of hydrogen to two molecules of water. When oxygen levels within the cell are high, transcription of COX5a, the aerobic subunit of the COX5 protein, is up-regulated as the rate of cellular respiration increases. Conversely, when oxygen levels are low, COX5a transcription decreases as the cell works to conserve oxygen by slowing the creation of water.

REFERENCES

1. Cumsky, M.G., et al. 1985. Two nonidentical forms of subunit V are functional in yeast cytochrome c oxidase. *Proc. Natl. Acad. Sci. USA* 82: 2235-2239.
2. Waterland, R.A., et al. 1991. The isoforms of yeast cytochrome c oxidase subunit V alter the *in vivo* kinetic properties of the holoenzyme. *J. Biol. Chem.* 266: 4180-4186.
3. Burke, P.V., et al. 1997. Effects of oxygen concentration on the expression of cytochrome c and cytochrome c oxidase genes in yeast. *J. Biol. Chem.* 272: 14705-14712.
4. Schmidt, T.R., et al. 2002. Amino acid replacement is rapid in primates for the mature polypeptides of COX subunits, but not for their targeting presequences. *Gene* 286: 13-19.
5. Williams, S.L., et al. 2004. Cytochrome c oxidase subassemblies in fibroblast cultures from patients carrying mutations in COX10, SCO1, or SURF1. *J. Biol. Chem.* 279: 7462-7469.

CHROMOSOMAL LOCATION

Genetic locus: COX5A (human) mapping to 15q24.2.

PRODUCT

COX5a 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 COX5a shRNA Plasmid (h): sc-62150-SH and COX5a shRNA (h) Lentiviral Particles: sc-62150-V as alternate gene silencing products.

For independent verification of COX5a (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-62150A, sc-62150B and sc-62150C.

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

COX5a siRNA (h) is recommended for the inhibition of COX5a 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

COX5a (A-5): sc-376907 is recommended as a control antibody for monitoring of COX5a 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 COX5a gene expression knockdown using RT-PCR Primer: COX5a (h)-PR: sc-62150-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.