SANTA CRUZ BIOTECHNOLOGY, INC.

COX5b siRNA (m): sc-72983

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, COX5 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. Two isoforms of COX5 exist, COX5a and COX5b. When oxygen levels within the cell are high, transcription of COX5a (the aerobic isoform) is up-regulated as the rate of cellular respiration increases. Conversely, when oxygen levels are low, COX5b (the hypoxic isoform) transcription increases and functions to maximize the turnover rate of the COX apoenzyme.

REFERENCES

1. Hodge, M.R., et al. 1989. Inverse regulation of the yeast COX5 genes by oxygen and heme. Mol. Cell. Biol. 9: 1958-1964.
2. Allen, L.A., et al. 1995. Isoforms of yeast cytochrome c oxidase subunit V affect the binuclear reaction center and alter the kinetics of interaction with the isoforms of yeast cytochrome c. J. Biol. Chem. 270: 110-118.
3. Bachman, N.J., et al. 1996. Phylogenetic footprinting of the human cytochrome c oxidase subunit VB promoter. Arch. Biochem. Biophys. 333: 152-162.
4. Burke, P.V., et al. 1998. Structure/function of oxygen-regulated isoforms in cytochrome c oxidase. J. Exp. Biol. 201: 1163-1175.
5. David, P.S., et al. 2005. Effects of a transition from normoxia to anoxia on yeast cytochrome c oxidase and the mitochondrial respiratory chain: implications for hypoxic gene induction. Biochim. Biophys. Acta 1709: 169-180.

CHROMOSOMAL LOCATION

Genetic locus: Cox5b (mouse) mapping to 1 B.

PRODUCT

COX5b 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 COX5b shRNA Plasmid (m): sc-72983-SH and COX5b shRNA (m) Lentiviral Particles: sc-72983-V as alternate gene silencing products.

For independent verification of COX5b (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-72983A, sc-72983B and sc-72983C.

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 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

COX5b siRNA (m) is recommended for the inhibition of COX5b 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

COX5b (C-5): sc-374416 is recommended as a control antibody for monitoring of COX5b 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 COX5b gene expression knockdown using RT-PCR Primer: COX5b (m)-PR: sc-72983-PR (20 µl). 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.