SANTA CRUZ BIOTECHNOLOGY, INC.

# NCB5OR siRNA (h): sc-75883

## BACKGROUND

NCB5OR, also referred to as CYB5R4 (cytochrome b5 reductase 4), is a flavohemoprotein that contains cytochrome b5 and chrome b5 reductase cytodomains. A member of the flavoprotein pyridine nucleotide cytochrome reductase family, NCB5OR is widely expressed and colocalizes with calreticulin to the endoplasmic reticulum (ER). NCB5OR has a cytochrome b5 heme-binding domain as well as one CS domain, two FAD and two iron binding motifs. NCB5OR reduces cytochrome c, methemoglobin, ferricyanide and molecular oxygen *in vitro*. NCB5OR is involved in the ER stress response pathway and plays a critical role in protecting pancreatic β-cells against oxidative stress by preventing excess buildup of reactive oxygen species (ROS). The absence of NCB5OR may cause Insulin-deficient diabetes.

## REFERENCES


1. Andersen, G., et al. 2004. Variation in NCB5OR: studies of relationships to type 2 diabetes, maturity-onset diabetes of the young, and gestational diabetes mellitus. Diabetes 53: 2992-2997.
2. Zhu, H., et al. 2004. NCB5OR is a novel soluble NAD(P)H reductase localized in the endoplasmic reticulum. J. Biol. Chem. 279: 30316-30325.
3. Kurian, J.R., et al. 2004. NADH cytochrome b5 reductase and cytochrome b5 catalyze the microsomal reduction of xenobiotic hydroxylamines and amidoximes in humans. J. Pharmacol. Exp. Ther. 311: 1171-1178.
4. Xie, J., et al. 2004. Absence of a reductase, NCB5OR, causes Insulin-deficient diabetes. Proc. Natl. Acad. Sci. USA 101: 10750-10755.
5. Larade, K. and Bunn, H.F. 2006. Promoter characterization and transcriptional regulation of NCB5OR, a novel reductase necessary for pancreatic β cell maintenance. Biochim. Biophys. Acta 1759: 257-262.
6. Larade, K., et al. 2007. The reductase NCB5OR is responsive to the redox status in β cells and is not involved in the ER stress response. Biochem. J. 404: 467-476.
7. Kurian, J.R., et al. 2007. Discovery and characterization of a cytochrome b5 variant in humans with impaired hydroxylamine reduction capacity. Pharmacogenet. Genomics 17: 597-603.


## CHROMOSOMAL LOCATION

Genetic locus: CYB5R4 (human) mapping to 6q14.2.

## PRODUCT

NCB5OR 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see NCB5OR shRNA Plasmid (h): sc-75883-SH and NCB5OR shRNA (h) Lentiviral Particles: sc-75883-V as alternate gene silencing products.

For independent verification of NCB5OR (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-75883A, sc-75883B and sc-75883C.

## 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

NCB5OR siRNA (h) is recommended for the inhibition of NCB5OR 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 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

NCB5OR (D-2): sc-390569 is recommended as a control antibody for monitoring of NCB5OR 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 NCB5OR gene expression knockdown using RT-PCR Primer: NCB5OR (h)-PR: sc-75883-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.

## PROTOCOLS

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