

CYB5R3 siRNA (h): sc-62173

BACKGROUND

CYB5R3 (NADH-cytochrome b5 reductase 3, diaphorase-1) is a 301 amino acid protein encoded by the human gene CYB5R3. CYB5R3 belongs to the flavoprotein pyridine nucleotide cytochrome reductase family and has two naturally occurring isoforms. Isoform 1 is anchored to the cytoplasmic side of the endoplasmic reticulum membrane and mitochondrion outer membrane, while isoform 2 is the soluble form found in erythrocytes. CYB5R3 is involved in the desaturation and elongation of fatty acids, cholesterol biosynthesis, drug metabolism and, in erythrocytes, methemoglobin reduction. A serine residue at position 117 seems to only be found in persons of African origin. The allele frequency is 0.23 in African Americans. It is not found in Caucasians, Asians, Indo-Aryans or Arabs. This difference seems to have no effect on the enzyme activity. Defects in CYB5R3 are the cause of hereditary methemoglobinemia (HM). There are three forms of this disease: type 1 (HM1), in which the enzyme is only deficient in erythrocytes with a mild cyanosis; type 2 (HM2), in which the enzyme is completely deficient; and type 3 (HM3), where the deficiency is seen in all blood cells. Type 2 is a severe form accompanied by mental retardation and neurological impairment.

REFERENCES

- Farahani, P., et al. 2004. Obesity in BSB mice is correlated with expression of genes for iron homeostasis and leptin. *Obes. Res.* 12: 191-204.
- Tonegawa, Y., et al. 2005. Evaluation of data in terms of two-dimensional random walk model: interaction between NADH-cytochrome b5 reductase and cytochrome b5. *Biomed. Res.* 26: 207-212.
- Roma, G.W., et al. 2005. Mutagenesis of Glycine 179 modulates both catalytic efficiency and reduced pyridine nucleotide specificity in cytochrome b5 reductase. *Biochemistry* 44: 13467-13476.
- Percy, M.J., et al. 2005. Recessive congenital methaemoglobinaemia: functional characterization of the novel D239G mutation in the NADH-binding lobe of cytochrome b5 reductase. *Br. J. Haematol.* 129: 847-853.

CHROMOSOMAL LOCATION

Genetic locus: CYB5R3 (human) mapping to 22q13.2.

PRODUCT

CYB5R3 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 CYB5R3 shRNA Plasmid (h): sc-62173-SH and CYB5R3 shRNA (h) Lentiviral Particles: sc-62173-V as alternate gene silencing products.

For independent verification of CYB5R3 (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-62173A, sc-62173B and sc-62173C.

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

CYB5R3 siRNA (h) is recommended for the inhibition of CYB5R3 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

CYB5R3 (G-11): sc-398043 is recommended as a control antibody for monitoring of CYB5R3 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 CYB5R3 gene expression knockdown using RT-PCR Primer: CYB5R3 (h)-PR: sc-62173-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.