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PCB siRNA (m): sc-45532

BACKGROUND

Pyruvate carboxylase (PCB) is a biotinylated mitochondrial enzyme that catalyzes the synthesis of oxaloacetate from pyruvate in a tissue specific manner. In addition to covalently binding the biotin cofactor, PCB contains consensus sequences for the attachment of ATP and the substrate pyruvate. The PCB gene is located on the long arm of chromosome 11q13.2. Mutations in PCB metabolism (pyruvate carboxylase deficiency) are known to cause lactic acidosis, hypoglycemia and mental retardation.

REFERENCES

1. Freytag, S.O., et al. 1984. Molecular cloning of a cDNA for human pyruvate carboxylase. Structural relationship to other biotin-containing carboxylases and regulation of mRNA content in differentiating preadipocytes. *J. Biol. Chem.* 259: 12831-12837.
2. MacKay, N., et al. 1994. cDNA cloning of human kidney pyruvate carboxylase. *Biochem. Biophys. Res. Commun.* 202: 1009-1014.
3. Wexler, I.D., et al. 1998. Molecular characterization of pyruvate carboxylase deficiency in two consanguineous families. *Pediatr. Res.* 43: 579-584.
4. Innocenti, A., et al. 2004. Carbonic anhydrase inhibitors: inhibition of the membrane-bound human isozyme IV with anions. *Bioorg. Med. Chem. Lett.* 4: 5769-5773.
5. Karnik, D., et al. 2004. Hyperammonemia with citrullinemia. *Indian Pediatr.* 41: 842-844.
6. Hall, P.R., et al. 2004. Transcarboxylase 5S structures: assembly and catalytic mechanism of a multienzyme complex subunit. *EMBO J.* 23: 3621-3631.

CHROMOSOMAL LOCATION

Genetic locus: Pcx (mouse) mapping to 19 A.

PRODUCT

PCB 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 PCB shRNA Plasmid (m): sc-45532-SH and PCB shRNA (m) Lentiviral Particles: sc-45532-V as alternate gene silencing products.

For independent verification of PCB (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-45532A, sc-45532B and sc-45532C.

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

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

PCB (D-9): sc-365673 is recommended as a control antibody for monitoring of PCB 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 PCB gene expression knockdown using RT-PCR Primer: PCB (m)-PR: sc-45532-PR (20 μ l, 440 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Ye, F., et al. 2011. Comparative proteome analysis of 3T3-L1 adipocyte differentiation using iTRAQ-coupled 2D LC-MS/MS. *J. Cell. Biochem.* 112: 3002-3014.

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.