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PCB shRNA (h) Lentiviral Particles: sc-45531-V

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 11. 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: PC (human) mapping to 11q13.2.

PRODUCT

PCB shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see PCB siRNA (h): sc-45531 and PCB shRNA Plasmid (h): sc-45531-SH as alternate gene silencing products.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

APPLICATIONS

PCB shRNA (h) Lentiviral Particles is recommended for the inhibition of PCB expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

PCB (E-1): sc-373937 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor PCB gene expression knockdown using RT-PCR Primer: PCB (h)-PR: sc-45531-PR (20 μ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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