SANTA CRUZ BIOTECHNOLOGY, INC.

PCCB siRNA (h): sc-76079

SANTA CRUZ BIOTECHNOLOGY

The Power to Question

BACKGROUND

Propionyl-CoA is an important intermediate of amino acid metabolism and is also produced by oxidation of odd-numbered fatty acids. Propionyl-CoA carboxylase (PCC) catalyzes the biotin-dependent carboxylation of propionyl-CoA to d-methylmalonyl-CoA. PCCB (propionyl coenzyme A carboxylase, β chain), also known as propanoyl-CoA:carbon dioxide ligase subunit β, is a 539 amino acid subunit of PCC that localizes to the mitochondrion matrix. Inherited mutations in the gene encoding PCCB result in mutations near the amino-terminus, which contains the biotin-binding site of the protein. This mutation leads to propionic acidemia type II (PA-2), an autosomal recessive disease characterized by neutropenia, hypogammagloubinemia, episodic vomiting, ketosis and lethargy, periodic thrombocytopenia, developmental retardation and general intolerance to dietary protein.

REFERENCES

1. Lamhonwah, A.M., Barankiewicz, T.J., Willard, H.F., Mahuran, D.J., Quan, F. and Gravel, R.A. 1986. Isolation of cDNA clones coding for the α and β chains of human propionyl-CoA carboxylase: chromosomal assignments and DNA polymorphisms associated with PCCA and PCCB genes. Proc. Natl. Acad. Sci. USA 83: 4864-4868.
2. Ohura, T., Ogasawara, M., Ikeda, H., Narisawa, K. and Tada, K. 1993. The molecular defect in propionic acidemia: exon skipping caused by an 8-bp deletion from an intron in the PCCB allele. Hum. Genet. 92: 397-402.
3. Tahara, T., Kraus, J.P., Ohura, T., Rosenberg, L.E. and Fenton, W.A. 1993. Three independent mutations in the same exon of the PCCB gene: differences between Caucasian and Japanese propionic acidaemia. J. Inherit. Metab. Dis. 16: 353-360.
4. Lamhonwah, A.M., Leclerc, D., Loyer, M., Clarizio, R. and Gravel, R.A. 1994. Correction of the metabolic defect in propionic acidemia fibroblasts by microinjection of a full-length cDNA or RNA transcript encoding the propionyl-CoA carboxylase β subunit. Genomics 19: 500-505.

CHROMOSOMAL LOCATION

Genetic locus: PCCB (human) mapping to 3q22.3.

PRODUCT

PCCB 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 PCCB shRNA Plasmid (h): sc-76079-SH and PCCB shRNA (h) Lentiviral Particles: sc-76079-V as alternate gene silencing products.

For independent verification of PCCB (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-76079A, sc-76079B and sc-76079C.

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

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

PCCB (E-12): sc-393929 is recommended as a control antibody for monitoring of PCCB 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 PCCB gene expression knockdown using RT-PCR Primer: PCCB (h)-PR: sc-76079-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.