SANTA CRUZ BIOTECHNOLOGY, INC.

PCCA siRNA (h): sc-76077

BACKGROUND

PCCA (propionyl coenzyme A carboxylase, α polypeptide) is a 703 amino acid protein that localizes to the mitochondrion matrix. PCCA is a mitochondrial, biotin-dependent enzyme involved in the catabolism of branched chain amino acids, odd chain fatty acids, and other metabolites. PCCA is responsible for the formation of carboxybiotin upon hydrolysis of ATP and contains a C-terminal biotin-binding domain and a biotin carboxylase domain. Inherited mutations in the gene that encodes PCCA result in a deficiency of the protein, which leads to propionic acidemia (PA), an autosomal recessive inborn error in the catabolism of methionine, isoleucine, threonine, and valine, odd-numbered chain length fatty acids and cholesterol. Two isoforms exists due to alternative splicing events.

REFERENCES

1. Kelson, T.L., Ohura, T. and Kraus, J.P. 1996. Chaperonin-mediated assembly of wild-type and mutant subunits of human propionyl-CoA carboxylase expressed in *Escherichia coli*. Hum. Mol. Genet. 5: 331-337.
2. Richard, E., Desviat, L.R., Perez, B., Perez-Cerdá, C. and Ugarte, M. 1997. Three novel splice mutations in the PCCA gene causing identical exon skipping in propionic acidemia patients. Hum. Genet. 101: 93-96.
3. Ugarte, M., Perez-Cerdá, C., Rodríguez-Pombo, P., Desviat, L.R., Perez, B., Richard, E., Muro, S., Campeau, E., Ohura, T. and Gravel, R.A. 1999. Overview of mutations in the PCCA and PCCB genes causing propionic acidemia. Hum. Mutat. 14: 275-282.
4. Clavero, S., Martínez, M.A., Perez, B., Perez-Cerdá, C., Ugarte, M. and Desviat, L.R. 2002. Functional characterization of PCCA mutations causing propionic acidemia. Biochim. Biophys. Acta 1588: 119-125.
5. Kim, S.N., Ryu, K.H., Lee, E.H., Kim, J.S. and Hahn, S.H. 2002. Molecular analysis of PCCB gene in Korean patients with propionic acidemia. Mol. Genet. Metab. 77: 209-216.

CHROMOSOMAL LOCATION

Genetic locus: PCCA (human) mapping to 13q32.3.

PRODUCT

PCCA 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 PCCA shRNA Plasmid (h): sc-76077-SH and PCCA shRNA (h) Lentiviral Particles: sc-76077-V as alternate gene silencing products.

For independent verification of PCCA (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-76077A, sc-76077B and sc-76077C.

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

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

PCCA (D-5): sc-374341 is recommended as a control antibody for monitoring of PCCA 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 PCCA gene expression knockdown using RT-PCR Primer: PCCA (h)-PR: sc-76077-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.