

P5CR siRNA (h): sc-93710

BACKGROUND

P5CR (pyrroline-5-carboxylate reductase), also known as PYCR1, is a 319 amino acid mitochondrial protein that belongs to the pyrroline-5-carboxylate reductase family. Existing as a homodecamer, P5CR is composed of five homodimers. Acting as a housekeeping enzyme that catalyzes the last step in proline biosynthesis, P5CR is involved in the cellular response to oxidative stress. Upon oxidative stress, P5CR interacts with both NAD and NADP. Defects in P5CR are the cause of cutis laxa autosomal recessive type 2B (ARCL2B), a multi-system disorder characterized by the appearance of premature aging, wrinkled and lax skin with reduced elasticity, joint laxity, craniofacial dysmorphic features, intrauterine growth retardation and some degree of postnatal growth deficiency. The gene that encodes P5CR consists of nearly 5,000 bases and maps to human chromosome 17q25.3.

REFERENCES

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2. Merrill, M.J., et al. 1989. Purified human erythrocyte pyrroline-5-carboxylate reductase. Preferential oxidation of NADPH. *J. Biol. Chem.* 264: 9352-9358.
3. Dougherty, K.M., et al. 1992. Cloning human pyrroline-5-carboxylate reductase cDNA by complementation in *Saccharomyces cerevisiae*. *J. Biol. Chem.* 267: 871-875.
4. Online Mendelian Inheritance in Man, OMIM[™]. 1993. Johns Hopkins University, Baltimore, MD. MIM Number: 179035. World Wide Web URL: <http://www.ncbi.nlm.nih.gov/omim/>
5. Basch, J.J., et al. 1996. Pyrroline-5-carboxylate reductase in lactating bovine mammary glands. *J. Dairy Sci.* 79: 1361-1368.
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7. Guernsey, D.L., et al. 2009. Mutation in pyrroline-5-carboxylate reductase 1 gene in families with cutis laxa type 2. *Am. J. Hum. Genet.* 85: 120-129.
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CHROMOSOMAL LOCATION

Genetic locus: PYCR1 (human) mapping to 17q25.3.

PRODUCT

P5CR 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 P5CR shRNA Plasmid (h): sc-93710-SH and P5CR shRNA (h) Lentiviral Particles: sc-93710-V as alternate gene silencing products.

For independent verification of P5CR (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-93710A, sc-93710B and sc-93710C.

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

P5CR siRNA (h) is recommended for the inhibition of P5CR 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

P5CR (A-12): sc-518219 is recommended as a control antibody for monitoring of P5CR 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 P5CR gene expression knockdown using RT-PCR Primer: P5CR (h)-PR: sc-93710-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.

PROTOCOLS

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