

# P5CRL siRNA (h): sc-77825

## BACKGROUND

Proteins containing L-proline play an important role in protein folding, structure and stability. Additionally, the presence of L-proline in certain proteins may serve as a sequence-recognition motif. Proline functions as a non-enzymatic antioxidant to minimize damage caused by reactive oxygen species (ROS) in microorganisms, animals and plants. In the last step of proline biosynthesis, pyrroline-5-carboxylate reductase (P5CR) catalyzes the reduction of aldehyde dehydrogenase 4A1 (ALDH4A1) to proline using NAD(P)H as the cofactor. P5CRL (pyrroline-5-carboxylate reductase-like), also known as P5CR 3, P5C reductase 3 or PYCRL, is 274 amino acids in length, belongs to the pyrroline-5-carboxylate reductase family and functions as a homodecamer, which is composed of five homodimers. P5CR may play a critical role in proline bio-synthesis.

## REFERENCES

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- Nocek, B., et al. 2005. Crystal structures of  $\delta$ 1-pyrroline-5-carboxylate reductase from human pathogens *Neisseria meningitidis* and *Streptococcus pyogenes*. *J. Mol. Biol.* 354: 91-106.
- Meng, Z., et al. 2006. Crystal structure of human pyrroline-5-carboxylate reductase. *J. Mol. Biol.* 359: 1364-1377.
- Yang, Y., et al. 2006. Purification and characterization of a functionally active *Mycobacterium tuberculosis* pyrroline-5-carboxylate reductase. *Protein Expr. Purif.* 45: 241-248.
- Meng, Z., et al. 2006. Purification, characterization, and crystallization of human pyrroline-5-carboxylate reductase. *Protein Expr. Purif.* 49: 83-87.
- Krishnan, N., et al. 2008. Proline modulates the intracellular redox environment and protects mammalian cells against oxidative stress. *Free Radic. Biol. Med.* 44: 671-681.
- Forlani, G., et al. 2008. Tailoring the structure of aminobisphosphonates to target plant P5C reductase. *J. Agric. Food Chem.* 56: 3193-3199.

## CHROMOSOMAL LOCATION

Genetic locus: PYCRL (human) mapping to 8q24.3.

## PRODUCT

P5CRL siRNA (h) is a pool of 2 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see P5CRL shRNA Plasmid (h): sc-77825-SH and P5CRL shRNA (h) Lentiviral Particles: sc-77825-V as alternate gene silencing products.

For independent verification of P5CRL (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-77825A and sc-77825B.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

P5CRL siRNA (h) is recommended for the inhibition of P5CRL 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  $\mu$ M in 66  $\mu$ 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

P5CRL (E-63): sc-100487 is recommended as a control antibody for monitoring of P5CRL 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  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 $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  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 P5CRL gene expression knockdown using RT-PCR Primer: P5CRL (h)-PR: sc-77825-PR (20  $\mu$ 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](http://www.scbt.com) for detailed protocols and support products.