SANTA CRUZ BIOTECHNOLOGY, INC.

P4HA1 siRNA (h): sc-90782

BACKGROUND

P4HA1 (prolyl 4-hydroxylase, α polypeptide I) and P4HA1 (prolyl 4-hydroxylase, α polypeptide II) are 534 and 535 amino acid proteins, respectively, that each localize to the lumen of the endoplasmic reticulum and contain one TPR repeat, as well as one PKHD domain. Using ascorbate and iron as cofactors, P4HA1 and P4HA2 catalyze the posttranslational formation of 4-hydroxyproline in collagens and other proteins, thereby playing an essential role in the proper folding of newly synthesized peptides. P4HA1 exists as a heterotetramer of two α-1 and two β chains, while P4HA2 exists as a heterotetramer of two α-2 chains and two β chains. Both P4HA1 and P4HA2 exists as multiple alternatively spliced isoforms.

REFERENCES

1. Helaakoski, T., et al. 1989. Molecular cloning of the α-subunit of human prolyl 4-hydroxylase: the complete cDNA-derived amino acid sequence and evidence for alternative splicing of RNA transcripts. Proc. Natl. Acad. Sci. USA 86: 4392-4396.
2. Nokelainen, M., et al. 2001. Characterization of the human and mouse genes for the α subunit of type II prolyl 4-hydroxylase. Identification of a previously unknown alternatively spliced exon and its expression in various tissues. Eur. J. Biochem. 268: 5300-5309.
3. Kukkola, L., et al. 2003. Identification and characterization of a third human, rat, and mouse collagen prolyl 4-hydroxylase isoenzyme. J. Biol. Chem. 278: 47685-47693.
4. Grimmer, C., et al. 2006. Regulation of type II collagen synthesis during osteoarthritis by prolyl-4-hydroxylases: possible influence of low oxygen levels. Am. J. Pathol. 169: 491-502.
5. Chen, L., et al. 2006. Human prolyl 4-hydroxylase α-1 transcription is mediated by upstream stimulatory factors. J. Biol. Chem. 281: 10849-10855.

CHROMOSOMAL LOCATION

Genetic locus: P4HA1 (human) mapping to 10q22.1.

PRODUCT

P4HA1 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 P4HA1 shRNA Plasmid (h): sc-90782-SH and P4HA1 shRNA (h) Lentiviral Particles: sc-90782-V as alternate gene silencing products.

For independent verification of P4HA1 (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-90782A, sc-90782B and sc-90782C.

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

P4HA1 siRNA (h) is recommended for the inhibition of P4HA1 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor P4HA1 gene expression knockdown using RT-PCR Primer: P4HA1 (h)-PR: sc-90782-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.