SANTA CRUZ BIOTECHNOLOGY, INC.

P4H-TM siRNA (h): sc-78320

BACKGROUND

The EF-hand domain is a twelve amino acid loop motif that is commonly found in proteins that participate in calcium-binding events within the cell. EF-hand domains generally exist in a pair that, together, form a stable four-helix bundle that enables the binding of calcium ions. P4H-TM, also known as P4HTM (prolyl 4-hydroxylase, transmembrane), PH-4, PHD4 or EGLN4, is a 502 amino acid single-pass type II membrane protein that localizes to the endoplasmic reticulum and contains one PKHD domain and two EF-hand domains. Expressed in a variety of tissues with highest expression in heart, brain, kidney, placenta and skeletal muscle, P4H-TM uses iron as a cofactor to catalyze the post-translational formation of 4-hydroxyproline in hypoxia-inducible factor (HIF) α proteins, thereby playing an important role in hypoxia adaptation and cellular oxygen sensing. Multiple isoforms of P4H-TM exist due to alternative splicing events.

REFERENCES

1. Kim, H.S., et al. 2000. Human CD1D associates with prolyl-4-hydroxylase during its biosynthesis. Mol. Immunol. 37: 861-868.
2. Oehme, F., et al. 2002. Overexpression of PH-4, a novel putative proline 4-hydroxylase, modulates activity of hypoxia-inducible transcription factors. Biochem. Biophys. Res. Commun. 296: 343-349.
3. Hirsilä, M., et al. 2003. Characterization of the human prolyl 4-hydroxylases that modify the hypoxia-inducible factor. J. Biol. Chem. 278: 30772-30780.
4. Pekkala, M., et al. 2004. The peptide-substrate-binding domain of collagen prolyl 4-hydroxylases is a tetratricopeptide repeat domain with functional aromatic residues. J. Biol. Chem. 279: 52255-52261.
5. Hirota, K. and Semenza, G.L. 2005. Regulation of hypoxia-inducible factor 1 by prolyl and asparaginyl hydroxylases. Biochem. Biophys. Res. Commun. 338: 610-616.
6. Koivunen, P., et al. 2007. An endoplasmic reticulum transmembrane prolyl 4-hydroxylase is induced by hypoxia and acts on hypoxia-inducible factor α. J. Biol. Chem. 282: 30544-30552.

CHROMOSOMAL LOCATION

Genetic locus: P4HTM (human) mapping to 3p21.31.

PRODUCT

P4H-TM 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 P4H-TM shRNA Plasmid (h): sc-78320-SH and P4H-TM shRNA (h) Lentiviral Particles: sc-78320-V as alternate gene silencing products.

For independent verification of P4H-TM (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-78320A, sc-78320B and sc-78320C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

P4H-TM siRNA (h) is recommended for the inhibition of P4H-TM 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 P4H-TM gene expression knockdown using RT-PCR Primer: P4H-TM (h)-PR: sc-78320-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

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