SANTA CRUZ BIOTECHNOLOGY, INC.

LEPREL1 siRNA (m): sc-146708

BACKGROUND

LEPREL1 (leprecan-like 1), also known as P3H2 (prolyl 3-hydroxylase 2 precursor) or MLAT4 (myxoid liposarcoma-associated protein 4), is a 708 amino acid protein that belongs to a family of collagen prolyl hydroxylases. LEPREL1 has a highly conserved C-terminal domain that contains critical catalytic residues found in lysyl and prolyl 4-hydroxylases, including two iron-binding histidines and an aspartic acid. LEPREL1 is expressed in heart, placenta, lung, liver, skeletal muscle and kidney. Expression is localized to the epithelia of bile ducts and to the sarcoplasm of heart muscle and skeletal muscle. LEPREL1 is required for proper collagen biosynthesis, folding, and assembly. LEPREL1 forms a complex with cartilage-associated protein (CRTAP) and cyclophilin B (CyPB) in the endoplasmic reticulum. This complex is responsible for 3-hydroxylation of proline residues of collagen type I, II and V, and is important for normal bone development.

REFERENCES

1. Vranka, J.A., Sakai, L.Y. and Bächinger, H.P. 2004. Prolyl 3-hydroxylase 1, enzyme characterization and identification of a novel family of enzymes. J. Biol. Chem. 279: 23615-23621.
2. Morello, R., Bertin, T.K., Chen, Y., Hicks, J., Tonachini, L., Monticone, M., Castagnola, P., Rauch, F., Glorieux, F.H., Vranka, J., Bächinger, H.P., Pace, J.M., Schwarze, U., Byers, P.H., Weis, M., Fernandes, R.J., Eyre, D.R., et al. 2006. CRTAP is required for prolyl 3-hydroxylation and mutations cause recessive osteogenesis imperfecta. Cell 127: 291-304.
3. Online Mendelian Inheritance in Man, OMIM™. 2006. Johns Hopkins University, Baltimore, MD. MIM Number: 610341. World Wide Web URL: http://www.ncbi.nlm.nih.gov/omim/
4. Marini, J.C., Cabral, W.A., Barnes, A.M. and Chang, W. 2007. Components of the collagen prolyl 3-hydroxylation complex are crucial for normal bone development. Cell Cycle 6: 1675-1681.
5. Cheung, M.S. and Glorieux, F.H. 2008. Osteogenesis imperfecta: update on presentation and management. Rev. Endocr. Metab. Disord. 9: 153-160.

CHROMOSOMAL LOCATION

Genetic locus: Leprel1 (mouse) mapping to 16 B1.

PRODUCT

LEPREL1 siRNA (m) is a target-specific 19-25 nt siRNA 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 LEPREL1 shRNA Plasmid (m): sc-146708-SH and LEPREL1 shRNA (m) Lentiviral Particles: sc-146708-V as alternate gene silencing products.

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.

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

LEPREL1 siRNA (m) is recommended for the inhibition of LEPREL1 expression in mouse 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

LEPREL1 (H-4): sc-377096 is recommended as a control antibody for monitoring of LEPREL1 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 LEPREL1 gene expression knockdown using RT-PCR Primer: LEPREL1 (m)-PR: sc-146708-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.