SANTA CRUZ BIOTECHNOLOGY, INC.

CRTAP siRNA (m): sc-142588

BACKGROUND

CRTAP (cartilage associated protein), also known as CASP or LEPREL3 (leprecan-like 3), is a secreted protein localizing to the extracellular space that plays a role in collagen post-translational modifications, extracellular fibril assembly and intracellular trafficking. CRTAP is widely expressed with predominant expression in articular chondrocytes. It contains a signal peptide and a tetratricopeptide-like helical domain and is essential for normal bone formation. In the endoplasmic reticulum (ER), CRTAP forms a complex with Gros1 and CyPB (cyclophilin B) and is required for the efficient 3-hydroxylation of target prolyl residues in Collagen Type I molecules, the major structural proteins of skin and bone. Mutations in the gene encoding CRTAP can lead to autosomal recessive osteogenesis imperfecta (OI) type 7 and type 2B. OI, also known as brittle bone disease, is characterized by bone fragility and susceptibility to fractures. OI type 7 is a mild form of this disorder, while OI type 2B is a neonatal lethal condition.

REFERENCES

1. Castagnola, P., et al. 1997. Cartilage associated protein (CASP) is a novel developmentally regulated chick embryo protein. J. Cell Sci. 110: 1351-1359.
2. Morello, R., et al. 1999. cDNA cloning, characterization and chromosome mapping of Crtap encoding the mouse cartilage associated protein. Matrix Biol. 18: 319-324.
3. Tonachini, L., et al. 1999. cDNA cloning, characterization and chromosome mapping of the gene encoding human cartilage associated protein (CRTAP). Cytogenet. Cell Genet. 87: 191-194.
4. Barnes, A.M., et al. 2006. Deficiency of cartilage-associated protein in recessive lethal osteogenesis imperfecta. N. Engl. J. Med. 355: 2757-2764.
5. Morello, R., et al. 2006. CRTAP is required for prolyl 3- hydroxylation and mutations cause recessive osteogenesis imperfecta. Cell 127: 291-304.
6. Martin, E. and Shapiro, J.R. 2007. Osteogenesis imperfecta:epidemiology and pathophysiology. Curr. Osteoporos. Rep. 5: 91-97.
7. Kwan, T., et al. 2007. Heritability of alternative splicing in the human genome. Genome Res. 17: 1210-1218.

CHROMOSOMAL LOCATION

Genetic locus: Crtap (mouse) mapping to 9 F3.

PRODUCT

CRTAP siRNA (m) 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 CRTAP shRNA Plasmid (m): sc-142588-SH and CRTAP shRNA (m) Lentiviral Particles: sc-142588-V as alternate gene silencing products.

For independent verification of CRTAP (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-142588A, sc-142588B and sc-142588C.

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

CRTAP siRNA (m) is recommended for the inhibition of CRTAP 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

CRTAP (E-1): sc-393136 is recommended as a control antibody for monitoring of CRTAP 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 CRTAP gene expression knockdown using RT-PCR Primer: CRTAP (m)-PR: sc-142588-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.

PROTOCOLS

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