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Ameloblastin siRNA (h): sc-44945

BACKGROUND

Dental enamel is a highly mineralized tissue with most of its volume occupied by large, highly organized, hydroxyapatite crystals. This structure is thought to be controlled through the interaction of many organic matrix molecules including Amelogenin, Ameloblastin, Enamelin, Tuftelin and several other enzymes. All of these secreted proteins are involved in the mineralization and enamel matrix formation in developing tooth enamel. Ameloblastin (AMBN), which localizes to the extracellular matrix, is an ameloblast-specific protein. It is detected in the sheath space between rod-interrod enamel and at the Tomes processes of secretory ameloblasts. Defects in the gene encoding for Ameloblastin, AMBN, can be seen in patients with ameloblastomas.

REFERENCES

1. MacDougall, M., et al. 2000. Cloning, characterization and immunolocalization of human Ameloblastin. *Eur. J. Oral Sci.* 108: 303-310.
2. Toyosawa, S., et al. 2000. Cloning and characterization of the human Ameloblastin gene. *Gene* 256: 1-11.
3. Mardh, C.K., et al. 2001. Human Ameloblastin gene: genomic organization and mutation analysis in amelogenesis imperfecta patients. *Eur. J. Oral Sci.* 109: 8-13.
4. Shintani, S., et al. 2005. Expression of Ameloblastin during enamel formation in a crocodile. Enamel formation, as well as adhesion between ameloblasts and the enamel. *J. Exp. Zool. B Mol. Dev. Evol.* 306: 126-133.
5. Torres-Quintana, M.A., et al. 2005. Ameloblastin and Amelogenin expression in postnatal developing mouse molars. *J. Oral Sci.* 47: 27-34.

CHROMOSOMAL LOCATION

Genetic locus: AMBN (human) mapping to 4q13.3.

PRODUCT

Ameloblastin 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Ameloblastin shRNA Plasmid (h): sc-44945-SH and Ameloblastin shRNA (h) Lentiviral Particles: sc-44945-V as alternate gene silencing products.

For independent verification of Ameloblastin (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-44945A, sc-44945B and sc-44945C.

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

APPLICATIONS

Ameloblastin siRNA (h) is recommended for the inhibition of Ameloblastin 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 μ M in 66 μ 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

Ameloblastin (H-2): sc-271012 is recommended as a control antibody for monitoring of Ameloblastin 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 Ameloblastin gene expression knockdown using RT-PCR Primer: Ameloblastin (h)-PR: sc-44945-PR (20 μ l, 512 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Tamburstuen, M.V., et al. 2011. Ameloblastin upstream region contains structural elements regulating transcriptional activity in a stromal cell line derived from bone marrow. *Eur. J. Oral Sci. Suppl.* 1: 286-292.

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.