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Amelogenin shRNA (h) Lentiviral Particles: sc-44845-V

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. The gene AMELX which encodes for the protein Amelogenin, is encoded on the X-chromosome. Amelogenin, also designated AMG, AMGX or AMEX, is involved in biomineralization and organization of developing enamel. It functions by regulating crystallite formation during the secretory stage of enamel development. Amelogenin, which localizes to the extracellular matrix, is expressed by ameloblasts and is the predominant protein in developing dental enamel. Defects in the AMELX gene can cause amelogenesis imperfecta hypoplastic type 1 (AIH1) which is an X-linked disease that affects the formation of tooth enamel.

REFERENCES

1. Nakahori, Y., et al. 1991. A human X-Y homologous region encodes "Amelogenin." *Genomics* 9: 264-269.
2. Catalano-Sherman, J., et al. 1993. Amino acid sequence of a major human Amelogenin protein employing Edman degradation and cDNA sequencing. *J. Dent. Res.* 72: 1566-1572.
3. Catalano-Sherman, J., et al. 1994. Production of a monoclonal antibody against human amelogenin. *Calcif. Tissue Int.* 54: 76-80.
4. Lagerstrom-Fermer, M., et al. 1995. Amelogenin signal peptide mutation: correlation between mutations in the amelogenin gene (AMGX) and manifestations of X-linked amelogenesis imperfecta. *Genomics* 26: 159-162.

CHROMOSOMAL LOCATION

Genetic locus: AMELX (human) mapping to Xp22.2.

PRODUCT

Amelogenin shRNA (h) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Amelogenin siRNA (h): sc-44845 and Amelogenin shRNA Plasmid (h): sc-44845-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

PROTOCOLS

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

APPLICATIONS

Amelogenin shRNA (h) Lentiviral Particles is recommended for the inhibition of Amelogenin expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Amelogenin (F-11): sc-365284 is recommended as a control antibody for monitoring of Amelogenin 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Amelogenin gene expression knockdown using RT-PCR Primer: Amelogenin (h)-PR: sc-44845-PR (20 μ l, 401 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.