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OSX shRNA (m) Lentiviral Particles: sc-45909-V

BACKGROUND

Osterix (OSX) is a zinc finger-containing transcriptional activator that is distinctly expressed in all developing bones and is important for osteoblast differentiation. In particular, OSX is implicated in the differentiation of osteoblasts, which are the specialized cells of bone formation. OSX is a nuclear protein that binds to GC box promoters elements and activates mRNA synthesis from genes containing functional recognition sites. The periosteal and mesenchymal cells of the membranous skeletal elements of OSX⁻ mice fail to differentiate into osteoblasts. Subsequently, the mesenchymal cells of OSX⁻ mice fail to deposit bone matrix and do not form bone. Cox-2 deficiency correlates with a decrease in OSX expression, suggesting that Cox-2 may induce OSX to mediate skeletal repair.

REFERENCES

1. Nakashima, K., et al. 2002. The novel zinc finger-containing transcription factor osterix is required for osteoblast differentiation and bone formation. *Cell* 108: 17-29
2. Yagi, K., et al. 2003. Bone morphogenetic protein-2 enhances osterix gene expression in chondrocytes. *J. Cell Biochem.* 88: 1077-1083
3. Lee, M.H., et al. 2003. BMP-2-induced osterix expression is mediated by Dlx-5 but is independent of RUNX2. *Biochem. Biophys. Res. Commun.* 309: 689-694.
4. Huang, L., et al. 2004. Expression of preosteoblast markers and Cbfa-1 and osterix gene transcripts in stromal tumour cells of giant cell tumour of bone. *Bone* 34: 393-401.
5. Ohyama, Y., et al. 2004. Spatiotemporal association and bone morphogenetic protein regulation of sclerostin and osterix expression during embryonic osteogenesis. *Endocrinology* 145: 4685-4692.

CHROMOSOMAL LOCATION

Genetic locus: Sp7 (mouse) mapping to 15 F3.

PRODUCT

OSX shRNA (m) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 2 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ 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 OSX siRNA (m): sc-45909 and OSX shRNA Plasmid (m): sc-45909-SH as alternate gene silencing products.

APPLICATIONS

OSX shRNA (m) Lentiviral Particles is recommended for the inhibition of OSX expression in mouse cells.

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.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ 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

OSX (F-3): sc-393325 is recommended as a control antibody for monitoring of OSX 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 OSX gene expression knockdown using RT-PCR Primer: OSX (m)-PR: sc-45909-PR (20 µl, 399 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° 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.

SELECT PRODUCT CITATIONS

1. Omoteyama, K., et al. 2010. The effects of Sp7/Osterix gene silencing in the chondroprogenitor cell line, ATDC5. *Biochem. Biophys. Res. Commun.* 403: 242-246.

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.