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MGP shRNA (m) Lentiviral Particles: sc-44627-V

BACKGROUND

Matrix Gla protein, or MGP, is involved in regulating calcification in the extracellular matrix, in particular in cartilage and arteries. MGP is a vitamin K-dependent protein containing five to six residues of γ -carboxy-glutamic acid (Gla), a Ca^{2+} binding amino acid requiring vitamin K-dependent γ carboxylase for its formation. In humans MGP is an 84 residue protein along with a 19 amino acid transmembrane signal peptide. A shortened 77 residue form of MGP is found in human bone extracts, likely formed by COOH-terminal processing by carboxypeptidase B-like enzymatic activity. High levels of expression occur in heart, kidney and lung, and over-expression of MGP occurs in the breast cancer cell line 600 PEI. Retinoic acid induces MGP expression in chondrocytes, fibroblasts and osteoblasts. Mutations in the gene coding for MGP can cause Keutel syndrome (KS), associated with abnormal cartilage calcification, substantiating the role of MGP in extracellular matrix calcification regulation. MGP can bind vitronectin and fibronectin via its C-terminus; phosphorylation of MGP occurs near the N-terminus at three serine residues, which are part of a tandemly repeated Ser-X-Glu sequence.

REFERENCES

- Price, P.A., et al. 1983. Matrix Gla protein, a new γ -carboxyglutamic acid-containing protein which is associated with the organic matrix of bone. *Biochem. Biophys. Res. Commun.* 117: 765-771.
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- Hale, J.E., et al. 1991. Carboxyl-terminal proteolytic processing of matrix Gla protein. *J. Biol. Chem.* 266: 21145-21149.
- Price, P.A., et al. 1994. Conserved phosphorylation of serines in the Ser-X-Glu/Ser(P) sequences of the vitamin K-dependent matrix Gla protein from shark, lamb, rat, cow, and human. *Protein Sci.* 3: 822-830.

CHROMOSOMAL LOCATION

Genetic locus: Mgp (mouse) mapping to 6 G1.

PRODUCT

MGP shRNA (m) 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 MGP siRNA (m): sc-44627 and MGP shRNA Plasmid (m): sc-44627-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.

APPLICATIONS

MGP shRNA (m) Lentiviral Particles is recommended for the inhibition of MGP expression in mouse 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

MGP (FL-103): sc-66965 is recommended as a control antibody for monitoring of MGP 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-rabbit IgG-HRP: sc-2004 (dilution range: 1:2000-1:100,000) or Cruz Marker™ compatible goat anti-rabbit IgG-HRP: sc-2030 (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-rabbit IgG-FITC: sc-2012 (dilution range: 1:100-1:400) or goat anti-rabbit IgG-TR: sc-2780 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor MGP gene expression knockdown using RT-PCR Primer: MGP (m)-PR: sc-44627-PR (20 μ l, 445 bp). Annealing temperature for the primers should be $55-60^\circ\text{C}$ and the extension temperature should be $68-72^\circ\text{C}$.

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.

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