

MGP siRNA (h): sc-44626

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

1. 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.
2. Cancela, L., et al. 1990. Molecular structure, chromosome assignment, and promoter organization of the human matrix Gla protein gene. *J. Biol. Chem.* 265: 15040-15048.
3. Chen, L., et al. 1990. Overexpression of matrix Gla protein mRNA in malignant human breast cells: isolation by differential cDNA hybridization. *Oncogene* 5: 1391-1395.
4. Hale, J.E., et al. 1991. Carboxyl-terminal proteolytic processing of matrix Gla protein. *J. Biol. Chem.* 266: 21145-21149.
5. 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.
6. Munroe, P.B., et al. 1999. Mutations in the gene encoding the human matrix Gla protein cause Keutel syndrome. *Nat. Genet.* 21: 142-144.

CHROMOSOMAL LOCATION

Genetic locus: MGP (human) mapping to 12p12.3.

PRODUCT

MGP 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 μM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see MGP shRNA Plasmid (h): sc-44626-SH and MGP shRNA (h) Lentiviral Particles: sc-44626-V as alternate gene silencing products.

For independent verification of MGP (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-44626A, sc-44626B and sc-44626C.

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

MGP siRNA (h) is recommended for the inhibition of MGP 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 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

MGP (A-11): sc-271906 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 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 MarkerTM 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 MGP gene expression knockdown using RT-PCR Primer: MGP (h)-PR: sc-44626-PR (20 μl , 285 bp). Annealing temperature for the primers should be $55-60^{\circ}\text{C}$ and the extension temperature should be $68-72^{\circ}\text{C}$.

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.