MGP

Enzyme immunoassay for the quantitative determination

of human Matrix Gla Protein

Cat. No. BI-20062

12 x 8 tests

For research use only

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1) Introduction

A. Biochemical aspects:

Human matrix Gla-protein (MGP) is an 84 aminoacid protein which was originally identified in bone. MGP belongs to the group generally referred to as the vitamin K-dependent proteins or Gla-proteins. These proteins have in common that they contain a number of residues of the unusual amino acid gammacarboxy glutamic acid (Gla), which is synthesized in a vitamin K-dependent posttranslational modification step. The biological activity of MGP is strictly dependent on the presence of its five Gla-residues.

The function of MGP was initially demonstrated in transgenic MGP-deficient mice, which were born normally, but which developed massive arterial calcifications during the first weeks of life, leading to aortic rupture and death within 8 weeks after birth It is now generally accepted that MGP is a potent inhibitor of soft tissue calcification. The importance of the Gla-residues for MGP function was demonstrated by treating rats with a vitamin K-antagonist, which also resulted in severe arterial calcifications.

MGP can be readily detected in tissue, where it accumulates in cartilage and vascular tissue, notably around sites at risk for calcification and around calcium salt deposits. Increased synthesis at these sites may be a tissue response on increased local calcium concentrations.

B. Clinical aspects:

The use of MGP as a biomarker is based on the assumption that its serum concentration reflects its *de novo* synthesis in tissue. In patients with existing calcifications, such as in atherosclerosis, in Mönckeberg's sclerosis of the vascular media and in heart valve calcifications, the high local MGP production would expectedly cause an increase of the circulating MGP levels. Another group of interest for serum MGP measurements are patients characterized by abnormal or excessive cartilage calcification (osteoarthritis, spondylitis, Bechterev's disease). Since many of the factors affecting MGP expression and serum MGP concentrations are presently unknown, this kit cannot be used for diagnostic purposes, but is a valuable tool for research purposes.

2.) Principle of the assay

The MGP test kit is a competitive enzyme immunoassay designed to determine human Matrix Gla Protein in serum, plasma or cell culture supernatants. The assay is based on the competitive reaction of the unlabelled MGP in the standards or samples and Biotin labelled peptide (tracer) for the limiting binding sites of the MGP specific antibody. Hence with increasing concentration of the peptide in the standard or sample, the binding of the competing tracer, which is detected with a horse radish peroxidase – streptavidine conjugate, is proportionally reduced. Thus the amount of colour developed is inversely proportional to the amount of MGP present in the standard or samples. A standard curve is plotted from the values measured and the concentrations of MGP in the samples are calculated from this curve.

3.) Contents of the kit

- 12 x 8 well microtiter strips in stripholder, packed in alubag with desiccant. Wells are coated with a monoclonal anti-human MGP antibody. Store unused strips with the desiccant at 4°C in the alu bag supplied. Strips are stable until expiry date stated on the label.

- 10x washing buffer; concentrate
  The bottle contains 100 ml of washing buffer concentrate

- Assay Buffer
  The bottle contains 25 ml of assay buffer, ready to use

- 6 standards (white caps)
  The vial contains synthetic MGP (3-15) calibrator peptide in human serum base, lyophilized, ranging from 0 to 90 nmol/l. Standards should be reconstituted in 250µl deionised H_2O. Reconstituted standards are stable at -20° or –70°C until expiry date stated on the label. Avoid repeated freeze-thaw cycles.

- 1 Control (yellow cap)
  The vial contains synthetic MGP (3-15) lyophilized in human serum. Control should be reconstituted in 250µl deionised H_2O. The concentration is stated on the label. Reconstituted control is stable at -20° or –70°C until expiry date stated on the label. Avoid repeated freeze-thaw cycles.

- 1 Tracer (glass vial, green solid)
  The vial contains biotin labelled synthetic MGP (3-15) lyophilized. Tracer should be reconstitued in 22ml assay buffer.Reconstituted tracer is stable at -20° or –70°C until expiry date stated on the label. Avoid repeated freeze-thaw cycles.

- Conjugate (red solution)
  The vial contains 24 ml streptavidin-HRP conjugate, ready to use.

- Substrate
  The vial contains 24 ml TMB solution, ready to use.

- Stop solution
  The vial contains 8 ml of stop solution, ready to use.

- 2 self-adhesive plastic films

- Protocol sheet
- Instructions for use (package insert)

4.) Additional material and equipment required

Distilled water
Variable volume pipettes in the range of 20 µl to 1000 µl
Multichannel or multipette
ELISA reader equipped with 450 nm filter
Graph paper or software for calculation of results

5.) Reagents and sample preparation -assay procedure

- Store samples at -20°C or –70°C if not assayed on the same day. Lipemic or hemolyzed samples may give erroneous results. Samples should be mixed well before assaying.

- We recommend duplicates for all values.

- Dilute washing buffer concentrate to 1000 ml (add the 100 ml of concentrate to 900 ml of distilled water) and mix well. Crystals in the buffer concentrate will dissolve at room temperature. Buffer is stable at 4°C until expiry date stated on the label.

5.A.) Measurement of MGP in human serum samples

1. Dissolve standards (white caps) and control (yellow cap) in 250µl of deionised water and leave at room temperature (18 - 26°C) for 30 min, shake well.
2. Dissolve tracer (glass vial, green solid) in 22 ml of assay buffer and leave at room temperature (18 - 26°C) for 30 min, shake well.
3. Mark positions for blank, standards, control, and samples on the protocol sheet supplied.

4. Pipette 20 µl of standards (white caps), control (yellow cap) or samples in the respective wells.
5. Add 200 µl tracer (green solution) to all wells, EXCEPT BLANK. Mix gently.
6. Cover strips with plastic film and incubate overnight (16 - 24 hours) at 4°C. Make sure all wells are sealed well with the plastic film to avoid evaporation.
7. Remove plastic film slowly and discard contents of the wells. Wash 5x with minimum 300 µl diluted wash buffer. Remove any remaining fluid by hitting plate against paper towel after the last wash.
8. Add 200 µl conjugate (red solution) to all wells and cover strips with plastic film.
9. Incubate for 60 min. at room temperature (18-26°C).
10. Remove plastic film slowly and discard contents of the wells. Wash 5x with minimum 300 µl diluted wash buffer. Remove any remaining fluid by hitting plate against paper towel after the last wash.
11. Add 200µl of substrate to all wells and incubate for 30 min. at room temperature (18-26°C) in the dark.
12. Add 50 µl stop solution to all wells, mix gently.
13. Determine absorption immediately with an ELISA reader at 450 nm against 620,630 or 690 nm as reference.
    If the correction wavelength of 620,630 or 690 nm is not available read only at 450nm.
14. If the optical density of the highest standard exceeds the measuring range of the photometer, absorption can be measured immediately at 405 nm against 620,630 or 690 as reference.

5.B.) Measurement of MGP in Cell Culture Supernatants:

Dissolve the highest standard (90 nmol/l) in 250µl of the used cell culture medium.
Prepare a 1+2 serial dilution of the 90 nmol/l standard with cell culture medium to obtain a calibration curve (e.g. 90/30/10/3.3/1.1 nmol/l). Cell culture medium is used as 0-standard.
Diluted standards are stable at -20° or –70°C until expiry date stated on the label. Avoid repeated freeze-thaw cycles
Follow the instructions for the further Assay procedure as described above (5A. 2, “Dissolve tracer ...”)

6.) Calculation of results

The extinction of the blank is subtracted from all other values. A calibration curve is constructed from the standards. Commercially available software can be used as well as semi- logarithmic graph paper. Results of the samples are read from this calibration curve.
The assay has been evaluated using a 4 PL algorithm. Different curve fitting methods need to be evaluated by the user.
Respective dilution factors have to be considered.

7.) Assay characteristics

Reference values: n=102, 7.0 nmol/l (range: 1.6-13.1 nmol/l)

Standard range: 0 to 90 nmol/l

Recommended matrix: serum

Detection Limit: (0 nmol/l + 3 SD): 0.3 nmol/l

Incubation time: overnight / 1h / 30 min

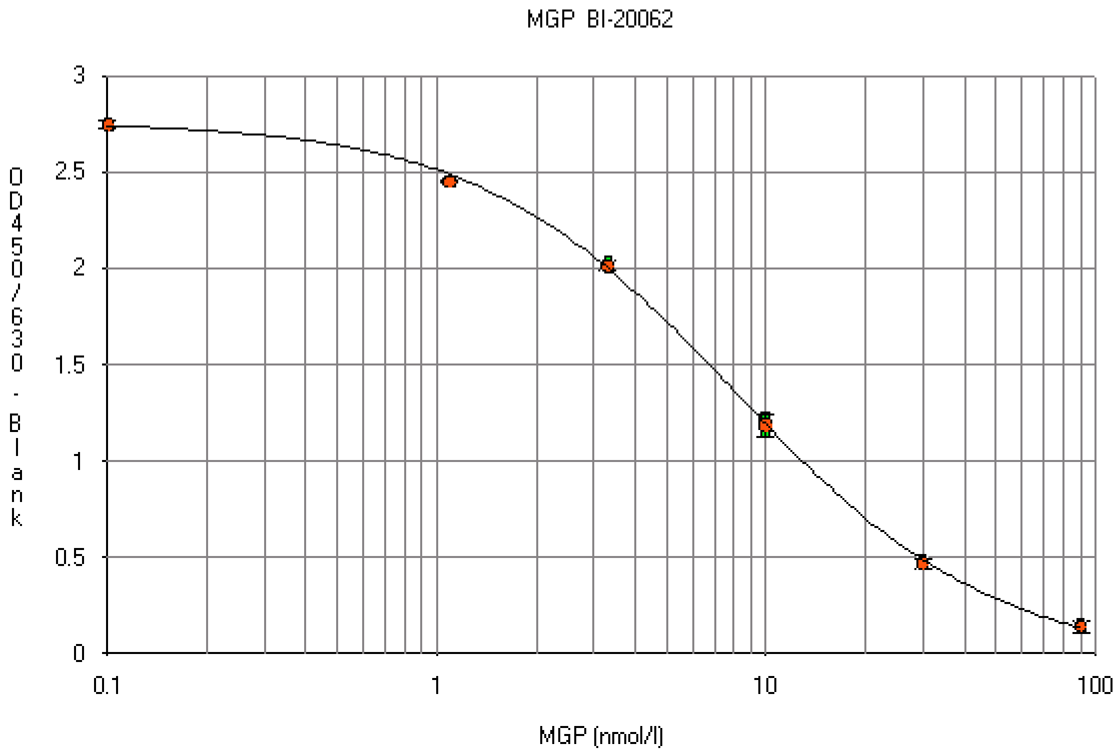
PRECISION:

Intra-Assay (Precision within an assay)
2 samples of known concentrations were tested 16 times to assess intra-assay precision

Inter-Assay (Precision between assay)
2 samples of known concentrations were tested in 10 assays to assess inter-assay precision

	#1	#2
Intra-Assay Precision n=16		
mean(nmol/l)	2,59	4,43
SD	0,15	0,24
CV %	6 %	5%
	#1	**#2**
Inter-Assay Precision n=10		
mean (nmol/l)	3,18	10,0
SD	0,58	0,70
CV %	18%	7%

Typical Calibration Curve:



Storage: 4°C

8.) Technical hints

- To avoid cross-contamination, change pipette tips between addition of standards, controls, samples, antibody, conjugate and substrate. Also use separate reservoirs for each reagent.
- When mixing reagents, always avoid foaming.
- Do not mix stoppers and caps of different reagents.
- Do not use reagents beyond expiration date.
- Do not mix or substitute reagents with those from other lots or sources.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Substrate solution should remain colourless until added to the plate.
- Stop solution should be added to the plate in the same order as the substrate solution.

9.) Precautions

All liquid reagents contain 0.01% Thimerosal or 0,01% Proclin 300 as preservative.

- Thimerosal is toxic! Avoid contact with skin and mucous membrane. Proclin 300 is not toxic in concentrations used in this kit. It may cause allergic skin reactions - avoid contact with skin or eyes.
- Protect reagents from direct sunlight.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics where reagents are used.
  Avoid all contact with the reagents by using gloves.
  Stop solution contains diluted sulfuric acid. Irritation to eyes and skin is possible. Flush with water after contact.

10. References:

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11.) Incubation scheme

	Blank	Std	Sample	Control
Standard/Sample/Control	-	20 µl	20 µl	20µl
Tracer	-	200 µl	200 µl	200 µl
	Incubate 16-24hrs at 4°C			
	Wash plate 5x			
HRP Conjugate	200µl	200µl	200µl	200µl
	Incubate 1h at RT			
	Wash plate 5x			
Substrate TMB	200µl	200µl	200µl	200µl
	Incubate 30min at RT			
Stop reagent	50µl	50µl	50µl	50µl