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Arbeitsanleitung / Manual

MPO ELISA Kit

Zur in vitro Bestimmung von Myeloperoxidase (MPO) in Serum und Plasma

MPO ELISA Kit

For the in vitro determination of Myeloperoxidase (MPO) in serum and plasma

Gültig ab / Valid from 22.10.2009

REF K 6631

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+2°C

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Content

1. INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the quantitative determination of MPO (Myeloperoxidase) in serum and plasma. It is for *in vitro* diagnostic use only.

2. INTRODUCTION

MPO is part of the defence mechanism of the polymorphonuclear leukocytes against exogenic substances. During bacterial infection, these leukocytes, are stimulated by chemotactically effective substances (leukotrienes, complement factors, bacterial toxins etc.). They move to the site of the infection and encapsulate the foreign substances. If the foreign agent is located in an intracellular vacuole, different substances are used for the intracellular digestion. Amongst these are MPO, cationic proteins, lysozyme, lactoferrin and some acidic hydrolases. A strong surge of oxidative metabolism takes place, producing a high number of oxygen radicals which leads to the destruction of foreign proteins. Some of these molecules can leak into the extracellular space during this process. This happens to a greater extent, when the leukocytes cannot encapsulate the foreign body because of its size or in cases where the neutrophils are destroyed (by bacterial toxins, crystalline substances etc.).

MPO, together with hydrogen peroxide and a halogen, forms a very strong anti microbial system, which can effectively combat a number of microorganisms. MPO is present at high concentration in neutrophil granulocytes, whereas hydrogen peroxide is produced during infection/ inflammation. The MPO system is inhibited by catalase, excess of hydrogen peroxide and other reducing substances (e.g. ascorbic acid, glutathione). In the absence of these agents other cells in the extracellular space can be affected (e.g. spermatocyte, erythrocytes, leukocytes, and tumor cells)

Apart from its implications in host defence, involvement of MPO has been described in numerous non-infectious diseases such as atherosclerosis, lung cancer, Alzheimer´s disease, and multiple sclerosis. MPO is present and active within atherosclerotic lesions. Numerous lines of evidence suggest mechanistic links between myeloperoxidase, inflammation and both acute and chronic manifestations of cardiovascular disease.

Brennan et al. (2003) showed that in 604 sequentially ascertained patients presenting with chest pain, a single initial measurement of plasma myeloperoxidase was an independent early predictor of myocardial infarction, as well as the risk of major adverse cardiac events in ensuing 30-day and 6-month periods. In contrast to troponin T, creatine kinase MB isoform, and C-reactive protein levels, MPO levels may identify patients at risk for cardiac events in the absence of myocardial necrosis.

Summary: The inflammatory protein myeloperoxidase is present, active and mechanistically poised to participate in the initiation and progression of cardiovascular disease. The many links between myeloperoxidase, oxidation and cardiovascular disease suggest this leukocyte protein may have clinical utility in risk stratification for cardiovascular disease status and outcomes.

Indications

- Marker for inflammatory activities in the gastrointestinal tract (Stool)
- Renal transplant rejection (Urine)
- Oxidative stress (Serum)
- For the differentiation between allergic and infectious asthma (bronchial lavage, respiratory condensate, sputum)
- Prediction of risk in patients with acute coronary syndromes (Serum)

3. MATERIAL SUPPLIED

Catalotue No	Content	Kit Components	Quantity
K 6631MTP	PLATE	One holder with precoated strips	12 x 8 wells
K 6631WP	WASHBUF	ELISA wash buffer concentrate 10x	100 ml
K 6631PV	SAMPLEBUF	Sample dilution buffer, ready-to-use	50 ml
K 6631ST	STD	MPO-Standard, lyophilized (see specification for concentration)	4 vials
K 6631KO	CTRL	Control, lyophilized (see specification for range)	4 vials
K 6631K	CONJ	Conjugate, rabbit anti-MPO peroxidase labelled antibody, concentrate	50 µl
K 6631TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	15 ml
K 6631AC	STOP	ELISA stop solution, ready to use	15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Laboratory balance
- Precision pipettors calibrated and tips to deliver 10-1000 µl
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Vortex-Mixer
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader at 450 nm
  (reference wave length 620 or 690 nm)

5. PREPARATION AND STORAGE OF REAGENTS

- To run the assay more than one time, make sure that the reagents are stored at the conditions stated on the label. **Prepare just the appropriate amount necessary for the assay.** The kit can be used up to 4 times within the expiry date stated on the label.
- The **ELISA WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml concentrate + 900 ml a. bidest.), mix well. Crystals can occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution of the buffer solutions. The **WASHBUF** is stable at **2-8°C** until the expiry date stated on the label. Diluted **buffer solution** can be stored in a closed flask at **2-8°C for one month.**
- The lyophilized **STD** (standard) and the lyophilized **CTRL** (control) are stable at **2-8°C** until the expiry date stated on the label. Standard and control have to be reconstituted with **SAMPLEBUF** (sample dilution buffer) (volume and concentration see product specification). Allow the vial content to solve for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted standard and control are **not stable.**

  Prepare a series of dilutions for the standard curve from the **MPO STD** (standard; S6) in **1:2** dilution steps by adding SAMPLEBUF (sample dilution buffer) as follows:

S6
250 µl S6 + **250 µl** SAMPLEBUF = **S5**
250 µl S5 + **250 µl** SAMPLEBUF = **S4**
250 µl S4 + **250 µl** SAMPLEBUF = **S3**
250 µl S3 + **250 µl** SAMPLEBUF = **S2**
SAMPLEBUF is used as standard S1, 0 ng/ml.

- The **CONJ** (conjugate) must be diluted **1:500** in diluted wash buffer (20 µl CONJ + 10 ml diluted wash buffer). The conjugate is stable at **2-8 °C** until expiry date stated on the label. **Diluted conjugate is not stable and can not be stored.**
- All other test reagents are ready to use. The test reagents are stable until the expiry date (see label of test package) when stored at **2-8°C.**

6. SAMPLE PREPARATION

Dilution of samples

Serum/plasma samples
Preanalytic handling

Significant differences in the MPO levels can be observed due to different sample preparation procedures, e.g. analysis of plasma or serum samples. The reasons are as follows:

- The granulocytes are activated during the serum clotting and release granulocyte-activating markers. The time between serum collecting and analysis as well as repeated freeze-thaw cycles don't cause a MPO concentration shift.
- On the contrary, in the case of plasma samples, varying the time between sampling and analysis or the number of freeze-thaw cycles will cause variation in the observed MPO levels. Therefore, the preanalytical conditions of plasma samples should be held constant. This is a general requirement independent of the test-system used.
- Fresh collected Serum/Plasma should be centrifuged within one hour. Store samples at -20 °C if not assayed on the same day. Lipemic or hemolytic samples may give erroneous results. Samples should be mixed well before assaying. We recommend duplicate analyses for each sample.

The preanalytical handling is critical for accurate and consistent/reproducible MPO measurement results.

- Shih et al. (2008) report that MPO concentrations were consistently higher in serum and heparin plasma samples than in samples in EDTA or citrate and recommend the

analysis of EDTA plasma samples. Furthermore, the authors investigated the effects of preanalytical handling, storage temperature and time for EDTA plasma, lithium-heparin and citrate preparation. Less than 10% differences were found after storage of samples at room temperature for 2 days, after storage at 2–8 °C for 8 days, and after 3 freeze-thaw cycles for all sample types

- Videm (1996) describes at heparin concentrations, as applied in clinical practice, a dose-dependent increase in granulocyte activation as measured by MPO release, quantitated in enzyme-immunoassay. Thus, direct effects of heparin on granulocytes, e.g. MPO release and concentration, should be taken into consideration for the evaluation of MPO results of samples from patients receiving systemic heparin therapy.

Serum samples
Prior to analyses the serum samples should be diluted **1:40** with **SAMPLEBUF** (sample dilution buffer).
For example: **25 µl** sample + **975 µl** SAMPLEBUF.

EDTA-plasma samples
Prior to analyses the EDTA-plasma samples should be diluted **1:10** with **SAMPLEBUF** (sample dilution buffer).
For example: **100 µl** sample + **900 µl** SAMPLEBUF.

7. ASSAY PROCEDURE

Principle of the test

The assay utilizes the two-site "sandwich" technique with two selected polyclonal antibodies that bind to human MPO.

Assay standards, controls and prediluted patient samples containing human MPO are added to wells of microplate that was coated with a high affine polyclonal anti-human MPO antibody. After the first incubation period, antibody immobilized on the wall of microtiter wells captures human MPO in the sample. Then a peroxidase-conjugated polyclonal anti-human MPO antibody is added to each microtiter well and a "sandwich" of capture antibody - human MPO – Peroxidase conjugate is formed. Tetramethylbenzidine (TMB) is used as a substrate for peroxidase. Finally an acidic stop solution is added to terminate the reaction. The color changes from blue to yellow. The intensity of the yellow color is directly proportional to the concentration of MPO in the sample. A dose response curve of the absorbance unit (optical density, OD at 450 nm) vs. concentration is generated, using the values obtained from the standard. MPO present in the patient samples, is determined directly from this curve.

Test procedure

Prior to use in the assay allow all reagents and samples to come to room temperature (18-26 °C) and mix well.
Mark the positions of STD /SAMPLE/CTRL (Standards/Sample/Control) on a protocol sheet.
Take the required microtiter strips out of the kit. Store unused strips covered at 2-8° C. Strips are stable until expiry date stated on the label.
Wash each well 5 times by dispending 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper.
Add 100 µl of STD/SAMPLE/CTRL (Standard/Sample/Control) in duplicate into respective wells. Use sample dilution buffer as STD 0 ng/ml.
Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) on a horizontal mixer
Aspirate the contents of each well. Wash 5 times by dispending 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper.
Add 100 µl CONJ (Conjugate) into each well.
Cover the plate tightly and incubate for 1 hour at room temperature (18-26°C) on a horizontal mixer.
Aspirate the contents of each well. Wash 5 times by dispending 250 µl of diluted WASHBUF (Wash buffer) into each well. After the final washing step the inverted microtiter plate should be firmly tapped on absorbent paper.
Add 100 µl of SUB (Substrate) into each well.
Incubate for 10 - 20 minutes at room temperature (18-26°C) in the dark.*
Add 50 µl of STOP (stop solution) into each well, mix thoroughly.
Determine absorption immediately with an ELISA reader at 450 nm against 620 nm (or 690 nm) as a reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as a reference

* The intensity of the color change is temperature sensitive. We recommend to observe the procedure of the color change and to stop the reaction upon good differentiation

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend to use the„4-Parameter-algorithm".

1. *4-Parameter-algorithm*
   It is recommended to use a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).
2. *Point-to-point-calculation*
   We recommend a linear ordinate for optical density and a linear abscissa for concentration.
3. *Spline-algorithm*
   We recommend a linear ordinate for optical density and a logarithmic abscissa for concentration. When using a logarithmic abscissa, the zero calibrator must be specified with a value less than 1 (e. g. 0.001).

   The plausibility of the pairs of values should be examined before the automatic evaluation of the results. If this option is not available with the used program, a control of the paired values should be done manually.

Serum/plasma samples

For the calculation of the MPO concentration in **serum** samples the result has to be multiplied by **40**.

For the calculation of the MPO concentration in **plasma** samples the result has to be multiplied by **10**.

9. LIMITATIONS

Serum/plasma with MPO levels greater than the highest standard value should be diluted with sample dilution buffer and re-assayed.

10. QUALITY CONTROL

Control samples should be analyzed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. The results for the patient sample may not be valid, if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Expected values

Normal ranges

MPO in serum (n = 42):	mean 340 ng/ml	(SD 176.7)
MPO in EDTA-plasma (n = 41):	mean 98.31 ng/ml	(SD 62.9)

Based on Immundiagnostik AG studies of evidently healthy persons a mean value was estimated.

We recommend each laboratory to establish its own norm concentration range.

11. PERFORAMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n=20)		
Sample	**MPO (ng/ml)**	**CV (%)**
1	147.1	4.3
2	288.6	4.8

Inter-Assay (n=20)		
Sample	**MPO (ng/ml)**	**CV (%)**
1	171.7	12
2	239.9	15

Recovery

2 samples were spiked with 3 different MPO standards and measured using this assay.

Sample (ng/ml)	Spike (ng/ml)	MPO expected (ng/ml)	MPO measured (ng/ml)
116	320	436	401
116	200	316	336
116	125	241	254
92	320	412	388
92	200	292	297
92	125	217	204

Sensitivity

The sensitivity was set as B_0 + 2SD. The zero-standard was measured 20 times.

Sample	MPO mean value (OD)	Standard variation (SD)	Detection limit (ng/ml)
1	0.013	0.003	1.6

Linearity

Two patient samples were diluted with sample dilution buffer and analyzed.The results are shown below:

Sample	Dilution	Expected (ng/ml)	Measured (ng/ml)	[%]
A	1:40	14.5	14.5	100
	1:80	7.2	7.1	98.6
	1:160	3.6	3.5	97.2
B	1:40	19.5	19.5	100
	1:80	9.75	10.1	103
	1:160	4.8	5.2	108

Cross reactivity

No cross reactivity with other plasma proteins in serum/plasma was observed.

Alpha-1-Antitrypsin	0 %
Albumin	0 %
CRP	0 %
Lysozym	0 %
sIgA	0 %
PMN-Elastase	0 %
Calprotectin	0 %

No cross reactivity with MPO in mouse serum was observed.

12. PRECAUTIONS

- For *in vitro* diagnostic use only.
- The quality control guidelines should be observed.
- Human material used in the kit components was tested and found to be negative for HIV, Hepatitis B and Hepatitis C. However, for safety reasons, all kit components should be treated as potentially infectious.
- Reagents of the kit package contain sodium azide or thimerosal as bactericides. Sodium azide and thimerosal are toxic. The substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulphuric acid, a strong acid. Even diluted, it still has to be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped out immediately with copious quantities of water.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay.
- Reagents should not be used beyond the expiration date shown on the kit label.
- Substrate solution should remain colourless until use.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- Avoid foaming when mixing reagents.
- The assay should always be performed according the enclosed manual.

14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- This assay was produced and put on the market according to the IVD guidelines of 98/79/EC.
- All reagents in the kit package are for *in vitro* diagnostic use only.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the components are defined by the producer. Any variation of the test procedure, which is not coordinated with the producer, may influence the results of the test. Immundiagnostik AG can therefore not be held responsible for any damage resulting from this.
- Warranty claims and complaints in respect of deficiencies must be lodged within 14 days after receipt of the product. The product shall be send to Immundiagnostik AG together with a written complaint.

15. REFERENCES

Publications based on the Immundiagnostik MPO-ELISA:

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Used Symbols:


Store at

REF Catalog Number

IVD In Vitro Diagnostic Device


No. of tests


Manufacturer


Use by

LOT Lot number