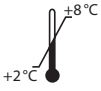
Manual

MPO ELISA Kit

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

Valid from 2014-10-23

REF **K 6631B**



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1. INTENDED USE

The described Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is intended for the quantitative determination of MPO (myeloperoxidase) in serum and plasma. 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 me-

chanistically 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

Cat. No.	Label	Kit components	Quantity
K 6631B MTP	PLATE	One holder with precoated strips	12 x 8 wells
K 6631B WP	WASHBUF	ELISA wash buffer concentrate 10x	1 x 100 ml
K 6631B K	CONJ	Conjugate, rabbit anti-MPO peroxidase labelled antibody, concentrate	1 x 50 µl
K 6631B ST	STD	MPO-Standard, lyophilized (see specification for concentration)	4 vials
K 6631B KO1	CTRL	Control, lyophilized	4 vials
K 6631B KO2	CTRL	Control, lyophilized	4 vials
K 6631B PV	SAMPLEBUF	Sample dilution buffer, ready to use	1 x 50 ml
K6631B TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
K6631B AC	STOP	ELISA stop solution, ready to use	1 x 15 ml

4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultra pure water*
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets

- Centrifuge, 3000 *g*
- Vortex
- Standard laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

* Immundiagnostik AG recommends the use of Ultra Pure Water (Water Type 1; ISO 3696), which is free of undissolved and colloidal ions and organic molecules (free of particles > 0.2 µm) with an electrical conductivity of 0.055 µS/cm at 25 °C (≥ 18.2 MΩ cm).

5. STORAGE AND PREPARATION OF REAGENTS

- To run the assay more than once, ensure that reagents are stored at the conditions stated on the label. **Prepare only the appropriate amount necessary for each run.** The kit can be used up to 4 times within the expiry date stated on the label.
- Reagents with a volume less than **100 µl** should be centrifuged before use to avoid loss of volume.
- The **ELISA wash buffer concentrate** (WASHBUF) should be diluted **1:10 in ultra pure water** before use (100 ml concentrate + 900 ml ultra pure water), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at room temperature or 37 °C before dilution of the buffer solutions. The **buffer concentrate** is stable at **2–8 °C** until the expiry date stated on the label. **Diluted buffer solution** (wash buffer) can be stored in a closed flask at **2–8 °C for one month**.
- The lyophilized **STD** (standard) and the lyophilized **CTRL** (controls) 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, concentration and dilution schema for the calibration curve 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 controls are not stable.**
- The **conjugate concentrate** (CONJ) must be diluted **1:301 in wash buffer** (40 µl CONJ + 12 ml wash buffer). The concentrate is stable at **2–8 °C** until the expiry date stated on the label. **Diluted conjugate is not stable and cannot be stored.**
- All other test reagents are ready to use. Test reagents are stable until the expiry date (see label of test package) when stored at **2–8 °C**.

6. STORAGE AND PREPARATION OF 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

Bring all **reagents and samples to room temperature** (15–30 °C) and mix well.

Take as many **microtiter strips** as needed from kit. Store unused strips in the aluminium foil bag with desiccant at 2–8 ° C. Strips are stable until expiry date stated on the label.

We recommend to carry out the tests in duplicate.

1.	Wash each well **5 x** by dispensing **250 µl of diluted WASHBUF** (wash-buffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
2.	Add **100 µl of STD** (Standard) **SAMPLE** (Sample) **CTRL** (Controls) into respective well.
3.	Cover the plate tightly and incubate for **1 hour** at room temperature (15-30°C) on a horizontal shaker.

4.	Discard the contents of each well. Wash each well **5 x** by dispensing **250 µl of diluted WASHBUF** (washbuffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
5.	Add **100 µl diluted CONJ** (conjugate) into each well.
6.	Cover the plate tightly and incubate for **1 hour** at room temperature (15-30°C) on a horizontal shaker.
7.	Discard the contents of each well. Wash each well **5 x** by dispensing **250 µl of diluted WASHBUF** (washbuffer) into each well. After the final washing step remove residual buffer by tapping the plate on absorbent paper.
8.	Add **100 µl** of **SUB** (substrate) into each well.
9.	Incubate for **10 - 20 min**.* at room temperature (15-30°C) in the dark.
10.	Add **100 µl of STOP** (stop solution) into each well, mix.
11.	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 observing the color change and stopping the reaction upon good differentiation.

For automated ELISA processors the given protocol may need to be adjusted according to the specific features of the respective automated platform.

For further details please contact your supplier or Immundiagnostik AG.

8. RESULTS

The following algorithms can be used alternatively to calculate the results. We recommend using the "4 parameter algorithm".

1. 4 parameter algorithm

It is recommended to use a linear ordinate for the optical density and a logarithmic abscissa for the concentration. When using a logarithmic abscissa, the zero standard must be specified with a value less than 1 (e. g. 0.001).

2. **Point-to-point calculation**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

3. **Spline algorithm**

   We recommend a linear ordinate for the optical density and a linear abscissa for the concentration.

The plausibility of the duplicate values should be examined before the automatic evaluation of the results. If this option is not available with the programme used, the duplicate values should be evaluated manually.

Serum

The obtained MPO levels of serum samples have to be multplied with the dilution factor of **40.**

Plasma

The obtained MPO levels of EDTA plasma samples have to be multplied with the dilution factor of **10.**

In case **another dilution factor** has been used, multiply the obtained result with the dilution factor used.

9. LIMITATIONS

Samples with concentrations above the measurement range must be further diluted and re-assayed. Please consider this greater dilution when calculating the results.

Samples with concentrations lower than the measurement range cannot be clearly quantified.

The upper limit of the measurement range can be calculated as:

highest concentration of the standard curve × *sample dilution factor to be used*

The lower limit of the measurement range can be calculated as:

LoB × *sample dilution factor to be used*

10. QUALITY CONTROL

Immundiagnostik recommends the use of external controls for internal quality control, if possible.

Control samples should be analysed with each run. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate sta-

tistical methods. The results for the patient samples may not be valid if within the same assay one or more values of the quality control sample are outside the acceptable limits.

Reference range

Based on Immundiagnostik studies of serum and EDTA-plasma samples of apparently healthy persons the following reference range was estimated.

MPO from serum (n = 20) median = 444 ng/ml

MPO from EDTA-plasma (n = 20) median = 108 ng/ml

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

Intra-Assay (n = 40)

Sample	MPO [ng/ml]	VK [%]
1	465,9	2,6
2	198,9	2,3

Inter-Assay (n = 13)

Sample	MPO [ng/ml]	VK [%]
1	459,0	4,2
2	198,3	4,1

Spiking Recovery

Two samples were spiked with different MPO concentrations and measured using this assay (n = 2).

Sample	Unspiked sample [ng/ml]	Spike [ng/ml]	MPO expected [ng/ml]	MPO measured [ng/ml]
A	5,1	2,6	7,7	6,6
	5,0	4,3	9,3	8,1
	4,8	7,9	12,7	10,6

Sample	Unspiked sample [ng/ml]	Spike [ng/ml]	MPO expected [ng/ml]	MPO measured [ng/ml]
B	5,0	2,6	7,6	6,8
	4,9	4,3	9,2	8,8
	4,7	7,9	12,6	11,7

Dilution recovery

Two samples (1 serum, 1 EDTA-plasma) were diluted and analyzed. The results are shown below (n = 2):

Sample	Dilution	MPO expected [ng/ml]	MPO measured [ng/ml]
A	1:80	-	8,9
	1:160	4,5	5,0
	1:320	2,5	2,7
	1:640	1,4	1,4
	1:1280	0,7	0,8
B	1:20	-	6,5
	1:40	3,2	3,6
	1:80	1,8	2,0
	1:160	1,0	1,1
	1:320	0,5	0,6

Analytical Sensitivity

Limit of blank, LoB 0.161 ng/ml

Limit of detection, LoD 0.294 ng/ml

The evaluation was performed according to the CLSI-Guideline:EP-17-A.

Specificity

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

- All reagents in the kit package are for *in vitro* diagnostic use only.
- Human materials used in kit components were 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.
- Kit reagents contain sodium azide or ProClin as bactericides. Sodium azide and ProClin are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- The stop solution consists of diluted sulphuric acid, a strong acid. Although diluted, it still must be handled with care. It can cause burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spill should be wiped up immediately with copious quantities of water. Do not breath vapour and avoid inhalation.

13. TECHNICAL HINTS

- Do not interchange different lot numbers of any kit component within the same assay. Furthermore we recommend not assembling wells of different microtiter plates for analysis, even if they are of the same batch.
- Control samples should be analyzed with each run.
- Reagents should not be used beyond the expiration date stated on 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.
- Do not mix plugs and caps from different 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 distributed according to the IVD guidelines of 98/79/EC.
- The guidelines for medical laboratories should be followed.
- 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 incorrect use.
- Warranty claims and complaints regarding deficiencies must be logged within 14 days after receipt of the product. The product should be send to Immundiagnostik AG along with a written complaint.

15. REFERENCES

General literature

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Used symbols:


Temperature limitation

Catalogue Number

In Vitro Diagnostic Medical Device

To be used with


Manufacturer

Contains sufficient for <n> tests

Lot number


Use by