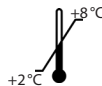


# IDK<sup>®</sup> MPO ELISA

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

Valid from 2019-03-20

**REF** KR6631B



**RUO**



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## 1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of MPO (myeloperoxidase) in serum and plasma.

For research use only. Not for use in diagnostic procedures.

## 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.

### 3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR6631B	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	ELISA wash buffer concentrate, 10x	1 x 100 ml
KR6631B	CONJ	Conjugate concentrate, rabbit anti-MPO, peroxidase labelled antibody	1 x 50 µl
KR6631B	STD	MPO-Standard, lyophilised (see specification for concentrations)	4 x 6 vials
KR6631B	CTRL1	Control, lyophilised (see specification for range)	4 x 1 vial
KR6631B	CTRL2	Control, lyophilised (see specification for range)	4 x 1 vial
KR6631B	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 100 ml
KR0002.15	SUB	Substrate (Tetramethylbenzidine), ready-to-use	1 x 15 ml
KR0003.15	STOP	Stop solution, ready-to-use	1 x 15 ml

For reorders of single components, use the catalogue number followed by the label as product number.

### 4. MATERIAL REQUIRED BUT NOT SUPPLIED

- Ultrapure water\*
- Laboratory balance
- Calibrated precision pipettors and 10–1000 µl single-use tips
- Foil to cover the microtiter plate
- Horizontal microtiter plate shaker
- Multi-channel pipets or repeater pipets
- Centrifuge, 3000 g
- Vortex
- Standard single-use laboratory glass or plastic vials, cups, etc.
- Microtiter plate reader (required filters see chapter 7)

\* Immundiagnostik AG recommends the use of ultrapure 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.
- **Preparation of the wash buffer:** The **wash buffer concentrate (WASHBUF)** has to be diluted with ultrapure water **1:10** before use (100 ml WASHBUF + 900 ml ultrapure water), mix well. Crystals could occur due to high salt concentration in the concentrate. Before dilution, the crystals have to be redissolved at room temperature or in a water bath at 37 °C. The **WASHBUF** is stable at **2–8 °C** until the expiry date stated on the label. **Wash buffer** (1:10 diluted WASHBUF) can be stored in a closed flask at **2–8 °C for 1 month**.
- The **lyophilised standards (STD)** and **controls (CTRL)** are stable at **2–8 °C** until the expiry date stated on the label. **Reconstitution** details are given in the **specification data sheet**. **Standards and controls** (reconstituted STD and CTRL) **are not stable and cannot be stored**.
- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:301** in wash buffer (40 µl CONJ + 12 ml wash buffer). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate** (1:301 diluted CONJ) **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) 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 up to 3 months 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

### **Serum samples**

EDTA plasma or serum samples must be diluted **1:40** before performing the assay,

e.g. **25 µl** sample + **975 µl** sample dilution buffer (SAMPLEBUF), mix well.

**100 µl** of the dilution are used in the test.

### **EDTA-plasma samples**

EDTA plasma or serum samples must be diluted **1:10** before performing the assay,

e.g. **100 µl** sample + **900 µl** sample dilution buffer (SAMPLEBUF), mix well.

**100 µl** of the dilution are used in the test.

## **7. ASSAY PROCEDURE**

### *Principle of the test*

This ELISA is designed for the determination of human MPO and utilises the two-site “sandwich” technique. Two selected polyclonal antibodies bind to human MPO.

Standards, controls and prediluted 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 immobilised 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 colour changes from blue to yellow. The intensity of the yellow colour 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 samples, is determined directly from this curve.

### *Test procedure*

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

Mark the positions of standards/controls/samples on a protocol sheet.

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

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.

We recommend to carry out the tests in duplicate.

1.	<b>Before use</b> , wash the wells <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each <b>100 µl standards/controls/diluted samples</b> into the respective wells.
3.	Cover the plate tightly and incubate for <b>1 hour</b> at room temperature (15-30 °C) on a <b>horizontal shaker*</b> .
4.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add <b>100 µl conjugate</b> (diluted CONJ) into each well.



6.	Cover the plate tightly and incubate for <b>1 hour</b> at room temperature (15-30°C) on a <b>horizontal shaker*</b> .
7.	Discard the content of each well and wash <b>5 times</b> with <b>250 µl wash buffer</b> . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add <b>100 µl substrate</b> (SUB) into each well.
9.	Incubate for <b>10–20 min**</b> at room temperature (15–30°C) in the <b>dark</b> .
10.	Add <b>100 µl stop solution</b> (STOP) into each well and mix well.
11.	Determine <b>absorption immediately</b> with an ELISA reader at <b>450 nm</b> 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 <b>405 nm</b> against 620 nm as a reference.

\* We recommend shaking the strips at 550 rpm with an orbit of 2 mm.

\*\* The intensity of the colour change is temperature sensitive. We recommend observing the colour change and stopping the reaction upon good differentiation.

## 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 results have to be multiplied by the **dilution factor of 40** to get the actual concentrations.

### Plasma

The obtained results have to be multiplied by the **dilution factor of 10** to get the actual concentrations.

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

## 9. LIMITATIONS

Samples with concentrations above the measurement range can be further diluted and re-assayed. Please consider this higher 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*

LoB see chapter "Performance Characteristics".

## 10. QUALITY CONTROL

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

We recommend each laboratory to establish its own reference range.

## 11. PERFORMANCE CHARACTERISTICS

### *Accuracy – Precision*

#### **Repeatability (Intra-Assay); n = 40**

The repeatability was assessed with 2 serum samples under **constant** parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [ng/ml]	CV [%]
1	465.93	2.6
2	198.94	2.3

#### **Reproducibility (Inter-Assay); n = 24**

The reproducibility was assessed with 4 serum samples under **varying** parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [ng/ml]	CV [%]
1	459.87	4.8
2	190.62	5.3
3	199.06	4.8
4	798.43	5.8

### *Analytical sensitivity*

The following value has been estimated based on the concentrations of the standard without considering possibly used sample dilution factors.

Limit of blank, LoB

0.128 ng/ml

## 12. PRECAUTIONS

- All reagents in the kit package are for research 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 analysed 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 to the enclosed manual.

### 14. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- The guidelines for laboratories should be followed.
- *IDK*<sup>®</sup> is a trademark of Immundiagnostik AG.
- 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 sent to Immundiagnostik AG along with a written complaint.

## 15. REFERENCES












### *General literature*

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### *Literature using Immundiagnostik IDK® MPO ELISA*

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**Used symbols:**

	Temperature limitation		Catalogue Number
	For research use only		To be used with
	Manufacturer		Contains sufficient for <n> tests
	Lot number		Use by
	Attention		Consult instructions for use
	Consult specification data sheet		