

# Myeloperoxidase (MPO) ELISA (Serum, Plasma)

Enzyme immunoassay for the in-vitro determination of Myeloperoxidase (MPO) in human serum and plasma.

**REF** **ID59341**

**Σ** **96**

For illustrative purposes only.

To perform the assay the instructions for use provided with the kit have to be used.

Distributed by:

## 1. INTENDED USE

The *Myeloperoxidase* Assay is intended for the quantitative determination of **Myeloperoxidase** in plasma and serum, designed to be also suitable for small series of specimen. For in vitro diagnostic use only.

## 2. SUMMARY AND EXPLANATION OF THE TEST

The granules of neutrophils (approx. 70% of the white blood cells) contain a large number of different enzymes. **Myeloperoxidase** (MPO) catalyzes the oxidation of substances through  $H_2O_2$ . The **MPO**  $H_2O_2$ -system has a toxic effect on many micro-organisms such as bacteria, fungi, viruses and mycoplasma. The efficiency of the bacteria-destructive Myeloperoxidase  $H_2O_2$ -system is increased by PMN-Elastase. **MPO** determination in the stool reflects the inflammatory activity of Crohn's disease or ulcerative colitis.

### Indication

- Marker for inflammatory activities in the gastrointestinal tract
- Renal transplant rejection
- Oxidative stress
- For the differentiation between allergic and infectious asthma

### 3. PRINCIPLE OF THE TEST

This Enzyme-Linked-Immuno-Sorbent-Assay (ELISA) is suitable for the quantitative determination of Myeloperoxidase from plasma, urine and stool. In a first incubation step, the Myeloperoxidase in the samples is bound to an available excess of antibodies against Myeloperoxidase, which are immobilised to the surface of the microtitre plates. To remove all unbound substances, a washing step is carried out. In a second incubation step, a Peroxidase-labelled Antibody against MPO is added. After another washing step, to remove all unbound substances, the solid phase is incubated with the substrate, Tetramethylbenzidine (TMB). An acidic stop solution is then added to stop the reaction. The color converts 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) vs. concentration is generated, using results obtained from the calibrators. MPO, present in the patient samples, is determined directly from this curve.

### 4. MATERIAL SUPPLIED

Catalogue No	Kit Components	Quantity
K 6631MTP	one holder with precoated strips	96
K 6631WP	ELISA wash buffer concentrate 10x	1 x 100 ml
K6631K	POD antibody, (polyclonal anti-MPO, Peroxidase-labelled)	1 x 50 µl
K 6631ST	Calibrator, lyophilized (25 ng/ml)	1 x 4 vials
K 6631ko	Control, lyophilized	4 x 1 vial
K 6631PV	Sample dilution buffer, ready to use	1 x 50 ml
K 6631TMB	TMB substrate (Tetramethylbenzidine)	1 x 15 ml
K 6631AC	ELISA stop solution, ready to use	1 x 7 ml

## 5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Deionized water
- Precision pipettes calibrated to deliver 10-100  $\mu$ l
- A multi-channel dispenser or repeating dispenser
- Centrifuge capable of 3000 x g
- Horizontal mixer
- Vortex
- Microplate reader 450 nm

## 6. PREPARATION AND STORAGE OF REAGENTS

- The **ELISA wash buffer concentrate** should be diluted with aqua dest. **1:10** before use (add 900 ml a. dest. to 100 ml concentrate). Crystals may be formed due to high salt concentration. The crystals have to be dissolved **before dilution of the buffer solutions** using a water bath (37°C). The buffer concentrates are stable at 2-8°C up to the expiry date stated on the label. Diluted solutions can be stored at 2-8°C for 1 month.
- The **calibrator concentrate and controls** have to be reconstituted with **500  $\mu$ l** aqua dest. Allow the vial to stand for 10 minutes and then mix thoroughly by gentle conversion to insure complete reconstitution. Reconstituted standards and controls are **not stable**.

The Calibration curve has to be diluted from the MPO calibrator concentrate (25 ng/ml concentrate = S1) 1:2 according to the following scheme:

**S1 (25 ng/ml)**

**250  $\mu$ l S1 + 250  $\mu$ l dilution buffer = S2 (12.5 ng/ml)**

**250  $\mu$ l S2 + 250  $\mu$ l dilution buffer = S3 (6.25 ng/ml)**

**250  $\mu$ l S3 + 250  $\mu$ l dilution buffer = S4 (3.13 ng/ml)**

**Dilution buffer is used as Standard 0.**

- The **Peroxidase-labelled antibody** conjugate must be diluted 1:500 in ELISA wash buffer (20 µl in 10 ml ELISA wash buffer). Undiluted antibody is stable at 2-8°C up to the expiry date stated on the label. **The diluted conjugate can not be stored and should be freshly prepared each time.**
- All other test reagents are ready for use. The test reagents are stable up to the date of expiry (see label of test package) when stored at 2-8°C. The antibody coated microtitre plate should be stored dry and dark at 2-8°C.

## 7. PRECAUTIONS

- For in vitro diagnostic use only.
- The calibrators and controls contain human source material which was tested and found to be non-reactive to HBsAg, anti-HIV-1/2. Since no method can offer complete assurance that hepatitis B virus, HIV-1/2, or other infectious agents are absent, these reagents should be handled as if potentially infectious.
- The stop Solution consists diluted sulfuric 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 out immediately with copious quantities of water. Do not breath vapor and avoid inhalation.
- Reagents should not be used beyond the expiration date stated on kit label.

## 8. SPECIMEN COLLECTION AND PREPARATION

### Plasma/Serum

Dilute all plasma samples 1:40 with sample dilution buffer (For example: dilute 25 µl of the sample in 975 µl sample dilution buffer).

## 9. ASSAY PROCEDURE

### *Procedural notes*

- Do not interchange different lot numbers of any kit component within the same assay.
- The quality control guidelines should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components are defined by the producer. Any variations of the test procedure, that are not coordinated with the producer, may influence the test results. The manufacturer can therefore not be held responsible for any damage.
- Carry out the assay with the actual manual delivered with the kit.

### *Test procedure*

Wash the precoated microtiter plate 5 x with 250 µl ELISA wash buffer. Carry out the tests in duplicate.

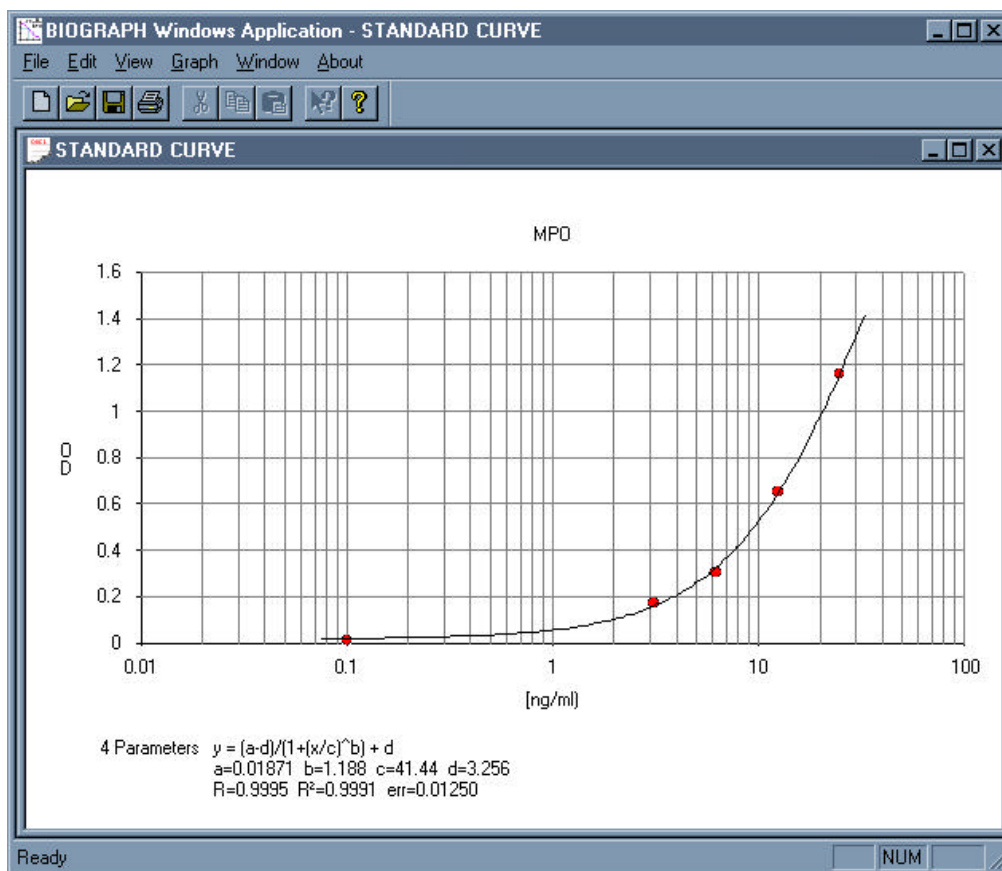
1. Add **100 µl** standard, control and prediluted patient samples.
2. Incubate for **1 hour**, shaking on a horizontal mixer, at room temperature.
3. Decant the content of the plate and wash the wells **5 x with 250 µl** ELISA wash buffer.
7. Add **100 µl** of Peroxidase-labelled antibody conjugate solution.
8. Incubate for **1 hour**, shaking on a horizontal mixer, at room temperature.
9. Decant the content of the plate and wash the wells **5 x with 250 µl** ELISA wash buffer.
10. Add **100 µl** of substrate solution (TMB substrate solution).
11. Incubate for **5-15** minutes at room temperature until colour differences are sufficient.
12. Add **50 µl** of stop solution and mix shortly.
13. Determine absorption with an ELISA reader at **450 nm** against 620 nm as reference. If no reference wavelength is available, read only at 450 nm. If the extinction of the highest standard exceeds the measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm as reference.

## 10. RESULTS

A calibration curve is constructed from the calibrators measured in the assay. Commercially available software can be used as well as graph paper. Results of the samples are read from this calibration curve.

THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline- or 4PL algorithm is recommended.

### Typical calibration curve



<b>Concentration [ng/ml]</b>	25	12.5	6.25	3.125	0
<b>OD mean value</b>	1.164	0.655	0.308	0.179	0.017

These data are for demonstration only and cannot be used instead of data obtained from the actual assay at the time of the assay.

**Plasma samples:**

The from the calibration curve calculated MPO concentration have to be multiplied by **40** to get the right concentration.

**11. LIMITATIONS**

Samples with Myeloperoxidase levels greater than the highest calibrator, should be diluted and re-assayed.

**12. QUALITY CONTROL**

**The manufacturer recommends commercial control samples for internal quality control.**

Control samples or serum pools should be analyzed with each run of calibrators and patient samples. Results, generated from the analysis of control samples, should be evaluated for acceptability using appropriate statistical methods. In assays in which one or more of the quality control sample values lie outside the acceptable limits, the results for the patient sample may not be valid.

*Expected values*

Myeloperoxidase concentration

Serum/Plasma: X (mean value n = 152) 121 ng/ml

### 13. PERFORMANCE CHARACTERISTICS

#### *Precision and reproducibility*

The precision (intra-assay variation) of the MPO ELISA test was calculated from 20 replicate determinations on each of one samples.

Intra-Assay CV n= 20

Sample	MPO mean value [ng/ml]	Intra-Assay CV [%]
1	147.1	4.3
2	288.6	4.8

The total precision (inter-assay variation) of the MPO ELISA test was calculated from data on 2 samples obtained in 20 different assays by three technicians on two different lots of reagents over a period of three months.

Inter-Assay CV n= 20

Sample	MPO mean value [ng/ml]	Inter-Assay CV [%]
1	171.7	12
2	239.9	15

*Recovery*

Two samples were spiked with MPO and measured with this assay.

Recovery n=2

Sample [ng/ml]	Spike [ng/ml]	MPO expected [ng/ml]	MPO measured [ng/ml]
116	500	616	514
116	320	436	401
116	200	316	336
116	125	241	254
92	500	592	504
92	320	412	388
92	200	292	297
92	125	217	204

*Sensitivity*

n=20

Sample	MPO mean value [OD]	Standard variation	Detection limit [ng/ml]
1	0.013	0.003	1.6

### *Cross reactivity*

No cross reactivity to other plasma proteins in stool.

Alpha-1-Antitrypsin	0 %
Albumin	0 %
CRP	0 %
Lysozyme	0 %
slgA	0 %
PMN-Elastase	0 %
Calprotectin	0 %

### *Sample dilution*

Linearity n= 2

Two patient serum samples were diluted with wash buffer. The results are shown below:

Sample	Dilution	Expected [ng/ml]	Measured [ng/ml]
A	1:40	14.5	14.5
	1:80	7.2	7.1
	1:160	3.6	3.5
B	1:40	19.5	19.5
	1:80	9.75	10.1
	1:160	4.8	5.2

## **14. REFERENCES**

1. Saiki T et al.: 1998; Kurume Med. J. 45, 69

## **15. 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.
- The test components which are made of human serum are tested for HBV and HIV and found to be negative. However, since no test method can offer complete assurance that infectious agents are absent, these reagents should be handled as recommended for any potentially infectious human serum or blood specimen. The normal precautions for laboratory working should be observed.
- Reagents of the test package contain sodium azide as a bactericide. Contact with skin or mucous membranes has to be avoided.
- All reagents in the test package are to be used for in-vitro diagnostics only.
- The reagents should not be used after the date of expiry (see label on the test package).
- Single components with different lot numbers should not be mixed or exchanged.
- The guidelines for medical laboratories should be observed.
- Incubation time, incubation temperature and pipetting volumes of the different components have been defined by the producer. Any alterations of the test procedure, that are not coordinated with the producer, may influence the results of the test. The manufacturer can therefore not be held reliable responsible for any damage.

# Symbols / Symbole / Symbôles / Símbolos / Símbolos / Σύμβολα

	Cat.-No.: / Kat.-Nr.: / No.- Cat.: / Cat.-No.: / N.º Cat.: / N.-Cat.: / Αριθμός-Κατ.:
	Lot-No.: / Chargen-Bez.: / No. Lot: / Lot-No.: / Lote N.º: / Lotto n.: / Αριθμός -Παραγωγή:
	Use by: / Verwendbar bis: / Utiliser à: / Usado por: / Usar até: / Da utilizzare entro: / Χρησιμοποιείται από:
	No. of Tests: / Kitgröße: / Nb. de Tests: / No. de Determ.: / N.º de Testes: / Quantità dei tests: / Αριθμός εξετάσεων:
	Concentrate / Konzentrat / Concentré / Concentrar / Concentrado / Concentrato / Συμπύκνωμα
	Lyophilized / Lyophilisat / Lyophilisé / Liofilizado / Liofilizado / Liofilizzato / Λυοφιλιασμένο
	In Vitro Diagnostic Medical Device. / In-vitro-Diagnostikum. / Appareil Médical pour Diagnostics In Vitro. / Dispositivo Médico para Diagnóstico In Vitro. / Equipamento Médico de Diagnóstico In Vitro. / Dispositivo Medico Diagnostico In vitro. / Ιατρική συσκευή για In-Vitro Διάγνωση.
	Evaluation kit. / Nur für Leistungsbewertungszwecke. / Kit pour évaluation. / Juego de Reactivos para Evaluació. / Kit de avaliação. / Kit di valutazione. / Κιτ Αξιολόγησης.
	Read instructions before use. / Arbeitsanleitung lesen. / Lire la fiche technique avant emploi. / Lea las instrucciones antes de usar. / Ler as instruções antes de usar. / Leggere le istruzioni prima dell'uso. / Διαβάστε τις οδηγίες πριν την χρήση.
	Keep away from heat or direct sun light. / Vor Hitze und direkter Sonneneinstrahlung schützen. / Garder à l'abri de la chaleur et de toute exposition lumineuse. / Manténgase alejado del calor o la luz solar directa. / Manter longe do calor ou luz solar directa. / Non esporre ai raggi solari. / Να φυλάσσεται μακριά από θερμότητα και άμεση επαφή με το φως του ηλίου.
	Store at: / Lagern bei: / Stocker à: / Almacene a: / Armazenar a: / Conservare a: / Αποθήκευση στους:
	Manufacturer: / Hersteller: / Fabricant: / Productor: / Fabricante: / Fabricante: / Παραγωγός:
	Caution! / Vorsicht! / Attention! / ¡Precaución! / Cuidado! / Attenzione! / Προσοχή!
<p>Symbols of the kit components see MATERIALS SUPPLIED.  Die Symbole der Komponenten sind im Kapitel KOMPONENTEN DES KITS beschrieben.  Voir MATERIEL FOURNI pour les symbôles des composants du kit.  Símbolos de los componentes del juego de reactivos, vea MATERIALES SUMINISTRADOS.  Para símbolos dos componentes do kit ver MATERIAIS FORNECIDOS.  Per i simboli dei componenti del kit si veda COMPONENTI DEL KIT.  Για τα σύμβολα των συστατικών του κιτ συμβουλευτείτε το ΠΑΡΕΧΟΜΕΝΑ ΥΛΙΚΑ.</p>	

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**LIABILITY:** Complaints will only be accepted in written and if all details of the test performance and results are included (complaint form available from IBL or supplier). Any modification of the test procedure or exchange or mixing of components of different lots could negatively affect the results. These cases invalidate any claim for replacement. Regardless, in the event of any claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the kit during transportation is not subject to the liability of the manufacturer.