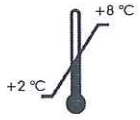
Manual

MPO ELISA Kit

For the in vitro determination of Myeloperoxidase (MPO) in stool and urine

Valid from 31.03.2010

REF **K 6630**



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1. INTENDED USE

The *Immundiagnostik* Assay is intended for the quantitative determination of **Myeloperoxidase** in urine and stool, 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 in 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 immobilized to the surface of the microtiter plates. To remove all unbound substances, a washing step is carried out. In a second incubation step, a Peroxidase-labeled 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

Cat. No	Content	Kit Components	Quantity
K 6630MTP	PLATE	One holder with precoated strips	96
K 6630WP	WASHBUF	ELISA wash buffer concentrate 10x	2 x 100 ml
K 6630ST	STD	Standards, lyophilized (see specification for range)	4 x 5 vials
K 6630KO1	CTRL	Control, lyophilized (see specification for range)	4 x 1 vial
K 6630KO2	CTRL	Control, lyophilized (see specification for range)	4 x 1 vial
K 6630A2	AB	Detection antibody, (mouse monoclonal anti-MPO antibody, biotinylated), concentrate	1 x 200 µl
K 6630K	CONJ	Conjugate, (streptavidin peroxidase labeled), concentrate	1 x 200 µl
K 6630PV	SAMPLEBUF	Sample dilution buffer, ready to use	1 x 50 ml
K 6630TMB	SUB	TMB substrate (Tetramethylbenzidine), ready to use	1 x 15 ml
K 6630AC	STOP	ELISA stop solution, ready to use	1 x 15 ml

5. MATERIAL REQUIRED BUT NOT SUPPLIED

- Bidistilled water (aqua bidest.)
- Laboratory balance
- Precision pipettors and disposable 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 or 405 nm (reference wave length 620 or 690 nm)

6. PREPARATION AND STORAGE OF REAGENTS

- To run assay more than once, ensure that reagents are stored at conditions stated on the label. **Prepare only the appropriate amount necessary for each assay.** 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 **WASHBUF** (wash buffer concentrate) should be diluted with aqua bidest. **1:10** before use (100 ml WASHBUF + 900 ml aqua bidest.), mix well. Crystals could occur due to high salt concentration in the stock solutions. The crystals must be redissolved at 37°C in a water bath before dilution. The **WASHBUF** (wash buffer concentrate) 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** (standards) must be reconstituted with **500 µl** aqua bidest. Allow the vial content to dissolve for 10 minutes and mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted standards are **not stable**.
- The lyophilized **CTRL** (controls) are stable at 2-8°C until the expiry date stated on the label. Reconstitute the controls (see product specification for volume and concentration), allow the vial content to solve for 10 minutes and then mix thoroughly by gentle inversion to insure complete reconstitution. Reconstituted controls are **not stable**.
- The **AB** (detection antibody, biotinylated) must be diluted **1:101** in wash buffer (e.g. 100 µl AB + 10 ml wash buffer). The undiluted AB is stable at **2-8 °C** until the expiry date given on the label. **Diluted antibody solution is not stable and can not be stored.**
- The **CONJ** (conjugate, POD-antibody) must be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The undiluted CONJ is stable at **2-8 °C** until the expiry date stated on the label. **Diluted conjugate is not stable and can not 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.**

7. PRECAUTIONS

- 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 thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Reagents should not be used beyond the expiration date shown on the kit label.

8. SPECIMEN COLLECTION AND PREPARATION

Faeces

Weigh ca. **100 mg** of the sample, note the exact sample amount, add **5 ml** of the wash buffer and mix well.
Centrifuge the sample suspension for 10 min at 3000 rpm. Transfer 1 ml of the supernatant into an Eppendorf tube and centrifuge again at 13.000 rpm for 5 min. The resulting supernatant can be stored at -20°C for about 1 month.

Centrifuge the supernatant at 13.000 rpm for 2 min before use. Dilute the supernatant **1:10** in wash buffer (100 µl supernatant + 900 µl wash buffer). Use **100 µl** of the end-dilution in the assay.

Immundiagnostik recommends the use of the sample tubes from Boehringer / Mannheim for sample preparation.

Urine

Urine must be stored at –20°C. Use urine samples diluted **1:10** with SAMPLEBUF (sample dilution buffer, e.g. 100µl urine + 900µl SAMPLEBUF).

9. ASSAY PROCEDURE

Procedural notes

- Do not mix different lot numbers of any kit component.
- Quality control guidelines 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 wrong use.
- The assay should always be performed according the enclosed manual.

Test procedure

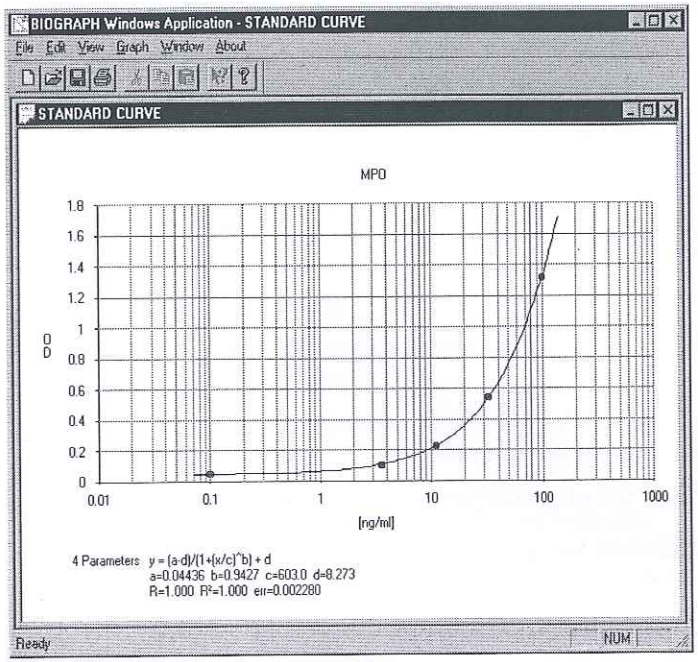
Wash the precoated microtiter plate 5 x with 250 µl ELISA wash buffer. Carry out the tests in duplicate.

1. Pipette **100 µl** of **STD** (standards), **CTRL** (controls) or **samples** into each well.
2. Incubate for **1 hour** at room temperature, shaking on a horizontal mixer.
3. Decant the contents of the plate and wash the cavities **5 x with 250** µl of washing buffer solution.
4. Add **100 µl** of diluted **AB** (detection antibody solution).
5. Incubate for **1 hour** at room temperature, shaking on a horizontal mixer.
6. Decant the contents of the plate and wash the cavities **5 x with 250** µl of washing buffer solution.
7. Add **100 µl** of diluted **CONJ** (conjugate solution).
8. Incubate for **1 hour** at room temperature, shaking on a horizontal mixer.
9. Decant the contents of the plate and wash the cavities **5x with 250 µl** of washing buffer solution.
10. Add **100 µl** of **SUB** (TMB-substrate) solution
11. Incubate approximately for **10 - 20 minutes** at room temperature, shaking slightly, until sufficient coloring is achieved.
12. Add **50 µl** of **STOP** (stop solution) and mix shortly.
13. 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 measurement range of the photometer, absorption must be measured immediately at 405 nm against 620 nm (or 690 nm) as reference.

10. RESULTS

A calibration curve is constructed from the calibrator values and the results of the samples are read from it. Commercially available software can be used as well as graph paper for evaluation.
THE CALIBRATION CURVE IS NOT LINEAR, therefore a spline- or 4PL-algorithm is recommended.

Typical calibration curve



Concentration [ng/ml]	100	33	11	3.6	0
OD mean value	1.322	0.543	0.232	0.106	0.048

The data are for demonstration only and cannot be used for the evaluation of test results.

Faeces

In order to determine the MPO concentration in faeces samples, calculate as described in the following example:

Sample amount: 80 mg (1ml stool = 1g) = 0,08 ml
Dilution step 1: 5ml / 0,08ml = 62,5
Dilution step 2: 10
Dilution factor: 62,5 x 10 = 625

The concentration read from the calibration curve must be multiplied by **625** to obtain the MPO concentration of the sample.

***Note*: The dilution factor varies depending on the stool amount weighed for the analysis. The concentration read from the calibration curve must be multiplied by the corresponding dilution factor.**

Urine samples

The concentration read from the calibration curve must be multiplied by **10** to obtain the MPO concentration of the sample.

11. LIMITATIONS

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

12. QUALITY CONTROL

Immundiagnostik AG recommends the use of commercial control samples for internal quality control if available.

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 samples 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

Myeloperoxidase concentration

Stool: < 2000 ng/g

The reference value should be used as a guideline only. It is recommended that each laboratory establishes an own expected range for its patient population.

13. PERFORMANCE CHARACTERISTICS

Precision and reproducibility

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

Intra-Assay CV n= 20

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

The total precision (inter-assay variation) of the Immundiagnostik 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

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

Cross reactivity

No cross reactivity to other plasma proteins in stool.

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

14. REFERENCES

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

15. GENERAL NOTES ON THE TEST AND TEST PROCEDURE

- For *in vitro* diagnostic use only.
- Quality control guidelines should be followed.
- 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 thimerosal as bactericides. Sodium azide and thimerosal are toxic. Substrates for the enzymatic color reactions are toxic and carcinogenic. Avoid contact with skin or mucous membranes.
- Stop solution is composed of sulfuric acid, which is a strong acid. Even diluted, it still must be handled with care. It can cause acid burns and should be handled with gloves, eye protection, and appropriate protective clothing. Any spills should be wiped out immediately with copious quantities of water.
- Do not mix different lot numbers of any kit component.
- Reagents should not be used beyond the expiration date shown on the kit label.
- The assay should always be performed according the enclosed manual.
- 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 wrong use.
- Warranty claims and complaints in respect of 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.