

IDK[®] MPO ELISA

***For the determination of myeloperoxidase (MPO)
in stool and urine***

Valid from 2019-07-18

REF KR6630

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1. INTENDED USE

This Immundiagnostik AG assay is an enzyme immunoassay intended for the quantitative determination of myeloperoxidase (MPO) in urine and stool. For research use only. Not for use in diagnostic procedures.

2. INTRODUCTION

The granules of neutrophils (approx. 70% of the white blood cells) contain a large number of different enzymes. Myeloperoxidase (MPO) catalyses 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.

3. MATERIAL SUPPLIED

Cat. No.	Label	Kit components	Quantity
KR6630	PLATE	Microtiter plate, pre-coated	12 x 8 wells
KR0001.C.100	WASHBUF	Wash buffer concentrate, 10 x	2 x 100 ml
KR6630	CONJ	Conjugate concentrate (peroxidase-labelled streptavidin)	1 x 200 µl
KR6630	AB	Detection antibody concentrate (mouse monoclonal anti-MPO antibody, biotinylated)	1 x 200 µl
KR6630	STDBUF	Standard dilution buffer, ready-to-use	1 x 35 ml
KR6630	STD	Standards, lyophilised (see specification for concentrations)	4 x 5 vials
KR6630	CTRL 1	Control, lyophilised (see specification for range)	4 x 1 vial
KR6630	CTRL 2	Control, lyophilised (see specification for range)	4 x 1 vial
KR6630	SAMPLEBUF	Sample dilution buffer, ready-to-use	1 x 50 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*
- Stool sample application system such as cat. no.: K 6998SAS
- 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
- 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 detection antibody:** Before use, the **detection antibody concentrate (AB)** has to be diluted **1:101** in wash buffer (100 µl AB + 10 ml wash buffer). The AB is stable at **2–8 °C** until the expiry date stated on the label. **Detection antibody** (1:101 diluted AB) **is not stable and cannot be stored**.

- **Preparation of the conjugate:** Before use, the **conjugate concentrate (CONJ)** has to be diluted **1:101** in wash buffer (100 µl CONJ + 10 ml wash buffer). The CONJ is stable at **2–8 °C** until the expiry date stated on the label. **Conjugate (1:101 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

Storage of urine samples

Urine should be stored at –20 °C until the measurement. MPO in urine is stable for 4 weeks at –20 °C.

Dilution of urine samples

Urine 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.

Storage of the stool samples

MPO in raw stool is stable for 10 weeks at –20 °C.

MPO in stool extract is not stable and cannot be stored.

Extraction of the stool samples

Wash buffer (1:10 diluted WASHBUF) is used as a **sample extraction buffer**. We recommend the following sample preparation:

Stool Sample Application System (SAS) (Cat. No.: K 6998SAS)

Stool sample tube – Instructions for use

Please note that the dilution factor of the final stool suspension depends on the amount of stool sample used and the volume of the buffer.

SAS with 0.75 ml sample extraction buffer:

Applied amount of stool: 15 mg

Buffer Volume: 0.75 ml

Dilution Factor: 1:50

Please follow the instructions for the preparation of stool samples using the SAS as follows:

- a) The raw stool sample has to be thawed. For particularly heterogeneous samples we recommend a mechanical homogenisation using an applicator, inoculation loop or similar device.
- b) Fill the **empty stool sample tube** with **0.75 ml sample extraction buffer** (1:10 diluted WASHBUF) before using it with the sample. **Important:** Allow the sample extraction buffer to reach room temperature.
- c) Unscrew the tube (yellow part of cap) to open. Insert the yellow dipstick into the sample. The lower part of the dipstick has notches which need to be covered completely with stool after inserting it into the sample. Place dipstick back into the tube. When putting the stick back into the tube, excess material will be stripped off, leaving 15 mg of sample to be diluted. Screw tightly to close the tube.
- d) Shake the tube well until no stool sample remains in the notches. Important: Please make sure that you have a maximally homogenous suspension after shaking. Especially with more solid samples, soaking the sample in the tube with sample extraction buffer for ~ 10 minutes improves the result.
- e) Allow sample to stand for ~10 minutes until sediment has settled. Floating material like shells of grains can be neglected.
- f) Carefully unscrew the complete cap of the tube including the blue ring plus the dipstick. Discard cap and dipstick. Make sure that the sediment will not be dispersed again.

Dilution I: 1:50

Dilution of stool samples

The supernatant of the sample preparation procedure (dilution I) is diluted **1:10 in wash buffer**. For example:

100 µl dilution I + **900 µl** wash buffer, mix well = **1:10 (dilution II)**. This results in a final dilution of **1:500**.

For analysis, pipet **100 µl** of **dilution II** per well.

7. ASSAY PROCEDURE

Principle of the test

This ELISA is designed for the quantitative determination of myeloperoxidase (MPO) 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 immobilised 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-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 colour converts 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) vs. concentration is generated, using results obtained from the calibrators. 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.	Before use , wash the wells 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
2.	Add each 100 µl standards/controls/prepared samples into the respective wells.
3.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a horizontal shaker* .

4.	Discard the content of each well and wash 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
5.	Add 100 µl detection antibody (diluted AB) into each well.
6.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a horizontal shaker* .
7.	Discard the content of each well and wash 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
8.	Add 100 µl conjugate (diluted CONJ) into each well.
9.	Cover the strips and incubate for 1 hour at room temperature (15–30 °C) on a horizontal shaker* .
10.	Discard the content of each well and wash 5 times with 250 µl wash buffer . After the final washing step, remove residual wash buffer by firmly tapping the plate on absorbent paper.
11.	Add 100 µl substrate (SUB) into each well.
12.	Incubate for 10–20 min** at room temperature (15–30 °C) in the dark .
13.	Add 100 µl stop solution (STOP) into each well and mix well.
14.	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.

* 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.

Urine

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

Stool samples

For determining the concentration of MPO in stool samples, calculate as described in the following example:

Sample amount: 15 mg (1 ml stool = 1 g) = 0.015 ml

Dilution I: 0.75 ml / 0,015 ml = 50

Dilution II: 10

Dilution factor: 50 x 10 = 500

The obtained results have to be multiplied by the **dilution factor of 500** 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 (see definition below) can be further diluted and re-assayed. Please consider this higher dilution when calculating the results.

Samples with concentrations lower than the measurement range (see definition below) 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:

Analytical sensitivity × sample dilution factor to be used

Analytical sensitivity 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.

Reference range

We recommend each laboratory to establish its own reference range.

11. PERFORMANCE CHARACTERISTICS

Accuracy – Precision

Repeatability (Intra-Assay); n = 12

The repeatability was assessed with 3 stool samples under **constant** parameters (same operator, measurement system, day and kit lot).

Sample	Mean value [µg/ml]	CV [%]
1	62.15	5.2
2	33.98	3.2
3	37.24	3.2

Reproducibility (Inter-Assay); n = 34

The reproducibility was assessed with 2 controls under **varying** parameters (different operators, measurement systems, days and kit lots).

Sample	Mean value [ng/ml]	CV [%]
1	5.01	12.8
2	21.21	9.1

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

0.137 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 colour 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 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.
- 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® 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 send to Immunodiagnostik AG along with a written complaint.

15. REFERENCES

Saiki, T., 1998. Myeloperoxidase concentrations in the stool as a new parameter of inflammatory bowel disease. *The Kurume medical journal*, **45**(1), pp.69–73.

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		