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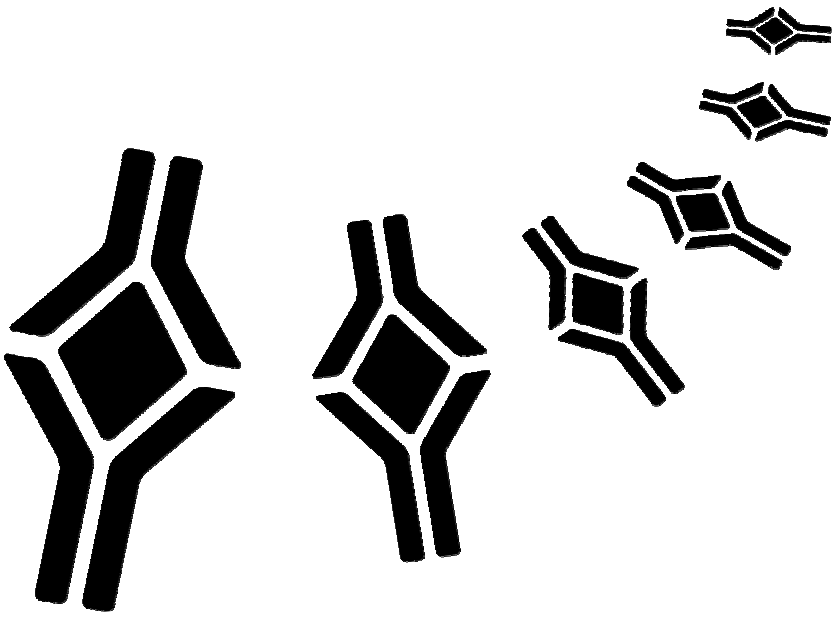

HUMAN MPO ELISA

Product Data Sheet

Cat. No.: RHK324R

For Research Use Only

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» **This kit is manufactured by:**
BioVendor – Laboratorní medicína, a.s.

» **Use only the current version of Product Data Sheet enclosed with the kit!**

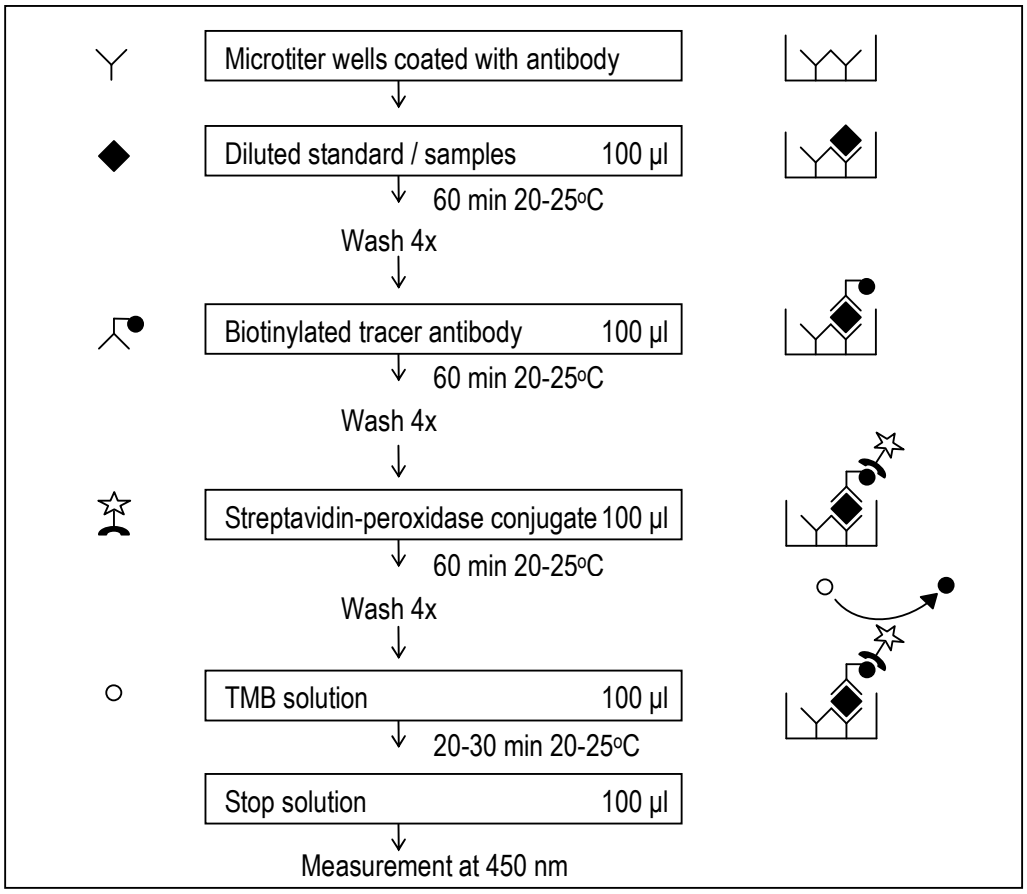
1 INTRODUCTION

Myeloperoxidase (MPO) is a glycoprotein with a alpha2beta2 heteromultimer expressed in all cells of the myeloid linage. MPO is abundantly present in azurophilic granules of polymorphonuclear neutrophils. It is an important enzyme used during phagocytic lysis of engulfed foreign particles which takes part in the defense of the organism through production of hypochlorous acid (HOCl), a potent oxidant. MPO is rapidly released by activated polymorphonuclear neutrophils. Involvement of MPO has been described in numerus diseases such as atherosclerosis, lung cancer, Alzheimer's disease and multiple sclerosis. Autoimmune antibodies to MPO are involved in Wegener's disease. Since the discovery of MPO deficiency, initially regarded as rare and restricted to patients suffering from severe infections, MPO has attracted more clinical attention.
The classical MPO assay is an enzymatic assay for activity of MPO. This classical MPO assay is hampered by the presence of inhibitory compounds in tissue homogenates and plasma. In this type of assays spiking often gives unreliable results. The human MPO ELISA is not influenced by inhibitors of the enzyme activity.

2 INTENDED USE

The human MPO ELISA kit is to be used for the *in vitro* quantitative determination of human MPO in plasma, sputum and cell culture supernatant samples. This kit is intended for laboratory research use only and is not for use in diagnostic or therapeutic procedures.
The analysis should be performed by trained laboratory professionals.

3 PROTOCOL OVERVIEW



4 PRINCIPLES OF THE TEST

- The human MPO ELISA is a ready-to-use solid-phase enzyme-linked immunosorbent assay based on the sandwich principle with a working time of 3½ hours.
- The efficient format of 2 plates with twelve disposable 8-well strips allows free choice of batch size for the assay.
- Samples and standards are incubated in microtiter wells coated with antibodies recognizing human MPO.
- Biotinylated tracer antibody will bind to captured human MPO.
- Streptavidin-peroxidase conjugate will bind to the biotinylated tracer antibody.
- Streptavidin-peroxidase conjugate will react with the substrate, tetramethylbenzidine (TMB).
- The enzyme reaction is stopped by the addition of oxalic acid.
- The absorbance at 450 nm is measured with a spectrophotometer. A standard curve is obtained by plotting the absorbance (linear) versus the corresponding concentrations of the human MPO standards (log).
- The human MPO concentration of samples, which are run concurrently with the standards, can be determined from the standard curve.

5 CONTENTS OF THE KIT

Item no.	Kit component	Quantity	Color code
Vial 1	**Wash buffer 20x**	**2 vials (20 ml)**	**Grey**
Vial 2	**Dilution buffer A 5x**	**1 vial (20 ml)**	**Gold**
Vial 3	**Dilution buffer B 10x**	**2 vials (5 ml)**	**Gold**
Vial 4	**Standard**	**2 vials, 0.5 ml lyophilized**	**Yellow**
Vial 5	**Tracer, biotinylated**	**2 vials, 1 ml lyophilized**	**Green**
Vial 6	**Streptavidin-peroxidase**	**1 vial, 1 ml lyophilized**	**Blue**
Vial 7	**TMB substrate**	**1 vial (20 ml)**	**Purple**
Vial 8	**Stop solution**	**1 vial (20 ml)**	**Red**
Item 9	**12 Microtiter strips, pre-coated**	**2 plates**	
Item 10	**Frame**	**1**	
Item 11	**Adhesive covers**	**4**	
Item 12	**Certificate of quality control**	**1**	
Item 13	**Manual**	**1**	
Item 14	**Data collection sheet**	**1**	

6 MATERIALS REQUIRED BUT NOT PROVIDED

- Calibrated micropipettes and disposable tips.
- Distilled or deionized water.
- Plate washer (automatic or manual).
  In case a plate washer is used the supplied wash buffer is not sufficient. Additional wash buffer can be ordered separately. Please contact your local distrobutor.
- Polypropylene tubes.
- Calibrated ELISA plate reader capable of measuring absorbance at 450 nm.

7 WARNINGS AND PRECAUTIONS

- For research use only, not for diagnostic or therapeutic use.
- This kit should only be used by qualified laboratory staff.
- Do not under any circumstances add sodium azide as preservative to any of the components.
- Do not use kit components beyond the expiration date.
- Do not mix reagents from different kits and lots. The reagents have been standardized as a unit for a given lot. Use only the reagents supplied by manufacturer.
- The assay has been optimized for the indicated standard range. Do not change the standard range.
- Standard, tracer and streptavidin-peroxidase vials should be opened after reconstitution. Open vials carefully: vials are under vacuum.
- Do not ingest any of the kit components.
- Kit reagents contain 2-chloroacetamide as a preservative. 2-Chloroacetamide is harmful in contact with skin and toxic if swallowed. In case of accident or if you feel unwell, seek medical advice immediately.
- The TMB substrate is light sensitive, keep away from bright light. The solution should be colorless until use.
- The stop solution contains 2% oxalic acid and can cause irritation or burns to respiratory system, skin and eyes. Direct contact with skin and eyes should be strictly avoided. If contact occurs, rinse immediately with plenty of water and seek medical advice.
- Incubation times, incubation temperature and pipetting volumes other than those specified may give erroneous results.
- Do not reuse microwells or pour reagents back into their bottles once dispensed.
- Handle all biological samples as potentially hazardous and capable of transmitting diseases.
- Hemolyzed, hyperlipemic, heat-treated or contaminated samples may give erroneous results.
- Use polypropylene tubes for preparation of standard and samples. Do not use polystyrene tubes or sample plates.

8 STORAGE INSTRUCTIONS

- Upon receipt, store individual components at 2 - 8°C. Do not freeze.
- Do not use components beyond the expiration date printed on the kit label.
- The standard, tracer and streptavidin-peroxidase are stable in lyophilized form until the expiration date indicated on the kit label, if stored at 2 - 8°C.
- The exact concentration of the standard is indicated on the label of the vial and the certificate of quality control.
- Once reconstituted the standard is stable for 24 hours, if stored at 2 - 8°C. For longer stability we recommend to store aliquots at –20°C. Stored at –20°C the standard will be stable for 1 month.
- Once reconstituted, tracer and streptavidin-peroxidase are stable for 1 month if stored at 2 - 8°C.
- Upon receipt, foil pouch around the plate should be vacuum-sealed and unpunctured. Any irregularities to aforementioned conditions may influence plate performance in the assay.
- Return unused strips immediately to the foil pouch containing the desiccant pack and reseal along the entire edge of the zip-seal. Quality guaranteed until expiration date if stored at 2 - 8°C.

9 SAMPLE HANDLING

Collection and handling

Plasma

Please be aware that human MPO is released from neutrophils into serum in the process of blood coagulation. This will lead to false positive results. It is therefore advised to use 'careful plasma', which can be obtained as follows.

Keep freshly collected blood on ice. Within 20 minutes after blood sampling, separate plasma by centrifugation: 1500xg at 4°C for 15 min. Remove plasma and transfer to fresh polypropylene tube. Be careful to not disturb white cells in the buffy coat. Recentrifuge the transferred plasma in order to avoid every contamination with white blood cells: 1500xg at 4°C for 15 min. Note that most reliable results are obtained with heparin plasma.

Storage

Store samples below -20°C, preferably at -70°C in polypropylene tubes. Storage at -20°C can affect recovery of human MPO. Use samples within 24 hours after thawing. Avoid multiple freeze-thaw cycles which may cause loss of human MPO activity and give erroneous results.

Do not use hemolyzed, hyperlipemic, heat-treated or contaminated samples.

Before performing the assay, samples should be brought to room temperature (18 – 25°C) and mixed gently. Prepare all samples (controls and test samples) prior to starting the assay procedure. Avoid foaming.

Dilution procedures

Plasma samples

Human MPO can be measured accurately if plasma samples are diluted at least 10x with supplied dilution buffer in polypropylene tubes.
Note that most reliable results are obtained with heparin plasma.

Sputum samples

Human MPO can be measured accurately if sputum samples are diluted at least 10x with supplied dilution buffer in polypropylene tubes.
Note that most reliable results are obtained if sputum samples are not treated with DTT.
Sputum samples should be kept on ice.

Remark regarding recommended sample dilution

The recommend dilution for samples should be used as a guideline. The recovery of human MPO from an undiluted sample is not 100% and may vary from sample to sample. When testing less diluted samples it is advisable to run recovery experiments to determine the influence of the matrix on the detection of human MPO.
Do not use polystyrene tubes or sample plates for preparation or dilution of the samples.

10 ASSAY PROCEDURE

9.1. General Remarks

1. All standards should be run with each series of unknown samples.
2. Each standard and sample should be assayed in duplicate each time the test is performed.
3. Standards should be subject to the same manipulations and incubation times as the samples being tested.

9.2. Guidelines for minimizing variation in ELISA

The ELISA technique is an extremely useful quantitative assay. However, variability introduced by a number of factors can lead to a diminished value of the results. In order to obtain the most accurate and meaningful data, it is important to be aware of these factors. The following guidelines will help you to realize the full potential of this assay system.

1. Bring all reagents to room temperature before use.
   Antibody-antigen and enzyme-substrate reactions are temperature dependent, and this can strongly influence the sensitivity of the assay. This is particularly important when short incubation times are used (eg: one hour or less at room temperature).
2. Mix all reagents well before use.
   Whenever mixtures or dilutions are made they should be mixed extremely well to provide homogenous solutions. During storage, density gradients can develop in some solutions, thus it is important to mix all reagents prior to use.

3. Be precise and accurate during pipetting.
   The accuracy of the assay is primarily determined by the precision by which the pipetting is performed. Do not underestimate the amount of variability which can be introduced during this stage of the ELISA procedure. Be precise when pipetting. Poor results may be the result of the following factors:a. The pipette-tip(s) are not fastened tightly to the pipette. Tips on multi-channel pipette should be checked individually to make sure that they are securely fitted.
   b. The pipette is filled too quickly; be aware of air bubbles in the pipette tip which may be formed when the liquid is drawn up too fast. (10 µl of air instead of sample gives a variation of 10%!)
   c. Droplets clinging to the outside of the pipette tip.
   d. The pipette tip is emptied too quickly.
   To reduce variation during the pipetting steps, work at an uniform pace - one that minimizes the time between pipetting the first and last wells but which does not introduce errors due to hasty pipetting. Also, the micropipettes should be checked periodically for proper function and calibration.
4. Incubate for times and at temperatures as specified in the protocol.
   If the incubation time is too short or the incubation temperature too low, this may result in a flat calibration curve accompanied by low absorbencies. In contrast, if the incubation time is prolonged excessively or the incubation temperature is too high again a flat calibration curve may be obtained.
5. Timing.
   Allowing wash buffer to stand too long in the wells, or allowing the wells to stand too long after aspiration of contents are all sources of inaccuracy and will result in unexpected variations in the test results.

6. Reading of plates.
   Check plate for presence of air bubbles and make sure that all are removed (without removing any solution), prior to reading the plate. The spectrophotometer wavelength should be set accurately to the optimum wavelength being 450 nm.
7. TMB.
   We recommend that disposable plastic tubes are employed when using TMB since the slightest residues from previous use may lead to color formation.
8. Incubate plates uniformly in case of 37°C incubation.
   Plates should be placed directly on a prewarmed surface for incubation at 37°C. Use a humidified incubator to reduce drying effects in the outside wells (“edge effects”) or place a wet tissue or blotter paper under the plates in non-humidified incubators. Avoid opening the incubator frequently during the incubation period. Do not stack plates.

9.3. Preparations

1. Allow all the reagents to equilibrate to room temperature (20 – 25°C) prior to use. Return to proper storage conditions immediately after use.
2. **Wash buffer:** Prepare wash buffer by mixing 40 ml of 20 x wash buffer with 760 ml of distilled or de-ionized water, which is sufficient for 2 x 96 tests. Where less volume is required, prepare the desired volume of wash buffer by diluting 1 part of the 20 x wash buffer with 19 parts of distilled or de-ionized water.
3. **Dilution buffer:** Prepare dilution buffer by mixing 20 ml of the 5x dilution buffer A with 30 ml of distilled or de-ionized water and 10 ml of 10x dilution buffer B with 40 ml distilled or de-ionized water. Combine both solutions equally and mix well. This 100 ml is sufficient for 2 x 96 tests. Where less volume is required, prepare the desired volume of dilution buffer by diluting 2 parts of the 5x dilution buffer A with 3 parts of distilled or de-ionized water and 1 part of 10x dilution buffer B with 4 parts distilled or de-ionized water. Combine both solutions equally and mix well. Concentrated dilution buffer may contain crystals. In case the crystals do not disappear at room temperature within 1 hour, concentrated dilution buffer can be warmed up to 37°C. Do not shake the solution.
4. **Standard solution:** The standard is reconstituted by injection of 0.5 ml of distilled or de-ionized water. Prepare each human MPO standard in polypropylene tubes by serial dilution of the reconstituted standard with dilution buffer as shown in Table 3.

Tube	Volume dilution buffer	Volume standard	Concentration (ng/ml)
1	See certificate of quality control	150 µl vial 4	100
2	225 µl	150 µl tube 1	40
3	225 µl	150 µl tube 2	16
4	225 µl	150 µl tube 3	6.4
5	225 µl	150 µl tube 4	2.6
6	225 µl	150 µl tube 5	1.0
7	225 µl	150 µl tube 6	0.4
8	225 µl	-	0

1. **Tracer solution:** The tracer is reconstituted by injection of 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml tracer with 11 ml dilution buffer, which is sufficient for 1 x 96 tests. Where less volume is required, prepare the desired volume of tracer by diluting 1 part of the reconstituted tracer with 11 parts of dilution buffer.
2. **Streptavidin-peroxidase solution:** The streptavidin-peroxidase is reconstituted by injection of 1 ml distilled or de-ionized water. Dilute the reconstituted 1 ml streptavidin-peroxidase with 23 ml dilution buffer, which is sufficient for 2 x 96 tests. Where less volume is desired, prepare the required volume of streptavidin-peroxidase solution by diluting 1 part of the reconstituted streptavidin-peroxidase with 23 parts of dilution buffer.

9.4. Test Protocol

Bring all reagents to room temperature (20 - 25°C) before use.

1. Determine the number of test wells required, put the necessary microwell strips into the supplied frame, and fill out the data collection sheet. Return the unused strips to the storage bag with desiccant, seal and store at 2 - 8°C.
2. Transfer 100 µl in duplicate of standard, samples, or controls into appropriate wells. Do not touch the side or bottom of the wells.
3. Apply an adhesive cover to the tray. Tap the tray to eliminate any air bubbles. Be careful not to splash liquid onto the cover.
4. Incubate the strips or plate for 1 hour at room temperature.
5. Wash the plates 4 times with wash buffer using a plate washer or as follows:
   a. Carefully remove the plate sealer, avoid splashing.
   b. Empty the plate by inverting plate and shaking contents out over the sink, keep inverted and tap dry on a thick layer of tissues.
   c. Add 200 µl of wash buffer to each well, wait 20 seconds, empty the plate as described in 5b.
   d. Repeat the washing procedure 5b/5c three times.
   e. Empty the plate and gently tap on thick layer of tissues.
6. Add 100 µl of diluted tracer to each well using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
7. Cover the tray with an adhesive cover. Incubate the tray for 1 hour at room temperature.
8. Repeat the wash procedure described in step 5.
9. Add 100 µl of diluted streptavidin-peroxidase to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
10. Cover the tray with an adhesive cover, incubate the tray for 1 hour at room temperature.
11. Repeat the wash procedure described in step 5.
12. Add 100 µl of TMB substrate to each well, using the same pipetting order as applied in step 2. Do not touch the side or bottom of the wells.
13. Cover the tray with a new adhesive cover, incubate the tray for 20 – 30 minutes at room temperature. Avoid exposing the microwell strips to direct sunlight. Covering the plate with aluminium foil is recommended.
14. Stop the reaction by adding 100 µl of stop solution with the same sequence and timing as used in step 12. Mix solutions in the wells thoroughly by gently swirling the plate. Gently tap the tray to eliminate any air bubbles trapped in the wells.
15. Read the plate within 30 minutes after addition of stop solution at 450 nm using a plate reader, following the instructions provided by the instrument's manufacturer.

10. INTERPRETATION OF RESULTS

1. Calculate the mean absorbance for each set of duplicate standards, control and samples.
2. If individual absorbance values differ by more than 15% from the corresponding mean value, the result is considered suspect and the sample should be retested.
3. The mean absorbance of the zero standard should be less than 0.3.
4. Create a standard curve using computer software capable of generating a good curve fit. The mean absorbance for each standard concentration is plotted on the vertical (Y) axis versus the corresponding concentration on the horizontal (X) axis (logarithmic scale). For an example of the standard curve see certificate of quality control included with the kit. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.
5. If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.
6. Samples that give a mean absorbance above the absorbance for the highest standard concentration are out of range of the assay. These samples should be retested at a higher dilution.

11 TECHNICAL HINTS

1. User should be trained and familiar with ELISA assays and test procedure.
2. If you are not familiar with the ELISA technique it is recommended to perform a pilot assay prior to evaluation of your samples. Perform the assay with a standard curve only following the instructions.
3. Improper or insufficient washing at any stage of the procedure will result in either false positive or false negative results. Completely empty wells before dispensing wash buffer, fill with wash buffer as indicated for each cycle and do not allow wells to sit uncovered or dry for extended periods.
4. Since exact conditions may vary from assay to assay, a standard curve must be established for every run. If the standard is out of range, the results of the test samples are not reliable. The test should be repeated.
5. Do not mix reagents from different batches, or other reagents and strips. Remainders should not be mixed with contents of freshly opened vials.
6. Each time the kit is used, fresh dilutions of standard, sample, tracer, streptavidin-peroxidase and buffers should be made.
7. Caps and vials are not interchangeable. Caps should be replaced on the corresponding vials.
8. To avoid cross-contaminations, change pipette tips between reagent additions of each standard, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
9. To ensure accurate results, proper adhesion of supplied covers during incubation steps is necessary.
10. The waste disposal should be performed according to your laboratory regulations.

12 QUALITY CONTROL

The certificate of quality control included in this kit is lot specific and is to be used to verify results obtained by your laboratory. The absorption values provided on the certificate of quality control are to be used as a guideline only. The results obtained by your laboratory may differ.
This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. For optimal performance of this kit, it is advised to work according to good laboratory practice.

13 SUMMARY OF THE PROCEDURE

- Fill in a data collection sheet.
- Prepare dilution buffer (vial 2 and vial 3) and wash buffer (vial 1).
- Reconstitute and dilute the standard (vial 4), tracer (vials 5) and streptavidin peroxidase conjugate (vial 6).
- Prepare dilution series of standard curve and samples.
- Transfer duplicate 100 µl aliquot of each standard, sample and controls to assigned wells.
- Apply adhesive cover and **incubate for 1 hour at room temperature.**
- Wash wells 4 times with 200 µl wash buffer.
- Add 100 µl of diluted tracer to each well.
- Apply adhesive cover and **incubate for 1 hour at room temperature.**
- Wash wells 4 times with 200 µl wash buffer.
- Add 100 µl of diluted streptavidin-peroxidase conjugate to each well.
- Apply adhesive cover and **incubate for 1 hour at room temperature.**
- Wash 4 times with 200 µl wash buffer.
- Add 100 µl TMB substrate solution (vial 7) to each well.
- Apply adhesive cover and **incubate for 20 to 30 minutes** in the dark **at room temperature.**
- Stop the reaction by adding 100 µl of stop solution (vial 8). Remove any air bubbles **without removing any solution.**
- Measure the absorbance at 450 nm.

14 TROUBLE SHOOTING

Suggestions summarized below in Table 4 can be used as a guideline in the case of unexpected assay results.

Low absorbance	High absorbance	Poor duplicates	All wells positive	All wells negative	Possible cause
•	•		•	•	Kit materials or reagents are contaminated or expired
•					Incorrect reagents used
•		•	•		Lyophilized reagents are not properly reconstituted
•	•	•	•	•	Incorrect dilutions or pipetting errors
•		•			Improper plastics used for preparation of standard and/or samples
•	•				Improper incubation times or temperature
		•			Especially in case of 37°C incubation: plates are not incubated uniformly
•					Assay performed before reagents were brought to room temperature
•	•	•	•	•	Procedure not followed correctly
				•	Omission of a reagent or a step
		•			Poor mixing of samples
	•		•		Low purity of water
	•	•			Strips were kept dry for too long during/after washing
	•	•	•		Inefficient washing
	•	•			Cross-contamination from other samples or positive control
		•	•		TMB solution is not clear or colorless
•	•				Wrong filter in the microtiter reader
	•	•			Airbubbles
		•			Imprecise sealing of the plate after use
•					Wrong storage conditions

15 EVALUATION OF RESULTS

- Working time of 3½ hours.
- Minimum concentration which can be measured is 0.4 ng/ml.
- Measurable concentration range of 0.4 to 100 ng/ml.
- Working volume of 100 µl/well.

Cross-reactivity
Potential cross-reacting proteins detected in the human MPO ELISA:

Cross reactant	Reactivity
Mouse MPO	negative
Rat MPO	negative
Rabbit MPO	negative
Bovine MPO	negative
Sheep MPO	negative
Swine MPO	negative
Horse MPO	negative

Cross-reactivity for other species or proteins/peptides has not been tested.

16 REFERENCES

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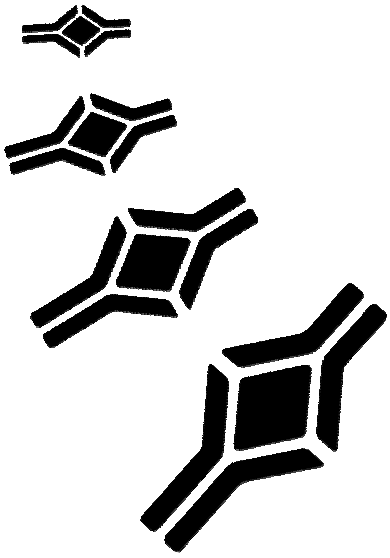

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