Mercodia MPO ELISA

Directions for Use

10-1176-01
Reagents for 96 determinations

For Research Use Only.
Not For Use in Diagnostic Procedures.

Manufactured by

Mercodia AB
Sylveniusgatan 8A
SE-754 50 Uppsala
Sweden

Explanation of symbols used on labels

	Reagents for 96 determinations
	Expiry date
	Store between 2–8°C
	Lot No.

Intended use

Mercodia MPO ELISA provides a method for the quantitative determination of human MPO in EDTA-plasma.

Summary and explanation of the test

Myeloperoxidase (MPO), an iron containing glycoprotein, is a covalently bound tetrameric complex with a molecular weight of 150 kDa. It is composed of two glycosylated alfa chains of MW 59-64 kDa and two unglycosylated beta chains of MW 14 kDa. MPO is found in abundance in the primary azurophilic granules of neutrophils and is present in monocytes.

In response to microbial invasion, MPO is released from the cytoplasmic granules of neutrophils into the phagosome and extracellular space, catalysing the conversion of hydrogen peroxide and chloride ions (Cl^-) into hypochlorous acid, a potent oxidizing agent.

Myeloperoxidase traditionally is used as a marker of airway inflammation caused by asthma or environmental irritants. It is also believed that MPO participates in different stages of atherogenesis and has a potential role in the promotion of atherosclerosis. Association between elevated MPO levels in serum and cardiovascular disease (CAD) supports an important role for MPO as an inflammatory marker in CAD, making it possible to identify patients at risk for cardiac events in the absence of mycardial necrosis.

Principle of the procedure

Mercodia MPO ELISA is a solid phase two-site enzyme immunoassay. It is based on the sandwich technique in which two monoclonal antibodies are directed against separate antigenic determinants on the MPO-molecule. During incubation, MPO in the sample react with anti-MPO antibodies bound to microtitration wells. After washing, peroxidase conjugated anti-MPO antibodies are added and after the second incubation and a simple washing step that removes unbounded enzyme labeled antibody, the bound conjugate is detected by reaction with 3,3',5,5'-tetramethylbenzidine (TMB). The reaction is stopped by adding acid to give a colorimetric endpoint that is read spectrophotometrically.

Warnings and precautions

- For Research Use Only. Not For Use in Diagnostic Procedures.
- Not for internal or external use in humans or animals.
- All patient specimens should be handled as of capable of transmitting infections.
- Each well can only be used once.
- The Stop Solution contains <5% Sulphuric acid.
  The Stop Solution is labeled:

Danger

H318 – Causes serious eye damage.
H315 – Causes skin irritation.
P280 – Wear protective gloves. Wear eye or face protection.
P264 – Wash hands thoroughly after handling.
P302 + P352 + P362 + P364 – IF ON SKIN: Wash with plenty of soap and water. Take off contaminated clothing and wash it before reuse.
P332 + P313 - If skin irritation occurs: Get medical attention.
P305 + P351 + P338 + P310 – IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or physician.

- The Enzyme Conjugate Buffer, Cal 0, 1, 2, 3, 4, 5, Wash Buffer, Assay Buffer and Sample Buffer contain <0.06% 5-chloro-2-methyl-4-isothiazolin-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

  The Enzyme Conjugate Buffer, the Calibrators, Wash Buffer, Assay Buffer and Sample Buffer are labeled:

Warning

H317 – May cause an allergic skin reaction.
P280 – Wear protective gloves.
P261 – Avoid breathing vapour.
P272 – Contaminated work clothing should not be allowed out of the workplace.
P302 + P352 – IF ON SKIN: Wash with plenty of soap and water.
P333 + P313 – If skin irritation or rash occurs: Get medical attention.
P501 – Dispose of contents and container in accordance with all local, regional, national and international regulations.

Material required but not provided

- Pipettes with appropriate volumes (repeating pipettes preferred for addition of Assay Buffer, enzyme conjugate 1X solution, Substrate TMB and Stop Solution)
- Tubes, beakers and cylinders for reagent preparation
- Tubes for sample dilution
- Redistilled water
- Magnetic stirrer
- Vortex mixer
- Microplate reader with 450 nm filter
- Microplate shaker (700–900 cycles per minute, orbital movement)
- Microplate washing device with overflow function (recommended but not required)

Reagents

Each MPO ELISA kit contains reagents for 96 wells, sufficient for 42 samples and one calibration curve in duplicate. For larger series of assays, use pooled reagents from packages bearing identical lot numbers. The expiry date for the complete kit is stated on the outer label. The recommended storage temperature is 2-8°C.

Coated Plate Mouse monoclonal anti-MPO For unused strips of microplate wells completely reseal the bag using adhesive tape and use within 2 months.	1 plate	96 wells 8-well-strips	Ready for use
Calibrators 1, 2, 3, 4, 5 Color coded yellow Concentration indicated on vial label Storage after reconstitution: 2-8°C for 2 months For storage of reconstituted Calibrators for more than 2 months, store at -20°C	5 vials	1000 µL	Lyophilized Add 1000 µL redistilled water per vial
Calibrator 0 Color coded yellow	1 vial	1000 µL	Ready for Use
Sample Buffer Color coded yellow	1 vial	12 mL	Ready for Use
Assay Buffer Color coded red	1 vial	12 mL	Ready for Use
Enzyme Conjugate 11X Peroxidase conjugated mouse monoclonal anti-MPO	1 vial	1.3 mL	Preparation, see below
Enzyme Conjugate Buffer Color coded blue.	1 vial	13 mL	Ready for use
Wash Buffer 21X Storage after dilution: 2–8°C for 2 months	1 bottle	50 mL	Dilute with 1000 mL redistilled water to make wash buffer 1X solution.
Substrate TMB Colorless solution *Note! Light sensitive!*	1 bottle	22 mL	Ready for Use
Stop Solution 0.5 M H_2SO_4	1 vial	7 mL	Ready for Use

Preparation of enzyme conjugate 1X solution

Prepare the needed volume of enzyme conjugate 1X solution by dilution of Enzyme Conjugate 11X (1+10) in Enzyme Conjugate Buffer or according to the table below. Mix gently. When preparing enzyme conjugate 1X solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial.

Number of strips	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips	1 vial	1 vial
8 strips	700 µL	7.0 mL
4 strips	350 µL	3.5 mL

Storage after dilution: 2–8°C for 2 months.

Specimen collection and handling

An important consideration in preanalytical handling conditions is the prevention of artificial release of MPO from neutrophils in the samples, which may lead to falsely increased results. This is especially important as MPO has shown potential as a marker for cardiovascular disease where reports have linked increased concentrations of circulating MPO with increased risk for coronary disease, increased risk in patients with acute coronary syndrome and clinical utility in identification and prognosis of heart failure patients. Higher MPO concentrations in heparin-plasma, citrate-plasma and serum can be explained by in vitro release of MPO from neutrophils, and that the increase is time dependent in room temperature after collection.

EDTA-plasma is recommended for MPO measurement because values are not confounded by poorly controllable *ex vivo* release of MPO from neutrophils and can therefore more accurately reflect the concentration of MPO in circulation.

EDTA-plasma

EDTA-plasma is the recommended sample type for MPO determintaions. Collect blood by venipuncture into tubes containing EDTA as anticoagulant and separate the plasma fraction by centrifugation.

Serum, heparin-plasma and citrate-plasma

Serum, heparin-plasma and citrate-plasma may be used in the assay. Serum or the presence of heparin or citrate anticoagulant will not effect the measurement of the analyte itself. However, when evaluating the results possible effects from preanalytical handling must be considered.

Storage

EDTA-plasma blood samples can be kept for 1 hour in room temperature before centrifuged at 1500g for 10 min. Separated plasma is either analyzed directly or stored frozen below -20°C until analysis.

Dilution of Samples

Normally, samples should be diluted 1:5 (50 µL sample + 200 µL Sample Buffer) before analysis.

Test procedure

All reagents and samples must be brought to room temperature before use. Prepare a calibrator curve for each assay run. The product has been optimized and validated without plate sealer.

1. Reconstitute Calibrator 1-5 with 1000 µL redistilled water to each vial.
2. Prepare enzyme conjugate 1X solution, wash buffer 1X solution and samples.
3. Prepare sufficient microplate wells to accommodate Calibrators, controls and samples in duplicate.
4. Pipette 25 µL each of Calibrators, controls and samples into appropriate wells.
5. Add 100 µL Assay Buffer to each well.
6. Incubate on a plate shaker (700-900 rpm) for 1 hour at room temperature (18-25°C).
7. Wash 6 times with 700 µL wash buffer 1X solution per well using an automatic plate washer with overflow- wash function, after final wash, invert and tap the plate firmly against absorbent paper. Do not include soak step in washing procedure.
   Or manually:
   Discard the reaction volume by inverting the microplate over a sink.
   Add 350 µL wash buffer 1X solution to each well. Discard the wash solution, tap firmly several times against absorbent paper to remove excess liquid.
   Repeat 5 times. Avoid prolonged soaking during washing procedure.
8. Add 100 µL enzyme conjugate 1X solution to each well.
9. Incubate on a plate shaker (700-900 rpm) for 1 hour at room temperature (18-25°C).
10. Wash as described in step 7.
11. Add 200 µL Substrate TMB to each well.
12. Incubate on the bench for 15 minutes at room temperature (18-25°C).
13. Add 50 µL Stop Solution to each well.
    Place plate on a shaker for approximately 5 seconds to ensure mixing.
14. Read optical density at 450 nm and calculate results.
    Read within 30 minutes.

Note! Be extra careful not to contaminate the Substrate TMB with enzyme conjugate solution.

Internal quality control

Internal serum pools with low, intermediate and high MPO concentrations should routinely be assayed as samples, and results charted from day to day. It is good laboratory practice to record the following data for each assay: kit lot number; dilution and/or reconstitution dates of kit components, OD values for the Calibrators and controls.

Laboratories should follow government regulations or accreditation requirements for quality control frequency.

Calculation of results

Note! The Calibrator 0 (negative control) should not be used in the calibrator curve calculation.

The concentration of MPO in unknown samples should if possible be calculated using computerized data reduction. Use the obtained absorbances versus the known concentrations of Calibrators 1-5 and cubic spline regression analysis to construct the calibrator curve. Multiply the calculated MPO concentrations with the dilution factor (e.g. x5).

Example of results

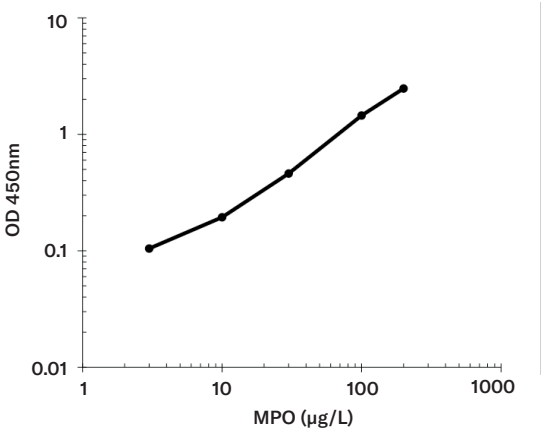
Wells	Identity	$A_{450\ nm}$	Conc. µg/L**
1A–B	Calibrator 0	0.066/0.062	
1C–D	Calibrator 1*	0.101/0.108	
1E–F	Calibrator 2*	0.195/0.193	
1G–H	Calibrator 3*	0.453/0.468	
2A–B	Calibrator 4*	1.479/1.422	
2C–D	Calibrator 5*	2.471/2.476	
2E–F	Sample 1	0.265/0.260	76
2G–H	Sample 2	0.817/0.821	272
3A–B	Sample 3	1.582/1.591	548

*Concentration stated on vial label

**Result multiplied by dilution factor (x5)

Example of calibrator curve

A typical calibrator curve is shown here. Do not use this curve to determine actual assay results.



Limitations of the procedure

As with all diagnostic tests, a definitive diagnosis should not be based on the results of a single test, but should be made by the physician after all clinical findings have been evaluated.

Grossly lipemic, icteric or hemolyzed samples do not interfere in the assay.

Expected values

Good practice dictates that each laboratory establishes its own expected range of values.

Performance characteristics

Detection limit

Detection limit is defined as the Capability of Detection according to ISO11843-Part 1. Capability of Detection should be seen as part of a method validation, rather than the lowest concentration that can be measured.

The detection limit is lower than the concentration of Calibrator 1 determined with the methodology described in ISO11843-Part 4. The concentration for samples with absorbance below Calibrator 1 should not be calculated, but instead expressed as less than or equal to (≤) the concentration indicated on the vial for Calibrator 1.

Hook effect

Samples with concentrations of up to 30 000 µg/L have been tested without giving falsely low results.

Recovery

Recovery upon addition is 85%-96% (mean 89%).
Recovery upon dilution is 97%-108% (mean 101%).

Precision

Each sample was analysed in 4 replicates on 33 different occasions

		Coefficient of variation	
Sample	Mean value µg/L	Repeatability %*	Within laboratory %**
1	11.58	4.4	9.9
2	34.93	3.0	8.6
3	119.21	3.1	5.5

*Within assay variation
**Total assay variation

Specificity

The following cross reactions have been found:

	Crossreaction
TPO	≤ 0.01%
CRP	≤ 0.01%
EPO	3.53%
Lysosyme	0.03%
Elastase	0.12%
alpha-1-antitrypsin	≤ 0.01%

Calibration

Mercodia MPO ELISA kit is calibrated against a highly purified, fully validated, commercial MPO preparation.

Warranty

The performance data presented here was obtained using the procedure indicated. Any change or modification in the procedure not recommended by Mercodia AB may affect the results, in which event Mercodia AB disclaims all warranties expressed, implied or statutory, including the implied warranty of merchantability and fitness for use.

Mercodia AB and its authorised distributors, in such event, shall not be liable for damages indirect of consequential.

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Further references can be found on our website: **www.mercodia.com**

Notes

Notes

Notes

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Experiment: **Date:** **KIT LOT#:**

Summary of protocol sheet
Mercodia MPO ELISA

Add Calibrators, controls* and samples	25 µL
Add Assay Buffer	100 µL
Incubate	1 hour at 18–25°C on a plate shaker (700–900 rpm)
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add enzyme conjugate 1X solution	100 µL
Incubate	1 hour at 18–25°C on a plate shaker (700–900 rpm)
Wash plate with wash buffer 1X solution	700 µL, 6 times
Add Substrate TMB	200 µL
Incubate	15 minutes at 18–25 °C
Add Stop Solution	50 µL Shake for 5 seconds to ensure mixing
Measure $A_{450\ nm}$	Evaluate results

*not included

For full details see page 7

For technical support please contact: support@mercodia.com

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