

Merodia

MPO ELISA

Directions for Use

10-1176-01
REAGENTS FOR 96 DETERMINATIONS





For Research Use Only
Not for Use in Diagnostic Procedures

Manufactured by

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Merodia 

EXPLANATION OF SYMBOLS USED ON LABELS

 $\Sigma = 96$	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 serum and plasma.

SUMMARY AND EXPLANATION OF THE TEST

Myeloperoxidase (MPO), an iron containing glycoprotein, is a covalently bounded 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 oxidant 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 myocardial 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 well. After washing, peroxidase conjugated anti-MPO antibodies are added and after the second incubation and a simple washing step that removes unbounded enzyme labelled 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.
- The content of this kit and their residues must not be allowed to come into contact with ruminating animals or swine.
- The Stop Solution in this kit contains 0.5 M H₂SO₄. Follow routine precautions for handling hazardous chemicals.
- All patient samples should be handled as capable of transmitting infections.

MATERIALS REQUIRED BUT NOT PROVIDED

- Pipettes for 25, 50, 100, 200, 250 and 500 μ l (repeat pipettes preferred for addition of enzyme conjugate solution, Substrate TMB and Stop Solution)
- Beakers and cylinders for reagent preparation
- Redistilled water
- Microplate reader (450 nm filter)
- Plate shaker (The recommended velocity is 700-900 cycles per minute, orbital movement)
- Microplate washing device

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)	1 plate	96 wells (8-well strips)	Ready for use
For unused microtitration wells, completely reseal the bag using adhesive tape and use within two months.			
Calibrators	5 vials	1 ml	Ready for use
Concentration indicated on vial label.			
Sample Buffer	1 vial	12 ml	Ready for use
Color coded yellow			
Assay Buffer	1 vial	12 ml	Ready for use
Color coded red			
Enzyme Conjugate 11X	1 vial	1.2 ml	Preparation, see below
(Peroxidase conjugated mouse monoclonal anti-MPO). <i>Note! Light sensitive!</i>			
Enzyme Conjugate Buffer	1 vial	12 ml	Ready for use
Color coded blue			
Wash Buffer 21X	1 bottle	40 ml	Dilute with 800 ml redistilled water to make wash buffer.
Storage after dilution: +2-8°C for 4 weeks.			
Substrate TMB	1 vial	22 ml	Ready for use
(TMB) Colorless solution. <i>Note! Light sensitive!</i>			
Stop Solution	1 vial	7 ml	Ready for use
0.5 M H ₂ SO ₄			

Preparation of enzyme conjugate solution

Prepare the needed volume of enzyme conjugate 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 solution for the whole plate, pour all of the Enzyme Conjugate Buffer into the Enzyme Conjugate 11X vial.

Number of strips	Callibrator Curve	Samples in duplicate	Enzyme Conjugate 11X	Enzyme Conjugate Buffer
12 strips (one plate)	1	42	1 vial	1 vial
8 strips	1	26	700 µl	7.0 ml
6 strips	1	18	500 µl	5.0 ml
4 strips	1	10	350 µl	3.5 ml

Storage after dilution: +2-8°C for four weeks.

SPECIMEN COLLECTION AND HANDLING

Serum

Collect blood by venipuncture, allow clotting for 60-120 minutes in room temperature (18-25°C), and separate the serum by centrifugation at 1000-1300 x g for 10 minutes in room temperature.

Note! Haemolysed serum cannot be used.

Plasma

Collect blood by venipuncture into tubes containing EDTA as anticoagulant and separate the plasma fraction.

Serum and plasma samples can be stored at 2-8°C up to 24 hours. For longer periods, store samples at -20°C. Avoid repeated freezing and thawing.

DILUTION OF SAMPLES

Samples should be diluted 1/11 v/v with Sample Buffer.
(25 µl sample + 250 µl Sample Buffer)

TEST PROCEDURE

All reagents and samples must be brought to room temperature before use. Prepare a calibrator curve for each assay run.

1. Prepare enzyme conjugate solution, wash buffer and samples.
2. Prepare sufficient microplate wells to accommodate Calibrators and samples in duplicate.
3. Pipette 25 μ l each of Sample Buffer, Calibrators and samples into appropriate wells.
4. Add 100 μ l Assay Buffer to each well.
5. Incubate on a plate shaker for 1 hour at room temperature (18-25°C).
6. Wash plate 6 times with automatic plate washer or:
Aspirate the reaction volume and fill each well completely with 350 μ l wash buffer.
Discard liquid completely. Repeat 5 times.
After final wash, invert and tap the plate firmly against absorbent paper.
7. Add 100 μ l enzyme conjugate solution to each well.
8. Incubate on a plate shaker for 1 hour at room temperature (18-25°C).
9. Wash as described above
After final wash, invert and tap the plate firmly against absorbent paper.
10. Add 200 μ l Substrate TMB.
11. Incubate for 15 minutes at room temperature (18-25°C).
12. Add 50 μ l Stop Solution.
Place plate on a shaker for approximately 5 seconds to ensure mixing.
13. Read optical density at 450 nm and calculate results.

INTERNAL QUALITY CONTROL

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

CALCULATION OF RESULTS

Note! Sample Buffer is used as a blank and is not used in calibrator curve calculation.

Computerised calculation

The concentration of MPO is obtained by computerized data reduction of the absorbance for Calibrators 1-5, versus the concentration using cubic spline regression.

Manual calculation

1. Plot the absorbance values obtained for the Calibrators 1-5, against the MPO concentration on a log-log paper and construct a calibrator curve.
2. Read the concentration of the unknown samples from the calibrator curve.
3. Multiply the concentration of the unknown samples with dilution factor (e.g. x11)

Example of worksheet

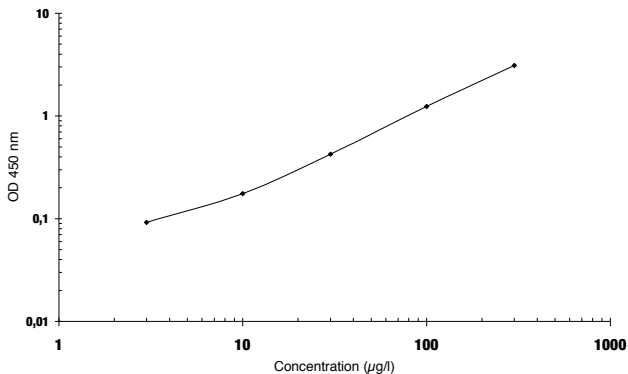
Wells	Identity	A ₄₅₀	Mean conc. µg/l*
1A-B	Sample Buffer	0.066/0.070	
1C-D	Calibrator 1 (3 µg/l)**	0.095/0.089	
1E-F	Calibrator 2 (10 µg/l)**	0.159/0.169	
1G-H	Calibrator 3 (30 µg/l)**	0.347/0.368	
2A-B	Calibrator 4 (100 µg/l)**	1.048/1.102	
2C-D	Calibrator 5 (300 µg/l)**	2.490/2.635	
2E-F	Unknown 1	0.181/0.184	131
2G-H	Unknown 2	0.403/0.443	390
3A-B	Unknown 3	1.211/1.233	1294

* Result multiplied by dilution factor (x11)

** Exact concentration indicated on vial label

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 or icteric 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

The detection limit is $\leq 3 \mu\text{g/l}$.

Recovery

Recovery upon addition is 84 – 98% (mean 89%)

Recovery upon dilution is 96-114% (mean 101%)

Precision

Each sample was analysed in 4-replicates on 33 different occasions

Sample	Mean value ($\mu\text{g/l}$)	within assay %	Coefficient of variation	
			between assay %	total assay %
1	11.58	4.4	9.7	9.9
2	34.93	3.0	8.5	8.6
3	119.21	3.1	5.3	5.5

Specificity

The following crossreactions have been found:

TPO	$\leq 0.01\%$
CRP	$\leq 0.01\%$
EPO	3.53%
Lysosym	0.03%
Elastas	0.12%
alfa 1-antitrypsin	$\leq 0.01\%$

CALIBRATION

MPO ELISA kit is calibrated against a highly purified, fully validated, commercial MPO preparation. The concentration of myeloperoxidase is expressed in µg/l.

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.

REFERENCES

- Baldus S. *et al.* Myeloperoxidase Serum Levels Predict Risk in Patients With Acute Coronary Syndromes. *Circulation* 2003, 108:1440-1445
- Brennan M-L. *et al.* Prognostic value of Myeloperoxidase in Patients with Chest Pain. *The New England Journal of Medicine* 2003, 349:1595-1604
- Carr A C. *et al.* Oxidation of LDL by Myeloperoxidase and Reactive Nitrogen Species. *Arterioscler Thromb Vasc Biol* 2000, 20:1716-1723
- Nambi V. The Use of Myeloperoxidase As a Risk marker for Atherosclerosis. *Current Atherosclerosis Reports* 2005, 7:127-131.
- Hazen S L. *et al.* Formation of Nitric Oxide-Derived Oxidants by Myeloperoxidase in Monocytes. *Circ. Res.* 1999, 85:950-958.
- Zhang R. *et al.* Association between myeloperoxidase levels and risk of coronary artery disease. *JAMA.* 2001, 286:2136-2142.

SUMMARY OF PROTOCOL SHEET

Add Sample Buffer, Calibrators, and samples	25 μ l
Add Assay Buffer	100 μ l
Incubate	1 hour at 18-25°C on a plate shaker
Wash plate with wash buffer	6 times
Add enzyme conjugate solution	100 μ l
Incubate	1 hour at 18-25°C on a plate shaker
Wash plate with wash buffer	6 times
Add Substrate TMB	200 μ l
Incubate	15 minutes
Add Stop Solution	50 μ l Shake for 5 seconds to ensure mixing
Measure A ₄₅₀	Evaluate results