FOR INFORMATIONAL USE ONLY ▪ DO NOT USE FOR PERFORMING ASSAY ▪ REFER TO MOST CURRENT PACKAGE INSERT ACCOMPANYING TEST KIT

MYELOPEROXIDASE (MPO) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: BC-1129

BioCheck, Inc
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Enzyme Immunoassay for the Quantitative Determination of Myeloperoxidase Concentration in Human Serum and Heparin-Plasma

FOR RESEARCH USE ONLY

Store at 2 to 8°C.

PROPRIETARY AND COMMON NAMES

BioCheck Myeloperoxidase Enzyme Immunoassay (MPO ELISA)

INTENDED USE

The MPO ELISA is intended for the quantitative determination of Myeloperoxidase (MPO) in human serum and heparin-plasma.

SUMMARY AND EXPLANATION OF TEST

Myeloperoxidase (MPO) plays an important role in host defense systems. This 140 kDa protein, composed of two heavy chains of 53kDa and two light chains of 15 kDa, was first discovered in the 1960s (1, 2). The release of MPO from the granules of neutrophils and monocytes in response to the activation of leukocytes allows the conversion of hydrogen peroxide and chloride ions into hypochlorous acid (HOCl), a strong oxidizing agent (1, 3). Although MPO serves an important purpose in the defense system, various studies show that MPO plays a role in several inflammatory conditions (1, 3, 4, and 5).

An elevated MPO level has been linked to coronary artery diseases. In atherosclerosis, the hardening of artery walls, high concentrations of MPO and its oxidation products can be found in areas of atheroma (4). MPO has also been linked to vulnerable plaque and endothelial dysfunction. Nitric oxide (NO) signals blood vessels to relax and dilate, but MPO's use of NO as a substrate can lead to atherosclerosis and endothelial dysfunction (4). MPO also affects cholesterol levels as well through the oxidation of LDL and HDL (4).

Other studies show that an elevated MPO level in serum and plasma indicate a higher risk for cardiovascular events in patients hospitalized for chest pain (3, 5). Patients with low CRP and TnT levels but a high MPO level are at greater cardiac risk than patients with low CRP, TnT and MPO levels (5). In the study by Baldus, results indicate that an elevated MPO level occurs before myocardial injury and the increased MPO level indicates unstable plaque formations, therefore, making MPO level a good predictor of potential negative cardiac events (5).

PRINCIPLE OF THE ASSAY

The MPO ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay (6). The assay system utilizes a unique monoclonal antibody directed against a distinct antigenic determinant on the MPO molecule. This mouse monoclonal anti-MPO antibody is used for solid phase immobilization (on the microtiter wells). Another mouse monoclonal anti-MPO antibody conjugated to horseradish peroxidase (HRP) is in the enzyme conjugate solution. The test samples are allowed to react sequentially with these two antibodies, resulting in the MPO molecules to be sandwiched between the solid phase and enzyme-linked antibodies. After two separate 90- minute incubations steps at room temperature with shaking, the wells are rinsed with Wash Buffer to remove unbound labeled antibodies. TMB Reagent is added and incubated for 20 minutes with shaking, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of MPO is directly proportional to the color intensity of the test samples. Absorbance is measured spectrophotometrically at 450 nm.

REAGENTS AND MATERIALS PROVIDED

1. *Antibody-Coated Wells (1 plate, 96 wells)*
   Microtiter wells coated with mouse monoclonal anti-MPO
2. *MPO Standard Stock, 40 ng/ml (lyophilized, 4 vials)*
   40 ng/ml MPO in bovine serum with preservatives.
   Reconstitution volume stated on the labels.
   One time use only; DO NOT REUSE after reconstitution.
3. *Standard Diluent (13 ml/vial, 1 vial)*
   For Standard Dilution Use ONLY
   Bovine Serum with preservatives
4. *Sample Diluent (50 ml/bottle, 1 bottle)*
   For Sample Dilution Use ONLY
   Contains phosphate buffer-BSA solution with preservatives
5. *Enzyme Conjugate Concentrate 25X (0.6 ml/vial, 1 vial)*
   Contains mouse monoclonal anti-MPO conjugated to horseradish peroxidase
6. *Enzyme Conjugate Diluent (13 ml/vial)*
   For Enzyme Conjugate Concentrate Dilution ONLY
   Tris Buffer with Preservatives
7. *20X Wash Buffer (50 ml/bottle, 1 bottle)*
   Phosphate buffer with detergents
8. *TMB Reagent (one-step) (11 ml/vial, 1 vial)*
   Contains one-step TMB solution
9. *Stop Solution 1N HCl (11ml/vial, 1 vial)*
   Contains diluted hydrochloric acid

MATERIALS REQUIRED BUT NOT PROVIDED

1. Distilled or deionized water
2. Precision pipettes: 5 µl, 10 µl, 100 µl and 1.0 ml
3. Disposable pipette tips

4. Microtiter well reader capable of reading absorbance at 450 nm.
5. EIA plate shaker capable of shaking microplates at 750 rpm
6. Vortex mixer, or equivalent
7. Absorbent paper
8. Graph paper

WARNINGS AND PRECAUTIONS

1. CAUTION: This kit contains human material. The source material used for manufacture of this component tested negative for HBsAg, HIV 1/2 and HCV by FDA-approved methods. However, no method can completely assure absence of these agents. Therefore, all human blood products, including serum and plasma samples, should be considered potentially infectious. Handling should be as defined by an appropriate national biohazard safety guideline or regulation, where it exists (7,8).
2. Avoid contact with 1N HCl. It may cause skin irritation and burns. If contact occurs, wash with copious amounts of water and seek medical attention if irritation persists.
3. Do not use reagents after expiration date and do not mix or use components from kits with different lot numbers.
4. Replace caps on reagents immediately. Do not switch caps.
5. Do not pipette reagents by mouth.
6. This test kit is for research use only.

STORAGE CONDITIONS

1. Store the unopened kit at 2-8°C upon receipt and when it is not in use, until the expiration shown on the kit label. Refer to the package label for the expiration date.
2. Keep microtiter plate in a sealed bag with desiccant to minimize exposure to damp air.

SPECIMEN COLLECTION AND PREPARATION

1. **Heparin Plasma Samples**: Whole blood should be collected in sodium heparin tubes using standard venipuncture techniques. Invert tube several times to adequately mix the blood with the anticoagulant. Blood tubes should be stored at room temperature for at least 2 hours, but no more than 5 hours before centrifuging samples at 2,500 rpm for 20 minutes at 4°C. Remove plasma supernatant and store at 2 - 8°C for up to 48 hours. Store at -20°C or below, for long term storage.
2. **Serum Samples**: Whole blood should be collected using standard venipuncture techniques. Invert tube several times to adequately mix the blood. Blood tubes should be stored at room temperature for at least 2 hours, but no more than 5 hours before centrifuging samples at 2,500 rpm for 20 minutes at 4°C. Remove serum supernatant and store at 2 - 8°C for up to 48 hours. Store at -20°C or below, for long term storage.
3. Avoid grossly hemolytic (bright red) samples (after centrifugation). Hemolyzed samples will give inaccurate results.
4. Specimens should not be repeatedly frozen and thawed prior to testing. DO NOT store in "frost free" freezers, which may cause occasional thawing. Specimens which have been frozen, and those which are turbid and/or contain particulate matter, must be centrifuged prior to use.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. **Reconstitution of MPO Standard Stock:**
   For each test run, reconstitute one vial of 40 ng/ml lyophilized Standard Stock with **the volume of dH_2O stated on the vial**. Allow the reconstituted material to stand at room temperature for about 20 minutes and mix gently.
3. **Preparation of MPO Standard Set (6 Standards):**

   Please use **Standard Diluent** for MPO standard preparation as follows:

Standard Concentration	Volume of MPO Standard	Volume of Standard Diluent
40 ng/ml	0.5 ml of 40 ng/ml	0 ml
20 ng/ml	0.5 ml of 40 ng/ml	0.5 ml
10 ng/ml	0.5 ml of 20 ng/ml	0.5 ml
5.0 ng/ml	0.5 ml of 10 ng/ml	0.5 ml
2.5 ng/ml	0.5 ml of 5.0 ng/ml	0.5 ml
0 ng/ml	0 ml	0.5 ml

***Reconstituted and diluted MPO standards should be discarded after use.** For future experiments, reconstitute a new vial of standard stock. This MPO kit can be used for up to a maximum of 4 separate test runs.

4. Patient serum and heparin-plasma should be diluted **100 fold** prior to use. Prepare a series of small tubes (i.e., 1.5 ml microcentrifuge tubes) and mix 5 µl of serum or heparin-plasma with 495 µl (0.495 ml) Sample Diluent.
5. Samples with expected MPO concentrations over 4,000 ng/ml may be quantitated by further dilution (10 fold) of the 100-fold diluted solution with sample diluent (i.e., 30 µl of the 100-fold diluted sample to 270 µl sample diluent).
6. **Working Conjugate Regent**: To prepare Working MPO Conjugate Reagent, dilute the Enzyme Conjugate Concentrate (25X) with the Enzyme Conjugate Diluent.

# of Strips	Volume of Conjugate Diluent	Volume of Conjugate Concentrate (25X)
4	3.84 ml	0.16 ml
6	5.76 ml	0.24 ml
8	7.68 ml	0.32 ml
10	9.60 ml	0.40 ml
12	11.52 ml	0.48 ml

***Do not reuse the Working Enzyme Conjugate Reagent. Make fresh dilution before each assay.**

7. **Working Wash Buffer**: Preparation of 1X Wash Buffer from 20X Stock. Add 50 ml of 20X Wash Buffer Stock to 950 ml of DI H_2O. The Working Wash Buffer is stable at 2-8°C for 30 days. NOTE: Any crystals that may be present due to high salt concentration must be redissolved at room temperature before making the dilution.

INSTRUMENTATION

A microtiter well reader with a bandwidth of 10 nm or less and an optical density range of 0 to 3 OD or greater at 450 nm wavelength is acceptable for absorbance measurement.

PROCEDURAL NOTES

1. Pipetting Recommendations (single and multi-channel): Pipetting of all standards, samples, and controls should be completed within 15 minutes.
2. All standards, samples, and controls should be run in duplicate concurrently so that all conditions of testing are the same.
3. It is recommended that the wells be read within 15 minutes following addition of Stop Solution.

ASSAY PROCEDURE

1. ***Reconstitute standard and serially dilute. See Reagent Preparation.***
2. ***Samples should be diluted 100-fold prior to use. See Reagent Preparation.***
3. Secure the desired number of coated wells in the holder.
4. Dispense 100 µl of MPO standards, and **DILUTED** specimens into the appropriate wells.
5. Incubate for 90 minutes at room temperature (18-25 °C) on an orbital shaker set at about 750 rpm.
6. Remove incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with 300 ul Working Wash Buffer. Strike the wells onto absorbent paper or paper towels to remove all residual water droplets.
7. Dispense 100 ul of MPO Enzyme Conjugate Reagent into each well.
8. Incubate for 90 minutes at room temperature (18-25 °C) on an orbital shaker set at about 750 rpm.
9. Remove incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with 300 ul Working Wash Buffer. Strike the wells onto absorbent paper or paper towels to remove all residual water droplets.
10. Dispense 100 µl TMB solution into each well.
11. Incubate for 20 minutes at room temperature (18-25 °C) on an orbital shaker set at about 750 rpm.
12. Stop the reaction by adding 100 µl of Stop Solution into each well.
13. Gently mix for 30 seconds. ***It is important to make sure that all the blue color changes to yellow color completely.***
14. Read absorbance at 450 nm with a microtiter well reader ***within 15 minutes.***

QUALITY CONTROL

Good laboratory practice requires that quality control specimens (controls) be run with each calibration curve to verify assay performance. To ensure proper performance, control material should be assayed repeatedly to establish mean values and acceptable ranges.

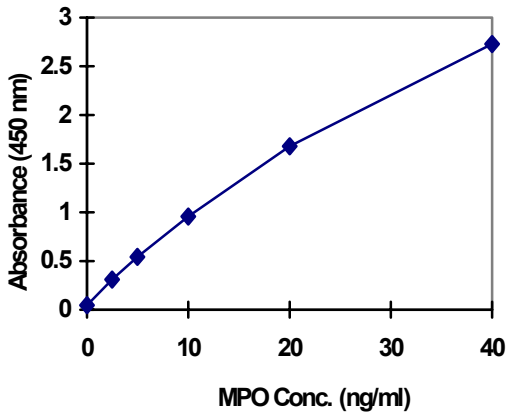
CALCULATION OF RESULTS

1. Calculate the mean absorbance value (OD_{450}) for each set of reference standards, controls and samples.
2. Construct a standard curve by plotting the mean absorbance obtained for each reference standard against its concentration in ng/ml on graph paper, with absorbance on the vertical (y) axis and concentration on the horizontal (x) axis.
3. The corresponding concentration of MPO (ng/ml) can be determined from the standard curve using the mean absorbance value for each sample. Depending on experience and/or the availability of computer capability, other methods of data reduction may be employed.
4. The obtained values of serum and heparin-plasma patient samples should be multiplied by the dilution factor of 100 to obtain MPO results in ng/ml.
5. Samples with MPO concentrations greater than 4,000 ng/ml should be further diluted 10-fold after the initial 100-fold dilution (total dilution 1:1,000), and the final MPO values should be multiplied by 1,000 to obtain MPO results in ng/ml.
6. NOTE: Patient serum or heparin-plasma samples with MPO concentrations less than 0.25 ng/ml before multiplying by dilution factor (1:100) should be reported as "< 25 ng/ml MPO."

EXAMPLE OF STANDARD CURVE

Results of a typical standard curve run with absorbency readings at 450 nm are shown on the Y axis against MPO concentrations shown on the X axis. **NOTE:** This standard curve is for the purpose of illustration only, and should not be used to calculate unknowns. Each laboratory must generate its own data and standard curve in each experiment.

MPO (ng/ml)	Absorbance (450 nm)
0	0.046
2.5	0.309
5.0	0.544
10.0	0.958
20.0	1.678
40.0	2.727



LIMITATIONS OF THE PROCEDURE

1. Serum or plasma samples demonstrating gross hemolysis should not be used with this test. Hemolyzed samples will give inaccurate results.
2. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the package insert instructions and with adherence to good laboratory practice.
3. The results obtained from the use of this kit should be used only as an adjunct to other diagnostic procedures and information available to the physician.
4. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
5. Patient samples may contain human anti-mouse antibodies (HAMA) that are capable of giving falsely elevated or depressed results with assays that utilize mouse monoclonal antibodies. This assay has been designed to minimize interference from HAMA-containing specimens. Nevertheless, complete elimination of this interference from all patient specimens cannot be guaranteed. A test result that is inconsistent with the clinical picture and patient history should be interpreted with caution.

EXPECTED VALUES

It is recommended that each laboratory establishes its own normal range based on the patient population. The MPO ranges obtained from apparently normal human samples in BioCheck MPO EIA and for a commercially available MPO EIA are as follows:

MPO Sample	Normal Range of BioCheck MPO EIA (ng/ml)	Normal Range of A Commercially Available MPO EIA (ng/ml)
1. Serum	63 to 320	89 to 322
2. Heparin-Plasma	37 to 124	25 to 63

PERFORMANCE CHARACTERISTICS

1. Minimum Detectable Sensitivity

The minimum detectable concentration of the MPO ELISA assay as measured by 2SD from the mean of a zero standard is estimated to be 0.25 ng/ml.

BioCheck's MPO ELISA test has a minimum detectablilty of 25 ng/ml after multiplying by the dilution factor of 1:100.

2. Precision

a. Intra-Assay Precision

Within-run precision was determined by 20 replicate determinations of four different serum samples in one assay. Within-assay variability is shown below:

Serum Sample	1	2	3	4
# Replicates	20	20	20	20
Mean MPO (ng/ml)	63	307	952	13,603
S.D.	3.11	5.81	21.37	225
C.V. (%)	4.9%	1.9%	2.2%	1.7%

b. Inter-Assay Precision

Between-run precision was determined by 5 replicate measurements of four different serum samples over a series of individually calibrated assays. Between-assay variability is shown below:

Serum Sample	1	2	3	4
# Replicates	20	20	20	20
Mean MPO (ng/ml)	63	520	1,057	12,083
S.D.	7.31	23.16	52.11	446
C.V. (%)	11.7%	4.5%	4.9%	3.4%

PERFORMANCE CHARACTERISTICS

3. Whole Plate Uniformity

Three levels of MPO concentration, one level for each plate, were pipetted into each well of a 96-well plate. The time to load the plate from the first well to the last well was 15 minutes.

MPO Conc. (ng/ml)	Ave. A_{450}	Std. Dev.	%CV
2	0.251	0.006	2.5%
20	1.614	0.031	1.9%
40	2.529	0.045	1.8%

4. Linearity and Recovery Studies

a. Linearity

5 patient serum samples were serially diluted to determine linearity. The mean recovery was 109.0%.

#	Dilution	Expected Conc. (ng/ml)	Observed Conc. (ng/ml)	% Expected
1	1:100	190	190	--
	1:200		194	102.1%
	1:400		202	106.3%
	1:800		216	113.7%
				Mean = 107.4%
2	1:100	613	613	--
	1:200		598	97.6%
	1:400		586	95.6%
	1:800		561	91.5%
				Mean = 94.9%
3	1:100	702	702	--
	1:200		734	104.6%
	1:400		766	109.1%
	1:800		768	109.4%
				Mean = 107.7%
4	1:100	1,016	1,016	--
	1:200		1,085	107.8%
	1:400		1,150	113.2%
	1:800		1,147	112.9%
				Mean = 111.3%
5.	1:800	18,266	18,266	--
	1:1,600		20,413	111.8%
	1:3,200		22,348	122.3%
	1:6,400		25,159	137.7%
				Mean = 123.9%

b. Recovery

Various patient serum samples of known MPO levels were combined and assayed in duplicate. The mean recovery was 101.9%.

PAIR NUMBER	EXPECTED [MPO] (ng/ml)	OBSERVED [MPO] (ng/ml)	% RECOVERY
1	16,318	15,931	97.6%
2	1,130	1,203	106.4%
3	946	1,034	109.3%
4	587	590	100.4%
5	346	335	96.7%
6	131	132	101.0%

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