



MPO ELISA

Catalog Number EA-0306

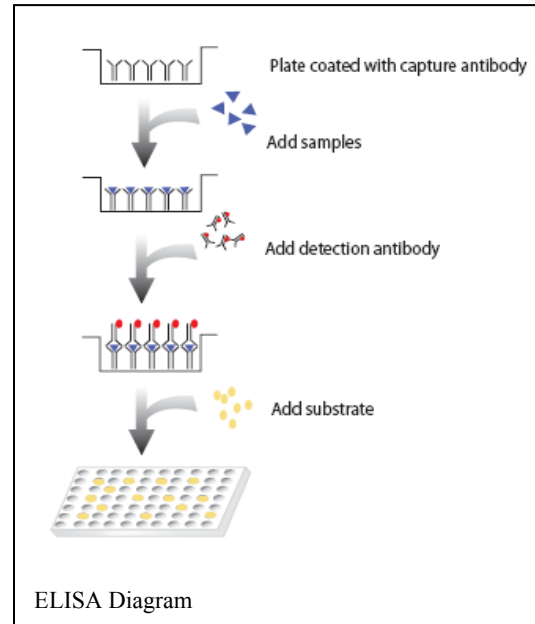
(For Research Use Only)

Introduction

Myeloperoxidase (MPO), released from the granules of neutrophils and monocytes in response to the activation of leukocytes, plays an important role in host defense systems and in several inflammatory conditions (1, 2-4). Inflammation is linked to adverse outcomes in acute coronary syndromes. Myeloperoxidase, an abundant leukocyte enzyme, is elevated in culprit lesions that have fissured or ruptured in patients with sudden death from cardiac causes. 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 (3). MPO has also been linked to vulnerable plaque and endothelial dysfunction. An elevated MPO level in serum and plasma indicate a higher risk for cardiovascular events in patients hospitalized for chest pain (2,4). 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 (4). 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 (4).

Principle of the assay

The MPO ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay utilizes a mouse monoclonal antibody against distinct determinants on MPO for immobilization on the microtiter wells and another mouse monoclonal anti-MPO antibody conjugated to horseradish peroxidase (HRP) for detection. The test sample is allowed to react simultaneously with these antibodies, resulting in MPO being sandwiched between the solid phase and enzyme-linked antibodies. After incubation, the wells are washed to remove unbound-labeled antibodies. A HRP substrate, TMB, is added to result in the development of a blue color. The color development is then 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 sample. Absorbance is measured spectrophotometrically at 450 nm.



Materials provided with the kit

- Antibody-coated microtiter plate with 96 wells
- 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.
- Standard Diluent (13 ml/vial, 1 vial) For Standard Dilution Use ONLY. Bovine Serum with preservatives
- Sample Diluent (50 ml/bottle, 1 bottle) For Sample Dilution Use ONLY. Contains phosphate buffer-BSA solution with preservatives
- Enzyme Conjugate Concentrate 25X (0.6 ml/vial, 1 vial) Contains mouse monoclonal anti-MPO conjugated to horseradish peroxidase
- Enzyme Conjugate Reagent, 18 ml.
- TMB Reagent (One-Step), 11 ml.
- Stop Solution (1N HCl), 11 ml.

Material required but not provided

- Microplate reader capable of measuring absorbance at 450 nm
- Deionized or distilled water.

Warning 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 samples, should be considered potentially infectious. It is recommended that the reagents and patient samples be handled according to the OSHA Standard on Bloodborne Pathogens (5) or other appropriate national biohazard safety guidelines or regulations (6-8).

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.

Reagent preparation

All reagents should be allowed to reach room temperature (18-25°C) before use.

Reconstitute each lyophilized standard with 1.0 ml distilled water. Allow the reconstituted material to stand for at least 20 minutes and mix gently. The Reconstituted standards will be stable for up to 8 hours when stored sealed at 2-8°C.

Discard the reconstituted Standards after 8 hours. To assure maximum stability of the reconstituted Standards, they should be aliquoted and frozen (-20°C or below) immediately after reconstitution has been achieved. Each aliquoted Standard should be frozen and thawed only once.

Assay procedure

1. Reconstitution of MPO Standard Stock: For each test run, reconstitute one vial of 40 ng/ml lyophilized Standard Stock with the volume of dH₂O stated on the vial. Allow the reconstituted material to stand at room temperature for about 20 minutes and mix gently.

2. 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.

3. Patient serum and heparin-plasma should be diluted 100 folds 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.

4. 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).

5. Working Conjugate Reagent: To prepare Working MPO Conjugate Reagent, dilute the Enzyme Conjugate Concentrate (25X) with the Enzyme Conjugate Diluent. Do not reuse the Working Enzyme Conjugate Reagent. Make fresh dilution before each assay.

6. Working Wash Buffer: Preparation of 1X Wash Buffer from 20X Stock. Add 50 ml of 20X Wash Buffer Stock to 950 ml of dH₂O. 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.

7. Samples should be diluted 100 folds prior to use.

8. Add 100µl of MPO standards, and diluted specimens into the appropriate wells.

9. Incubate for 90 minutes at room temperature (18-25°C) on an orbital shaker set at about 750 rpm.

10. Remove incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with 300µ Working Wash Buffer. Strike the wells onto absorbent paper or paper towels to remove all residual water droplets.

11. Add 100µl of MPO Enzyme Conjugate Reagent into each well.

12. Incubate for 90 minutes at room temperature (18-25°C) on an orbital shaker set at about 750 rpm.

# 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

13. Remove incubation mixture by flicking plate contents into a waste container. Rinse and flick the microtiter wells 5 times with 300 µl Working Wash Buffer. Strike the wells onto absorbent paper or paper towels to remove all residual water droplets.

14. Add 100µl TMB solution into each well.

15. Incubate for 20 minutes at room temperature (18-25°C) on an orbital shaker set at about 750 rpm.

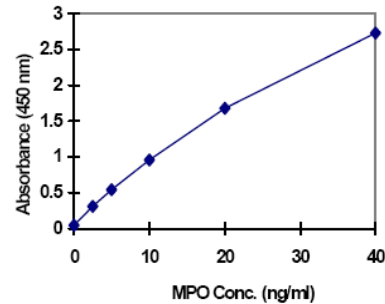
16. Stop the reaction by adding 100µl of Stop Solution into each well.

17. Gently mix for 30 seconds. It is important to make sure that all the blue color changes to yellow color completely.

18. Read absorbance at 450 nm with a microtiter well reader within 15 minutes.

Example of standard curve

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



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