MYELOPEROXIDASE (MPO) ENZYME IMMUNOASSAY TEST KIT
Catalog Number: EA101012

Enzyme Immunoassay for the Quantitative Determination of Myeloperoxidase Concentration in Serum and Plasma

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

PRINCIPLE OF THE ASSAY

The MPO ELISA is based on the principle of a solid phase enzyme-linked immunosorbent assay. 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 monoclonal antibody conjugated to horseradish peroxidase (HRP) is in the enzyme conjugate solution. The test samples are allowed to react sequentially with the 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. A 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. *40 ng/vial MPO Standard /lyophilized, 4 vials)*
   40 ng/vial MPO in bovine serum with preservatives; lyophilized; 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 (11 ml/vial, 1 vial)*
   Contains one-step TMB solution
9. *Stop Solution (11ml/vial, 1 vial))*
   Contains diluted hydrochloric acid (1N HCl)

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.

REAGENT PREPARATION

1. All reagents should be allowed to reach room temperature (18-25°C) before use.
2. For each test run, reconstitute one vial of 40 ng/vial lyophilized standard with volume of dH_20 indicated on label. Allow the reconstituted material to stand for at least 20 minutes and mix gently.
3. **Please Note:**
   a. MPO Standards should be diluted with **STANDARD DILUENT**.
   b. Patient samples should be diluted with **SAMPLE DILUENT**.
4. **Prepare two-fold serial dilutions with the 40 ng/ml standard with STANDARD DILUENT.**
   a. 40 ng/ml: DO NOT Dilute after reconstitution
   b. 20 ng/ml: 0.5 ml of 40 ng/ml + 0.5 ml of **Standard Diluent**
   c. 10 ng/ml: 0.5 ml of 20 ng/ml + 0.5 ml of **Standard Diluent**
   d. 5 ng/ml: 0.5 ml of 10 ng/ml + 0.5 ml of **Standard Diluent**
   e. 2.5 ng/ml: 0.5 ml of 5 ng/ml + 0.5 ml of **Standard Diluent**
   f. 0 ng/ml: 0.5 ml of **Standard Diluent**

   ***Reconstituted standard should be discarded after use.** For future experiments, reconstitute a new vial of 40 ng/vial standard. This MPO kit can be used up to a maximum of 4 times.
5. ***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.***
6. ***EDTA-plasma and citrate-plasma should be diluted 10 fold prior to use. Prepare a series of small tubes (i.e. 1.5 ml microcentrifuge tubes) and mix 50 ul of EDTA or citrate-plasma sample with 450 ul (0.450 ml) of Sample Diluent.***

7. 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., 10 µl of the 100-fold diluted sample to 90 µl sample diluent).

8. **Working Conjugate Reagent:** To prepare Working MPO Conjugate Reagent, dilute the Enzyme Conjugate Concentrate (25X) with Enzyme Conjugate Diluent.
   Example: For 4 strips, prepare 4 ml of Working Enzyme Conjugate Reagent. Add 0.16 ml of Conjugate Concentrate (25X) to 3.84 ml of Conjugate Diluent.
   Do not reuse the Working Enzyme Conjugate Reagent. Make a fresh dilution before each assay.

9. **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.

ASSAY PROCEDURE

1. ***Reconstitute standard and serially dilute. See Reagent Preparation.***
2. ***Serum and heparin-plasma samples should be diluted 100-fold prior to use. Patient EDTA and Citrate plasma should be diluted 10-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 Working 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*.

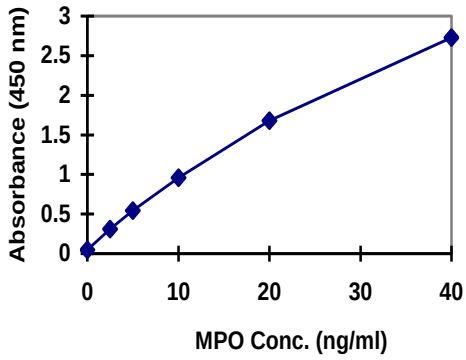
CALCULATION OF RESULTS

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 samples should be multiplied by the dilution factor of 100 to obtain MPO results in ng/ml.**

EXAMPLE OF STANDARD CURVE

Results of a typical standard run with absorbency readings at 450 nm 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



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