*Oxis*Research™

A Division of OXIS Health Products, Inc.

BIOXYTECH® MPO-EIA™

Assay For Human Myeloperoxidase
For Research Use Only. Not For Use In Diagnostic Procedures.
Catalog Number 21013

INTRODUCTION

The Analyte

Human myeloperoxidase (MPO) is a hemoprotein with a molecular weight of 140 kDa. It is composed of two heavy subunits of 53 kDa and of two light subunits of 15 kDa. Each MPO molecule contains two prosthetic porphyrins which play an essential role in the catalytic cycle.

MPO is stored in primary granules (azurophilic) of neutrophils. It is a major component of the bactericidal armamentarium of neutrophils, due to its capacity to catalyze the production of hypochlorous acid (HOCl), a powerful oxidant. HOCl is derived from chloride ion (Cl^-) and hydrogen peroxide (H_2O_2). In a number of inflammatory situations, MPO is released into the extracellular medium where its measurement can be used as an index of neutrophil activation.

Principles of the Procedure

The MPO-EIA assay system is a "sandwich" ELISA. Antigen captured by a solid phase monoclonal antibody is detected with a biotin-labeled goat polyclonal anti-MPO. An avidin alkaline phosphatase conjugate then binds to the biotinylated antibody. The alkaline phosphatase substrate *p*-nitrophenyl phosphate (pNPP) is added and the yellow product (*p*-nitrophenol) is monitored at 405 nm.

REAGENTS

Materials Provided (for 96 tests)

- Sample Diluting Buffer (1) — Phosphate buffer, containing bovine serum albumin (BSA), Tween-20 and sodium azide. This solution is used to dilute biological samples and standards. 3 x 25 mL
- MPO Standard (2) — Purified MPO in lyophilized form. 1 vial
- Washing Buffer (3) — Tris-HCl buffer, containing NaCl, Tween-20 and sodium azide. 100 mL, 20x concentrated
- Anti-MPO Solution (4) — 75 µL concentrated solution of goat polyclonal antibody to MPO in phosphate buffer, containing NaCl, BSA, glycerol and sodium azide. 1 microtube
- Avidin Alkaline Phosphatase (5) — 75 µL concentrated solution of avidin-coupled alkaline phosphatase in Tris-HCl buffer, containing $MgCl_2$, BSA, and sodium azide. 1 microtube
- Diluting Buffer (6) — Phosphate buffer for (4) and (5), containing NaCl, BSA, and sodium azide. 3 x 8 mL
- pNPP Diluting Buffer (7) — Diethanolamine buffer, containing $MgCl_2$ and sodium azide. 20 mL
- Stop Solution (8) — Sodium hydroxide, containing EDTA. 20 mL
- pNPP Tablets (9) — 4 tablets
- Microplate (10) — Divided into 6 sections of 16 wells each.
- Plate Sealers (11) — Four adhesive plate sealing tape sheets.

Materials Required But Not Provided

- Deionized water
- Test tubes and beakers
- Adjustable pipettes (50-1000 µL)
- 37°C Incubator
- Paper towels
- Stir bar/stir plate or vortex mixer
- Microplate reader capable of absorbance measurements at 405 nm

Warnings and Precautions

- For *in vitro* use only.
- Use pipettes with disposable tips to avoid bacterial contamination.
- In case of accidental contact with skin, eyes, or mucous membranes with Stop Solution (8), wash the exposed area thoroughly with water for 15 minutes.
- Final concentrations of sodium azide are 0.2% or lower. Sodium azide has been reported to form explosive lead or copper azides in laboratory plumbing. Waste solutions should be diluted with water prior to disposal.
- Human MPO was purified from human source material. MPO Standard (2) solution should be handled with the same precautions required for other blood products of human origin.

Reagent Storage and Handling

The MPO-EIA™ kit should be stored at 4°C before and after each use. Once the MPO standard solution is prepared, aliquots should be made and stored at -70°C if not used within 3 days. Buffers (1) and (6) should be used within 5 days of opening the bottles.

PROCEDURE

Reagent Preparation

Solutions (1), (3), (6), (7), and (8) should be placed at room temperature for 30 minutes prior to use.

MPO Standard (2):

WARNING: The Standard is stored under vacuum – open the stopper slowly to prevent loss of material.

Prepare a solution of MPO Standard (50 ng/mL) by adding the required volume of Sample Diluting Buffer (1) listed on the vial label. This solution may be stored at 4°C for 3 days. For longer storage, immediately prepare aliquots and store at -70°C. Avoid freeze thaw cycles.

Washing Buffer (20x) (3):

This solution should be diluted with deionized water prior to use. Depending on the volume required, dilute this buffer as described in Table 1.

Table 1: Dilution volumes for wash buffer

Required volume of Buffer	Volume of Wash Buffer (20x) (3)	Volume of deinonized water
250 mL	12.5 mL	237.5 mL
500 mL	25 mL	475 mL
1000 mL	50 mL	950 mL

The diluted washing buffer is stable for 5 days at room temperature.

Anti-MPO Solution (4) and Avidin Alkaline Phosphatase (5):

These solutions must be freshly diluted 1:250 with Diluting Buffer (6) prior to use. Examples of dilutions are described in Table 2.

Table 2: Dilution volumes for solutions (4) and (5)

Number of 16-well-strips used	Volume of Diluting Buffer (6)	Volume of (4) or (5)
2	4 mL	16 µL
4	8 mL	32 µL
6	12 mL	48 µL

pNPP Tablets (9):

Fifteen minutes before use (but not sooner), begin dissolving the required number of tablets in pNPP Diluting Buffer (7). Examples are described in Table 3. A stir bar and stir plate, vortex mixer, or constant swirling are required to dissolve the tablets.

Table 3: Dilution volumes for pNPP tablets and buffer

Number of 16-well strips used	Volume of pNPP Diluting Buffer (7)	Number of pNPP Tablets
2	5 mL	1
4	10 mL	2
6	15 mL	3

Set Up Summary

- Place solutions (1), (3), (6), (7) and (8) at room temperature 30 minutes prior to assay.
- Perform dilutions of the Standard by either of the following methods:
  - Perform serial dilutions of the 50 ng/mL MPO Standard with Sample Diluting Buffer (1) to obtain concentrations of 50, 25, 12.5, 6.2, 3.1 and 1.6 ng/mL.
  - Manually dilute the 50 ng/mL MPO Standard in test tubes with Sample Diluting Buffer (1) to obtain concentrations of 50, 35, 25, 15, 10, 5 and 1.5 ng/mL.
- Dilute Washing Buffer (3) solution with water (Table 1).
- Dilute Anti-MPO Solution (4) with Diluting Buffer (6) (Table 2).
- Dilute Avidin Alkaline Phosphatase (5) solution with Diluting Buffer (6) (Table 2).
- Dilute samples in Sample Diluting Buffer if necessary.
- Prepare pNPP solution using pNPP Tablets (9) and pNPP Diluting Buffer (7). This solution should not be prepared until 15 minutes prior to use.
- Use only the number of plate strips required for the number of samples. Store unused strips in the resealable foil pouch provided. Store at 2-8°C.

Note: Reagents (3), (4) and (5) are NOT “working solutions”!

Assay

1. Add 100 µL of standard or sample to each well, as appropriate.
2. Cover the plate and incubate 2 hours at 37°C.
3. Wash wells 5 times with Washing Buffer (3).
4. Add 100 µL anti-MPO Solution (4).
5. Cover the plate and incubate 1 hour at 37°C.
6. Wash wells 5 times with Washing Buffer (3).
7. Add 100 µL Avidin Alkaline Phosphatase (5) solution.
8. Cover the plate and incubate 1 hour at 37°C.
9. Wash wells 5 times with Washing Buffer (3).
10. Add 100 µL pNPP solution.
11. Cover the plate and incubate at 37°C until the absorbance of the wells with 50 ng/mL Standard is 1.5 - 2.0, approximately 10 - 20 minutes.
12. Add 50 µL of Stop Solution (8).
13. Mix and read absorbance at 405 nm.

Calculations

A standard curve is obtained by plotting the absorbance values at 405 nm as a function of the standard MPO concentrations, see Figure 1. Standard curves should be calculated by 4 parameter curve fit:

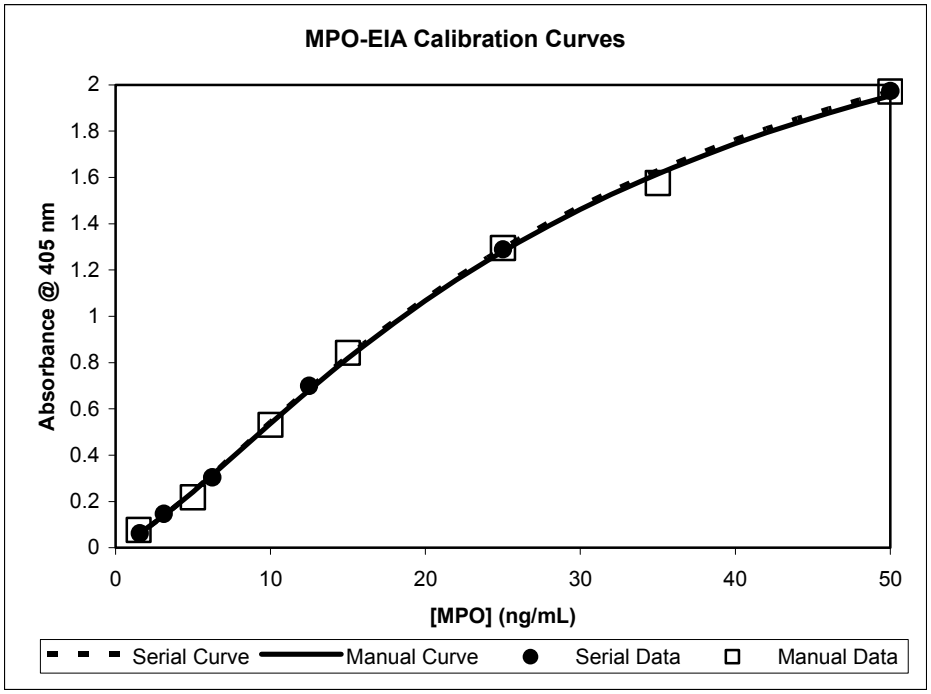
$$A_{405} = \frac{(A-D)}{\left(1+\frac{[MPO]}{C}\right)^{B}} + D$$

Figure 1

Examples of standard curves illustrating the two standard dilution methods – serial dilution and manual dilution. Parameters for 4 parameter curve fit are:

Serial Dilution: A=2.9610 B=-1.3676 C=30.3156 D=0.0127; R^2 = 0.9999

Manual Dilution: A=2.9182 B=-1.3780 C=30.1595 D=0.0166; R^2 = 0.9989



Sample values are obtained by:

- Automatic data reduction by a plate reader, or
- Solving the equation for [MPO]:

$$[MPO] = C\left(\sqrt[B]{\frac{(A-D)}{A_{405}-D}+1}\right)$$

Sensitivity

From 36 repeated measurements performed on a sample blank, on the same day using the same microplate, the detection limit of the assay is 1.5 ng/mL.

NOTES

Examples of Sample Preparation

Samples should be diluted, if necessary, using Sample Diluting Buffer (1). A sample volume of 100 µL is used for each measurement.

Plasma

1. Draw blood in EDTA or heparin tube.
2. Centrifuge whole blood within 6 hours of draw at 3000 x *g* for 10 minutes at 4°C.
3. Remove the plasma supernatant. Plasma samples can be stored at 4°C for 24 hours. For longer storage, samples should be stored at -20°C. Avoid repeated freezing/thawing.
4. Plasma MPO concentrations will vary from undetectable to excess depending on the state of neutrophil activation. Therefore, OXIS recommends a 1/10 dilution as a starting point only. It may be necessary to dilute plasma samples more or less with Sample Diluting Buffer (1) in order to obtain a concentration within the assay range. Detection and subsequent quantification of MPO will depend on the optimization of the dilution range for the specific system.
5. Use 100 µL of diluted sample for the assay. The MPO value in plasma can be biased if anti-MPO auto-antibodies are present.

Urine

1. Centrifuge at 1000 x *g* for 10 minutes at 4°C.
2. Remove supernatant. The supernatant can be stored at 4°C for 3 days.
3. Use 100 µL of the supernatant for each measurement. At MPO concentrations above 50 ng/mL, dilute the sample with Sample Diluting Buffer (1).

Broncho-Alveolar Lavage, Cerebrospinal Fluid, Supernatants after centrifugation of cell cultures:

The MPO assay can be performed without prior dilution of the medium unless the measured concentration of MPO in such samples is higher than 100 ng/mL. For higher concentrations of MPO, the samples should be diluted with Sample Diluting Buffer (1).

Cellular extracts (example HL-60)

1. Collect HL-60 cells (2×10^6) in phosphate-buffered saline (PBS).
2. Wash cells 3 times in PBS by centrifugation at 2000 *g* for 5 minutes at 4°C.
3. Resuspend the final pellet in 1 mL of 20 mM phosphate buffer, pH 7.4, containing 0.1% Tween detergent.
4. Transfer cell suspension into a microtube.
5. Break cells by freezing/thawing 3 times at -70°C.
6. Centrifuge the final suspension at 12000 x *g* for 15 minutes at 4°C.
7. Remove supernatant and dilute 10 times with Sample Diluting Buffer (1).
8. Use 100 µL of diluted sample for the measurement of MPO concentration.

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