
OxisResearch™

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 composed of two heme-containing heavy subunits of 53 kDa and of two light subunits of 15 kDa (1). Agner (2) defines an extinction coefficient of 180,000 based upon the heme absorbance at 430 nm, which upon calculation implies a molecular weight of the whole molecule as 149,000. MALDI-TOF analysis of the reference material employed by Oxis measures the molecular weight of purified myeloperoxidase as 145,000, with an extinction coefficient at 430 nm of 182,000 for the whole molecule. Each MPO molecule contains two prosthetic porphyrins, which play an essential role in the catalytic cycle. It has been reported that the enzyme exists in three forms.

MPO is stored in primary granules (azurophilic) of neutrophils (3). 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 (H₂O₂). 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.

MPO is a specific marker for polymorphonuclear cells (PMNs). It is released extracellularly via degranulation after PMN activation. Thus, extracellular MPO is an index of PMN activation in inflammatory processes that lead to disease pathology, such as cardiovascular disease (4).

Antibodies to MPO are present in human plasma, and have been characterized as part of the Anti-neutrophil cytoplasmic antibodies (ANCA) set of autoantibodies (5)

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. 2 x 25 mL
- MPO Standard (2) Purified MPO in lyophilized form, containing 150 ng MPO, with stabilizers. 1 vial
- Washing Buffer (3) Tris-HCl buffer, containing NaCl, Tween-20 and sodium azide. 1 x 100 mL, 20x concentrated
- Anti-MPO Solution (4) Concentrated solution of biotinylated goat polyclonal antibody to MPO in phosphate buffer, containing NaCl, BSA, glycerol and sodium azide. 1 x 75 µL
- Avidin Alkaline Phosphatase (5) Concentrated solution of avidin-coupled alkaline phosphatase in stabilizing buffer. 1 x 3.0 mL.
- Diluting Buffer (6) Phosphate buffer for (4), containing NaCl, BSA, and sodium azide. 1 x 12 mL
- pNPP substrate (7) 1 x 12 mL.
- Stop Solution (8) Sodium hydroxide, containing EDTA. 1 x 20 mL
- Microplate (9) Divided into 6 sections of 16 wells each.
- Plate Sealers (10) Two adhesive plate sealing tape sheets.

Materials Required But Not Provided

- Deionized water
- Test tubes and beakers
- Adjustable pipettes (0-1000 µL)
- EIA plate shaker
- Paper towels
- 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. Unused wells should also be stored at 4°C and fully sealed in foil bag. Do not freeze. Once the MPO standard solution is prepared, aliquots should be made and stored at -20°C if not used within 1 day. Solution 4 and solution 5, once diluted, should be used immediately and not stored for more than an hour.

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 deionized water 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 -20°C or colder. 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 deionized 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 at least 5 days at room temperature.

Anti-MPO Solution (4):

This solution 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 solution (4)

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

Avidin Alkaline Phosphatase solution(5):

This solution requires a 1:4 dilution with deionized water just prior to use

Table 3: Dilution volumes for solution (5)

Number of 16-well strips used	Volume of solution (5)	Volume of deionized water
2	1 mL	3 ml
4	2 mL	6 ml
6	3 mL	9 ml

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 25, 12.5, 6.2, 3.12 and 1.56, and 0 ng/mL.
 - ◆ Manually dilute the 50 ng/mL MPO Standard in test tubes with Sample Diluting Buffer (1) to obtain concentrations of 25, 12.5, 6.2, 3.12 and 1.56, and 0 ng/mL.

Dilute samples in Sample Diluting Buffer if necessary.

- Dilute Washing Buffer (3) solution with water (Table 1).
- Dilute Anti-MPO Solution (4) with Diluting Buffer (6) (Table 2), just prior to use
- Dilute Avidin Alkaline Phosphatase (5) solution with dH₂O (Table 3), just 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 (2), (3), (4), and (5) are NOT “ready-to-use working solutions,” and must be prepared.

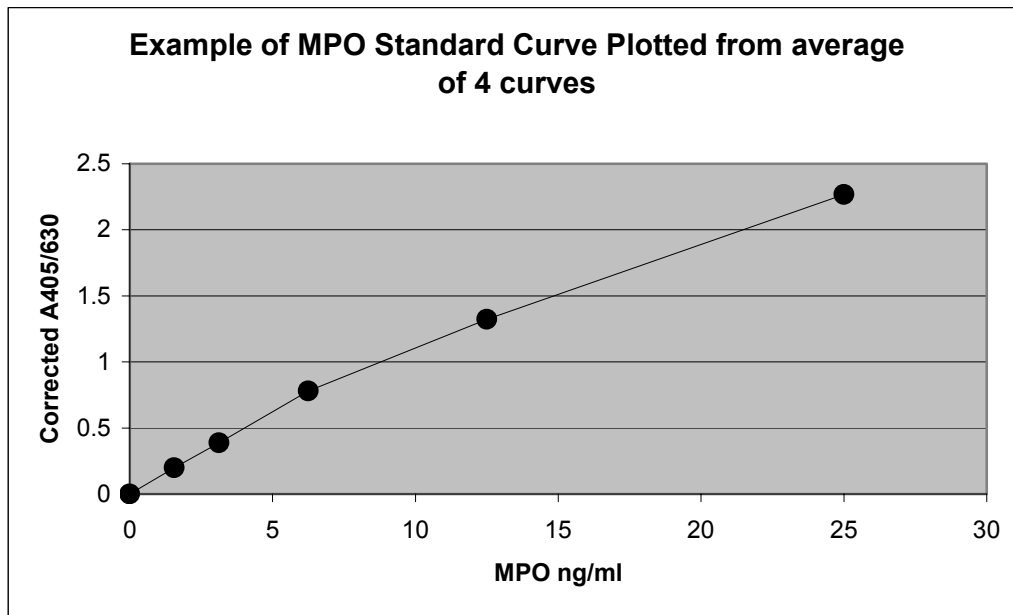
Assay

1. Add 100 µL of standard or sample to each well, as appropriate.
2. Cover the plate with strips and shake for 60 minutes at RT.
3. Wash wells 5 times with Washing Buffer (3).
4. Add 100 µL diluted anti-MPO Solution (4) to each well.
5. Cover the plate and shake 30 minutes at RT.
6. Wash wells 5 times with Washing Buffer (3).
7. Add 100 µL of diluted Avidin Alkaline Phosphatase (5) solution to each well.
8. Cover the plate and shake 30 minutes at RT.
9. Wash wells 5 times with Washing Buffer (3).
10. Add 100 µL of pNPP solution (7) to each well.
11. Allow wells to develop on a shaker at RT until the absorbance of the 25 ng/ml standard, is 1.5 - 2.0, approximately 20 - 30 minutes.
12. Add 50 µL of Stop Solution (8) to each well.
13. Mix and read absorbance at 405 nm, or 405 nm with 630 nm reference.

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 can be fit using appropriate data reduction methods, such as 4 parameter, or second order polynomial.

Figure 1



Units

The units used in the kit are ng/ml. To convert to molar units use the conversion equation:

$$1.0 \text{ pM} = 145,000 \text{ pg/L} = 145 \text{ pg/mL}$$

$$1.0 \text{ ng/mL} = 1.0 \text{ pM} / 0.145 \text{ ng/ml} = 6.9 \text{ pM}$$

The recommended calibration standard levels are therefore:

25 ng/ml = 172.3 pM, 12.5 ng/ml = 86.2 pM, 6.25 ng/ml = 43.1 pM, 3.13 ng/ml = 21.6 pM, 1.56 ng/ml = 10.8 pM

Interferences

The measurement of MPO directly in human plasma is subject to non-recovery on dilution and after spiking with purified MPO (6). Please refer to the OXIS Technical bulletin located at www.oxisresearch.com.

Sensitivity

Sensitivity was determined from eight individual measurements performed on standard curves from 4 separate runs, on the same day. The sensitivity signal was determined as 3.3 times the mean SD of the 0 ng/ml Standard from the individual curves. A linear extrapolation was made of this signal with the mean of the 1.56 ng/ml signal from the 4 curves, and an interassay sensitivity of 0.17 ng/ml (1.2 pM) MPO was determined.

NOTES

Examples of Sample Preparation

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

Plasma

1. Draw blood in heparin tube, mix adequately.
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 – 70°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 or 1:16 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 μ L of diluted sample, per well, for the assay.

For further information on interferences that may affect plasma samples, please consult the technical bulletin section of our website at www.oxisresearch.com/specifications/bulletins.shtml.

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 25 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 μ L of diluted sample for the measurement of MPO concentration.

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