

## Instructions for Use

Version: 1.0.1  
Revision date: 3-Apr-23

### Myeloperoxidase (MPO) Assay Kit

**Catalog No.:** abx294012

**Size:** 96 tests

**Detection Range:** 19.42 U/L – 893.41 U/L

**Sensitivity:** 19.42 U/L

**Storage:** Store all components at 4°C for up to 12 months.

**Application:** For detection and quantification of MPO activity in serum, plasma, milk, cell culture supernatants and tissue samples.

#### Introduction

Myeloperoxidase (MPO) is a heme-containing cationic glycoprotein that belongs to the heme peroxidase family. MPO is a dimer formed by polymerization of two subunits, with each subunit containing a heavy chain and a light chain. MPO contains anti-microbial properties and plays a role in the generation of oxidants and host defense mechanisms in neutrophilic granulocytes. MPO catalyses the breakdown of Hydrogen Peroxide (H<sub>2</sub>O<sub>2</sub>) to oxidize chloride and hypochlorous acid, which reacts with o-dianisidine to produce a yellow product.

Abbexa's MPO Assay Kit is a quick, convenient, and sensitive method for measuring and calculating MPO activity. The absorbance should be measured at 460 nm. The intensity of the color is proportional to the activity of MPO which can then be calculated.

#### Kit components

1. 96-well microplate
2. Reagent 1: 2 x 20 ml
3. Reagent 2: 2 vials
4. Reagent 3: 2 vials
5. Reagent 4: 6ml
6. Reagent 5: 2 x 1.2 ml
7. Reagent 6: 2 vials
8. Reagent 7: 0.1 ml
9. Reagent 8: 1 ml
10. Plate sealer: 2

#### Materials Required But Not Provided

1. Microplate reader (460 nm)
2. Distilled water
3. Pipette and pipette tips
4. Vials/tubes
5. Sonicating water bath
6. Vortex mixer

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### Protocol

#### A. Preparation of reagents and samples

##### 1. Reagents

- **Reagent 1:** Dilute with double distilled water at a ratio of 1:9. Mix thoroughly. Prepare immediately before carrying out the assay.
- **Reagent 2:** Dissolve a vial of Reagent 2 in 60 ml of pre-prepared Reagent 1 working solution to prepare Reagent 2 Working Solution. Mix thoroughly. Prepare immediately before carrying out the assay.
- **Reagent 3:** Dissolve a vial of Reagent 3 in 3 ml of Reagent 4 to prepare Reagent 3 Working Solution. Mix thoroughly. Prepare immediately before carrying out the assay and mix fully.
- **Chromogenic Reagent:** Dissolve a vial of Reagent 6 in 12.5 ml of Reagent 1. Add 12.5  $\mu$ l of Reagent 7. Mix thoroughly and prepare immediately before carrying out the assay. Store in the dark.

##### 2. Samples

Isolate the test samples soon after collecting and analyze immediately or aliquot and store at -20°C or -80°C for long term storage. Avoid multiple freeze-thaw cycles.

The following sample preparation methods are intended as a guide and may be adjusted as required depending on the specific samples used.

- **Serum:** Samples should be collected into a serum separator tube. Coagulate the serum by leaving the tube undisturbed in a vertical position overnight at 4°C or at room temperature for up to 1 hr. Centrifuge at approximately 2000  $\times$  g for 15 mins at 4°C. If a precipitate appears, centrifuge again. Take the supernatant, keep on ice and pretreat immediately, assay immediately, or aliquot and store at -80°C for up to 1 month.
- **Plasma:** Collect plasma using heparin as the anticoagulant. Centrifuge for 10 mins at 1000-2000  $\times$  g at 4°C, within 30 mins of collection. If precipitate appears, centrifuge again. Avoid hemolytic samples. Take the supernatant (avoid taking the middle layer containing white blood cells and platelets), keep on ice and pretreat immediately, or aliquot and store at -80°C for up to 1 month.
- **Tissue Homogenates:** Weigh the tissue homogenate. For each 1 g of homogenate, add 19 ml homogenization medium. Homogenize by hand, using a mechanical homogenizer, or by ultrasonication. Collect the supernatant, store on ice and pretreat immediately.
- **Milk samples:** Collect fresh milk sample, centrifuge at 4 °C for 10 minutes at 10,000  $\times$  g. Discard the upper layer and collect the middle layer. Store on ice and pretreat immediately.
- **Cell culture supernatants:** Collect cells into a centrifuge tube and wash with PBS. Centrifuge at 1000  $\times$  g for 10 min and discard the supernatant. Add 300-500  $\mu$ l of normal saline (0.9% NaCl) or PBS (0.01 M, pH 7.4) per 1  $\times$  10<sup>6</sup> cells, then sonicate in an ice water bath. Centrifuge at 10,000  $\times$  g at 4 °C for 10 min. Take the supernatant into a new centrifuge tube (while kept on ice) and analyze immediately.
- **Note:** Fresh samples or recently obtained samples are recommended to prevent degradation and denaturalization that may lead to erroneous results.
- Lysis buffers may interfere with the kit. It is therefore recommended to use mechanical lysis methods for cell lysates and tissue homogenates.
- Samples should not contain detergents such as SDS Tween-20, NP-40 and Triton X-100, or reducing agents such as DTT.

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### 3. Sample pretreatment

- **Serum, plasma, cell culture supernatants and milk samples:** Add 45 µl of sample and 45 µl of Reagent 2 Working Solution to a vial. Mix fully, and incubate at 37°C for 15 minutes. Take the processed sample for assay immediately.
- **Tissue Homogenates:** Add 90 µl of tissue homogenate and 10 µl of Reagent 3 Working Solution to a vial. Mix fully, and incubate at 37°C for 15 minutes. Take the processed sample for assay immediately.

### B. Assay Procedure

1. Set the Sample and Control tubes. We recommend setting up each sample in duplicate.
2. Add 350 µl of distilled water to the Control tubes.
3. Add 20 µl of Sample to all tubes.
4. Add 20 µl of Reagent 5 to all tubes.
5. Add 350 µl of pre-prepared Chromogenic Agent working solution to the Sample tubes.
6. Mix thoroughly and incubate in a water bath at 37 °C for 30 minutes.
7. Add 5 µl of Reagent 8 to all tubes.
8. Mix thoroughly and incubate in a water bath at 60 °C for 10 minutes. Centrifuge at 3000 x g for 10 minutes and take 300 µl of supernatant.
9. Measure the OD values at 460 nm with a microplate reader.

For Reference Only

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### C. Calculation of Results

#### 1. Serum, plasma, cell culture supernatants and milk samples:

One unit of MPO activity is defined as the quantity of MPO in 1 L of sample that catalyzes the consumption of 1  $\mu\text{mol/L}$   $\text{H}_2\text{O}_2$  at 37°C in 30 minutes.

$$\begin{aligned} \text{MPO activity(U/L)} &= \frac{\Delta A}{11.3 \times b} \times \frac{V_1 \times V_{\text{Total}}}{V_{\text{Sample}}} \times f \times 1000 \\ &= \frac{174.78 \times \Delta A}{V_{\text{Sample}}} \times f \end{aligned}$$

#### 2. Tissue samples:

One unit of MPO activity is defined as the quantity of MPO in 1 g wet weight of tissue sample that catalyzes the consumption of 1  $\mu\text{mol/L}$   $\text{H}_2\text{O}_2$  at 37°C in 30 minutes.

$$\begin{aligned} \text{MPO activity(U/L)} &= \frac{\Delta A}{11.3 \times b} \times \frac{V_3 \times V_{\text{Total}}}{V_2 \times W \times 0.9} \\ &= \frac{1.492 \times V_3 \times \Delta A}{W} \end{aligned}$$

where:

$\Delta A$	OD value of the sample ( $\text{OD}_{\text{Sample}} - \text{OD}_{\text{Control}}$ )
$b$	optical path of cuvette, 1 cm
$V_{\text{Total}}$	Total volume of the reaction system, 0.395 ml
$V_{\text{Sample}}$	Volume of sample added in sample pretreatment for serum, plasma, and milk, 0.045 ml
$V_1$	Total volume in sample pretreatment step, 0.1 ml
$V_2$	Volume of sample added to the reaction system
$V_3$	Volume of reagent 2 working solution added to tissue sample
$f$	dilution factor of the sample prior to assay
$W$	wet weight of sample (g)
11.3	Constant
1000	Conversion factor (1 L = 1000 ml)
0.9	Ratio of sample volume to total volume in sample pretreatment (0.09 ml / 0.1 ml = 0.9)