



# Myeloperoxidase (MPO) Assay Kit

(CAT/NO.:BC100 Size:100T/48S Colorimetric method)

## 1. COMPOSITION & PREPARATION (The kit is valid for 6 months)

**Reagent 1:** Buffer stock solution 35 ml×1 bottle, prepare buffer working solution according to volume you need, can be stored at 4 °C .

**Buffer working solution preparation:** Dilute buffer stock solution with double distilled water at ratio of 1:9, can be stored at 4 °C for 1 month.

**Reagent 2:** Powder×2 vials, can be stored at 4 °C . When use, add 60ml buffer working solution in each vial, you can place it in 37 °C water bath in order to dissolve complete. Prepared reagent can be stored at 4 °C for 2 weeks.

**Note:** If you use tissue sample and need to measure other factors besides MPO, then you should use 2 times concentrated solution (you can add 1 vial Reagent 2 powder in 30ml buffer working solution).

**Reagent 3:** Powder×3 vials, Diluent 6ml×3 vials, can be stored at 4 °C . When use, please pour 1 vial powder in 1 vial diluent to dissolve. It is suggested to prepare this reagent 1 day before using, completely dissolved reagent can be stored at 4 °C for 2 weeks.

**Reagent 4:** Solution 24ml×1 bottle, it will solidify in cold weather. Before use, shake it in water above 37 °C until it dissolves and becomes transparent. Store at room temperature.

**Reagent 5:** Powder×2 vials, can be stored at 4 °C .

**Reagent 6:** Solution 0.5cm×1 vial, can be stored at 4 °C .

**Chromogenic agent preparation:** When use, add 1 vial Reagent 5 powder in 100ml buffer working solution, shake sufficiently until powder dissolves completely, add 0. 1ml Reagent 6, mix sufficiently, prepared chromogenic agent can be stored at 4 °C away from light.(Discard after the color turns dark red)

**Reagent 7:** Solution 6ml×1 vial, can be stored at 4 °C

## 2. Required instruments and reagents

Visible spectrophotometer and 1cm optical path cuvette, distilled water, vortex mixer, 37 °C and 60 °C water bath or constant temperature chamber.

## 3. MPO determination of tissue samples:

### Sample pretreatment:

- (1) Serum (plasma) sample: Mix serum (plasma) with reagent 2 at a 1:1 ratio and vortex thoroughly.
- (2) Tissue Sample: Accurately weigh the tissue. Using the prepared reagent II solution as the homogenizing medium, add the homogenizing medium at a weight-to-volume



ratio of 1:19 to prepare a 5% tissue homogenate (a 10% homogenate can also be prepared as needed). Centrifugation is not required. (The tissue homogenate should be as homogenous as possible, without large pieces of tissue.) [Note]: If you need to measure other indicators besides myeloperoxidase, the tissue homogenate preparation can be done according to the following steps:

- ① When preparing reagent 2, it should be concentrated by one-fold, that is, each vial of reagent 2 powder should be added to 30mL of buffer solution;
- ② Prepare a homogenate concentrated by one time using physiological saline as the homogenizing medium, i.e., a 10% homogenate (brain tissue is prepared into a 20% homogenate). Do not centrifuge. Take out a portion and add a 1:1 concentration of reagent II solution, mix well, and then perform the measurement.

### Operation table:

	Control tube	Measurement tube
Processed samples (mL)	0.18	0.18
Reagent 3 (mL)	0.02	0.02
Mix thoroughly and incubate in a 37°C water bath for 15 minutes.		
distilled water (mL)	3	
color developer (mL)		3
Mix well and incubate in a 37°C water bath for 30 minutes.		
Reagent 4 (mL)	0.2	0.2
Reagent 7 (mL)	0.05	0.05
Mix well, incubate in a 60°C water bath for 10 minutes, and immediately measure the absorbance of each tube at 460nm with a 1cm optical path using a spectrophotometer with distilled water as the zero point.		

[Note 1]: When the weather is cold, the reaction solution will solidify and the absorbance will increase. You can place a water bath at 25-37°C or a beaker containing hot water at 25-37°C next to the colorimeter and put each test tube into the water bath or beaker in turn for 1-2 minutes. After the solidification disappears, you can perform colorimetric analysis.

[Note 2]: When mixing, it is best to use a vortex mixer to ensure that the liquid is thoroughly mixed from top to bottom (make sure that the liquid at the bottom is also rotated to the top; it is recommended not to use tubes with pointed bottoms, especially not 1.5mL EP tubes, as they are very difficult to mix).

### Serum (plasma) sample calculation:

1. Unit definition: One unit of enzyme activity (U) is defined as 1 mol of H<sub>2</sub>O<sub>2</sub> decomposed in a reaction system at 37°C per liter of serum (plasma).

2. Calculation formula:



$$\text{MPO Activity (U/L)} = \frac{A_{\text{Measurement}} - A_{\text{Control}}}{11.3 \times V_{\text{Sample}}}$$

$V_{\text{sample}}$ : The amount of serum contained in the sample (in liters),  
 $V_{\text{sample}}$  = serum concentration after pretreatment 0.5 (mL/mL) × sample volume ( $0.18 \times 10^{-3}$ L).

### 3. Calculation example:

Take 0.3 mL of serum and add 0.3 mL of reagent II, mix thoroughly, and take 0.18 mL for testing. The OD value of the test tube is 0.053, and the OD value of the control tube is 0.013. The calculation is as follows:

$$\text{MPO Activity (U/L)} = \frac{0.053 - 0.013}{11.3 \times 0.5 \times 0.18} \times 1000 = 39.33 \text{ U/L}$$

### Tissue sample calculation:

1. Unit definition: One unit of enzyme activity (U) is defined as 1 mol of H<sub>2</sub>O<sub>2</sub> decomposed in a reaction system at 37°C for every gram of wet tissue slide.
2. Calculation formula:

$$\text{MPO Activity (U/g Weight)} = \frac{A_{\text{Assay}} - A_{\text{Control}}}{11.3 \times W}$$

11.3 is the reciprocal of the slope (a constant);

W is the sample volume (g), and W = homogenate concentration (5% or 10%) × sample volume (0.18 mL).

### 3. Calculation example:

Example 1: Take 0.18 mL of 5% mouse myocardial homogenate and perform the above steps for detection. The absorbance of the control tube is 0.030, and the absorbance of the test tube is 0.164. The calculation is as follows:

$$\text{MPO Activity (U/g Weight)} = \frac{0.164 - 0.030}{11.3 \times 0.05 \times 0.18} = 1.317 \text{ U/g Weight}$$

Example 2: Take 0.18 mL of 5% rat liver homogenate and perform the above steps for detection. The absorbance of the control tube is 0.028, and the absorbance of the test tube is 0.183. The calculation is as follows:

$$\text{MPO Activity (U/g Weight)} = \frac{0.183 - 0.028}{11.3 \times 0.05 \times 0.18} = 1.524 \text{ U/g Weight}$$

Example 3: Take 0.18 mL of 10% rat brain homogenate and perform the above steps for detection. The absorbance of the control tube is 0.002, and the absorbance of the test tube is

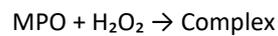


0.012. Calculate as follows:

$$\text{MPO Activity (U/g Weight)} = \frac{0.012 - 0.002}{11.3 \times 0.1 \times 0.18} = 0.0492 \text{ U/g Weight}$$

#### 4. Measurement principle

Neutrophils contain myeloperoxidase, and the amount of this enzyme in each cell is fixed, approximately 5% of the cell's dry weight. This enzyme has the ability to reduce hydrogen peroxide. This characteristic can be used to analyze enzyme activity and quantitatively determine the number of neutrophils. The principle is as follows:



A yellow compound is generated by hydrogen donation from the hydrogen donor o-anisidine. The amount of product A generated is determined by colorimetric analysis at 460 nm, thereby deduce the activity of MPO, the amount of H<sub>2</sub>O<sub>2</sub> reduction, and the number of white blood cells.