Myeloperoxidase Peroxidation Fluorometric Assay Kit

Introduction

Myeloperoxidase (MPO) is an important member of the heme peroxidase-cyclooxygenase superfamily, which is expressed in a variety of inflammatory cells such as neutrophils, activated microglia, monocytes, and is essential for immune surveillance and host defense. Studies have shown that MPO plays a key role in a variety of diseases and is a potential biomarker and therapeutic target for inflammatory and oxidative stress-related diseases.

MPO can catalyze two types of redox reactions, including chlorination and peroxidation. The Myeloperoxidase Peroxidation Fluorometric Assay Kit can be used to quantitatively detect MPO peroxidation activity in cell and tissue samples. The peroxidase in the sample can catalyze non-fluorescent ADHP to the fluorescent product Resorufin (excitation/emission wavelength = 535/587 nm), and the fluorescence intensity of Resorufin is proportional to the total peroxidase activity in the sample. A specific MPO inhibitor is also provided in the kit that specifically inhibits the peroxidation activity of MPO in the sample, thereby distinguishing MPO-mediated peroxidation from the activity of other peroxidases in the sample.

In addition, MPO activity can also be detected by chlorination, the Myeloperoxidase Chlorination Fluorometric Assay Kit (Cat. No. K2289).

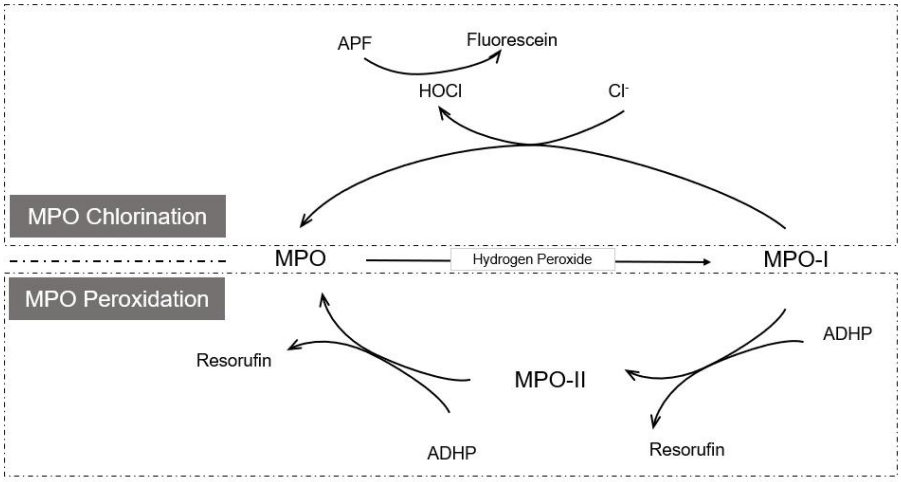

Figure 1: MPO catalytic mechanism

Hydrogen Peroxide + Peroxidation Substrate ⟶ Fluorescence Signal F_1

Hydrogen Peroxide + Peroxidation Substrate + MPO Inhibitor ⟶ Fluorescence Signal F_2

Fluorescence Signal For MPO Peroxidation = $F_1 - F_2$

Figure 2: The detection principle of this product

Composition and Storage

Components \ Size	100 Tests	Storage
Hydrogen Peroxide	1 mL	-20°C
MPO Assay Buffer	50 mL	-20°C
MPO Inhibitor (10×)	100 µL	-20°C
Peroxidation Substrate	200 µL	-20°C away from light
Resorufin Standard (5 mM)	50 µL	-20°C away from light
Shipping: Blue ice	Shelf life: 1 year	

Protocol

1. **Sample Preparation:**

   1) For cell samples, harvest 6 × 10^6 cells by centrifugation at 1000 g for 5 min at 4°C. Add 500 µL of MPO Assay Buffer to resuspend the cell pellet and incubate on ice for 10 min. Centrifuge at 10,000 g for 10 min at 4°C, and collect the supernatant on ice for later use.

   2) For tissue samples, weigh approximately 20 mg of tissue, wash 1-2 times with PBS, then add 500 µL of MPO Assay Buffer and homogenize on ice. After the homogenization, centrifuge at 10000 g for 10 min at 4°C, and collect the supernatant on ice for later use.

***Note:**

a) Protease inhibitor is recommended to be added to the MPO Assay Buffer during sample preparation, and the Protease Inhibitor Cocktail (EDTA-Free, 100X in DMSO) (Cat. No. K1007) can be selected.

b) It is recommended to use fresh cell or tissue lysate for the assay. If it cannot be used in time, the supernatant can be stored at -80°C. However, this may affect the results of the test.

2. **MPO Inhibitor Preparation:** Dilute the appropriate amount of MPO Inhibitor (10×) to 1 × with MPO Assay Buffer. For example, dilute 50 µL of MPO Inhibitor (10×) in 450 µL of MPO Assay Buffer.

3. **Standard Preparation:**

Keep standards on ice throughout.

1) Dilute 2 µL of Resorufin Standard (5 mM) in 998 µL of MPO Assay Buffer to make the Resorufin Standard (10 pmol/µL).

2) Dilute 50 µL of Resorufin Standard (10 pmol/µL) in 450 µL of MPO Assay Buffer to make the Resorufin Standard (1 pmol/µL).

3) Refer to the table below to prepare the standards in 96-well plates, and it is recommended to prepare at least two parallel wells for each set of standards.

Standard #	1 pmol/μL Resorufin Standard (μL)	MPO Assay Buffer (μL)	Resorufin (pmol/well)
1	0	60	0
2	4	56	4
3	8	52	8
4	12	48	12
5	16	44	16
6	20	40	20

4. MPO Activity Assay:

1) Transfer samples to the 96-well plates containing standards, and each sample needed to be divided into two groups, the experimental group and inhibitor group. Transfer samples referring to the table below. It is also recommended to prepare at least two parallel wells for each group.

Experimental group	Take 2-50 μL of the sample and make up the volume to 60 μL with MPO Assay
Inhibitor group	Take 2-50 μL of sample, make up to 50 μL with MPO Assay Buffer, and add 10 μL of MPO Inhibitor (1×)
Standard group	60 μL of standards at different concentrations

2) Prepare enough reaction solution for the assay. 40 μL of reaction solution is required per well.

	Reaction mix (μL)
MPO Assay Buffer	37
Peroxidation Substrate	1
Hydrogen Peroxide	2

3) Add 40 μL of reaction solution to each well and mix well.

4) At 37°C, detect the fluorescence signal (Ex/Em=535/587 nm). For samples, it is recommended to directly detect the kinetic curve for 5-20 min. For the standard curve set, the endpoint method can be used directly.

***Note:** For samples, the required incubation time depends on the specific experiment, and it is recommended to measure the kinetics curve directly.

5. Result analysis:

1) Subtract the background value of standard #1 (at 0 Resorufin) from all standard readings, and then plot the standard curve.

2) Calculate the MPO activity in the sample:

$$\Delta F = (FS(T2) - FS(T1)) - (RI(T2) - RI(T1))$$

Note: choose two time points (T1 and T2), FS is the fluorescence signal value of the experimental group,

and FI is the fluorescence signal value of the inhibitor group

Apply ΔF to the standard curve to get A pmol of resorufin in the sample during the reaction time (ΔT=T2-T1).

$$\text{MPO activity} = A \div \Delta T \div V \times B = \text{pmol/min/mL} = \mu\text{U/mL}$$

Note: V is the volume of sample added per well, mL; B is the dilution factor, if the sample is not diluted, B = 1

Unit Definition: a unit of MPO is the amount of enzyme that produces 1.0 μmol of resorufin per min at pH 7.0.

Note

1. Before the formal experiment, it is recommended to perform several dilutions of the sample to ensure the readings are within the standard value range.
2. For your safety and health, please wear lab coats and gloves during the experiment.
3. For research use only. Not to be used in clinical diagnostic or clinical trials.

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