Protocol

Cat. No. K2289

Myeloperoxidase Chlorination 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 Chlorination Fluorometric Assay Kit provides a rapid, simple, and sensitive method for detecting myeloperoxidase (MPO) activity by chlorination. MPO can catalyze the production of hypochlorous acid (HClO). Subsequently, HClO can react with the fluorescent probe APF to form Fluorescein, which can be detected at excitation/emission wavelength = 485/525 nm and its fluorescence signal intensity is positively correlated with MPO activity. In addition, MPO activity can also be detected by the detection of MPO peroxidation, the Myeloperoxidase Peroxidation Fluorometric Assay Kit (Cat. No. K2285).

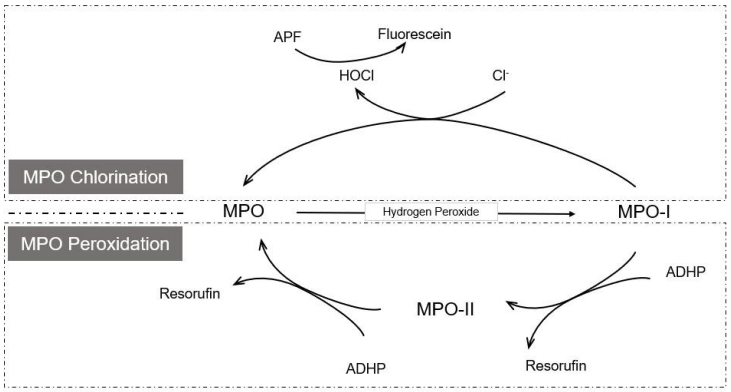


Figure 1: MPO catalytic mechanism

Composition and Storage

Components \ Size	100 Tests	Storage
MPO Assay Buffer	25 mL	-20°C
Hydrogen Peroxide	50 µL	-20°C
MPO Probe	200 µL	-20°C away from light
Fluorescein Standard (1 mM)	50 µL	-20°C away from light
Shipping: Blue ice	**Shelf life: 1 year**	

Protocol

1. Sample Preparation:

1) For cell samples, harvest 2×10^6 cells by centrifugation at 1,000 g for 5 min at 4°C. Wash the cells one time with pre-chilled PBS. Resuspend cells by adding 100 µL of pre-chilled MPO Assay Buffer to the cell pellet, pipetting several times with a pipette tip and incubating 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 10 mg of tissue, wash 1-2 times with pre-chilled PBS, then add 100 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.

3) For blood, take 2 mL of blood, and lyse red blood cells with Red Blood Cell Lysis Buffer. Incubated at room temperature for 10 min. After incubation, centrifuge at 400 g for 5 min. Discard the supernatant, and wash the pellet one time with PBS. Centrifuge at 400 g for 5 min to remove PBS, resuspend the pellet with 200 µL of MPO Assay Buffer, and incubate on ice for 10 min. Centrifuge at 10,000 g for 10 min at 4°C and carefully transfer the supernatant. Dilute the supernatant 10 times with MPO Assay Buffer and take 1-10 µL of the diluted supernatant on ice for later use.

***Note:** Fresh samples should be used whenever possible, and follow-up experiments should be performed as soon as possible after sample preparation. If it cannot be used in time, it can be stored at -80°C first. However, this may affect the results of the test.

2. Standard Preparation:

Always prepare a fresh set of standards for every use. Prepare standards immediately prior the assay as it needs to be read quickly after preparation.

1) Dilute 5 µL of Fluorescein Standard (1 mM) in 995 µL MPO Assay Buffer, mixing to make the Fluorescein Standard (5 µM).

2) Refer to the table below to prepare the standard in a 96-well plate, 100 µL per well is required. Each dilution has enough amount of standard to set up duplicate readings.

	5 µM Fluorescein Standard (µL)	MPO Assay Buffer (µL)	Final standard in well (µL)	Fluorescein (pmol/well)
1	0	300	100	0
2	6	294	100	10
3	12	288	100	20
4	18	282	100	30
5	24	276	100	40
6	30	270	100	50

3) After preparation, incubate at room temperature for 5 min.

4) Measure the fluorescence signal (Ex/Em=484/525 nm).

3. MPO Activity Assay:

1) Take 1-50 μL of the sample and make up the volume to 50 μL with MPO Assay Buffer. It is also recommended to prepare at least two parallel wells for each sample.

2) Prepare the reaction solution referring to the table below, 50 μL of reaction solution is required per well. Prepare enough reaction solution for the assay.

	Reaction mix (μL)
MPO Assay Buffer	46
MPO Probe	2
Hydrogen Peroxide	2

3) Add 50 μL of reaction solution to each well and mix well.

4) Measure the fluorescence signal (Ex/Em=484/525 nm) in kinetic mode, every 2-3 min, for at least 30 min.

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

4. 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:

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

Note: T1 and T2 are two time points

Apply ΔF to the standard curve to get A pmol of Fluorescein 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

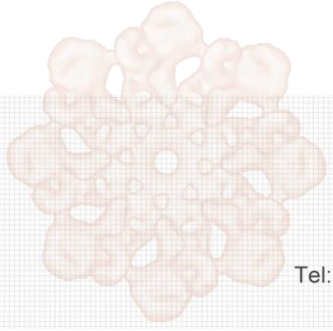
Unit Definition: Under these conditions, a unit of MPO is the amount of enzyme that produces 1.0 μmol of Fluorescein per min.

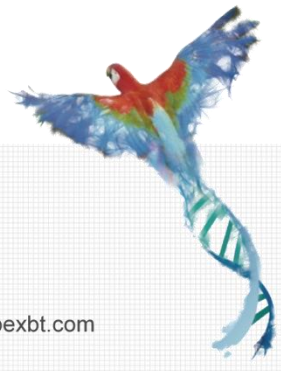
Note

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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