Myeloperoxidase Chlorination and Peroxidation Inhibitor Screening Assay Kit

Introduction

Myeloperoxidase (MPO) is a crucial enzyme within the heme peroxidase superfamily, predominantly expressed in neutrophils, monocytes, and macrophages. It plays a vital role in innate immunity but is also a significant driver of inflammatory and oxidative stress-related pathologies, making it a promising biomarker and therapeutic target. The development of effective MPO inhibitors is a major focus in pharmaceutical research.

MPO can catalyze two types of redox reactions, including chlorination and peroxidation. The detection principle of peroxidation activity is that the peroxidase in the sample can catalyze the reaction of hydrogen peroxide and non-fluorescence ADHP to obtain the fluorescent product Resorufin (Ex/Em = 535/587 nm), and the fluorescence intensity of Resorufin is directly proportional to the total peroxidase activity in the sample.

In addition, MPO activity can also be detected by chlorination. MPO can catalyze hydrogen peroxide and chloride ions (Cl^-) to produce hypochlorous acid (HClO). HClO can then react with the fluorescent probe APF to produce fluorescein. The resulting fluorescence signal (Ex/Em = 485/525 nm) is proportional to MPO chlorination activity.

This kit provides two distinct, well-established methods for quantifying MPO activity, making it particularly suitable for high-throughput screening of inhibitors.

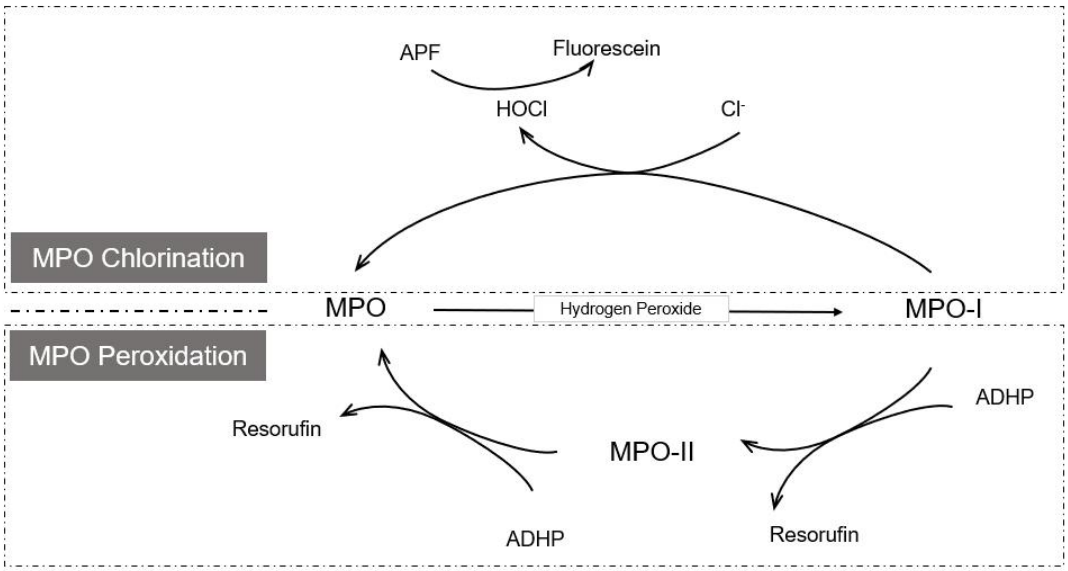

Figure 1: MPO catalytic mechanism

Components and Storage

Components \ Size	100 Tests	Storage
MPO Assay Buffer	50 mL	-20°C
MPO Chlorination Substrate	100 µL	-20°C away from light
ADHP Assay Reagent	1 vial	-20°C away from light
Myeloperoxidase Assay Reagent	50 µL	-80°C
MPO Inhibitor	300 µL	-20°C
MPO Hydrogen Peroxide	100 µL	-20°C away from light
Shipping: Dry ice	Shelf life: 1 year	

Protocol

1. Preparation before the experiment

1) Dissolve the ADHP Assay Reagent completely in 500 µL of DMSO and aliquot for storage. Prior to use, dilute the reagent 10-fold with MPO Assay Buffer to prepare the ADHP working solution.

***Note:** The ADHP working solution should be prepared freshly and used within 15 min to avoid an increase in background signal.

2) Thaw the Myeloperoxidase Assay Reagent (1 mg/mL human MPO) on ice and mix gently. Dilute the reagent 80-fold with MPO Assay Buffer to obtain a final concentration of 12.5 µg/mL. For example, add 25 µL of MPO to 1975 µL of MPO Assay Buffer. MPO (12.5 µg/mL) is stable on ice for up to 1 h and should be used promptly.

3) The MPO Inhibitor is supplied as a 50 mM stock solution. First, dilute 10 µL of the inhibitor in 490 µL of MPO Assay Buffer to obtain a 1 mM intermediate solution. Further dilute the 1 mM solution to a final working concentration of 110 µM.

***Note:** The 110 µM MPO Inhibitor working solution should be used within 4 h.

4) Prepare a 3% MPO Hydrogen Peroxide solution by performing a 10-fold dilution of the MPO Hydrogen Peroxide with MPO Assay Buffer. Further dilute the 3% solution to a final concentration of 5 mM. For example, add 10 µL of 3% H_2O_2 to 1.74 mL of MPO Assay Buffer.

***Note:** The 5 mM H_2O_2 working solution must be used within 2 h.

2. MPO chlorination activity assay

1) Prepare a sufficient volume of the reaction solution based on the number of samples. The following recipe is for 50 reactions. The reaction solution color is yellow.

Number of samples	50
MPO Assay Buffer	2.44 mL

MPO Chlorination Substrate	40 μL
5 mM MPO Hydrogen Peroxide	20 μL

2) Set up groups in a 96-well plate according to the table below. The positive control is recommended to verify system, but is optional. The compound can be dissolved in ethanol, methanol, or DMSO, but it needs to be diluted into MPO Assay Buffer before being added to the sample well.

Background control	Add 60 μL MPO Assay Buffer
100% activity control	Add 50 μL MPO Assay Buffer and 10 μL MPO (12.5 μg/mL)
Positive control	Add 40 μL MPO Assay Buffer,10 μL MPO (12.5 μg/mL) and 10 μL MPO Inhibitor (110 μM)
Sample	Add 40 μL MPO Assay Buffer,10 μL MPO (12.5 μg/mL) and 10 μL compound

3) Add 50 μL of the reaction solution to each well and mix well. Cover the plate and incubate for 10 min on a shaker at room temperature and protected from light.

4) Remove the plate cover. Measure the fluorescence signal with a microplate reader (Ex/Em=484/525 nm).

3. MPO peroxidation activity assay

1) Prepare a sufficient volume of the reaction solution based on the number of samples. The following recipe is for 50 reactions.

Number of samples	50
MPO Assay Buffer	2.24 mL
ADHP working solution	250 μL
5 mM MPO Hydrogen Peroxide	10 μL

2) Set up groups in a 96-well plate according to the table below. The positive control is recommended to verify system, but is optional. The compound can be dissolved in ethanol, methanol, or DMSO, but it needs to be diluted into MPO Assay Buffer before being added to the sample well.

Background control	Add 60 μL MPO Assay Buffer
100% activity control	Add 50 μL MPO Assay Buffer and 10 μL MPO (12.5 μg/mL)
Positive control	Add 40 μL MPO Assay Buffer,10 μL MPO (12.5 μg/mL) and 10 μL MPO Inhibitor (110 μM)
Sample	Add 40 μL MPO Assay Buffer,10 μL MPO (12.5 μg/mL) and 10 μL compound

3) Add 50 μL of the reaction solution to each well and mix well. Cover the plate and incubate for 5 min on a shaker at room temperature and protected from light.

4) Remove the plate cover. Measure the fluorescence signal with a microplate reader (Ex/Em=535/585 nm).

4. Analysis of results:

1) Subtract the reading of the background control from all readings

2) Calculate the inhibition efficiency of a compound according to the following formula:

% Inhibition rate = (100% activity control - sample) ÷ 100% activity control × 100%

3) If multiple concentrations of the compound are tested simultaneously, the IC_{50} value can be determined by plotting the inhibition rate against the logarithm of the compound concentration.

Note

1. For your safety and health, please wear lab coats and gloves during the experiment.
2. For research use only. Not to be used in clinical diagnostic or clinical trials.

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