Technical Bulletin

Myeloperoxidase (MPO) Assay Kit

Catalogue number MAK516

Product Description

Myeloperoxidase (MPO; EC 1.11.2.2) is a peroxidase enzyme and can be found in neutrophil, monocytes, and some soft tissue macrophages. MPO has an ability to use chloride as a cosubstrate with hydrogen peroxide to generate hypochlorous acid, a powerful antimicrobial agent produced by neutrophils. However, an excessive production of hypochlorous acid can lead to oxidative stress and tissues damage. Inflammation may also result when MPO oxidizes various substances such as phenols and anilines. Studies show that increased MPO levels may increase the risk of myocardial infarction and cardiovascular disease.

Our Myeloperoxidase (MPO) Assay Kit is based on the MPO enzyme reaction with hydrogen peroxide (H_2O_2) which oxidizes the dye reagent to a highly fluorescent product. The fluorescence intensity of this product, measured at λex = 530 nm / λem = 585 nm, is proportional to the total peroxidation activity in the sample. The provided MPO inhibitor is used to suppress peroxidase activity due to MPO in order to differentiate other peroxidase activities that may be present in the samples.

The linear detection range of the kit is 0.0025 to 2 U/L for 10 min reaction at 25 °C.
The Myeloperoxidase Assay Kit is suitable for MPO peroxidation activity determination in biological samples (for example, cell lysates, tissues, etc.).

Components

The kit is sufficient for 100 fluorometric assays in 96-well plates.

- Assay Buffer 10 mL
  Catalogue Number MAK516A
- Resorufin 1.5 mL
  Catalogue Number MAK516B
- 20× MPO Inhibitor 120 µL
  Catalogue Number MAK516C
- Dye Reagent 120 µL
  Catalogue Number MAK516D
- 3% Stabilized H_2O_2 100 µL
  Catalogue Number MAK516E

Reagents and Equipment Required but Not Provided

- Pipetting devices and accessories (for example, multichannel pipettor)
- Fluorescent multiwell plate reader
- Black, flat-bottom 96-well or 384-well plates. Cell culture or tissue culture treated plates are not recommended.
- 1.5 mL microcentrifuge tubes
- Phosphate Buffered Saline (PBS) pH 7.4
- Dounce tissue grinder set
  (Catalog Number D9063 or equivalent)
- 20 mM PBS, pH 7.4
- Refrigerated microcentrifuge capable of RCF ≥ 14,000 × g
- 50 mM potassium phosphate solution (pH 7.5)
- Rubber policeman or cell scraper

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

The kit is shipped on wet ice. Store components at -20 °C.

Preparation Instructions

Sample Preparation

Tissue:

1. Prior to dissection, rinse tissue in phosphate buffered saline (pH 7.4) to remove blood.
2. Homogenize tissue (50 mg) with a Dounce homogenizer in ~200 µL cold 20 mM PBS, pH 7.4.
3. Freeze the homogenized tissue at -80 °C to lyse the cells.
4. After freezing, thaw and centrifuge Samples at 14,000 x g for 20 min at 4 °C.
5. Remove supernatant for assay.

Cell Lysate:

1. Collect cells by centrifugation at 2,000g for 5 min at 4 °C.
2. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman or cell scraper. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5).
3. Centrifuge at 14,000g for 10 min at 4 °C. Remove supernatant for assay.

All Samples can be stored at -20 to -80 °C for at least one month.

Procedure

All Samples and Standards should be run in duplicate.

Briefly centrifuge small vials prior to opening. Equilibrate all components to room temperature prior to use.

Note: This procedure is written for 96-well plates.

1. Prepare a 1× MPO Inhibitor by diluting enough 20× MPO Inhibitor 20- fold in purified water for the number of assays.
2. Prepare a 30 µM Resorufin Premix by mixing 15 µL of the provided Resorufin and 235 µL water.

Transfer 100 µL water and 100 µL 30 µM Resorufin into two separate wells of a black flat-bottom 96-well plate.

3. For each Sample prepare 2 parallel wells.
   - Add 20 µL of samples to each well.
   - Add 20 µL of 1× MPO inhibitor to one of each Sample well and add 20 µL of assay buffer to the other Sample well.
4. Incubate the Samples at room temperature for 10 minutes.
5. Prepare the 0.007% H_2O_2 solution.
   - Prepare a 0.07% solution by mixing 4.7 µL of 3% H_2O_2 and 195.3 µL of purified water.
   - Prepare a 0.007% H_2O_2 solution by combining 60 µL of 0.07% H_2O_2 solution and 540 µL of purified water.

Note: Use this solution within 1 hour of preparation.

6. Prepare enough working reagent for the number of Samples being assayed according to Table 1.

Table 1.

Reagent	Volume
Assay Buffer	60 µL
0.007% H_2O_2	1 µL
Dye Reagent	1 µL

Measurement

1. Add 60 µL Working Reagent to all sample and inhibitor wells.
2. Tap plate briefly to mix.
3. Read fluorescence λ_{ex}=530 nm / λ_{em} 585 nm at 0 min (ΔR0) and 10 min (ΔR10) at room temperature.

Results

1. Subtract ΔR0 from ΔR10 for each Standard and Sample to obtain ΔR.
2. Plot the standard ΔR's and determine the slope.
3. The MPO activity is calculated as follows.
   MPO Activity (µL)

$$\frac{\Delta R_{Sample} - \Delta R_{INB}}{R_{Resorufin} - R_{H2O}} \times \frac{\text{Resorufin Concentration}}{t} \times \frac{\text{Reaction Volume}}{\text{Sample Volume}} \times DF$$

where,
ΔR_{Sample} = Fluorescence readings of the Sample
ΔR_{INB} = Fluorescence readings of the Sample Inhibitor
$R_{Resorufin}$ = Fluorescence readings of the Resorufin
R_{H2O} = Fluorescence readings of the Water
DF = Sample dilution factor
(DF = 1 for undiluted Samples)
Resorufin concentration (µM) = 30
t (minutes) = reaction time
Reaction Volume(µL) = 100
Sample Vol = 20 µL

Notes: if ΔR_{Sample} values are higher than that of the $R_{Resorufin}$, dilute sample in Assay Buffer and repeat the assay. Multiply the results by the dilution factor, DF. Unit definition: one unit of enzyme will catalyze the formation of 1 µmole resorufin per min under the assay conditions.

Figure 1.

Typical MPO raw kinetic fluorometric data.

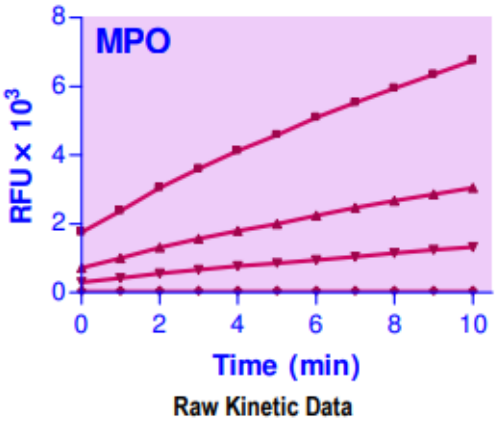
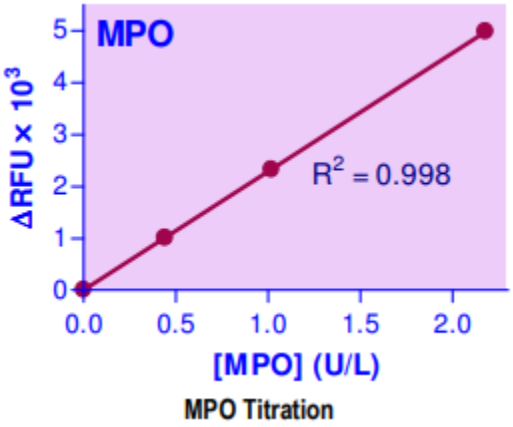


Figure 2.

Typical Fluorometric Standard Curve.



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