MPO / Myeloperoxidase Assay Kit (Fluorometric)

MPO / Myeloperoxidase Assay Kit (Fluorometric) is a detection kit for the quantification of Myeloperoxidase peroxidation in cell lysate and tissue.

Catalog number: ARG82771

Package: 100 tests

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INTRODUCTION

Myeloperoxidase (MPO) is a peroxidase enzyme that in humans is encoded by the MPO gene on chromosome 17. MPO is most abundantly expressed in neutrophil granulocytes (a subtype of white blood cells), and produces hypohalous acids to carry out their antimicrobial activity, including hypochlorous acid, the sodium salt of which is the chemical in bleach. It is a lysosomal protein stored in azurophilic granules of the neutrophil and released into the extracellular space during degranulation. Neutrophil myeloperoxidase has a heme pigment, which causes its green color in secretions rich in neutrophils, such as mucus and sputum. The green color contributed to its outdated name verdoperoxidase. [Provide by Wikipedia: Myeloperoxidase]

PRINCIPLE OF THE ASSAY

This MPO / Myeloperoxidase Assay Kit (Fluorometric) is a simple fluorometric assay that measures the amount of MPO present in cell lysate and tissue sample. This assay 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/em = 530/585 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.

MATERIALS PROVIDED & STORAGE INFORMATION

The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life of 6 months after receipt.

Component	Quantity	Storage information
Assay Buffer	10 mL	-20°C
Resorufin	1.5 mL	-20°C
20X MPO Inhibitor	120 µL	-20°C
Dye Reagent	120 µL	-20°C
3% stabilized H_2O_2	100 µL	-20°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Fluorescence microplate reader capable of reading excitation at 530 nm and emission at 585 nm
- Centrifuge and centrifuge tube
- Deionized or distilled water
- Black flat-bottom 96 well microplate
- Pipettes, pipette tips and multichannel micropipette reservoir

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Reagents are for research use only. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.
- This assay is based on a kinetic reaction. To ensure identical incubation time, addition of Working Reagent to samples should be quick and mixing should be brief but thorough. Use of a multi-channel pipettor is recommended. Assays can be executed at any desired temperature (E.g., 25°C or 37°C).
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

Tissue: prior to dissection, rinse tissue in 1X PBS (pH 7.4) to remove blood. Homogenize tissue (50 mg) with a Dounce homogenizer in ~200 µL cold 20 mM PBS, pH 7.4. Freeze the homogenized tissue at -80°C to lyse the cells. After freezing, thaw and centrifuge samples at 14,000 x g for 20 minutes at 4°C. Collection supernatant for assay.

Cell lysate: collect cells by centrifugation at 2,000 x g for 5 minutes at 4°C. For adherent cells, do not harvest cells using proteolytic enzymes; rather use a rubber policeman. Homogenize or sonicate cells in an appropriate volume of cold buffer containing 50 mM potassium phosphate (pH 7.5). Centrifuge at 14,000 x g for 10 minutes at 4°C. Collection supernatant for assay.

Note:

- Samples not measured the same day should be stored frozen, preferably at-20 to-80°C for at least one month.

REAGENT PREPARATION

- **1X MPO Inhibitor:** dilute 20X MPO Inhibitor with distilled water to 1X MPO Inhibitor. (E.g., Mix 10 µL of 20X MPO Inhibitor with 190 µL of distilled water)
- **Working Resorufin:** prepare 30 µM Resorufin Premix by mixing 15 µL of provided Resorufin and 235 µL of distilled water.
- **0.007% H_2O_2:** prepare 0.07% H_2O_2 by mixing 4.7 µL of 3% H_2O_2 with 195.3 µL of distilled water. Then to 0.007% H_2O_2 by mixing 60 µL 0.07% H_2O_2 with 540 µL distilled water. Use the 0.007% H_2O_2 within one hour.
- **Working Reagent:** for each reaction, mix 60 µL of Assay Buffer, 1 µL of 0.007% H_2O_2 and 1 µL of Dye Reagent.

ASSAY PROCEDURE

Prior to the assay, equilibrate all components to room temperature. Briefly centrifuge tubes before opening.

	H_2O well	Resorufin well	Inhibitor well	Sample well
Distilled water	100 µL			
Working Resorufin		100 µL		
Each Sample			20 µL	20 µL
1X MPO Inhibitor			20 µL	
Assay Buffer				20 µL
Incubate for **10 minutes** at **room temperature**.				
Working Reagent			60 µL	60 µL
Tap plate to mix briefly. Read the fluorescence intensity at **λex/em = 530/585 nm** at **0 minute** and **10 minutes** at **room temperature**.				

CALCULATION OF RESULTS

1. The MPO activity in a sample is calculated as follows:

   MPO Activity (U/L)

   = $[(\Delta R_{SAMPLE} - \Delta R_{Inhibitor}) / (R_{Resorufin} - R_{H2O})]$ x [Resorufin (µM) / t (min)] x (Reaction Vol / Sample Vol) x n

   = $[(\Delta R_{SAMPLE} - \Delta R_{Inhibitor}) / (R_{Resorufin} - R_{H2O})]$ x 15 x n

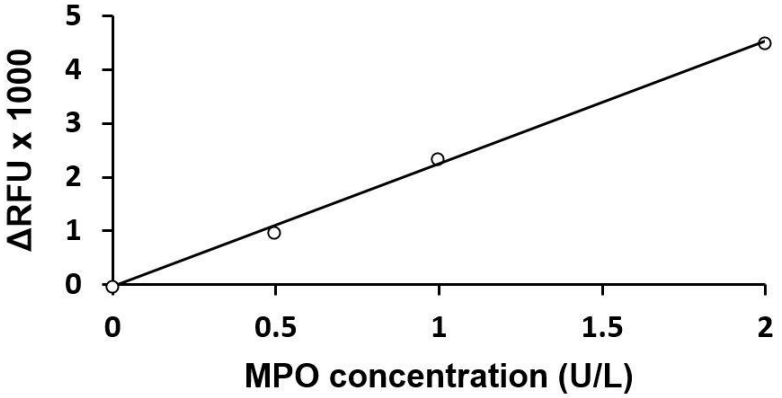
   Note:

   - R_{SAMPLE}, $R_{Inhibitor}$, $R_{Resorufin}$ and R_{H2O} : the fluorescence intensity values of Sample, Inhibitor, Resorufin and H_2O well, respectively.
   - $\Delta R_{SAMPLE} = R_{Sample,10min} - R_{Sample,0min}$ and $\Delta R_{Inhibitor} = R_{Inhibitor,10min} - R_{Inhibitor,0min}$.
   - [Resorufin] = 30 µM, Reaction Vol = 100 µL, Sample Vol = 20 µL, Reaction time (t) = 10 min.
   - 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, n.

2. Unit definition: one unit of enzyme will catalyze the formation of 1 µmole resorufin per min under the assay conditions.

EXAMPLE OF RESULT

The following figures demonstrate typical results with the MPO / Myeloperoxidase Assay Kit (Fluorometric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

0.0025 U/L