Product Manual

Myeloperoxidase Fluorometric Detection Kit

Catalog #: ADI-907-029

Kit for Detection of Myeloperoxidase Activity

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Product Manual

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Please read entire booklet before proceeding with the assay.

Please contact Enzo Life Sciences Technical Support if necessary.

INTRODUCTION

The Myeloperoxidase fluorometric detection kit is a complete it for the determination of Myeloperoxidase activity in neutrophils, macrophages, polymorphonuclear leukocytes, and tissue extracts. This kit provides a quick one step homogenous assay that is adaptable to kinetic and high throughput applications.

Myeloperoxidase (MPO) is a highly cationic glycosolated hemoprotein that has a molecular weight of 144kDa. The hemoprotein consists of two dimers linked via a disulfide bridge. Each dimer is composed of a heavy (53kDa) and light (15kDa) subunit. Each heavy chain contains an independently acting protoporphyrin group containing a central iron[1-4]. MPO is present in the azurophilic granules of polymorphonuclear leukocytes (PMNs) and is unique to neutrophils and monocytes. However, monocytes contain only one third of the MPO found in PMN's. MPO utilizes hydrogen peroxide produced by the neutrophils to oxidize a variety of aromatic compounds to give substrate radicals for bactericidal activity[4]. This enzyme is unique however in that it can oxidize chloride ions to produce a strong nonradical oxidant, HOCl. HOCl is the most powerful bactericidal produced by neutrophils[4]. Excessive production of these radicals can cause oxidative stress leading to oxidative tissue injury.

Applications:

- Detection of MPO activity in neutrophils and macrophages.
- Detection of PMN infiltration in tissue samples (inflammation and innate host defense mechanisms).
- Acute and chronic inflammatory disorders due to oxidative tissue damage.
- MPO activity in acute and chronic manifestations of cardiovascular disease.

PRINCIPLE

The MPO Fluorometric Detection Kit utilizes a non-fluorescent detection reagent, which is oxidized in the presence of hydrogen peroxide and MPO to produce its fluorescent analog.[5-13]

Reaction:

H_2O_2 + Detection reagent (non-fluorescent)+ MPO --> fluorescent analog

- Excitation 530-571nm
- Emission 590-600nm

MATERIALS SUPPLIED

1. **MPO Assay Buffer Concentrate**

   60 mL, Product No. 80-1706
   A 10X buffer concentrate

2. **Detection Reagent (ADHP)**

   One Lyophilized vial for 500 assays, Product No. 80-1703

3. **3% Hydrogen Peroxide**

   1 mL of a stabilized 3% solution, Product No. 80-1704

4. **Myeloperoxidase Enzyme**

   1 vial. See vial label for units of activity and concentration, Product No. 80-1707

STORAGE

All components of this kit are stable at 4°C until the kit's expiration date. Once a vial of the Detection Reagent (ADHP) is opened, it should be used promptly since it is subject to oxidation.

OTHER MATERIALS NEEDED

1. Dimethyl sulfoxide (DMSO)
2. Ethanol
3. Black 96-well plates (clear bottom optional for bottom reading instruments).
4. Fluorescence plate reader
5. Deionized water
6. NEM : N-Ethylmaleimide (Sigma Cat# E1271)
7. HTA-Br: hexadecyltrimethylammonium (Sigma Cat# H9151).
8. Catalase Inhibitor: 3-Amino-1,2,4-triazole (Sigma Cat# A8056).
9. Eosinophil peroxidase inhibitor (Sigma Cat# R5645).

REAGENT PREPARATION

1. **MPO Assay Buffer**

   Prepare a 1X working solution of the assay buffer by diluting 4ml of MPO Assay Buffer Concentrate in 36ml of deionized water. This can be stored at 4°C until the kit expiration date, or 6 months, whichever is earlier.

2. **Hydrogen Peroxide (H_2O_2)**

   Prepare a 20mM solution of H_2O_2 by diluting 22.7 µL of the 3% (0.88M) solution provided in 977 µL of the assay buffer. Once diluted, the H_2O_2 should be used promptly as it degrades rapidly. Make enough H_2O_2 for one days work, discard remaining solution.

3. **Detection reagent**

Avoid freeze / thaw cycles

   Prepare a 10 mM stock solution of the Detection Reagent by dissolving the contents of the vial in 500 µL DMSO. Once opened, this should be used promptly and any remaining reagent should be aliquoted and refrozen at -80°C. Avoid repeated freeze / thaw cycles.

4. **Myeloperoxidase**

   The Myeloperoxidase Enzyme can be diluted in the assay buffer to construct a standard curve (see below). Refer to the vial for the concentration of MPO.

5. **MPO Standard Curve**

   Prepare standard curve of MPO by serially diluting the enzyme in the assay buffer. Refer to the MPO vial for units of activity and concentration. Use the table below for the recommended Standard Curve concentration.

   If Catalase inhibitor is added to samples, add a final concentration of 20 mM of the inhibitor to the assay Buffer used to construct the Standard Curve. The Inhibitor tends to slightly inhibit MPO activity and will cause depressed sensitivity (see results in Typical Data).

6. Prepare 5ml reaction cocktail (for 100 assays) as follows:
   - 50 µl of Detection Reagent (50 µM final)
   - 5 µL of 20 mM Hydrogen peroxide
   - 4.875 mL of 1X assay buffer

   Optional: Eosinophil peroxidase inhibitor at 0.1-1.0 µM/well[13]. Make the inhibitor at double the concentration in the reaction cocktail as this will be diluted 1:2 in the well. High concentrations of Eosinophil Peroxidase inhibitor will slightly inhibit MPO activity.

SAMPLE HANDLING

Tissue Preparation

1. Prior to tissue extraction exsanguinate the animal to remove red blood cells from tissue. Homogenize tissue using standard techniques. 1X Assay Buffer may be used as the homogenization buffer.

   Optional: Add a final concentration of 10 mM NEM to the homogenization buffer. Tissue or cells that contain endogenous reductants (Glutathione: GSH) will interfere with the assay. Addition of NEM will block reductant interference and increase MPO yield.

2. Centrifuge the homogenates at 8000 – 12000 x g at 4°C for 15-20 minutes; remove the supernatant.
3. Add 1 mL of solubilization buffer to the pellet. Solubilization buffer can be prepared by making a 0.5% HTA-Br (w/v) solution in the assay buffer.
4. Homogenize and sonicate samples for 30 seconds. Submit samples to two cycles of freeze / thaw. Centrifuge at 8000 x g for 20 minutes at 4°C. The supernatant can be assayed immediately or frozen at –70°C for later use.

   Note: If catalase activity is present in the homogenates block by incubating homogenates with a final concentration of 20 mM 3-Amino-1,2,4-triazole (Sigma Cat# A8056) for 60 minutes prior to running assay.

Cell Preparation

Tissue or cells that contain endogenous reductants (Glutathione: GSH) will interfere with the assay. Addition of NEM will block reductant interference and increase MPO yield. If catalase activity is present in the cells, block by incubating cells, prior to solubilizing cells, with a final concentration of 20 mM 3-Amino-1,2,4-triazole (Sigma Cat# A8056). Incubate cells with inhibitor for 30 minutes at 37°C and wash cells to remove excess inhibitor.

1. Collect blood in heparin tubes and isolate PMN using standard techniques.
2. After PMN isolation, wash the PMS in PBS.
3. After the final wash, decant the supernatant and solubilize the cells in 1mL of ice cold 0.5% HTA-Br (w/v) (prepared in the assay buffer).
4. Supernatants are ready to assay for MPO activity. Samples may require additional dilution depending on activity; optimal dilution must be determined by the investigator.

ASSAY PROCEDURE

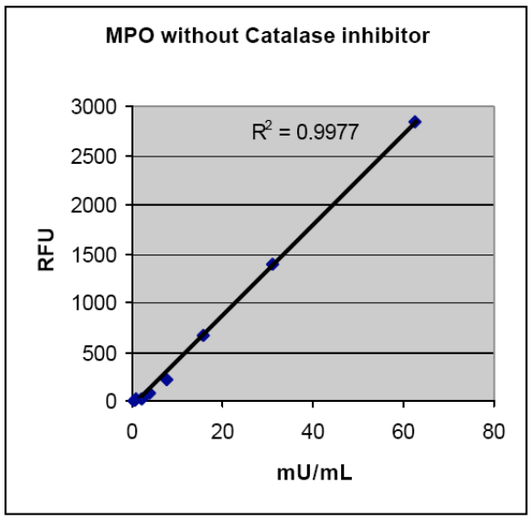
1. Pipette 50 µL of sample or standard to the bottom of a 96 well black plate.
2. Pipet 50 µL of the Reaction Cocktail to each well.
3. Incubate at room temperature in the dark for 30-60 minutes.
4. Measure the fluorescence at excitation: 530-570 nm and emission at 590-600 nm in a fluorescent plate reader.

TYPICAL RESULTS

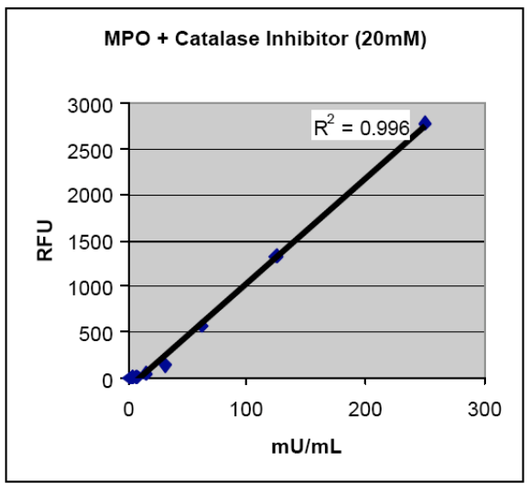
The results shown below are for illustration only and should not be used to calculate results from another assay.

MPO Standard Curve With and Without Catalase Inhibitor

MPO standard curves below were serially diluted in the assay buffer with and without a final concentration of 20 mM catalase inhibitor. 50 µL of MPO standard and 50 µL of RC was added to individual wells of a 96 well black plate. The plate was incubated at room temperature in the dark for 30 minutes. Next the wells were read using Ex: 530nm and Em: 590nm. There is approximately 50% reduction in signal in the presence of 20mM catalase inhibitor.



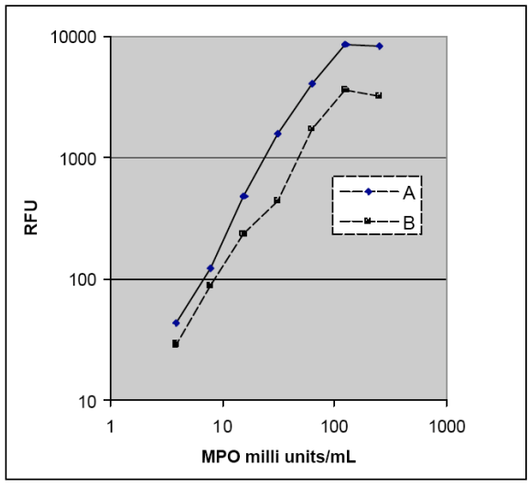
mU/mL	MPO RFU
62.5	2838
31.25	1401
15.625	668
7.8125	207
3.90625	71
1.953125	27
0.976563	14
0.488281	6
0.244141	4



mU/mL	MPO + Inhib RFU
250	2783
125	1401
62.5	567
31.25	143
15.625	46
7.8125	12
3.90625	6
1.953125	2

MPO DETECTION WITH AND WITHOUT EPO INHIBITOR

Reaction cocktail (RC) was prepared as described **A**: without EPO inhibitor and **B** with EPO inhibitor (100uM final). Next 50mL of MPO standard and 50 mL of RC was added to individual well of a 96 well black plate. The plate was incubated at room temperature in the dark for 30 minutes. Next the wells were read using Ex: 530nm and Em: 590nm. There is approximately 50% reduction in signal in the presence of 100mM EPO inhibitor.



CALCULATION OF RESULTS

Make sure to multiply sample concentrations by the dilution factor used during sample preparation.

Several options are available for the calculation of MPO activity in samples. We recommend that the data be handled by an immunoassay software package utilizing a 4 parameter logistic curve fitting program. If data reduction software is not readily available, the MPO activity can be calculated as follows:

1. Calculate the average net RFU for each standard and sample by subtracting the average 0 U/mL RFU from the average RFU for each standard and sample.
2. Using linear graph paper, plot the Average Net RFU for each standard versus MPO Activity (U/mL) in each standard. Approximate a line through the points. The concentration of the unknowns can be determined by interpolation.

Samples with concentrations outside of the standard curve range will need to be reanalyzed using a higher dilution.

Reaction cocktail was prepared as described A: without Eosinophil Peroxidase inhibitor and B with Eosinophil Peroxidase inhibitor (1µM final). There is approximately 50% reduction in signal in the presence of 1µM Eosinophil Peroxidase inhibitor.

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GLOBAL HEADQUARTERS
Enzo Life Sciences Inc.
10 Executive Boulevard
Farmingdale, NY 11735
Toll-Free:1.800.942.0430
Phone:631.694.7070
Fax: 631.694.7501
info-usa@enzolifesciences.com

EUROPE/ASIA
Enzo Life Sciences (ELS) AG
Industriestrasse 17
CH-4415 Lausen
Switzerland
Phone:+41/0 61 926 89 89
Fax:+41/0 61 926 89 79
info-ch@enzolifesciences.com

For local distributors and detailed product information visit us online:
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