Amplite™ Fluorimetric Myeloperoxidase Assay Kit

Red Fluorescence

Ordering Information:	*Storage Conditions:*	*Instrument Platform:*
Product Number: 11301 (200 assays)	Keep at -20°C and protect from light	Fluorescence microplate readers

Introduction

Myeloperoxidase (MPO), most abundantly present in neutrophils and monocytes, is a green hemoprotein having peroxidase activity. It catalyzes the reaction of hydrogen peroxide and halide ions to form cytotoxic acids and other intermediates; and plays an important role in the oxygen-dependent killing of tumor cells and microorganisms. MPO deficiency is a hereditary deficiency of the enzyme, which predisposes to immune deficiency. However, elevated MPO levels significantly increases the risk for cardiovascular mortality. The fact that circulating levels of MPO have been shown to predict risks for major adverse cardiac events and that levels of MPO-derived chlorinated compounds are specific biomarkers for disease progression, has attracted considerable interest in the development of therapeutic MPO inhibitors.

Our Amplite™ Fluorimetric Myeloperoxidase Assay Kit provides a quick and sensitive method for the measurement of myeloperoxidase in solution and in cell lysates. It can be performed in a convenient 96-well or 384-well microtiter-plate format and easily adapted to automation without a separation step. The kit uses our Amplite™ Red substrate which enables a dual recordable mode. The signal can be easily read by either a fluorescence microplate reader at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540 nm/590 nm) or an absorbance microplate reader at 576±5 nm. With the Amplite™ Myeloperoxidase Assay Kit, we have detected as little as 0.1 mU/mL myeloperoxidase in a 100 µL reaction volume.

Kit Components

Components	**Amount**
Component A: Amplite™ Red (light sensitive)	1 vial
Component B: Assay Buffer	20 mL
Component C: H_2O_2	100µL (3%)
Component D: Myeloperoxidase Standard	1 vial (10 mU, lyophilized)
Component E: DMSO	1vial (200 µL)

Assay Protocol for One96-Well Plate

Brief Summary

MPO standards or test samples (50 µL) → Add MPO assay mixture (50 µL) → Incubate at room temperature for 30-60 min → Read fluorescence intensity at Ex/Em = 540/590 nm (cut off 570 nm)

Note: Thaw all the kit components to room temperature before starting the experiment.

1. Prepare stock solutions:

1.1 Amplite™ Red stock solution (250X): Add 40 µL of DMSO (Component E) into the vial of Amplite™ Red substrate (Component A). The stock solution should be used promptly. Any remaining solution should be aliquoted and frozen at -20°C.
Note 1: Avoid repeated freeze-thaw cycles.
Note 2: The Amplite™ Red substrate is unstable in the presence of thiols such as dithiothreitol (DTT) and 2-mercaptoethanol. The final concentration of DTT or 2-mercaptoethanol in the reaction should be no higher than 10 µM. The Amplite™ Red substrate is also unstable at high pH (>8.5). Therefore, the reaction should be performed at pH 7–8. The provided assay buffer, pH 7.4, is recommended.

Ordering:sales@aatbio.com; Tel: 800-990-8053 or 408-733-1055; Fax: 408-733-1304
Technical Support:support@aatbio.com; Tel: 08-733-1055

1.2 10 mM H_2O_2 stock solution (500X): Add 10 µL of 3% H_2O_2 (0.88 M, Component C) into 870 µL of Assay Buffer (Component B).
Note: The diluted H_2O_2 solution is not stable. The unused portion should be discarded.

1.3 200 mU/mL myeloperoxidase stock solution: Add 50 µL of Assay Buffer (Component B) into the vial of Myeloperoxidase Standard (Component D).
Note: One vial contains approximately 5~10 mU myeloperoxidase. The unused MPO stock solution should be divided into single use aliquots and stored at -20°C.

2. Prepare assay mixture:

Prepare assay mixture according to Table 1 and protect from light.

Table 1. Assay mixture for one 96-well plate

Components	**Volume**
Amplite™ Red Stock Solution (250X, from Step 1.1)	20 µL
H_2O_2 (500X, from Step 1.2)	10µL
Assay Buffer (Component B)	5 mL
Total volume	5.03 mL

3. Prepare serially diluted MPO standards (0 to 10 mU/mL):

3.1 Add 20 µL of 200 mU/mL MPO stock solution (from Step 1.3) into 380 µL of Assay Buffer (Component B) to get 10 mU/mL MPO standard solution.

3.2 Take 150 µL of 10 mU/mL MPO standard solution to perform 1:3 serial dilutions to get 3, 1, 0.3, 0.1, 0.03, 0.01and 0 mU/mL serially diluted MPO standards.

3.3 Add MPO standards and/or MPO-containing test samples into a 96-well solid black microplate as described in Tables 2 and 3

Table 2. Layout of MPO standards and test samples in a 96-well solid black microplate

BL	BL	TS	TS	….	….						
MPO1	MPO1	….	….	….	….						
MPO2	MPO2										
MPO3	MPO3										
MPO4	MPO4										
MPO5	MPO5										
MPO6	MPO6										
MPO7	MPO7										

Note: MPO =myeloperoxidase standards, BL=blank control, TS = test samples.

Table 3. Reagent composition for each well

MPO Standard	**Blank Control**	**Test Sample**
Serial Dilutions* (50 µL)	Assay Buffer (Component B): 50 µL	50 µL

**Note 1: Add the serially diluted myeloperoxidase standards from 0.01 mU/mL to 10 mU/mL into each well from MPO1 to MPO7 in duplicate.*
Note 2: High concentration of MPO may cause reduced fluorescence signal due to the over oxidation of Amplite™ Red substrate (to a non-fluorescent product).

4. Run MPO assay:

4.1 Add 50 µL of assay mixture (from Step 2) into each well of the MPO standards, blank control, and test samples (see Step 3, Table 2) to make the total MPO assay volume of 100 µL/well.
Note: For a 384-well plate, add 25 µL of sample and 25 µL of assay mixture into each well.

4.2 Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.

4.3 Monitor the fluorescence intensity with a fluorescence plate reader at Ex/Em = 530-570 nm/590-600 nm (optimal Ex/Em = 540/590 nm, cut off = 570 nm).
Note: The contents of the plate can also be transferred to a white clear bottom plate and read by an absorbance microplate reader at the wavelength of 576±5 nm. The absorption detection has lower sensitivity compared to fluorescence reading.

5. Run Data Analysis:

The fluorescence in blank wells (with the assay buffer only) is used as a control, and is subtracted from the values for those wells with myeloperoxidase reactions. The typical data are shown in Figure 1 (MPO standard curve).
Note: The fluorescence background increases with time, thus it is important to subtract the fluorescence intensity value of the blank wells for each data point.

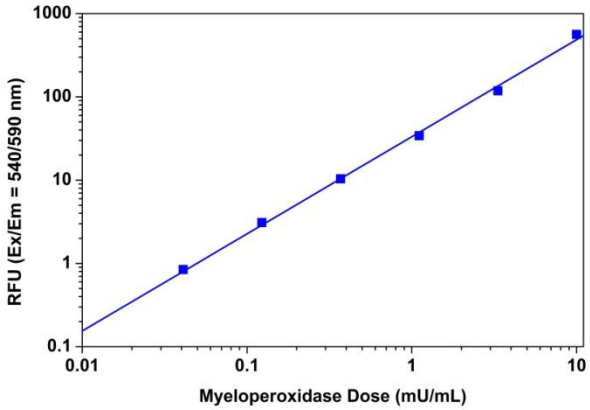

Figure 1. Myeloperoxidase dose response was measured with Amplite™ Fluorimetric Myeloperoxidase Assay Kit in a 96-well black solid plate using a Gemini fluorescence microplate reader (Molecular Devices). As low as 0.1 mU/mL myeloperoxidase was detected with 60 minutes incubation time (n=3).

References

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