Amplite™ Fluorimetric Myeloperoxidase Assay Kit *Red Fluorescence*

Catalog number: 11301
Unit size: 200 Tests

Component	Storage	Amount
Component A: Amplite™ Red (light sensitive)	Freeze (<-15 °C), Minimize light exposure	1 vial
Component B: Assay Buffer	Freeze (<-15 °C)	20 mL
Component C: H2O2	Refrigerate (2-8 °C), Minimize light exposure	100 µL (3%)
Component D: Myeloperoxidase Standard	Freeze (<-15 °C), Minimize light exposure	1 vial (10 mU, lyophilized)
Component E: DMSO	Freeze (<-15 °C)	1 vial (200 µL)

OVERVIEW

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. There are considerable interests in the development of therapeutic MPO inhibitors. Our Amplite™ 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 or an absorbance microplate reader. With the Amplite™ Myeloperoxidase Assay Kit, we have detected as little as 0.1 mU/ml myeloperoxidase in a 100 µL reaction volume. The kit can be automated for high throughput screenings of MPO inhibitors.

AT A GLANCE

Protocol summary

1. MPO standards or test samples (50 µL)
2. Add MPO working solution (50 µL)
3. Incubate at room temperature for 30 - 60 min
4. Read fluorescence intensity at Ex/Em = 540/590 nm (cut off 570 nm)

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

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	540 nm
Emission:	590 nm
Cutoff:	570 nm
Recommended plate:	Solid black

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

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.

Note 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.

2. H_2O_2 stock solution (500X, 10 mM):
Add 10 µL of 3% H_2O_2 (0.88M, 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.

3. Myeloperoxidase (MPO) standard solution (200 mU/mL):
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.

PREPARATION OF STANDARD SOLUTION

MPO standard

For convenience, use the Serial Dilution Planner:
https://www.aatbio.com/tools/serial-dilution/11301

Add 20 µL of 200 mU/mL MPO standard solution into 380 µL of Assay Buffer (Component B) to get 10 mU/mL MPO standard solution (MPO7). Take 10 mU/mL MPO standard solution to perform 1:3 serial dilutions to get remaining serially diluted MPO standards (MPO6 - MPO1).

PREPARATION OF WORKING SOLUTION

Add 20 µL of Amplite™ Red Stock Solution (250X) and 10 µL of H_2O_2 (500X) into 5 mL of Assay Buffer (Component B) to make a total volume of 5.03 mL MPO working solution. Protect from light.

SAMPLE EXPERIMENTAL PROTOCOL

Table 1. Layout of MPO standards and test samples in a 96-well solid black microplate. MPO= myeloperoxidase standards (MPO1 - MPO7, 0.01 to 10 mU/mL); BL=blank control; TS = test samples.

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

Table 2. Reagent composition for each well. Note that high concentration of MPO

may cause reduced fluorescence signal due to the over oxidation of Amplite™ Red substrate (to a non-fluorescent product).

Well	Volume	Reagent
MPO1 - MPO7	50 µL	Serial Dilution (0.01 to 10 mU/mL)
BL	50 µL	Assay Buffer (Component B)
TS	50 µL	test sample

1. Prepare myeloperoxidase standards (MPO), blank controls (BL), and test samples (TS) according to the layout provided in Tables 1 and 2. For a 384-well plate, use 25 µL of reagent per well instead of 50 µL.
2. Add 50 µL of MPO working solution to each well of myeloperoxidase standard, blank control, and test samples to make the total MPO assay volume of 100 µL/well. For a 384-well plate, add 25 µL of MPO working solution into each well instead, for a total volume of 50 µL/well.
3. Incubate the reaction for 30 to 60 minutes at room temperature, protected from light.
4. Monitor the fluorescence intensity with a fluorescence plate reader at Excitation = 530 - 570 nm, Emission = 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 that of the fluorescence reading.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Myeloperoxidase samples. We recommend using the Online Linear Regression Calculator which can be found at:

https://www.aatbio.com/tools/linear-logarithmic-semi-log-regression-online-calculator

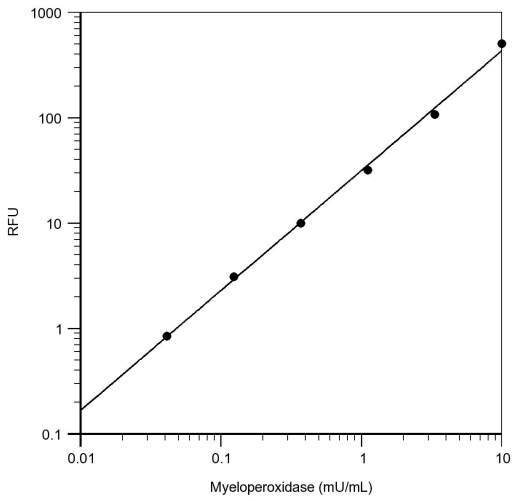

Figure 1. Myeloperoxidase dose response was measured with Amplite™ Fluorimetric Myeloperoxidase Assay Kit in a 96-well solid black plate using a Gemini fluorescence microplate reader (Molecular Devices).

DISCLAIMER