EnzChek® Myeloperoxidase (MPO) Activity Assay Kit

Table 1. Contents and Storage Information

Material	Amount	Concentration	Storage	Stability
3′-(*p*-aminophenyl) fluorescein (APF) (Component A) *	50 µL	5 mM solution in dimethylformamide (DMF)	• Store at 4°C • Desiccate • Protect from light	When stored as directed, the kit components are stable for at least 6 months.†
Amplex® UltraRed reagent (Component B) *	1 mg	NA		
Human myeloperoxidase (MPO) standard (Component C)	100 µL	10 µg/mL		
Chlorination inhibitor (Component D)	25 mg	NA		
Peroxidation inhibitor (Component E)	8 mg	NA		
Hydrogen peroxide (H_2O_2) (Component F)	0.5 mL	3% (882 mM) stabilized solution		
1X phosphate-buffered saline (PBS), pH 7.2 (Component G)	50 mL	1X		
Dimethyl sulfoxide (DMSO) (Component H)	0.5 mL	NA		

* APF and the Amplex® UltraRed reagent are somewhat air sensitive. Protect them from light. † The entire kit can be stored under the conditions listed. For optimal storage conditions of individual kit components, refer to the labels on the vials. NA = Not applicable.

Number of Assays: Each kit contains sufficient materials for performing 200 assays for chlorination activity and 200 assays for peroxidation activity in a 96-well fluorescence microplate format (100 µL per assay).

Spectral Data: Fluorescein ~490/515 nm. Amplex® UltraRed product ~568/581 nm.

Introduction

Myeloperoxidase (MPO, EC 1.11.1.7) is the most abundant protein in neutrophils and is also present in monocytes. In neutrophils, it is stored in azurophilic granules and released during phagocytosis. It is well established that MPO-derived oxidants damage cells and tissue.[1] Hypochlorous acid (HOCl), the major strong oxidant produced by neutrophils, oxidizes proteins, lipids, lipoproteins, and nucleic acids[2,3,4] with a multitude of pathological consequences.[5,6] MPO is also experimentally and clinically important for distinguishing myeloid from lymphoid leukemia and has been advocated as a prognostic marker of cardiovascular disease.[7]

The ferric, or native, MPO reacts with hydrogen peroxide (H_2O_2) to form the active redox and enzyme intermediate compound MPO-I, which oxidizes chloride (Cl^-) to HOCl (Figure 1). These reactions make up the chlorination cycle. MPO also oxidizes a variety of substrates, including phenols and anilines, via the classic peroxidation cycle (Figure 1). The relative concentrations of chloride and the reducing substrate (AH) determine whether MPO uses hydrogen peroxide for chlorination or peroxidation.[8] Assays based on measurement of chlorination activity are more specific for MPO than those based on peroxidase substrates such as tetramethylbenzidine (TMB).[8]

The EnzChek® Myeloperoxidase Activity Assay Kit provides assays for rapid and sensitive determination of both chlorination and peroxidation activities of MPO in solution and in cell lysates. For detection of chlorination, the kit provides nonfluorescent 3´-(*p*-aminophenyl) fluorescein (APF), which is selectively cleaved by hypochlorite (^{-}OCl) to yield fluorescein.[9] Peroxidation is detected using nonfluorescent Amplex® UltraRed reagent, which is oxidized by the H_2O_2-generated redox intermediates MPO-I and MPO-II to form a fluorescent product. The EnzChek® Myeloperoxidase Activity Assay Kit can be used to continuously detect these activities at room temperature over a broad dynamic range (1.5 to 200 ng/mL). The speed (30 minutes), sensitivity, and mix-and-read convenience make this kit ideal for measuring MPO activities and for high-throughput screening for MPO-specific inhibitors.

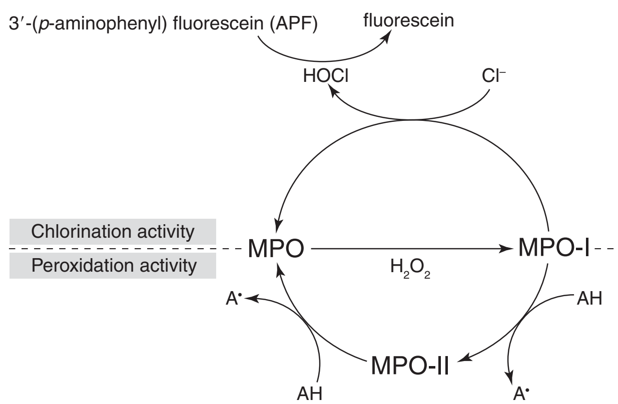

Figure 1. Schematic diagram for detection of chlorination and peroxidation activity of MPO using the EnzChek® Myeloperoxidase Activity Assay Kit. AH represents the nonfluorescent Amplex® UltraRed substrate, and A· represents its fluorescent oxidation product.

Before You Begin

Materials Required But Not Provided

- 96-well microplates
- Methanol

Assay Limitations

Chlorination and peroxidation activity cannot be measured simultaneously in the same sample using APF and Amplex® UltraRed reagent. Each assay for chlorination *or* peroxidation requires 50 µL of diluted cell lysate (see protocol step 2.1).

The kit is designed for detection of MPO activity in solution and cell lysates, not in serum samples.

Solution Preparation

Allow all kit reagents to warm to room temperature before opening the vials.

1.1 Prepare a **fresh** 5 mM H_2O_2 solution for each experiment by adding 5 µL of Component F to 870 µL of 1X PBS (Component G). Component F is provided in sufficient quantity for multiple preparations of 5 mM H_2O_2. Do not store unused quantities of 5 mM H_2O_2 for more than a day. Discard any unused 5 mM H_2O_2 solution after a day.

1.2 Prepare 100X Amplex® UltraRed stock solution by dissolving the contents of the vial supplied (Component B) in 0.34 mL of DMSO (Component H). To prepare sufficient 2X Amplex® UltraRed reagent working solution for the analysis of 20 samples, add 20 µL of 100X Amplex® UltraRed stock solution and 4 µL of 5 mM H_2O_2 to 976 µL of 1X PBS. Store unused portions of the 100X Amplex® UltraRed stock solution in the dark at –20°C.

1.3 Make a 2X APF working solution by mixing 4 µL of Component A and 4 µL of 5 mM H_2O_2 into 992 µL of 1X PBS. This working solution is sufficient to assay 20 samples.

1.4 Add 2 mL of 1X PBS to the vial of chlorination inhibitor (Component D) to make a 10X solution. The solution can be stored at –20°C when not in use.

1.5 Add 5 mL of methanol to the vial of peroxidation inhibitor (Component E) to make a 10X solution. The solution can be stored at –20°C when not in use.

Experimental Protocols

Preparing Cell Lysates

2.1 Prepare clarified cell lysates by any standard method (e.g., freeze/thaw), and dilute samples to a total of 50 µL each in 1X PBS.

2.2 To prepare inhibited control samples for the cell lysates, include 10 µL of 10X chlorination inhibitor (for APF assays) or 10X peroxidation inhibitor (for Amplex® UltraRed assays) in the 50 µL sample volume.

2.3 Follow steps 4.2–4.6 for the chlorination assay or 5.2–5.6 for the peroxidase assay.

Preparing MPO Standards

3.1 Prepare 125 µL of 200 ng/mL MPO by adding 2.5 µL of Component C to 122.5 µL of 1X PBS.

3.2 In a column of 8 wells (e.g., wells A1–H1), make a dilution series with 200, 100, 50, 25, 12.5, 6.25, 3.13, and 0 ng/mL MPO in 1X PBS, with 50 µL in each well.

Chlorination Activity Assay

4.1 Pipet 50 µL of each experimental sample or MPO standard into wells of a 96-well microplate.

4.2 Using a multichannel pipet, add 50 µL of 2X APF working solution to all sample and standard wells.

4.3 Incubate the microplate at room temperature for up to 30 minutes, protected from light. The assay is continuous, so fluorescence may be measured (see step 4.4) at multiple time points. The reactions can be stopped at any time point, if desired, by adding 10 µL of 10X chlorination inhibitor to all of the samples and the standards.

4.4 Measure the fluorescence intensity of each sample using excitation at 485 nm and emission at 530 nm.

4.5 Subtract the fluorescence intensity of the zero-MPO standard from all of the experimental samples and standards.

4.6 Determine the MPO concentration of the experimental samples from your own standard curve. Figure 2 shows a typical standard curve for this assay.

Peroxidation Activity Assay

5.1 Pipet 50 µL of each experimental sample or MPO standard into wells of a 96-well microplate.

5.2 Using a multichannel pipet, add 50 µL of 2X Amplex® UltraRed reagent working solution to all sample and standard wells.

5.3 Incubate the microplate at room temperature for up to 30 minutes, protected from light. The assay is continuous, so fluorescence may be measured (see step 5.4) at multiple time points. The reactions can be stopped at any time point, if desired, by adding 10 µL of 10X peroxidation inhibitor to all of the samples. Add the inhibitor to the standards as well.

5.4 Measure the fluorescence intensity of each sample using excitation at 530 nm and emission at 590 nm.

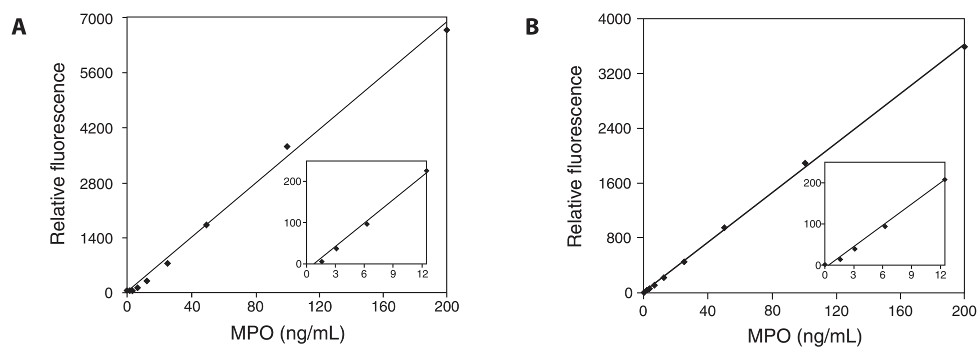

Figure 2. Typical standard curves for detection of MPO using the APF-based chlorination assay (panel A) and Amplex® UltraRed–based peroxidation assay (panel B). Reactions were incubated at room temperature for 30 minutes. Values on the x-axes are concentrations of MPO in the standards prior to adding the detection reagent. Fluorescence was measured with a fluorescence microplate reader using fluorescence excitation and emission at 485 and 530 nm, respectively, for the APF assay, or excitation and emission at 530 and 590 nm, respectively, for the Amplex® UltraRed assay. The background fluorescence measured for each zero-MPO control reaction was subtracted from each fluorescence measurement before plotting.

5.5 Subtract the fluorescence intensity of the zero-MPO standard from all of the experimental samples and standards.

5.6 Determine the MPO concentration of the experimental samples from your own standard curve. Figure 2 shows a typical standard curve for this assay.

References

1. Toxicology 181-182, 223 (2002); **2.** Am J Physiol 267, H1597 (1994); **3.** Arterioscler Thromb Vasc Biol 20, 1716 (2000); **4.** J Biol Chem 278, 23522 (2003); **5.** Annu Rev Med 46, 193 (1995); **6.** Physiol Rev 84, 138 (2004); **7.** N Engl J Med 349, 1595 (2003); **8.** Methods Enzymol 233, 502 (1994); **9.** J Biol Chem 278, 3170 (2003).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat #	Product Name	Unit Size
E33856	EnzChek® Myeloperoxidase (MPO) Activity Assay Kit *400 assays* *for myeloperoxidase chlorination and peroxidation activity*	1 kit
A36003	3'-(*p*-aminophenyl) fluorescein (APF) *5 mM solution in DMF*	470 µL
A36006	Amplex® UltraRed reagent	5 x 1 mg

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