Zen™ Myeloperoxidase (MPO) ELISA Kit

Table 1. Contents and Storage Information.

Material	Amount	Concentration	Storage	Stability
Amplex® UltraRed reagent (Component A)	2 vials	NA	• 2–6°C • Desiccate • Protect from light	When stored as directed, the kit components are stable for at least 6 months.*
Dimethylsulfoxide (DMSO) (Component B)	200 µL	NA		
10X phosphate-buffered saline (PBS), pH 7.2 (Component C)	80 mL	NA		
Goat anti–rabbit IgG, horseradish (HRP) conjugate (Component D)	100 µg	NA		
Amplex® stop reagent (Component E)	25 mg	NA		
Hydrogen peroxide (H_2O_2), stabilized (Component F)	100 µL	~3% solution		
MPO standard (Component G)	100 µL	10 µg/mL		
Bovine serum albumin (BSA) (Component H)	1.2 g	NA		
Tween 20 (Component I)	1 mL			
Mouse anti–MPO antibody (primary capture antibody) (Component J)	200 µL	50 µg/mL		
Rabbit anti–MPO antibody (secondary capture antibody) (Component K)	2 mL	10 µg/mL		
Zen™ microplates (Component L)	2 plates	NA		

* 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.

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

Introduction

Myeloperoxidase (MPO, EC 1.11.1.7) is a lysosomal hemeoprotein located in the azurophilic granules of polymorphonuclear (PMN) leukocytes and monocytes. It is a dimeric protein composed of two 59 kDa and two 13.5 kDa subunits.[1] MPO is a unique peroxidase that catalyzes the conversion of hydrogen peroxide (H_2O_2) and chloride to hypochlorous acid, the major strong oxidant with powerful antimicrobial activity and broad-spectrum reactivity with biomolecules.[1,2] MPO has been considered as an important marker for inflammatory and autoimmune diseases and cancer.[1,2,3,4] Due to its role in the pathology of atherogenesis, MPO is also thought to be an important cardiac biomarker.

The Zen™ Myeloperoxidase (MPO) ELISA Kit provides a comprehensive set of components for accurate and sensitive quantitation of MPO in a variety of biological samples, including human serum. The assay is based on Amplex® UltraRed reagent, a fluorogenic substrate for horseradish peroxidase (HRP) that reacts with H_2O_2 in a 1:1 stoichiometric ratio to produce the Amplex® UltraRed product, a brightly fluorescent and strongly absorbing product (excitation/emission maxima ~568/581 nm) (Figure 1). Because the Amplex® UltraRed product has long-wavelength emission, there is little interference from the blue or green autofluorescence found in most biological samples. With a high extinction coefficient, good quantum efficiency, and resistance to autooxidation, the fluorescence-based Amplex® UltraRed reagent delivers better sensitivity and a broader assay range than colorimetric reagents. Each kit contains sufficient reagents for ~200 assays in a microplate format, using a 100 µL per well reaction volume. The microplate-based formulation provides speed and convenience yet is highly sensitive, and it can be used to detect MPO over a broad dynamic range (0.2 to 100 ng/mL) at room temperature.

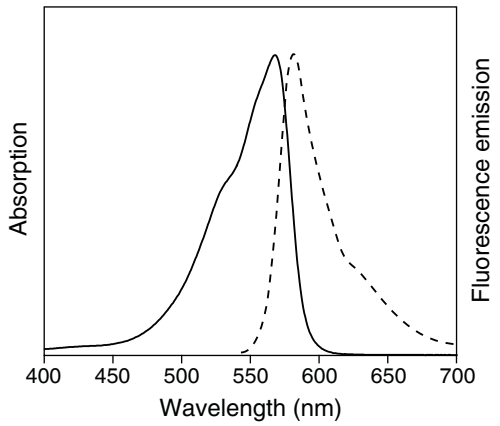

Figure 1. Normalized absorption and fluorescence emission spectra for the Amplex® UltraRed reaction product.

Before You Begin

The quantities of solutions and reagents given in *Solution Preparation* are sufficient for ~100 assays, or one 96-well microplate. To run smaller numbers of assays in a partial plate, solution and reagent quantities may be scaled down, or solutions can be stored as indicated.

Solution Preparation

Before opening any vial in this kit, allow its contents to warm to room temperature.

Caution: Components B, D, and E are irritants, and Component E is a potential mutagen. Follow safe laboratory practices, and handle these chemicals with appropriate precautions.

1.1 Prepare 500 mL of 1X PBS by adding 50 mL of 10X PBS (Component C) to 450 mL of distilled and deionized water. This 1X PBS will be used in the preparation of other buffers, as well as in the final Amplex® UltraRed reaction.

1.2 Prepare 300 mL of 1X PBST by adding 300 µL of Tween 20 (Component I) to 300 mL of 1X PBS. Shake well to mix. This solution is sufficient for 100 assays using the protocol described here. Solution left over can be stored at 4°C for future assays.

1.3 Prepare 100 mL of 1X PBS-BSA by adding 1 g of BSA (Component H) to 100 mL of 1X PBS. Dissolve completely. Store at 4°C when not in use.

1.4 Prepare 50 mL of 0.1X PBS-BSA by mixing 5 mL of 1X PBS-BSA with 45 mL of 1X PBS.

1.5 Dilute the MPO standard (component G) 1:100 in 0.1X PBS-BSA to make a 100 ng/mL MPO stock solution. Divide this MPO stock solution into aliquots (e.g., 500 µL) and store at 4°C when not in use.

1.6 Reconstitute the goat anti–rabbit IgG HRP conjugate (Component D) by adding 0.5 mL of 1X PBS-BSA directly to the Component D vial, to create a 200 µg/mL stock solution. This stock solution may be stored at 4°C after adding thimerosal (not included in the kit) to a final concentration of 0.02%. Alternatively, the stock solution can be frozen, without thimerosal.

1.7 Prepare an Amplex® UltraRed reagent solution by adding 60 µL of DMSO (Component B) to one vial of Amplex® UltraRed reagent (Component A). Vortex well to dissolve. Protect this solution from light and store at –20°C when not in use.

1.8 Prepare 10 mL of Amplex® stop solution by adding 1 mL of 1 M NaOH to the vial of Amplex® stop reagent (Component E), and then once it is fully dissolved, adding it to 9 mL of 1X PBS. This solution is stable for one month at 4°C when protected from light. Discard this solution if it starts to turn amber in color.

Experimental Protocol

The following protocol describes a typical sandwich ELISA in 96-well format designed for use with a fluorescence microplate reader. This protocol is provided for your convenience but may be replaced with any standard sandwich ELISA protocol at your discretion. However, steps 2.9–2.15 of this protocol should be followed as written.

2.1 Hydrate the Zen™ microplate plate by dispensing 200 µL 1X PBST into each well. Incubate the plate on a plate shaker at room temperature for 5 minutes at ~500 rpm.

2.2 Following the hydration, empty the wells. The wells can be emptied by simply inverting the plate over a sink or waste receptacle. For optimal assay sensitivity, remove as much fluid as possible (for example, by pipetting the fluid out carefully, without touching the bottoms of the wells). As a final measure, invert the plate on a paper towel, and firmly tap the plate to remove any remaining buffer.

2.3 Prepare a 500 ng/mL solution of the mouse anti-MPO primary capture antibody. For 100 assays, make 10 mL by adding 100 µL of Component J to 10 mL of 0.1X PBS-BSA.

For each sample, dispense 100 µL of the primary capture antibody solution into a well of the Zen™ plate. Incubate the plate on a plate shaker for 1 hour at ~500 rpm at room temperature.

2.4 Near the end of the 1 hour incubation, prepare a series of MPO standards from the MPO stock solution made in step 1.5 and using 0.1X PBS-BSA as a diluent, ranging from 100 ng/mL to 0 ng/mL. We recommend 8 to 10 MPO concentrations—e.g., 100, 50, 25, 12.5, 6.25, 3.125, 1.5, 0.75, 0.375, and 0 ng/mL—and duplicates for each concentration. Each assay well uses 100 µL of MPO-containing sample or standard (see step 2.6), so one 500 µL aliquot of the 100 ng/mL MPO stock solution is sufficient to make duplicate sets of the concentrations listed above. Keep the diluted standards on ice until step 2.6.

2.5 Dilute your samples (sera or cell lysates) 1:1, 1:10, 1:100, and 1:1,000 in 0.1X PBS-BSA. The goal is to obtain at least one dilution that contains an amount of MPO that falls within the dynamic range of the assay. If you know from past experience the approximate MPO content of samples that you work with often, the samples can be diluted at different ratios at your discretion.

2.6 Following the 1 hour incubation, empty the wells and wash three times with 200 µL of 1X PBST. After the final wash, invert the plate over a paper towel and firmly tap the plate to remove any solution from the wells.

Dispense 100 µL each of the MPO standards and diluted samples into microplate wells. Incubate on a plate shaker for 1 hour at ~500 rpm at room temperature.

2.7 Empty the wells and wash them three times with 200 µL of 1X PBST. After the final wash, invert the plate on a paper towel, and firmly tap the plate to remove any remaining buffer from the wells.

2.8 Prepare a 1.0 µg/mL solution of the rabbit anti-MPO secondary capture antibody. For 100 assays, make 10 mL by adding 1 mL of Component K to 9 mL of 0.1X PBS-BSA.

Dispense 100 µL of the secondary capture antibody solution into each assay well. Incubate on a plate shaker for 30 minutes at ~500 rpm at room temperature.

2.9 Empty the wells and wash them three times with 200 µL of 1X PBST. After the final wash, invert the plate on a paper towel, and firmly tap the plate to remove any remaining buffer from the wells.

2.10 Make 10 mL of 100 ng/mL goat anti–rabbit IgG-HRP by adding 5 µL of the goat anti–rabbit IgG-HRP stock solution (prepared in step 1.6) to 10 mL of 0.1X PBS-BSA.

Add 100 µL of this solution to each microplate well. Incubate on a plate shaker for 30 minutes at ~500 rpm at room temperature.

2.11 Empty the wells and wash them three times with 200 µL of 1X PBST. The stringency of the assay may be adjusted by washing more or fewer times with 1X PBST, or by agitating the 1X PBST in the wells during the wash steps. After the final wash, empty the wells, invert the plate on a paper towel, and firmly tap the plate to remove any remaining buffer from the wells.

To obtain optimal sensitivity from the assay, **protect the plate from light after the final wash**. Antibodies exposed to UV light can produce trace amounts of singlet oxygen, which can interfere with detection of Amplex® UltraRed reagent.

2.12 Make 10 mL of reagent mix by adding 50 µL of the Amplex® UltraRed reagent stock solution (prepared in step 1.7) and 22.7 µL of 3% H_2O_2 (Component F; check the label for the actual H_2O_2 concentration, and adjust as necessary) to 10 mL of 1X PBS. Protect the reagent mix from light and use it within 1 hour.

2.13 Using a multichannel pipet, add 100 µL of the reagent mix to each assay well. This initiates the detection reaction.

2.14 Incubate the plate at room temperature or 37°C, protected from light until the fluorescence measurement is taken. For most reactions, a 30 minute incubation is sufficient. The plate can also be read continuously for up to an hour. If desired, 20 µL of Amplex® stop solution (prepared in step 1.8) may be added to each assay well. This will arrest the reactions, providing a stable signal that may be read for at least 2 hours if the plate is protected from light and kept at room temperature.

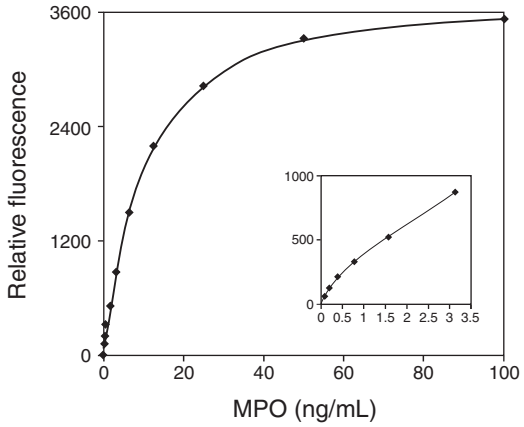

Figure 2. Typical standard curve for detection of MPO using the Zen™ Myeloperoxidase (MPO) ELISA Kit. The sandwich ELISA was carried out as described in the protocol using a mouse anti-MPO primary capture antibody, MPO standards ranging from 0.2 ng/mL to 100 ng/mL, and a rabbit anti-MPO secondary capture antibody.

2.15 Read the fluorescence of the microplate wells using filters for 530 nm (excitation) and 590 nm (emission).

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

References

1. Methods Enzymol 233, 502 (1994); **2.** Am J Physiol 267, H1597 (1994); **3.** Inflammmation: Basic Principles and Clinical Correlates, p. 391. Raven Press, New York (1988); **4.** Annu Rev Med 46, 193 (1995).

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

Cat. no.	Product Name	Unit Size
Z33857	Zen™ Myeloperoxidase (MPO) ELISA Kit *200 assays*	1 kit
E33856	EnzChek® Myeloperoxidase (MPO) Activity Assay Kit *400 assays* *for myeloperoxidase chlorination and peroxidation activity*	1 kit
A33851	Amplex® ELISA Development Kit for Mouse IgG *with Amplex® UltraRed reagent* *500 assays*	1 kit
A33852	Amplex® ELISA Development Kit for Rabbit IgG *with Amplex® UltraRed reagent* *500 assays*	1 kit
A36006	Amplex® UltraRed reagent	5 x 1 mg
A33855	Amplex® Red/UltraRed stop reagent *500 tests* *set of 5 vials*	1 set

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