Enzyme Immunoassay for
Human Myeloperoxidase

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INTRODUCTION

Human myeloperoxidase (MPO) is a hemoprotein composed of two heme-containing heavy subunits of 53 kDa and of two light subunits of 15 kDa. MPO is stored in the granules of neutrophils and monocytes, and is released in response to leukocyte activation. MPO acts as a catalyst in the production of hypochlorous acid (HOCl), a powerful oxidant produced from chloride ion (Cl^-) and hydrogen peroxide (H_2O_2). Although MPO is important to the immune system, it also plays a role in several inflammatory conditions.

Elevated MPO levels have been linked to coronary artery diseases. High concentrations of MPO and its oxidation products are found in areas of plaque deposits present in atherosclerosis. MPO also affects cholesterol levels through the oxidation of LDL and HDL.

As a specific marker for polymorphonuclear cells (PMNs), MPO is released extracellularly via degranulation after PMN activation. Thus, extracellular MPO is an index of PMN activation in inflammatory processes that lead to disease pathology, such as cardiovascular disease. Elevated levels of MPO present in serum and plasma indicate a greater risk of myocardial injury and unstable plaque formations. Therefore, MPO levels can be used to predict potentially negative cardiac events.

Antibodies to MPO are present in human plasma, and have been characterized as part of the anti-neutrophil cytoplasmic antibodies (ANCA) set of autoantibodies.

PRINCIPLES OF PROCEDURE

The Human MPO EIA Kit is a sandwich ELISA (Enzyme-Linked Immunosorbent Assay) that measures MPO in plasma/serum samples. The MPO in the sample interacts with and binds to the anti–MPO antibody coated on the wells. The plate is then washed to remove all unbound materials and anti–MPO, conjugated with HRP (horseradish peroxidase), is added to the plate wells. The HRP conjugate binds to the MPO that is bound to the anti–MPO of the plate. After washing the plate again, TMB is added, which will produce a blue hue. A darker blue color corresponds to a higher concentration of MPO. After reaction has occurred with the conjugate a stop solution is added, producing a yellow hue. The absorbance is measured at 450 nm.

MATERIALS PROVIDED

Component	Description	Volume	Storage	Cat. No.
5x EIA Buffer	Buffer used to dilute the conjugate, standard, and samples.	50 mL	4°C	MP14a
20x Wash Buffer	Buffer used to wash the plate.	50 mL	4°C	MP14b
Stop Solution	0.3 M sulfuric acid for stopping the reaction.	12 mL	4°C	MP14c
TMB	TMB substrate used for color development.	12 mL	4°C	MP14d
Anti-MPO-HRP	Anti-MPO horseradish peroxidase conjugate.	200 µL	4°C	MP14e
MPO Standard	Lyophilized human myeloperoxidase.	1 vial	4°C	MP14f
Coated Plate	96-well microplate coated with an anti-Hu MPO antibody.	1 plate	4°C	MP14g

MATERIALS NEEDED BUT NOT PROVIDED

1. Microplate reader with a 450 nm filter
2. Adjustable micropipettes (10 – 1000 μL) and tips
3. Deionized water
4. Vortex
5. Plate cover or film
6. Plate shaker/rotator

STORAGE

1. Store the components of this kit at the temperatures specified on the labels.
2. Unopened reagents are stable until the indicated kit expiration date.
3. Store diluted reagents at 4°C and use within one week.
4. Desiccant packet must remain in the pouch with unused strips. Keep pouch sealed when not in use to maintain a dry environment. Remove excess air before sealing.

WARNINGS AND PRECAUTIONS

1. Use aseptic technique when opening and dispensing reagents.
2. The MPO Standard has been purified from human source material. Necessary precautions should be taken when handling, treating it as potentially infectious.
3. This kit is designed to work properly as provided and instructed. Additions, deletions or substitutions to the procedure or reagents are not recommended, as they may be detrimental to the assay.

PROCEDURAL NOTES

1. Allow kit components to come to room temperature before use.
2. The HRP conjugate is most stable in its concentrated form. Dilute only the volume necessary for the amount of strips currently being used.
3. After reconstitution of the MPO Standard, aliquots should be made and frozen at -80°C. Avoid multiple freeze/thaw cycles.
4. To minimize errors in absorbance measurements due to handling, wipe the exterior bottom of the microplate wells with a lint-free paper towel prior to inserting into the plate reader.

SAMPLE COLLECTION AND STORAGE

This ELISA is meant for the measurement of myeloperoxidase in human serum and plasma.

1. Draw blood into a serum separator tube for serum or a heparin tube for plasma and mix gently.
2. Centrifuge whole blood within 6 hours of draw at 3000 x *g* for 10 minutes at 4ºC.
3. Remove the serum supernatant. Samples can be stored at 4ºC for 24 hours. For longer storage, samples should be stored at -20ºC or -80ºC. Avoid repeated freeze/thaw cycles.

SAMPLE PREPARATION

Thaw samples completely. Dilute the samples in 1x EIA Buffer. 1:4 and 1:10 are recommended.

REAGENT PREPARATION

1. **5x EIA Buffer:** Dilute the appropriate amount to 1x with deionized water prior to use.
2. **20x Wash Buffer:** Add 50 mL of 20x Wash Buffer to 950 mL of deionized water prior to use.
3. **Anti-MPO-HRP:** Dilute 1:100 - 100 μL of Conjugate into 9.9 mL total volume of 1x EIA Buffer.
4. **MPO Standard:** Reconstitute with 1 mL deionized water.

STANDARD CURVE PREPARATION

The MPO Standard is provided as a lyophilized powder, and needs to be reconstituted with 1 mL of deionized water prior to use. Use the following table to construct a six-point standard curve.

Table 1: Standard Curve Preparation

Standard	MPO Conc. (ng/mL)	Vol. of 1x EIA Buffer (μL)	Transfer Volume (μL)	Transfer Source	Final Volume (μL)
S_5	30	538	62	Stock	300
S_4	15	300	300	S_5	300
S_3	7.5	300	300	S_4	300
S_2	3.75	300	300	S_3	300
S_1	1.875	300	300	S_2	600
B_0	0	300	-	-	300

ASSAY PROCEDURE

1. Add 100 μL of Standards and Samples to the corresponding wells on the microplate in duplicate. See **Scheme I** for a sample plate layout. Cover and incubate at room temperature for one hour.
2. Wash the plate four times with 300 μL of 1x Wash Buffer per well.
3. Add 100 μL of diluted Anti-MPO-HRP to each well. Cover and incubate at room temperature for 20 minutes.
4. Wash the plate four times with 300 μL of 1x Wash Buffer per well.
5. Add 100 μL of TMB to each well. Place covered plate on a plate shaker and incubate at room temperature for 10 minutes.
6. Add 100 μL of Stop Solution to each well.
7. Read the plate at 450 nm.

Scheme I: Sample Plate Layout

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
A	B_0	B_0	U_3	U_3	U_{11}	U_{11}	U_{19}	U_{19}	U_{27}	U_{27}	U_{35}	U_{35}
B	S_1	S_1	U_4	U_4	U_{12}	U_{12}	U_{20}	U_{20}	U_{28}	U_{28}	U_{36}	U_{36}
C	S_2	S_2	U_5	U_5	U_{13}	U_{13}	U_{21}	U_{21}	U_{29}	U_{29}	U_{37}	U_{37}
D	S_3	S_3	U_6	U_6	U_{14}	U_{14}	U_{22}	U_{22}	U_{30}	U_{30}	U_{38}	U_{38}
E	S_4	S_4	U_7	U_7	U_{15}	U_{15}	U_{23}	U_{23}	U_{31}	U_{31}	U_{39}	U_{39}
F	S_5	S_5	U_8	U_8	U_{16}	U_{16}	U_{24}	U_{24}	U_{32}	U_{32}	U_{40}	U_{40}
G	U_1	U_1	U_9	U_9	U_{17}	U_{17}	U_{25}	U_{25}	U_{33}	U_{33}	U_{41}	U_{41}
H	U_2	U_2	U_{10}	U_{10}	U_{18}	U_{18}	U_{26}	U_{26}	U_{34}	U_{34}	U_{42}	U_{42}

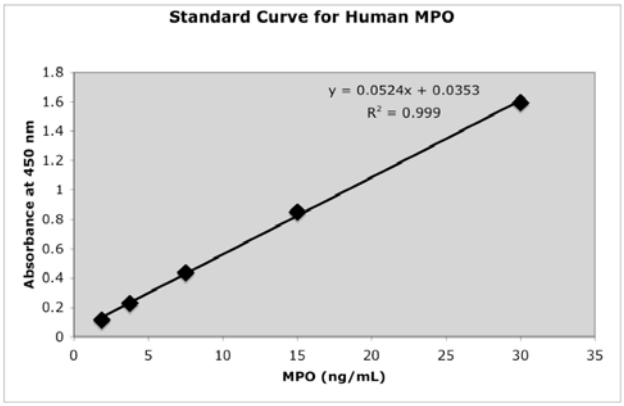
CALCULATIONS

1. Subtract the average background absorption (B_0) from each of the mean absorbance values (A_{450}) for the standards and samples.
2. Graph the standard curve by plotting the A_{450} for each standard concentration on the y-axis against the MPO concentration on the x-axis. Draw the curve by using a linear regression curve-fitting routine. Most programs can do this for you. See **Figure 1** for a sample standard curve.
3. Using the standard curve and the resulting linear regression equation, the concentration of MPO in each sample can be determined using the A_{450} of each sample.

Linear Regression Equation: $y = mx + b$
Where: $y = A_{450}$ x = MPO Concentration
m = slope b = y-axis intercept

4. If the samples were diluted, the concentration determined from the standard curve must be multiplied by the dilution factor.

Figure 1: Typical Standard Curve

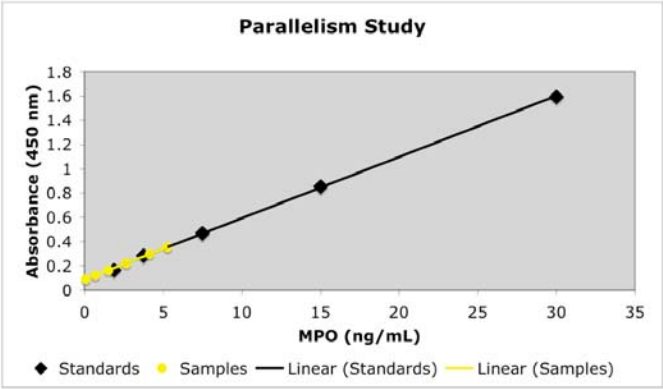


NOTES

1. The normal ranges for serum samples and heparin–plasma samples are 2.5–100 ng/mL and 37-124 ng/mL, respectively.
2. Badly hemolyzed serum or plasma samples should not be used, as they may give inaccurate results.
3. Insufficient washing will result in poor precision and false absorbance readings.

PERFORMANCE CHARACTERISTICS

Parallelism:
Performed using a sequence of serial dilutions.



Standards	Sample
y = 0.0505x + 0.0869	y = 0.0505x + 0.0869

Recovery:
Sample was diluted 1:10 in 1x EIA Buffer prior to starting the assay. Three analyzed volumes of MPO were added to dispensed volumes of the diluted sample.

	% Recovery
Concentration 1	83
Concentration 2	90
Concentration 3	180

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