

INTRODUCTION

Myeloperoxidase (MPO) is predominantly expressed in neutrophils. At sites of infection or inflammation, neutrophils degranulate and release MPO. By virtue of its peroxidase activity, it generates anti-microbial hypochlorous acids (ref 1). In humans it serves as a biomarker of infectious diseases and inflammatory conditions (ref 1).

PRINCIPLE OF THE ASSAY

The assay uses two mouse monoclonal antibodies developed at Life Diagnostics. MPO-20-4D1 is used as coating antibody and HRP conjugated MPO-20-7A8 is used for detection. Standards and diluted samples (100 μ l) are incubated in anti-MPO coated microtiter wells for 45 minutes. After washing the wells, HRP-conjugate (100 μ l) is added and incubated for 45 minutes. If MPO molecules are present, they are sandwiched between the capture and detection antibodies. The wells are then washed to remove unbound HRP-conjugate. TMB is added and incubated for 20 minutes. If MPO is present, a blue color develops. Color development is stopped after 20-minutes by addition of Stop Solution, changing the color to yellow. Absorbance is measured at 450 nm. The concentration of MPO is proportional to absorbance and is derived from a standard curve.

MATERIALS**Materials provided with the kit:**

- Anti-MPO coated plate (12 x 8-well strips)
- Anti-MPO HRP conjugate, 11 ml
- MPO stock, 1 vial. **Store at -20°C**
- 20x Wash Solution: TBS50-20, 50 ml
- Diluent: YD50-1, 50 ml
- TMB: TMB11-1, 11 ml
- Stop Solution: SS11-1, 11 ml

Materials required but not provided:

- Pipettors and tips
- Distilled or deionized water
- Polypropylene tubes or 96-well polystyrene plates
- Vortex mixer
- Absorbent paper or paper towels
- Plate incubator/shaker
- Plate washer
- Plate reader capable of measuring absorbance at 450 nm
- Graphing software

STORAGE

Store the standard stock vial at -20°C. The remainder of the kit should be stored at 4°C and the microtiter plate should be kept in a sealed bag with desiccant. The kit will remain stable for six months from the date of purchase.

GENERAL INSTRUCTIONS

1. All reagents should be allowed to reach room temperature before use.
2. It is important that standards and samples be added to the ELISA plate quickly. If testing large numbers of samples, rather than pipetting standards and samples from individual tubes into the ELISA plate, we recommend the following: pipette an excess volume of standards and samples into wells of a blank polystyrene 96-well plate¹. Then use an 8 or 12-channel multi-pipettor to quickly transfer 100 μ l aliquots to the wells of the antibody-coated plate.
3. The wash procedure is critical. Insufficient washing will result in poor precision and falsely elevated absorbance readings.
4. Laboratory temperature will influence absorbance readings. The assay was calibrated using a shaking incubator set at 150 rpm and 25°C. Performing the assay at lower temperatures and mixing speeds may result in lower absorbance values.

WASH SOLUTION

The Wash Solution is provided as a 20x stock. Prior to use, dilute the contents of the bottle (50 ml) with 950 ml of distilled or deionized water. Unused wash buffer may be stored at 4°C for one week.

DILUENT

The Diluent (YD50-1) is formulated for measurement of MPO in serum. It is supplied ready to use. DO NOT substitute other buffers.

STANDARD

1. The stock is lyophilized. It is comprised of pure MPO in a stabilizing matrix. Reconstitute it with deionized water as described on the vial label and gently mix. Prepare the 5 ng/ml standard as described on the label.
2. Label seven polypropylene tubes as 2.5, 1.25, 0.625, 0.313, 0.156, 0.078 and 0 ng/ml. Dispense 0.25 ml of diluent into each.
3. Pipette 0.25 ml of the 5 ng/ml MPO standard into the tube labeled 2.5 ng/ml and mix. This provides the 2.5 ng/ml MPO standard.
4. Similarly prepare the 1.25 to 0.078 ng/ml standards by two-fold serial dilution.

IMPORTANT – If future use of the stock is intended, the capped vial must be frozen at or below -20°C within 30 minutes of reconstitution.

SAMPLES

The assay is intended for measurement of MPO in monkey serum. In serum from sick monkeys, we found MPO levels ranging from 1 to 90 ng/ml. A dilution of 40-fold in YD50-1 worked well for most samples, but optimal dilutions should be determined by the end user. To avoid matrix effects, do not use dilutions lower than 20-fold. Diluent YD50-1 must be used for sample dilution.

¹ Standards and sample dilutions may also be prepared directly in a blank polystyrene plate.

HRP CONJUGATE

The HRP conjugate is provided ready to use. Equilibrate it to room temperature prior to use. Use 100 µl per well.

PROCEDURE

1. Secure the desired number of 8-well strips in the cassette. Unused strips should be stored in a sealed bag with desiccant at 4°C.
2. Dispense 100 µl of standards and samples into appropriate wells. We recommend that standards and samples be tested in duplicate.
3. Incubate on a plate shaker at 150 rpm and 25°C for 45 minutes.
4. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
5. Dispense 100 µl of diluted HRP conjugate into the wells.
6. Incubate on a plate shaker at 150 rpm and 25°C for 45-minutes.
7. Empty and wash the microtiter wells 5 times with 1x Wash Solution using a plate washer (400 µl/well).
8. If necessary, strike the wells sharply onto absorbent paper or paper towels to remove all residual droplets.
9. Dispense 100 µl of TMB into each well.
10. Incubate on an orbital micro-plate shaker at 150 rpm at 25°C for 20 minutes.
11. After 20 minutes stop the reaction by adding 100 µl of Stop Solution to each well.
12. Gently mix. It is important to make sure that all the blue color changes to yellow.
13. Read absorbance at 450 nm² with a plate reader within 5 minutes.

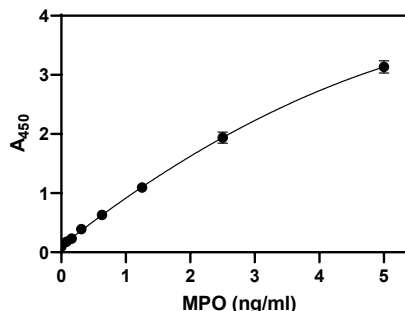
RESULTS

1. Using curve fitting software, construct a standard curve by plotting absorbance values of the standards versus the MPO concentration. We suggest fitting data to a second order polynomial equation.
2. Derive the concentration of MPO in the samples and multiply by the dilution factor to determine the concentration in the original sample.
3. If the absorbance values of samples fall outside the standard curve, samples should be further diluted appropriately and re-tested.

TYPICAL STANDARD CURVE

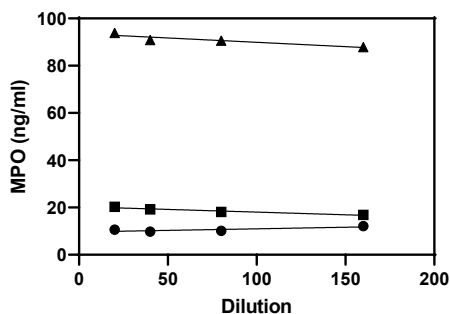
A typical standard curve is shown below. This curve is for illustration only.

MPO (ng/ml)	A ₄₅₀
5	3.135
2.5	1.940
1.25	1.093
0.625	0.632
0.313	0.391
0.156	0.232
0.078	0.177
0	0.096



PERFORMANCE

Linearity: To assess the linearity of the assay, serum samples with MPO concentrations of 10.7, 18.6 and 89.5 ng/ml diluted with YD50-1 to give values within range of the assay.



REFERENCES

1. Khan AA, Alsahli MA, and Rahmani AH. Myeloperoxidase as an active disease biomarker: Recent biochemical and pathological perspectives. Med. Sci. 2018, 6, 33; doi:10.3390/medsci6020033

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For technical assistance please email us: techsupport@lifediagnosics.com

² If absorbance of the high standard is ≥ 4 when measured at 450 nm, absorbance of all standards and samples should be read at 405 nm.