

## HUMAN MYELOPEROXIDASE (MPO) ANTIBODY SET FOR ELISA

FOR THE QUANTITATIVE DETERMINATION  
OF HUMAN MYELOPEROXIDASE (MPO)  
CONCENTRATIONS IN SERUM AND  
PLASMA



FOR RESEARCH USE ONLY. NOT FOR USE  
IN DIAGNOSTIC PROCEDURES.

### PURCHASE INFORMATION:

ELISA NAME	HUMAN MYELOPEROXIDASE ANTIBODY SET FOR ELISA
Catalog No.	SK00172-08
Lot No.	
Formulation	960 T
Standard range	0.39 – 400 ng/mL
Sensitivity	0.05 ng/mL
Sample Volume	100 µl
Sample Type	Serum and Plasma
Specificity	Human MPO only
Intra-assay Precision	6-8%
Inter-assay Precision	10-12%
Storage	2 °C-8 °C

### ORDER CONTACT:

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## INTRODUCTION

Human MPO Antibody Set for ELISA contains basic components required for the development of sandwich ELISA to measure human MPO in serum and plasma. It contains natural human MPO and antibodies raised against this protein. It has been shown to accurately quantify natural human MPO. Each kit contains sufficient material to run ELISAs on approximately 960-well plates, provided that the following conditions are met:

- The assay is run as summarized in the Assay Procedure.
- The recommended buffers, diluents, substrates and solutions are used.

## PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. An antibody specific for MPO will be coated onto a microplate. Standards and samples are pipetted into the wells and any MPO present is bound by the immobilized antibody. After washing away any unbound substances, an antibody specific for MPO is added to the wells. Following a wash to remove any unbound antibody reagent, Anti Rabbit IgG HRP conjugate is added to the wells. After washing away any unbound enzyme, a substrate solution is added to the wells and color develops in proportion to the amount of MPO bound in the initial step. The color development is stopped and the intensity of the color is measured.

## MATERIALS PROVIDED

DESCRIPTION	CODE	QUANTITY
<b>Capture Antibody</b> – 1.05mL/vial of 100-fold concentrated purified monoclonal antibody against human MPO in PBS, lyophilized	172-08-01	1 vial
<b>MPO Standard</b> – 5 µg/vial of natural human MPO in a buffered protein base with preservatives; lyophilized.	172-08-02	1 vial
<b>Detection Antibody</b> – 1.05 mL/vial of 100-fold concentrated of purified antibody against MPO with preservatives; lyophilized.	172-08-03	1 vial
<b>Anti Rabbit IgG-HRP Conjugate</b> -1.2 mL/vial, 100-fold concentrated solution of	ARIGHRP	1 vial

Goat Anti Rabbit IgG conjugate to HRP

## STORAGE

**Unopened Components: Unopened Capture Antibody, Standard and Detection Antibody** Store at 2 - 8° C for up to 1 month. For longer storage, unopened Standard, Capture Antibody and Detection Antibody Concentrate should be stored at -20 or -70 °C for up to 6 months. Do not use kit past expiration date.

**Opened / Reconstituted Reagents:** Aliquot the reconstituted standard, which can be stored for up to 2 months at -70° C. Diluted standard working solution should be prepared and used immediately. Anti Rabbit IgG - HRP Conjugate 100-fold concentrated may be stored at 2 - 8°C for up to 6 months.

## SOLUTIONS REQUIRED

- PBS (137 mM NaCl, 2.7 mM KCl, 8.1 mM Na<sub>2</sub>PO<sub>4</sub>, 1.5 mM KH<sub>2</sub>PO<sub>4</sub>, pH 7.4, 0.2 µm filtered)
- Wash Buffer (0.05% Tween-20 in PBS, pH 7.4)
- Blocking Buffer (BSA base. Code:BS-01)
- Diluent Solution (DB08) **Note:** DB08 is for standard, detection antibody and ARIGHRP dilution.
- Substrate Solution (TMB)
- STOP SOLUTION (0.5N HCl)
- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Microplate shaker (250-300rpm).
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 100 mL and 500 mL graduated cylinders.

**\*Note: Wash Buffer, Blocking Buffer, Diluent Solutions (DB06 and DB08), Substrate Solution and Stop Solution are available for purchase online at [www.aviscerabioscience.com](http://www.aviscerabioscience.com)**

## REAGENT PREPARATION

**Bring all reagents to room temperature before use.**  
**Capture Antibody** - Reconstitute the **Capture Antibody** with 1.05 mL of PBS to produce a 100-fold concentrated stock solution. Pipette 9.9 mL of the

appropriate PBS into a 15 mL centrifuge tube and transfer 100  $\mu$ L of 100-fold concentrated stock solution to prepare working solution. **Note:** Capture Antibody should be diluted without any carry proteins.

**MPO Standard** - Reconstitute the **MPO Standard** with 1.0 mL of Diluent Solution (**DB08**). This reconstitution produces a stock solution of 5  $\mu$ g/mL. Allow the standard to sit for a minimum of 15 minutes with gentle agitation prior to aliquot. Aliquot and store at -70  $^{\circ}$ C for up to 2 months. A six point standard curve using 4-fold serial dilutions in Diluent Solution is suggested. A high standard of 400 ng/ml is recommended.

**Detection Antibody** - Reconstitute the **Detection Antibody** with 1.05 mL of Diluent Solution (**DB08**) to produce a 100-fold concentrated stock solution. Pipette 9.9 mL of the Diluent Solution into a 15 mL centrifuge tube and transfer 100  $\mu$ L of 100-fold concentrated stock solution to prepare working solution.

**Goat Anti Rabbit IgG-HRP Conjugate** - Pipette 9.9 mL of Diluent Solution (**DB08**) into a 15 mL centrifuge tube and transfer 100  $\mu$ L of 100-fold concentrated stock solution to prepare working solution. **Note:** 1x working solution of Anti Rabbit IgG-HRP Conjugate should be used within a few days.

## ASSAY PROCEDURE

**Bring all reagents and samples to room temperature before use. It is recommended that blank, standards and samples be assayed in duplicates.**

1. Prepare all reagents and working standards as directed in the previous sections.
2. Coat a 96-well microplate with 100  $\mu$ L per well of Capture Antibody Working Solution. Seal it and incubate overnight at 2-8  $^{\circ}$ C.
3. Aspirate each well and wash, repeating the process two times for a total of three washes. Wash by filling each well with Wash Buffer (300  $\mu$ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by

aspirating or decanting. Invert the plate and blot it against clean paper towels.

4. Add 150  $\mu$ L per well of Blocking Buffer to each well. Seal plate and incubate for 5 hours at room temperature or overnight at 2-8  $^{\circ}$ C.
5. Aspirate each well and let it dry.
6. Add 100  $\mu$ L of standard dilutions and samples per well. Seal plate and incubate for 2 hours on micro-plate shaker at room temperature.
7. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (300  $\mu$ L) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
8. Add 100  $\mu$ L of Detection Antibody working solution to each well. Cover with sealer. Incubate for 2 hours on micro-plate shaker at room temperature.
9. Repeat the aspiration/wash as in step 6.
10. Add 100  $\mu$ L of **Anti Rabbit IgG-HRP Conjugate working solution** to each well. Incubate for 60 minutes on micro-plate shaker at room temperature. **Protect from light.**
11. Repeat the aspiration/wash as in step 6.
12. Add 100  $\mu$ L of Substrate Solution to each well. Incubate for 5-7 minutes at room temperature. **Protect from light.**
13. Add 100  $\mu$ L of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green, or if the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
14. Determine the optical density of each well within 15 minutes, using a micro-plate reader set to 450 nm.

## CALCULATION OF RESULTS

Average the duplicate readings for each standard and sample and subtract the average zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a log-log curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the MPO concentrations versus the log of the O.D. and the

best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Calculation of samples with a concentration exceeding that of standard 400 ng/mL may result in inaccurate, low human MPO levels. Such samples require further external predilution according to expected human MPO values with Dilution Buffer in order to precisely quantify the actual human MPO level.

**TYPICAL DATA**

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.

MPO (ng/mL)	Absorbance 450nm (Corrected)
Blank	0 (0.061)
0.39	0.026
1.56	0.091
6.25	0.288
25	0.728
100	1.177
400	1.574

- Lot:

**CALIBRATION**

This immunoassay is calibrated against a highly purified natural human MPO.

**SENSITIVITY**

Twenty-five assays were evaluated and the minimum detectable dose (MDD) of MPO was 50 pg/mL.

**SPECIFICITY**

PROTEIN	CROSSREACTIVITY (%)
Human MPO	100
Human Fetuin A	0
Human CRP	0
Human Gas 6	0

**SUMMARY OF ASSAY PROCEDURE**

