EAGLE BIOSCIENCES

Myeloperoxidase (MPO) Serum / Plasma ELISA Kit

Catalog Number:
MPO31-K01 (1 x 96 wells)
For Research Use Only. Not for use in diagnostic procedures.
v. 2.1 (26 SEP 23)

EAGLE BIOSCIENCES, INC.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
WWW.EAGLEBIO.COM

INTENDED USE

The Eagle Biosciences Human Myeloperoxidase (MPO) Serum/Plasma ELISA Assay Kit (enzyme-linked immunoassay kit) is intended for the quantitative determination of human myeloperoxidase (MPO) levels in serum and EDTA-plasma samples. The test is useful for detecting elevated levels of myeloperoxidase in serum and plasma samples. The Eagle Biosciences Human Myeloperoxidase (MPO) Serum/Plasma ELISA Assay Kit is for research use only and not to be used in diagnostic procedures.

INTRODUCTION

Myeloperoxidase (MPO) is a specific polymorphonuclear enzyme that is most abundantly expressed in neutrophil granulocytes. It functions in the oxygen-dependent killing of microorganisms and was released from primary granules of neutrophils during acute inflammation. MPO is the product of a single gene, which is about 11 kb in size, composed of 11 introns and 12 exons, and located in the long arm of chromosome 17 in segment q12-24. The mature 150 kDa MPO protein is a dimer consisting of two 15 kDa light chains and two heavy chains of variable degrees of glycosylation.

MPO is related to both inflammation and oxidative stress. It is a sensitive predicator for myocardial infarction in presenting with chest pain. Studies have indicated that MPO is causally linked to atherosclerosis. Moreover, a combination test of MPO and CRP (C-reactive protein) provides added benefit for risk prediction of cardiovascular mortality than just measuring CRP alone. This assay utilizes a specific monoclonal antibody to capture MPO in test samples to ensure that only myeloperoxidase is detected.

PRINCIPLE OF THE ASSAY

The Eagle Biosciences Human Myeloperoxidase (MPO) Serum/Plasma ELISA Assay Kit is designed, developed and produced for the quantitative measurement of human myeloperoxidase in serum and plasma samples. The assay utilizes the two-site "sandwich" technique with selected antibodies that bind to different epitopes of myeloperoxidase

Assay standards, controls and diluted samples are added directly to wells of a microtiter plate that is coated with antibody to myeloperoxidase. After an incubation period, the plate is washed and horseradish peroxidase (HRP) conjugated human myeloperoxidase antibody is added to each well. After the second incubation period, a "sandwich" of solid-phase monoclonal antibody - human myeloperoxidase – HRP conjugated antibody" is formed. The unbound antibodies and buffer matrix are removed in the subsequent washing step. For the detection of this immunocomplex, the well is then incubated with a substrate solution in a timed reaction and the absorbances are then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to the wall of each microtiter well is directly proportional to the amount of human myeloperoxidase in the test sample. A standard curve is generated by plotting the absorbance versus the respective human myeloperoxidase concentration for each standard on a point-to-point or 4-parameter curve fitting. The concentration of human myeloperoxidase in test samples is determined directly from this standard curve.

REAGENTS: Preparation and Storage

This MPO ELISA Assay kit must be stored at 2 – 8°C upon receipt. For the expiration date of the kit refer to the label on the kit box. All components of the MPO ELISA Assay Kit are stable until the expiration date.

Allow all reagents of the MPO ELISA Assay Kit to come to room temperature prior to use. Reagents from different kit lot numbers should not be combined or interchanged.

1. **Myeloperoxidase Antibody Coated Microplate**
   One microplate with twelve by eight strips (96 wells total) coated with myeloperoxidase antibody. The plate is framed and sealed in a foil zipper bag with a desiccant. This reagent should be stored at 2 – 8 °C and is stable until the expiration date on the MPO ELISA Assay Kit box.

2. **Myeloperoxidase Tracer Antibody**
   One vial containing 0.6 mL HRP labeled anti-human myeloperoxidase antibody in a stabilized protein matrix. This reagent must be diluted with Tracer Antibody Diluent before use. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the MPO ELISA Assay Kit box.

3. **ELISA Wash Concentrate**
   One bottle contains 30 mL of 30-fold concentrate. Before use, the contents must be diluted with **870 mL** of demineralized water and mixed well. Upon dilution, this yields a working wash solution containing a surfactant in phosphate buffered saline with a non-azide, non-mercury preservative. The diluted wash solution may be stored at room temperature and is stable until the expiration date on the MPO ELISA Assay Kit box.

4. **ELISA HRP Substrate**
   One bottle contains 12 mL of tetramethylbenzidine (TMB) with hydrogen peroxide. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the MPO ELISA Assay Kit box.

5. **ELISA STOP SOLUTION**
   One bottle contains 12 mL of 2N Hydrochloric Acid (HCl). This reagent may be stored at 2 – 8°C or room temperature and is stable until the expiration date on the MPO ELISA Assay Kit box.

6. **Myeloperoxidase Standard Concentrate**
   One vial containing human myeloperoxidase in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. **Refer to the vial for exact concentration of the standard.** This reagent should be stored at 2 – 8°C and is stable until the expiration date on the kit box. Refer to assay procedure section for dilution direction.

7. **Myeloperoxidase Controls**
   Three vials containing human myeloperoxidase in a lyophilized bovine serum based matrix with a non-azide, non-mercury preservative. **Refer to vials for exact concentration range for each control.** Both controls should be stored at 2 – 8 °C and are stable until the expiration date on the MPO ELISA Assay Kit box.

8. **Tracer Antibody Diluent**
   One vial containing 12 mL ready to use buffer. It should be used only for tracer antibody dilution according to the assay procedures. This reagent should be stored at 2 – 8°C and is stable until the expiration date on the MPO ELISA Assay Kit box.

9. **MPO Sample Diluent Concentrate**
   Two bottles containing 30 mL of ready-to-use reagent for serum/EDTA-plasma sample dilution. The Sample Diluent must be stored at 2 – 8°C and is stable until the expiration date on the MPO ELISA Assay Kit box.

SAFETY PRECAUTIONS

The Human Myeloperoxidase (MPO) Serum/Plasma ELISA Assay Kit reagents must be used in a professional laboratory environment and is for Research Use Only and is not to be used in diagnostic procedures. Only source material from which reagents of bovine serum was derived in the contiguous 48 United States. It was obtained only from donor health animals maintained under veterinary supervision and found free of contagious diseases. Wear gloves while performing this assay and handle these reagents as if they were potentially infectious. Avoid contact with reagents containing TMB, hydrogen peroxide, or sulfuric acid. TMB may cause irritation to skin and mucous membranes and cause an allergic skin reaction. TMB is a suspected carcinogen. Sulfuric acid may cause severe irritation on contact with skin. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale fumes. On contact, flush with copious amounts of water for at least 15 minutes. Use Good Laboratory Practices**.**

MATERIALS REQUIRED BUT NOT PROVIDED

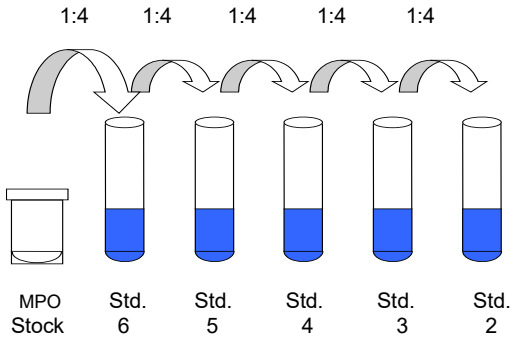
- Precision single channel pipettes capable of delivering 50 µL, 100 µL, 500 µL, etc.
- Disposable pipette tips suitable for above volume dispensing.
- Disposable 12 x 75 mm or 13 x 100 glass tubes
- Disposable plastic 100 mL and 1000 mL bottle with caps.
- Aluminum foil.
- Deionized or distilled water.
- Plastic microtiter well cover or polyethylene film.
- ELISA multichannel wash bottle or automatic (semi-automatic) washing system.
- Spectrophotometric microplate reader capable of reading absorbance at 405/650 nm

SPECIMEN COLLECTION

Only 50 µL of human serum or EDTA-plasma is required for myeloperoxidase measurement in duplicate. No special preparation of individual is necessary prior to specimen collection. In the case of serum, whole blood should be collected and must be allowed to clot for a minimum of 30 minutes at room temperature before the serum is separated by centrifugation (850 – 1500xg for 10 minutes). The serum should be separated from the clot within one hour of blood collection and transferred to a clean test tube. Serum or plasma samples should be stored at 2 - 8ºC if the assay is to be performed within 72 hours. Otherwise, samples should be stored at -20ºC or below until measurement. Avoid repeated (more than three times) freezing and thawing of specimen.

REAGENT PREPARATION

1. Prior to use allow all reagents to come to room temperature. Regents from different kit lot numbers should not be combined or interchanged.
2. ELISA Wash Concentrate must be diluted to working solution prior use. Please see REAGENTS section for details.
3. Reconstitute assay standard by adding **2.0 mL** of demineralized water to standard vial. Separately, reconstitute controls by adding **1.0 mL** of demineralized water to control vials. Allow the standard and controls to sit undisturbed for 5 minutes, and then mix well by inversions or gentle vortexing. One must make sure that all solid is dissolved completely prior to use. These reconstituted standard and controls may be stored at 2–8°C for up to 3 days or at –10°C or below for long-term storage. Do not exceed 3 freeze-thaw cycles.
4. Dilute the reconstituted standard 1:4 using the diluted MPO sample diluent to obtain a level six standard by mixing the concentrated MPO standard with MPO sample diluents. For example: mix **300 µL** of concentrated MPO standard with **900 µL** of the MPO sample diluent. Continue diluting standards down to level two as it is shown below. Level one standard is the diluted MPO sample diluent.



5. Place a sufficient number of myeloperoxidase antibody coated microwell strips in a holder to run human myeloperoxidase standards, controls and unknown samples in duplicate.

6. Test Configuration

ROW	STRIP 1	STRIP 2	STRIP 3	STRIP 4
A	STD 1	STD 5	C 3	SAMPLE 4
B	STD 1	STD 5	C 3	SAMPLE 4
C	STD 2	STD 6	SAMPLE 1	SAMPLE 5
D	STD 2	STD 6	SAMPLE 1	SAMPLE 5
E	STD 3	C 1	SAMPLE 2	SAMPLE 6
F	STD 3	C 1	SAMPLE 2	SAMPLE 7
G	STD 4	C 2	SAMPLE 3	SAMPLE 7
H	STD 4	C 2	SAMPLE 3	Etc.

7. Prepare Tracer Antibody working solution by 1:21 fold dilution of the Myeloperoxidase Tracer Antibody by adding the tracer antibody into the Tracer Antibody Diluent. Following is a table that outlines the relationship of strips used and antibody mixture prepared.
   NOTE: *the tracer antibody should be prepared just prior to the end of the first incubation cycle.*

Dilution Scheme	Tracer Antibody Diluent	Tracer Antibody
1	1 mL	50 µL
2	2 mL	100 µL
3	3 mL	150 µL
4	4 mL	200 µL
5	5 mL	250 µL
6	6 mL	300 µL
7	7 mL	350 µL
8	8 mL	400 µL
9	9 mL	450 µL
10	10 mL	500 µL
11	11 mL	550 µL
12	12 mL	600 µL

Note*: this antibody working solution should be freshly prepared just before pipetting the tracer antibody to the washed wells.*

Patient Sample Preparation

Each serum and/or plasma sample has to be diluted 1:5 using MPO Sample Diluent for dilution. For example: 50 µL of human serum or plasma diluted in 200 µL of the diluted MPO Sample Diluent will yield a 1:5 dilution and a sufficient sample amount for myeloperoxidase measurement in duplicate.

Assay Procedure

1. Add **100 µl** of Standards, Controls and diluted samples (diluted beforehand 1:5 with assay buffer) into the designated microwells.
2. Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for **1.5 hr. ± 5 minutes** at 400 to 450 rpm.
3. Just prior to the end of the incubation time, dilute the proper amount of Tracer Antibody for the assay.
4. Wash each well five times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.

5. Add **100 µL** of above Tracer Antibody to each well.
6. Seal the plate wells securely, cover with foil or other material to protect from light, and rotate on an ELISA plate shaker (small orbit radius) for **45 minutes ± 5 minutes** at 400 to 450 rpm.
7. Wash each well five times by dispensing 350 µL of working wash solution into each well and then completely aspirating the contents. Alternatively, an automated microplate washer can be used.
8. Add **100 µL** of ELISA HRP Substrate into each of the wells.
9. Cover the plate with aluminum foil or other material to avoid exposure to light. Incubate plate static, at room temperature for **20 minutes**.
10. Immediately add **100 µL** of ELISA Stop Solution into each of the wells. Mix gently.
11. Read the absorbance at 405 nm with reference filter at 620 nm or 650 nm.

PROCEDURAL NOTES

1. It is recommended that all standards, controls and unknown samples be assayed in duplicate. The average absorbance reading of each duplicate should be used for data reduction and the calculation of results.
2. Keep light sensitive reagents in the original amber bottles.
3. Store any unused antibody coated strips in the foil zipper bag with desiccant to protect from moisture.
4. Careful technique and use of properly calibrated pipetting devices are necessary to ensure reproducibility of the test.
5. Incubation times or temperatures other than those stated in this insert may affect the results.
6. An orbital mixer with a larger orbit radius (e.g. > 1 cm) may be used at speeds of 150 to 200 rpm.
7. Avoid air bubbles in the microwell as this could result in lower binding efficiency and higher CV% of duplicate reading.
8. All reagents should be mixed gently and thoroughly prior to use. Avoid foaming.
9. When adapting this assay to an automated ELISA system such as DS-2 (Diamedix Corp., Miami), a procedural validation is necessary if there is any modification of the assay procedure.

INTERPRETATION OF RESULTS

It is recommended to use a point-to-point or 4-parameter standard curve fitting.

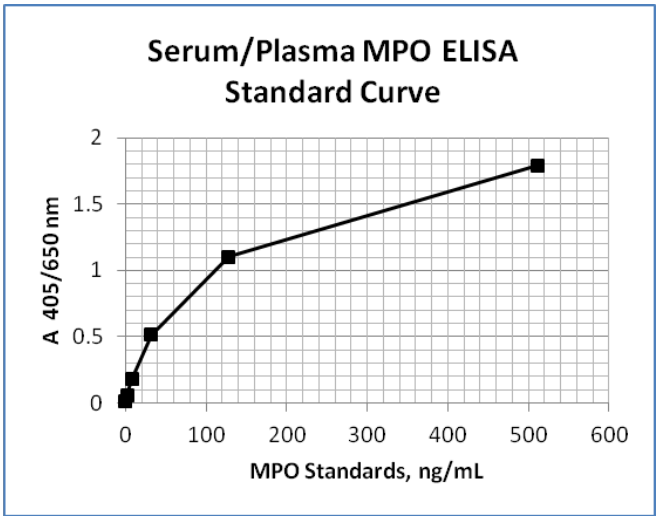
1. Calculate the average absorbance for each pair of duplicate test results.
2. Subtract the average absorbance of the level 1 standard (0 ng/mL) from the average absorbance of all other readings to obtain corrected absorbance.
3. The standard curve is generated by the corrected absorbance of all standard levels on the ordinate against the standard concentration on the abscissa using point-to-point or log-log paper. Appropriate computer assisted data reduction programs may also be used for the calculation of results.

The serum/plasma human myeloperoxidase concentrations for the controls and the samples are read directly from the standard curve using their respective corrected absorbance.

EXAMPLE DATA AND STANDARD CURVE

Typical absorbance data and the resulting standard curve from this serum/plasma human myeloperoxidase ELISA are represented. **This curve should not be used in lieu of standard curve run with each assay.**

Well I.D.	OD 405/650 nm Absorbance			Results
	Readings	Average	Corrected	
Std-1: 0 ng/mL	0.015 0.014	0.015	0.000	
Std-2: 2 ng/mL	0.057 0.061	0.059	0.044	
Std-3: 8 ng/mL	0.179 0.181	0.180	0.165	
Std-4: 32 ng/mL	0.520 0.509	0.514	0.499	
Std-5: 128 ng/mL	1.097 1.109	1.103	1.088	
Std-6: 512 ng/mL	1.781 1.801	1.791	1.776	
Control 1	0.104 0.103	0.104	0.089	4.2 ng/mL
Control 2	0.330 0.345	0.338	0.323	19.3 ng/mL
Control 3	0.873 0.898	0.886	0.871	92.6 ng/mL



EXPECTED VALUES

Serum/EDTA-Plasma samples from normal healthy adults ages 20 – 60 were collected and measured with this ELISA. Because each of the serum and plasma samples were initially diluted 1:5, the measured value of each sample has to be multiplied by 5 for the true result.

The recommended **normal cut-off** for serum myeloperoxidase concentration by using this ELISA is 220 ng/mL, and the normal cut off for EDTA-plasma myeloperoxidase concentration is 40 ng/mL. We strongly recommend for each clinical laboratory to establish its own normal cut-off level by measuring normal serum or EDTA-plasma samples with this ELISA.

LIMITATION OF THE PROCEDURE

1. For sample values reading greater than highest standard, it is recommend to re-assay samples with dilution (i.e. 1:10 or 1:100 with MPO Sample Diluent).
2. Water deionized with polyester resins may inactivate the horseradish peroxidase enzyme.
3. Since there is no Gold Standard concentration or international standard available for MPO measurement, the values of assay standards were established and validated by Eagle Biosciences. Results obtained with different assay methods or kits cannot be used interchangeably.
4. Bacterial or fungal contamination of serum/plasma specimens or reagents, or cross contamination between reagents may cause erroneous results.

QUALITY CONTROL

To assure the validity of the results each assay should include adequate controls with known MPO levels. We recommend that all assays include the laboratory's own MPO controls in addition to those provided with this kit.

PERFORMANCE CHARACTERISTICS

Sensitivity

The analytical sensitivity (LLOD) of the human myeloperoxidase ELISA as determined by the 95% confidence limit on 16 duplicate determination of zero standard is approximately 0.65 ng/mL.

High Dose "hook" effect

This assay has showed that it did not have any high dose "hook" for myeloperoxidase level up to 50,000 ng/mL.

Precision

The intra-assay precision was validated by measuring two sample extracts in a single assay with 12 replicate determinations.

Sample #	Mean Myeloperoxidase Value in Serum (ng/mL)	CV (%)
1	53.1	7.9
2	78.3	5.1

Sample #	Mean Myeloperoxidase Value in Plasma (ng/mL)	CV (%)
1	29.8	7.5
2	66.7	5.3

The inter-assay precision was validated by measuring two controls in duplicate in six individual assays.

Sample #	Mean Myeloperoxidase Value (ng/mL)	CV (%)
1	21.8	10.4
2	92.5	5.2

Linearity

Two serum samples were diluted 1:5 with MPO sample diluent, spiked with various volumes of myeloperoxidase concentrations and tested. The results of serum myeloperoxidase percent recovery in the value of ng/mL are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
Neat A	58.6	-
1:2	29.0	99
1:4	16.9	115
Neat B	91.6	-
1:2	54.4	119
1:4	28.3	124

Two EDTA-plasma samples were diluted 1:5 with MPO sample diluent, spiked with various volumes of myeloperoxidase concentrations and tested. The results of plasma myeloperoxidase percent recovery in the value of ng/mL are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	RECOVERY %
Neat A	26.5	-
1:2	15.1	114
1:4	7.9	120
Neat B	65.4	-
1:2	31.1	95
1:4	19.1	117

Spiked Recovery

Three diluted serum samples and three assay standards (8, 32, 128 ng/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	EXPECTED VALUE (ng/mL)	RECOVERY %
Neat A	29.3	-	-
Std-3	21.6	18.7	116
Std-4	27.9	30.7	91
Std-5	58.9	78.7	75
Neat B	44.5	-	-
Std-3	27.8	26.3	106
Std-4	35.7	38.3	93
Std-5	62.0	86.3	72
Neat C	61.4	-	-
Std-3	33.8	34.7	97
Std-4	45.5	46.7	97
Std-5	77.3	94.7	82

Three diluted plasma samples and three assay standards (8, 32, 128 ng/mL) were combined at equal volumes and tested. The results are as follows:

DILUTION	OBSERVED VALUE (ng/mL)	EXPECTED VALUE (ng/mL)	RECOVERY %
Neat A	13.7	-	-
Std-3	10.9	10.9	100
Std-4	20.7	22.9	90
Std-5	51.9	70.9	73
Neat B	27.7	-	-
Std-3	20.0	17.8	112
Std-4	27.9	29.8	93
Std-5	57.9	77.8	74
Neat C	52.0	-	-
Std-3	30.3	30.0	101
Std-4	37.3	42.0	89
Std-5	76.5	90.0	85

REFERENCES

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6. Carr AC, et al. Myeloperoxidase binds to low-density lipoprotein: potential implications for atherosclerosis. FEBS Lett 2000;487:176-80.

Warranty Information

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For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.