ATHENS RESEARCH & TECHNOLOGY

ARBOR ASSAYS™

Human Myeloperoxidase (MPO)
Enzyme Immunoassay Kit

1 Plate Kit Catalog Number K060-H1

Sample Types Validated:

Serum, Platelet-Poor Heparin Plasma, Saliva, Urine and Tissue Culture Media

Please read this insert completely prior to using the product.
For research use only. Not for use in diagnostic procedures.
Not for human diagnostic use.

www.ArborAssays.com | **www.AthensResearch.com**

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BACKGROUND

Myeloperoxidase (MPO) is a tetrameric heme-containing protein abundantly produced in neutrophil granulocytes where it plays an important anti-microbial role. Normally stored in azurophilic granules, MPO is released into the extracellular space during degranulation. There, as part of the neutrophils "respiratory burst", it produces hypochlorous acid from hydrogen peroxide and Cl^-. MPO also uses hydrogen peroxide to oxidize tyrosine to the tyrosyl radical. Both hypochlorous acid and tyrosyl are cytotoxic and when present can kill bacteria and other pathogens. Hereditary deficiency of myeloperoxidase predisposes individuals to immune deficiency.

Studies have shown an association between elevated MPO levels and coronary artery disease[1], and in 2003 it was suggested that MPO may serve as a sensitive predictor of myocardial infarction in patients complaining of chest pain[2]. Since that time the clinical utility of MPO testing in cardiac patients has been solidly established in the literature with well over 100 papers published. In 2010 Heslop et al. further refined the clinical application by determining that measuring both MPO and C-reactive protein (CRP) provided more accurate prediction of mortality risk than measuring just CRP alone[3].

1. Zhang et al., "Association between myeloperoxidase levels and risk of coronary artery disease." JAMA, 2001; 286: 2136-2142.
2. Brennan et al. "Prognostic value of myeloperoxidase in patients with chest pain". New Engl. J. Med., 2003; 349: 1595-1604.
3. Heslop, CL, Frolich, JJ, and Hill, JS, "Myeloperoxidase and C-reactive protein have combined utility for long-term prediction of cardiovascular mortality after coronary angiography". J. Am. Coll. Of Cardiol., 2010; 55: 1102-1109.

ASSAY PRINCIPLE

The human Myeloperoxidase (MPO) EIA kit is designed to quantitatively measure MPO present in a variety of samples and tissue culture media. Please read the complete kit insert before performing this assay. A myeloperoxidase standard is provided to generate a standard curve for the assay and all samples should be read off the standard curve. Standards or diluted samples are pipetted into a clear microtiter plate coated with a monoclonal antibody to capture MPO present in the sample. After a 60 minute incubation, the plate is washed. A peroxidase conjugated MPO antibody is added and the plate is again incubated for 60 minutes and washed. Substrate is then added to the plate, which reacts with the bound MPO conjugated antibody. After a third incubation, the reaction is stopped and the intensity of the generated color is detected in a microtiter plate reader capable of measuring 450 nm wavelength. The concentration of myeloperoxidase in the sample is calculated, after making suitable correction for dilution, using software available with most plate readers.

RELATED PRODUCTS

Arbor Assay Kits	Catalog No.
Allopregnanolone EIA & CLIA Kits	K044-H1/H5, K044-C1/C5
Atrial Natriuretic Peptide (ANP) EIA Kits	K026-H1/H5
Endothelin-1 (ET-1) EIA Kit	K045-H1
Nitric Oxide (NO) Colorimetric Detection Kit	K023-H1
Hemoglobin Colorimetric Detection Kit	K013-H1
Prostaglandin E_2 (PGE_2) Multi-Format Kits	K051-H1/H5
Protein Kinase A (PKA) Activity Kit	K027-H1
ST2 Human EIA Kit	K055-H1

Athens Research and Technology Reagents	Catalog No.
Myeloperoxidase, Human Neutrophils	16-14-130000
High Density Lipoprotein (HDL), Human Plasma	12-16-080412
Ceruloplasmin, Human Plasma	16-16-030518
Superoxide Dismutase (SOD), Human Erythrocytes	16-05-191504
Elastase, Human Neutrophils	16-14-051200
Eosinophil Peroxidase (EPO), Human Eosinophils	16-15-160518
Lactoferrin, Human Milk	16-13-120103

SUPPLIED COMPONENTS

Mouse anti-Myeloperoxidase Clear Coated 96 Well Plate
Clear plastic microplate with break-apart strips coated with monoclonal antibody to human myeloperoxidase.
One Plate Catalog Number C223-1EA

Human Myeloperoxidase Standard
Myeloperoxidase at 200 ng/mL in a special stabilizing solution.
70 µL Catalog Number C225-70UL

Myeloperoxidase Conjugate
An antibody to human myeloperoxidase labeled with peroxidase.
5 mL Catalog Number C224-5ML

Assay Buffer Concentrate
A 5X concentrate that should be diluted with deionized or distilled water.
28 mL Catalog Number X132-28ML

Wash Buffer Concentrate
A 20X concentrate that should be diluted with deionized or distilled water.
30 mL Catalog Number X135-30ML

TMB Substrate
11 mL Catalog Number X133-11ML

Stop Solution
A 1M hydrochloric acid solution. **CAUSTIC**.
5 mL Catalog Number X134-5ML

Plate Sealer
2 each Catalog Number X002-1EA

STORAGE INSTRUCTIONS

Once opened the kit can be stored at 4°C up to the expiration date on the kit label.

OTHER MATERIALS REQUIRED

Distilled or deionized water.

Polypropylene or glass test tubes. Polypropylene standards correlate 90% to those made in glass.

Repeater pipet and disposable tips capable of dispensing 100 µL and 50 µL.

A microplate washer.

Colorimetric 96 well microplate reader capable of reading optical density at 450 nm.

Software for converting raw relative optical density readings from the plate reader and carrying out four parameter logistic curve (4PLC) fitting. Contact your plate reader manufacturer for details.

PRECAUTIONS

As with all such products, this kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

The antibody coated plate needs to be stored desiccated. The silica gel pack included in the foil ziploc bag will keep the plate dry. The silica gel pack will turn from blue to pink if the ziploc has not been closed properly.

This kit utilizes a peroxidase-based readout system. Buffers, including other manufacturers' wash buffers, containing sodium azide will inhibit color production from the enzyme. Make sure **all** buffers used for samples are **azide free**. Ensure that any plate washing system is rinsed well with deionized water prior to using the supplied Wash Buffer as prepared on Page 8.

The Stop Solution is acid. The solution should not come in contact with skin or eyes. Take appropriate precautions when handling this reagent.

SAMPLE TYPES

This assay has been validated for human serum, platelet-poor heparin plasma, saliva, urine and tissue culture media (TCM) samples only. Samples containing visible particulate should be centrifuged prior to using. This assay has low or no reactivity to mouse MPO. The end user should test this kit for application in their samples.

SAMPLE PREPARATION

Serum and Platelet-Poor Heparin Plasma Samples

Serum and plasma samples must be diluted a **minimum** of 1:15 or greater in diluted Assay Buffer.

Platelet-Poor Plasma samples are prepared from fresh plasma within 1 hour after collection. Centrifuge the plasma at 1,500 x g for 15 minutes at 4°C. Transfer the plasma into a clean tube and repeat the centrifugation at 1,500 x g for 15 minutes at 4°C to remove any red cells or platelets. Carefully transfer the top 3/4 volume of supernatant without disturbing any cells at the bottom of the tube, aliquot and freeze at -20°C until use.

Saliva Samples

Saliva samples must be diluted a **minimum** of 1:2 or greater in diluted Assay Buffer. See our Saliva Sample Handling Instructions at www.arborassays.com/assets/saliva-sample-protocol.pdf.

Urine Samples

Urine samples must be diluted a **minimum** of 1:4 or greater in diluted Assay Buffer. Normal urine has very low levels of myeloperoxidase which is too low to detect and is not recommended as a sample type for screening.

Tissue Culture Media Samples

TCM samples should either be diluted in diluted Assay Buffer, or read off a standard curve generated in TCM. RPMI-1640, diluted 1:4 in Assay Buffer, was validated in this kit.

The end user must determine the appropriateness of the TCM solution used and the minimum dilution required if diluting into diluted Assay Buffer.

Any samples with concentrations outside the standard curve range should be diluted further with Assay Buffer, as appropriate, to obtain readings within the standard curve range.

Use all samples within 2 hours of dilution.

REAGENT PREPARATION

Allow the kit reagents to come to room temperature for 30 minutes. Ensure that all samples have reached room temperature and have been diluted as appropriate prior to running them in the kit.

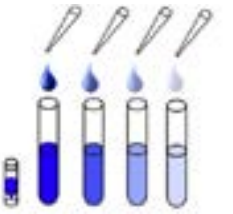
Assay Buffer

Dilute Assay Buffer Concentrate 1:5 by adding one part of the concentrate to four parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Wash Buffer

Dilute Wash Buffer Concentrate 1:20 by adding one part of the concentrate to nineteen parts of deionized water. Once diluted this is stable at 4°C for 3 months.

Standard Preparation



Label test tubes as 1 through 7. Pipet 380 µL of Assay Buffer into tube 1. Pipet 200 µL of Assay Buffer into tubes 2 to 7. Carefully add 20 µL of the 200 ng/mL MPO standard to tube 1 and vortex completely. Take 200 µL of the MPO solution in tube 1 and add it to tube 2 and vortex completely. Repeat the serial dilutions for tubes 3 through 7. The concentration of Myeloperoxidase in the tubes 1 through 7 will be 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL.

Use all Standards within 2 hours of preparation.

	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6	Std 7
Assay Buffer Volume (µL)	**380**	200	200	200	200	200	200
Addition	**Stock**	Std 1	Std 2	Std 3	Std 4	Std 5	Std 6
Volume of Addition (µL)	**20**	200	200	200	200	200	200
Final Conc (ng/mL)	10	5	2.5	1.25	0.625	0.313	0.156

ASSAY PROTOCOL

We recommend that all standards and samples be run in duplicate to allow the end user to accurately determine Myeloperoxidase concentrations.

1. Use the plate layout sheet on the back page of the insert to aid in proper sample and standard identification. Determine the number of wells to be used and return unused wells to foil pouch with desiccant. Seal the ziplock plate bag and store at 4˚C.
2. Pipet standards or samples down the plate strip columns (A to H) to ensure maximum use of the strip wells.
3. Pipet 50 µL of samples or standards into wells in the plate. Pipet 50 µL of diluted Assay Buffer into the zero standard wells. Cover the plate with the plate sealer and shake at room temperature for 60 minutes. *NOTE: Incubation without shaking reduces overall signal and increases zero standard signal.*
4. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
5. Add 50 µL of the Myeloperoxidase Conjugate to each well, using a repeater pipet.
6. Cover the plate with the plate sealer and shake at room temperature for 60 minutes.
7. Aspirate the plate and wash each well 4 times with 300 µL wash buffer. Tap the plate dry on clean absorbent towels.
8. Add 100 µL of the TMB Substrate to each well, using a repeater pipet.
9. Incubate the plate at room temperature for 30 minutes, without shaking.
10. Add 50 µL of the Stop Solution to each well, using a repeater pipet.
11. Read the optical density generated from each well at 450 nm.
12. Use the plate reader's built-in 4PLC software capabilities to calculate Myeloperoxidase concentration for each sample.

NOTE: If you are using only part of a strip well plate, at the end of the assay throw away the used wells and retain the plate frame for use with the remaining unused wells.

CALCULATION OF RESULTS

Average the duplicate OD readings for each standard and sample. Create a standard curve by reducing the data using computer software capable of generating a four-parameter logistic curve (4PLC) fit. The sample concentrations should be multiplied by the dilution factor to obtain neat sample values.

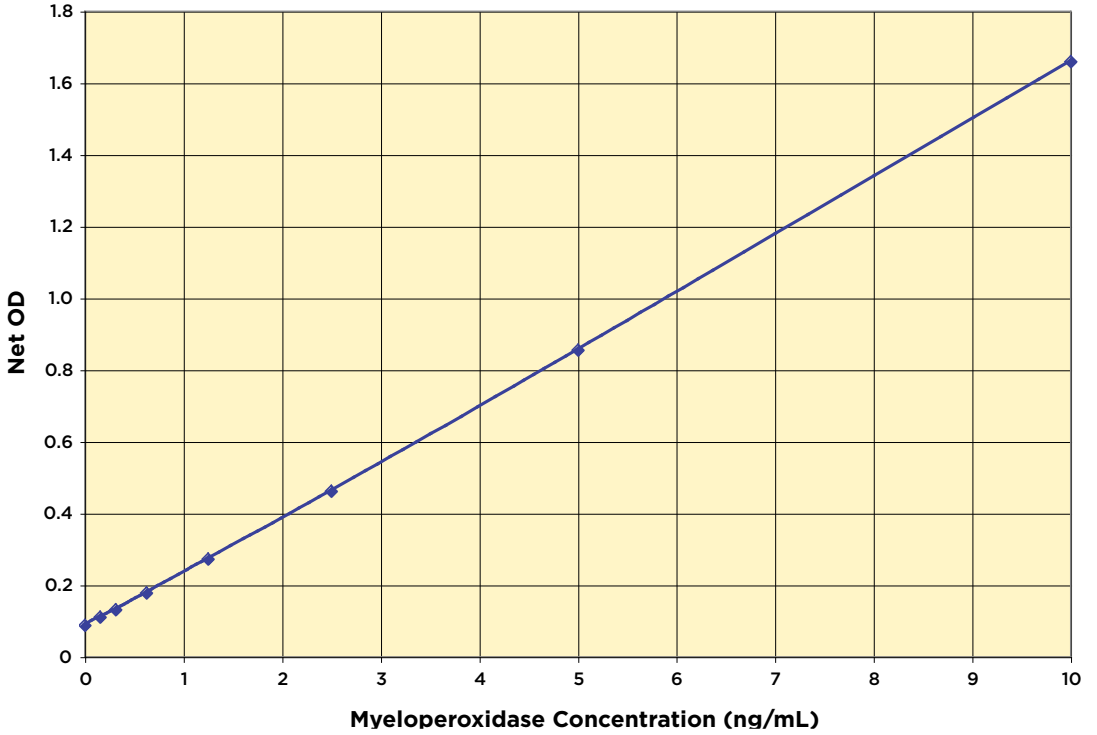
Or use the online tool from MyAssays to calculate the data:
https://www.myassays.com/arbor-assays-human-myeloperoxidase-(mpo)-eia-kit-k060.assay

TYPICAL DATA

Sample	Mean OD	Myeloperoxidase Conc. (ng/mL)
Standard 1	1.660	10
Standard 2	0.856	5
Standard 3	0.462	2.5
Standard 4	0.273	1.25
Standard 5	0.179	0.625
Standard 6	0.132	0.313
Standard 7	0.111	0.156
Zero	0.089	0
Sample 1	0.641	3.64
Sample 2	0.384	1.99

Always run your own standard curve for calculation of results. Do not use this data.

Typical Standard Curve



Always run your own standard curve for calculation of results. Do not use this data.

VALIDATION DATA

Sensitivity and Limit of Detection

Sensitivity was calculated by comparing the OD's for nineteen wells run for each of the zero and standard 7. The detection limit was determined at two (2) standard deviations from the zero along the standard curve.

Sensitivity was determined as 0.068 ng/mL.

The Limit of Detection for the assay was determined in a similar manner by comparing the OD's for nineteen replicates for each of the zero standard and a low concentration serum sample.

Limit of Detection was determined as 0.074 ng/mL.

Linearity

Linearity was determined by taking two diluted samples, one with a low myeloperoxidase level and one with a higher level, and mixing them in the ratios given below. The measured concentrations were compared to the values determined for each diluted sample.

SERUM

High Sample (5.7 ng/mL)	Low sample (1.19 ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	4.80	4.81	100.3%
60%	40%	3.90	4.00	102.7%
40%	60%	2.99	2.94	98.2%
20%	80%	2.09	1.96	93.7%
			Mean Recovery	**98.7%**

PLASMA

High Sample (7.59 ng/mL)	Low sample (1.01 ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	6.27	6.33	100.9%
60%	40%	4.96	4.86	98.1%
40%	60%	3.64	3.76	103.3%
20%	80%	2.32	2.39	102.6%
			Mean Recovery	**101.2%**

SALIVA

High Sample (5.71 ng/mL)	Low sample (1.77 ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	4.92	4.78	97.1%
60%	40%	4.13	3.89	94.2%
40%	60%	3.34	3.14	94.0%
20%	80%	2.56	2.34	91.7%
			Mean Recovery	**94.2%**

URINE

High Sample (5.72 ng/mL)	Low sample (0.39 ng/mL)	Expected Conc. (ng/mL)	Observed Conc. (ng/mL)	% Recovery
80%	20%	4.65	4.74	101.8%
60%	40%	3.59	3.71	103.4%
40%	60%	2.52	2.46	97.7%
20%	80%	1.45	1.42	97.3%
			Mean Recovery	**100.1%**

Intra Assay Precision

Three serum samples were diluted with diluted Assay Buffer and run in replicates of twenty in an assay. The mean and precision of the calculated MPO concentrations were:

Sample	Myeloperoxidase Conc. (ng/mL)	%CV
1	6.26	2.5
2	4.11	2.4
3	2.35	2.3

Inter Assay Precision

Three serum samples were diluted with Assay Buffer and run in duplicates in twenty assays run over multiple days by five operators. The mean and precision of the calculated MPO concentrations were:

Sample	Myeloperoxidase Conc. (ng/mL)	%CV
1	5.64	7.8
2	3.74	7.6
3	2.12	11.8

SAMPLE VALUES

Thirteen human heparin plasma samples were tested in the kit. Normal human plasma levels ranged from 45.8 to 120.4 ng/mL with an average of 70.5 ng/mL.

Human serum samples were tested in the kit. Serum levels ranged from 15.6 to 95.2 ng/mL with an average of 67.8 ng/mL.

A number of human saliva samples were tested in the kit. Saliva levels ranged from 2.0 to 414.1 ng/mL with an average of 116.7 ng/mL.

Human urine samples were also tested in the kit. Urine levels ranged from 0.9 to 22.9 ng/mL with an average of 6.7 ng/mL.

CROSS REACTIVITY

The following cross reactants were tested in the assay and cross reactivity calculated within the standard curve.

Steroid	Cross Reactivity (%)
human MPO	100%
mouse MPO	not detected
lactoferrin	2.09%
eosinophil peroxidase	0.44%
neutrophil elastase	0.17%
hmn serum albumin	not detected
erythropoietin	not detected
bovine lactoperoxidase	not detected
eosinophil derived neurotoxin	not detected
plasma kininogen HMW	not detected
bovine glutahione peroxidase	not detected

LIMITED WARRANTY

Arbor Assays and Athens Research and Technology warrant that at the time of shipment this product is free from defects in materials and workmanship. This warranty is in lieu of any other warranty expressed or implied, including but not limited to, any implied warranty of merchantability or fitness for a particular purpose.

We must be notified of any breach of this warranty within 5 days of receipt of the product. No claim shall be honored if we are not notified within this time period, or if the product has been stored in any way other than outlined in this publication. The sole and exclusive remedy of the customer for any liability based upon this warranty is limited to the replacement of the product, or refund of the invoice price of the goods.

CONTACT INFORMATION

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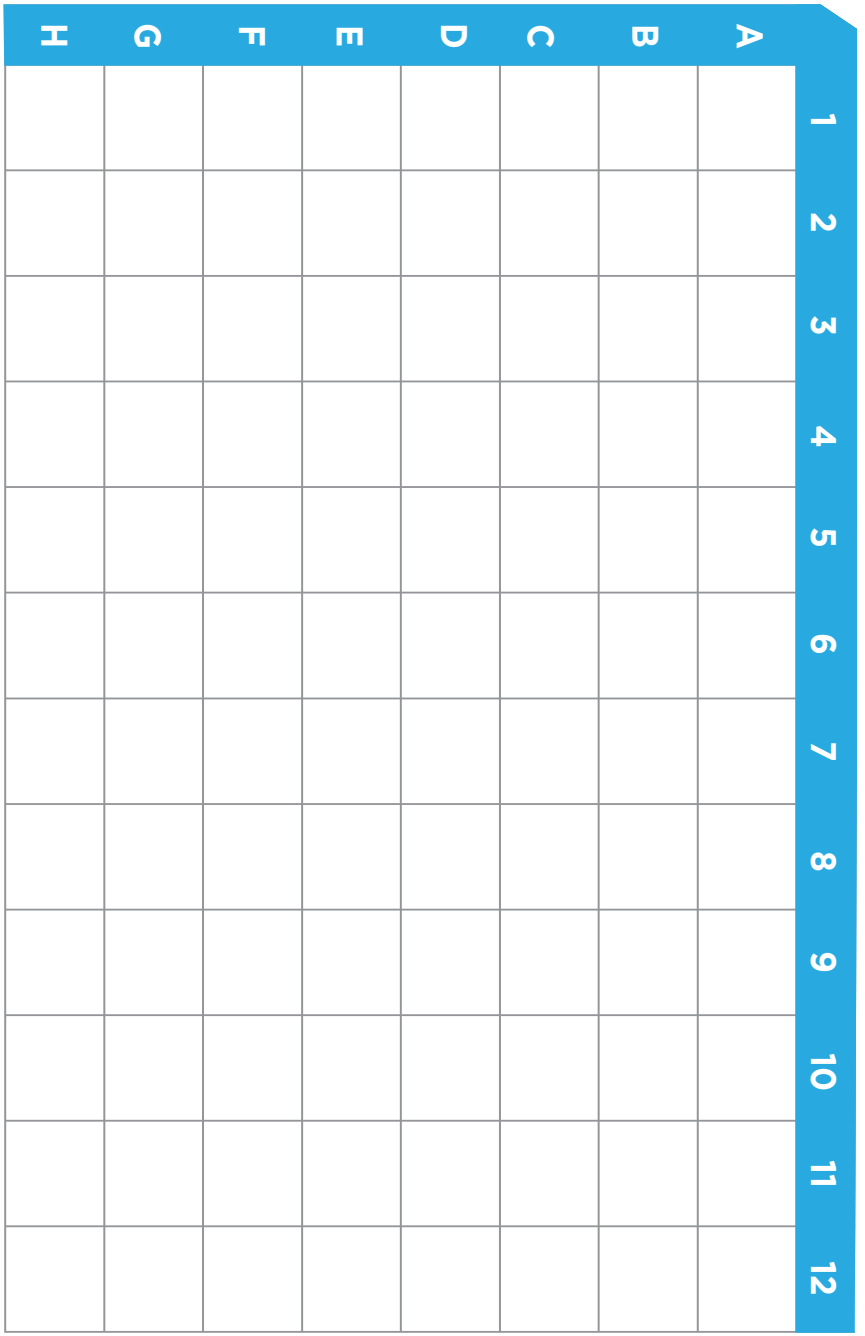

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