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ab119605 Myeloperoxidase (MPO) Human ELISA Kit

For quantitative detection of Human Myeloperoxidase (MPO) in cell culture supernatants, cell lysate, tissue homogenates, serum, plasma (heparin, EDTA), saliva and urine.

This product is for research use only and is not intended for diagnostic use.

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1. Overview

Abcam's Human Myeloperoxidase (MPO) *in vitro* ELISA (Enzyme-Linked Immunosorbent Assay) kit is designed for the accurate quantitative measurement of Myeloperoxidase in cell culture supernatants, cell lysate, tissue homogenates, serum, plasma (heparin, EDTA), saliva and urine.

A Myeloperoxidase specific mouse monoclonal antibody has been precoated onto 96-well plates. Standards and test samples are added to the wells and incubated. A biotinylated detection polyclonal antibody from goat specific for Myeloperoxidase is then added followed by washing with PBS or TBS buffer. Avidin-Biotin-Peroxidase Complex is added and unbound conjugates are washed away with PBS or TBS buffer. TMB is then used to visualize the HRP enzymatic reaction. TMB is catalyzed by HRP to produce a blue color product that changes into yellow after adding acidic stop solution. The density of yellow coloration is directly proportional to the Human Myeloperoxidase amount of sample captured in plate.

Myeloperoxidase is a mammalian phagocyte hemoprotein thought to primarily mediate host defense reactions. It is abundantly expressed in neutrophils and secreted during their activation. Myeloperoxidase is part of the host defense system of Human polymorphonuclear leukocytes, responsible for microbicidal activity against a wide range of organisms. It is located in the nucleus as well as in the cytoplasm. Intranuclear MPO may help to protect DNA against damage resulting from oxygen radicals produced during myeloid cell maturation and function. The standard product used in this kit is the product of gene recombination, consisting of 697(A49-S745) amino acids with the molecular mass of 80KDa.

2. Protocol Summary

Prepare all reagents, samples, and standards as instructed

Add standard or sample to each well used.

Incubate at room temperature.

Add prepared biotin antibody to each well. Incubate at room temperature.

Add prepared Avidin-Biotin-Peroxidase Complex (ABC). Incubate at room temperature.

Add TMB to each well. Incubate at room temperature. Add Stop Solution to each well. Read

3. Precautions

Please read these instructions carefully prior to beginning the assay.

- All kit components have been formulated and quality control tested to function successfully as a kit.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipet by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

4. Storage and Stability

Store kit at 20°C immediately upon receipt. Avoid multiple freeze-thaw cycles. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in the Materials Supplied section.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

6. Materials Supplied

Item	Quantity	Storage Condition
Anti-Human Myeloperoxidase antibody Microplate (12 x 8 wells)	96 wells	-20°C
Lyophilized recombinant Human Myeloperoxidase standard	2 x 20 ng	-20°C
Biotinylated anti-Human Myeloperoxidase antibody	130 µL	-20°C
Avidin-Biotin-Peroxidase Complex (ABC)	130 µL	-20°C
Sample Diluent Buffer	30 mL	-20°C
Antibody Diluent Buffer	12 mL	-20°C
ABC Diluent Buffer	12 mL	-20°C
TMB Color Developing Agent	10 mL	-20°C
TMB Stop Solution	10 mL	-20°C
Plate Seal	4 units	-20°C

7. Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully perform this assay:

- Standard microplate reader.
- Automated plate washer (optional).
- Adjustable pipettes and pipette tips. Multichannel pipettes are recommended when large sample sets are being analyzed.
- 100 mL and 1 liter graduated cylinders.
- Eppendorf tubes.
- Washing buffer, either neutral PBS or TBS (see Section 9 for recipes).

8. Technical Hints

- This kit is sold based on number of tests. A 'test' simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.
- To determine the appropriate sample dilution to use in this ELISA a pilot experiment using standards and a small number of samples is recommended
- The TMB Color Developing agent is colorless and transparent before use
- Before using the kit, briefly centrifuge the tubes in case any of the contents are trapped in the lid
- It is recommended to assay all standards, controls and samples in duplicate
- Do not let the 96-well plate dry out as this will inactivate active components on plate
- To avoid cross contamination do not reuse tips and tubes
- In order to avoid marginal effects of plate incubation due to temperature difference (reaction may be stronger in the marginal wells), it is suggested that the diluted ABC and TMB solution be pre-warmed in 37°C for 30 minutes before using

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use. The kit contains enough reagents for 96 wells.
- Prepare only as much reagent as is needed on the day of the experiment.

9.1 1X Biotinylated anti-Human Myeloperoxidase

Biotinylated anti-Human Myeloperoxidase antibody must be diluted in 1:100 with the antibody Diluent buffer and mixed thoroughly. (i.e. Add 1 µL Biotinylated anti-Human Myeloperoxidase antibody to 99 µL antibody Diluent buffer.) The total volume should be: 100 µL /well x (the number of wells). (Allow 100 µL - 200 µL extra for pipetting error).

9.2 1X Avidin-Biotin-Peroxidase Complex

Avidin- Biotin-Peroxidase Complex (ABC) must be diluted in 1:100 with the ABC Diluent Buffer and mixed thoroughly. (i.e. Add 1 µL ABC to 99 µL ABC Diluent Buffer.) The total volume should be: 100 µL/well x (the number of wells). (allow 100 µL - 200 µL extra for pipetting error).

9.3 0.01 M TBS

Add 1.2 g Tris, 8.5 g NaCl; 450 µL of purified acetic acid or 700 µL of concentrated hydrochloric acid to distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

9.4 0.01 M PBS

Add 8.5 g NaCl, 1.4 g Na2HPO4 and 0.2 g NaH2PO4 to distilled water and adjust pH to 7.2 - 7.6. Finally, adjust the total volume to 1 L with distilled water.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.
- Reconstitution of the Human Myeloperoxidase standard should be prepared no more than 2 hours prior to the experiment. Two tubes of Myeloperoxidase standard (20 ng per tube) are included in each kit. Use one tube for each experiment.
- The following section describes the preparation of a standard curve for duplicate measurements (recommended).

10.1 Prepare a 20 ng/mL **Standard #1** by reconstituting the Myeloperoxidase standard with addition of 1 mL Sample Diluent Buffer. Hold at room temperature for 10 minutes. This 20 ng/mL Standard #1 should be stored at 4°C for up to 12 hours, or at -20°C for up to 48 hours. Avoid repeated freeze-thaw cycles.

10.2 Label seven tubes with #2 - 8.

10.3 Add 300 µL Sample Diluent Buffer into tubes #2 - 8.

10.4 Prepare **Standard #2** by transferring 300 µL from Standard #1 to tube #2. Mix thoroughly and gently.

10.5 Prepare **Standard #3** by transferring 300 µL from Standard #2 to tube #3. Mix thoroughly and gently.

10.6 Prepare **Standard #4** by transferring 300 µL from Standard #3 to tube #4. Mix thoroughly and gently.

10.7 Using the table below as a guide, repeat for tubes #5 through #7.

10.8 **Standard #8** contains no protein and is the Blank control.

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Starting Conc. (pg/mL)	Final Conc. (pg/mL)
1	Step 10.1			20,000
2	300 µL Standard #1	300	20,000	10,000
3	300 µL Standard #2	300	10,000	5,000
4	300 µL Standard #3	300	5,000	2,500
5	300 µL Standard #4	300	2,500	1,250
6	300 µL Standard #5	300	1,250	625
7	300 µL Standard #6	300	625	312
8 (Blank)	N/A	300		0

11. Sample Preparation

11.1 Cell Culture Supernatants:

Remove particulates by centrifugation, assay immediately or aliquot and store samples at -20°C.

11.2 Tissue Homogenates:

Rinse tissue with PBS to remove excess blood, chopped into 1-2 mm pieces, and homogenize with a tissue homogenizer in PBS or in lysate solution (i.e. Add 10ml lysate solution to 1g tissue). Centrifuge at approximately 5,000 x g for 5 min. Assay immediately or aliquot and store homogenates at -20°C. Avoid repeated freeze-thaw cycles.

11.3 Cell Lysates:

Collect cells and rinse cells with PBS. Homogenize and lyse cells thoroughly in lysate solution. Centrifuge cell lysates at approximately 10,000 x g for 5 min to remove debris. Aliquots of the cell lysates were removed and assayed.

11.4 Serum:

Allow the serum to clot in a serum separator tube (about 4 hours) at room temperature. Centrifuge at approximately 1,000 x g for 15 min. Analyze the serum immediately or aliquot and store frozen at -20°C.

11.5 Plasma:

Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 min at 1,500 x g within 30 min of collection. Assay immediately or aliquot and store samples at -20°C.

11.6 Saliva:

Collect saliva using a collection device without any protein binding or filtering capabilities such as a Salivette or aliquot and store samples at -20°C.

11.7 Urine:

Aseptically collect the first urine of the day; micturate directly into a sterile container. Remove particular impurities by centrifugation, assay immediately or aliquot and store samples at -20°C.

General Sample information:

The user needs to estimate the concentration of the target protein in the sample and select the correct dilution factor so that the diluted target protein concentration falls near the middle of the linear regime in the standard curve.

Dilute the samples using the provided Sample Diluent Buffer. The following is a guideline for sample dilution. Several trials may be necessary to determine the optimal dilution factor. The sample must be thoroughly mixed with the Sample Diluent Buffer before assaying.

- High target protein concentration (200 – 2,000 ng/mL). The working dilution is 1:100. i.e. Add 1 µL sample into 99 µL Sample Diluent Buffer
- Medium target protein concentration (20 - 200 ng/mL). The working dilution is 1:10. i.e. Add 10 µL sample into 90 µL Sample Diluent Buffer
- Low target protein concentration (312 - 20,000 pg/mL). The working dilution is 1:2. i.e. Add 50 µL sample to 50 µL Sample Diluent Buffer
- Very Low target protein concentration (≤ 312 pg/mL). No dilution necessary, or the working dilution is 1:2.

12. Plate Preparation

- The 96 well plate strips included with this kit are supplied ready to use. It is not necessary to rinse the plate prior to adding reagents
- Unused well strips should be returned to the plate packet and stored at 4°C
- For each assay performed, a minimum of 2 wells must be used as blanks, omitting primary antibody from well additions
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates)
- Well effects have not been observed with this assay. Contents of each well can be recorded on the template sheet included in the Resources section

13. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.

13.1 Prepare all reagents, working standards, and samples as directed in the previous sections.

13.2 Add 100 µL of prepared standards and diluted samples to appropriate wells.

13.3 Seal the plate with a new plate seal and incubate at 37°C for 90 minutes.

13.4 Remove the cover, discard contents of each well, and blot the plate onto paper towels or other absorbent material. Do NOT let the wells completely dry at any time.

13.5 Add 100 µL of 1X Biotinylated anti-Human Myeloperoxidase antibody into each well, seal with a new plate seal and incubate the plate at 37°C for 60 minutes.

13.6 Wash the plate three times with 300 µL 0.01 M TBS or 0.01 M PBS, and each time let the washing buffer stay in the wells for one minute. Discard the washing buffer and blot the plate onto paper towels or other absorbent material.

Δ Note For automated washing, aspirate all wells and wash THREE times with PBS or TBS buffer, overfilling wells with each wash. Blot the plate onto paper towels or other absorbent material.

13.7 Add 100 µL of 1X Avidin-Biotin-Peroxidase Complex working solution into each well, seal with a new plate seal and incubate the plate at 37°C for 30 minutes.

13.8 Wash plate five times with 0.01M TBS or 0.01M PBS, and each time let washing buffer stay in the wells for 1 - 2 minutes. Discard the washing buffer and blot the plate onto paper towels or other absorbent material. (See Step 13.6 for plate washing method).

13.9 Add 90 µL of prepared TMB color developing agent into each well, seal the plate with a new plate seal and incubate plate at 37°C in dark for 25 - 30 minutes

Δ Note The optimal incubation time should be determined by end user. The shades of blue should be seen in the wells with the four most concentrated Human Myeloperoxidase standard solutions; the other wells show no obvious color.

13.10 Add 100 µL of prepared TMB Stop Solution into each well. The color changes into yellow immediately.

13.11 Read the O.D. absorbance at 450 nm in a microplate reader within 30 minutes after adding the stop solution.

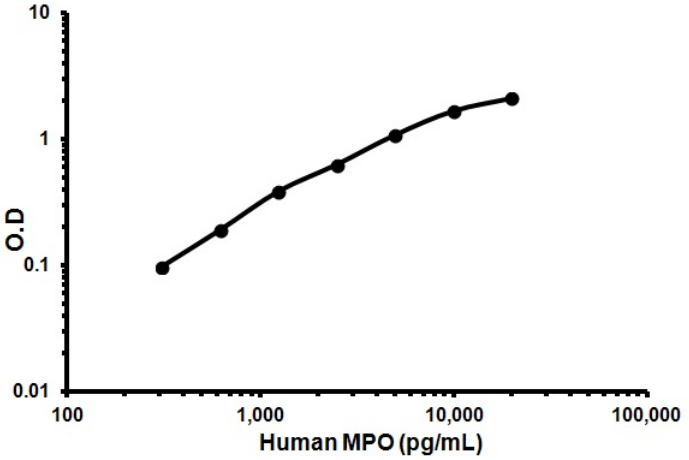
14. Calculations

For calculation, the relative O.D.450 = (the O.D.450 of each well) – (the O.D.450 of Zero well). The standard curve can be plotted as the relative O.D.450 of each standard solution (Y) vs. the respective concentration of the standard solution (X). The Human Myeloperoxidase concentration of the samples can be interpolated from the standard curve.

Δ Note If the samples measured were diluted, make sure to account for this in your calculations.

15. Typical Data

Typical standard curve – data provided **for demonstration purposes only.** A new standard curve must be generated for each assay performed.



Conc. (pg/mL)	O.D. 450nm
0	0.020
312	0.097
625	0.190
1,250	0.385
2,500	0.628
5,000	1.076
10,0000	1.668
20,0000	2.105

Figure 1. Example of Myeloperoxidase standard curve. The standard curve was prepared as described in Section 10.

16. Typical Sample Values

RANGE – 312 - 20,000 pg/mL

SENSITIVITY – < 10 pg/mL

17. Assay Specificity

This kit detects for natural Human Myeloperoxidase.

No detectable cross-reactivity with other relevant proteins.

Please contact our Technical Support team for more information.

18. Troubleshooting

Problem	Reason	Solution
Poor standard curve	Inaccurate pipetting	Check pipettes
	Improper standards dilution	Prior to opening, briefly spin the stock standard tube and dissolve the powder thoroughly by gentle mixing
Low Signal	Incubation times too brief	Ensure sufficient incubation times; change to overnight standard/sample incubation
	Inadequate reagent volumes or improper dilution	Check pipettes and ensure correct preparation
Samples give higher value than the highest standard	Starting sample concentration is too high.	Dilute the specimens and repeat the assay
Large CV	Plate is insufficiently washed	Review manual for proper wash technique. If using a plate washer, check all ports for obstructions
	Contaminated wash buffer	Prepare fresh wash buffer
Low sensitivity	Improper storage of the kit	Store the all components as directed.

19. Notes

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