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ab155458 – MPO (Myeloperoxidase) Mouse ELISA Kit

For the quantitative measurement of mouse MPO (Myeloperoxidase) in cell culture supernatants and plasma.

∆Notes: We have not been able to detect the endogenous Mouse MPO (Myeloperoxidase) in normal serum with ab155458, only in serum spiked with Mouse MPO (Myeloperoxidase).

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

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1. Overview

Abcam's MPO (Myeloperoxidase) Mouse ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay designed for the quantitative measurement of mouse MPO (Myeloperoxidase) in plasma and cell culture supernatants. We have not been able to detect the endogenous Mouse MPO (Myeloperoxidase) in normal serum with ab155458, only in serum spiked with Mouse MPO (Myeloperoxidase).

This assay employs an antibody specific for mouse MPO coated on a 96-well plate. Standards and samples are pipetted into the wells and MPO present in a sample is bound to the wells by the immobilized antibody. The wells are washed and biotinylated anti-mouse MPO antibody is added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin is pipetted to the wells. The wells are again washed, a TMB substrate solution is added to the wells and color develops in proportion to the amount of MPO bound. The Stop Solution changes the color from blue to yellow, and the intensity of the color is measured at 450nm.

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 Streptavidin solution. Incubate at room temperature.

Add TMB One-Step Development Solution to each well. Incubate at room temperature.

Add Stop Solution to each well. Read immediately.

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.

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
MPO Microplate(12 x 8 wells)	96 wells	-20°C
20X Wash Buffer concentrate	25 mL	-20°C
Recombinant Mouse MPO Standard	2 vials	-20°C
Assay Diluent C	30 mL	-20°C
5X Assay Diluent B	15 mL	-20°C
Biotinylated anti-mouse MPO	2 vials	-20°C
400X HRP-Streptavidin Concentrate	200 µL	-20°C
TMB One-Step Substrate Reagent	12 mL	-20°C
Stop Solution	8 mL	-20°C

7. Materials Required, Not Supplied

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

- 1 Microplate reader capable of measuring absorbance at 450 nm.
- Precision pipettes to deliver 2 µL to 1 mL volumes.
- Adjustable 1-25 mL pipettes for reagent preparation.
- 100 mL and 1 liter graduated cylinders.
- Absorbent paper.
- Distilled or deionized water.
- Log-log graph paper or computer and software for ELISA data analysis.
- Tubes to prepare standard or sample dilutions.

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.
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Make sure all buffers and solutions are at room temperature before starting the experiment.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Make sure you have the right type of plate for your detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.
- Complete removal of all solutions and buffers during wash steps. When preparing your standards, it is very critical to briefly spin down the vial first. The powder may drop off from the cap when opening it if you do not spin down. Be sure to dissolve the powder thoroughly when reconstituting. After adding Assay Diluent to the vial, we recommend inverting the tube a few times, then flick the tube a few times, and then spin it down; repeat this procedure 3-4 times. This is a technique we find very effective for thoroughly mixing the standard without too much mechanical force.
- Do not vortex the standard during reconstitution, as this will destabilize the protein.
- Once your standard has been reconstituted, it should be used right away or else frozen for later use.

- Keep the standard dilutions on ice while during preparation, but the ELISA procedure should be done at room temperature.
- Be sure to discard the working standard dilutions after use – they do not store well.

9. Reagent Preparation

- Equilibrate all reagents to room temperature (18-25°C) prior to use.

9.1 1X Diluent B

Dilute 5X Assay Diluent 5-fold with deionized or distilled water before use.

9.2 1X Wash Buffer Solution

If the 20X Wash Buffer Concentrate contains visible crystals, warm to room temperature and mix gently until dissolved. Dilute 20 mL of 20X Wash Buffer Concentrate into deionized or distilled water to yield 400 mL of 1X Wash Buffer.

9.3 1X Biotinylated MPO Detector Antibody

Briefly spin the Biotinylated anti-mouse MPO vial before use. Add 100 µL of 1X Assay Diluent B into the vial to prepare a detection antibody concentrate. Pipette up and down to mix gently (the concentrate can either be stored at 4°C for 5 days or aliquoted and frozen at -20°C for 2 months). The detection antibody concentrate should be diluted 80-fold with 1X Assay Diluent B and used in Assay Procedure.

9.4 1X HRP-Streptavidin Solution

Briefly spin the 400X HRP-Streptavidin concentrate vial and pipette up and down to mix gently before use. The 400X HRP-Streptavidin concentrate should be diluted 400-fold with 1X Assay Diluent B.

For example: Briefly spin the 400X HRP vial and pipette up and down to mix gently. Add 30 µL of 400X HRP-Streptavidin concentrate into a tube with 12 mL 1X Assay Diluent B to prepare a 400-fold diluted HRP-Streptavidin solution (don't store the diluted solution for next day use). Mix well.

10. Standard Preparation

- Prepare serially diluted standards immediately prior to use. Always prepare a fresh set of standards for every use.
- Standard (recombinant protein) should be stored at -20°C or -80°C (recommended at -80°C) after reconstitution.

10.1 Briefly spin the vial of MPO Standard. Prepare a 400 ng/mL **Stock Standard** by adding 400 µL Assay Diluent C (for plasma samples) or 1X Assay Diluent B (for cell culture supernatants) into the vial (see table below).

10.2 Ensure the powder is thoroughly dissolved by gentle mixing.

10.3 Label tubes #1-8.

10.4 Prepare **Standard #1** by adding 180 µL 400 ng/mL **Stock Standard** to 300 µL Assay Diluent C or 1X Assay Diluent B into tube #1. Mix thoroughly and gently.

10.5 Pipette 300 µL Assay Diluent C or 1X Assay Diluent B into each tube.

10.6 Prepare **Standard #2** by transferring 200 µL from tube #1 to tube #2, mix thoroughly.

10.7 Prepare **Standard #3** by transferring 200 µL from tube #2 to tube #3, mix thoroughly.

10.8 Using the table below as a guide, prepare further serial dilutions.

10.9 Assay Diluent C or or 1X Assay Diluent B serves as the zero standard (0 ng/mL).

Standard #	Volume to dilute (µL)	Volume Diluent (µL)	Total Volume (µL)	Starting Conc. (ng/mL)	Final Conc. (ng/mL)
1	180	300	480	400	150
2	200	300	500	150	60
3	200	300	500	60	24
4	200	300	500	24	9.6
5	200	300	500	9.6	3.84
6	200	300	500	3.84	1.54
7	200	300	500	1.54	0.61
8	0	300	300	0	0

11. Sample Preparation

General Sample Information:

- If your samples need to be diluted, Assay Diluent C should be used for dilution of plasma samples. 1X Assay Diluent B should be used for dilution of culture supernatants.
- Suggested dilution for normal plasma: 2-10 fold.
- Please note that levels of the target protein may vary between different specimens. Optimal dilution factors for each sample must be determined by the investigator.

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 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.

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 Add 100 µL of each standard (see Standard Preparations, section 10) and sample into appropriate wells. Cover well and incubate for 2.5 hours at room temperature or over night at 4°C with gentle shaking.

13.2 Discard the solution and wash 4 times with 1X Wash Solution. Wash by filling each well with Wash Buffer (300 µL) using a multi-channel Pipette 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.

13.3 Add 100 µL of 1X prepared biotinylated antibody (see Reagent Preparation, section 9) to each well. Incubate for 1 hour at room temperature with gentle shaking.

13.4 Discard the solution. Repeat the wash as in step 13.2.

13.5 Add 100 µL of prepared Streptavidin solution (see Reagent Preparation, section 9) to each well. Incubate for 45 minutes at room temperature with gentle shaking.

13.6 Discard the solution. Repeat the wash as in step 13.2.

13.7 Add 100 µL of TMB One-Step Substrate Reagent to each well. Incubate for 30 minutes at room temperature in the dark with gentle shaking.

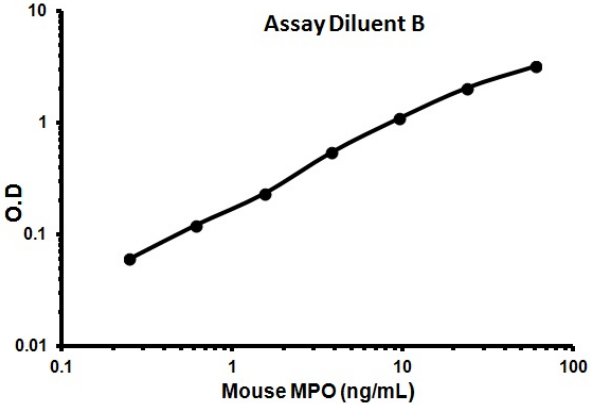
13.8 Add 50 µL of Stop Solution to each well. Read at 450 nm immediately.

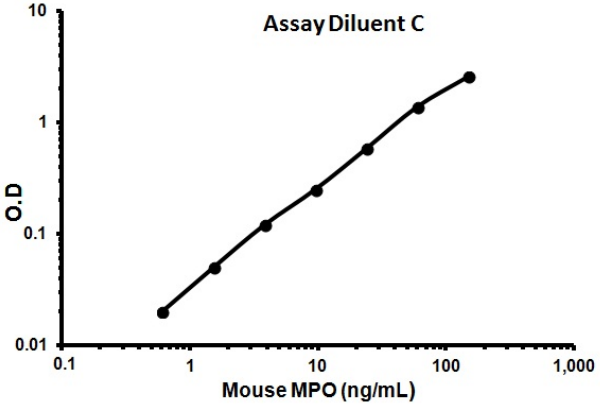
14. Calculations

Calculate the mean absorbance for each set of duplicate standards, controls and samples, and subtract the average zero standard optical density. Plot the standard curve on log-log graph paper, with standard concentration on the x-axis and absorbance on the y-axis. Draw the best-fit straight line through the standard points.

15. Typical Data

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





Conc. (ng/mL)	O.D. Assay Diluent B	Conc. (ng/mL)	O.D. Assay Diluent C
0.25	0.06	0.61	0.02
0.61	0.12	1.54	0.05
1.54	0.23	3.84	0.12
3.84	0.54	9.60	0.25
9.60	1.09	24.00	0.58
24.00	2.04	60.00	1.38
60.00	3.17	150.00	2.57

16. Typical Sample Values

SENSITIVITY –

The minimum detectable dose (MDD) of MPO is typically less than 0.6 ng/mL.

RECOVERY –

Recovery was determined by spiking various levels of mouse MPO into mouse plasma and cell culture media. Mean recoveries are as follows:

Sample Type	Average % Recovery	Range (%)
Plasma	86.89	68-105
Cell culture supernatant	105.5	96-112

Linearity of Dilution

Plasma Dilution	Average % Expected Value	Range (%)
1:2	94.68	83-106
1:4	123.5	114-135

Cell Culture Supernatant Dilution	Average % Expected Value	Range (%)
1:2	113.3	105-120
1:4	114.7	96-126

PRECISION –

	Intra-Assay	Inter-Assay
CV	<10%	<12%

17. Assay Specificity

This ELISA pair antibody detects mouse MPO. Other species not determined yet.

18. Species Reactivity

This kit recognizes MPO (Myeloperoxidase).

19. Troubleshooting

Problem	Reason	Solution
Low Precision	Use of expired components	Check the expiration date listed before use. Do not interchange components from different lots
	Improper wash step	Check that the correct wash buffer is being used. Check that all wells are empty after aspiration. Check that the microplate washer is dispensing properly. If washing by pipette, check for proper pipetting technique
	Splashing of reagents while loading wells	Pipette properly in a controlled and careful manner
	Inconsistent volumes loaded into wells	Pipette properly in a controlled and careful manner. Check pipette calibration. Check pipette for proper performance
	Insufficient mixing of reagent dilutions	Thoroughly agitate the lyophilized components after reconstitution. Thoroughly mix dilutions
	Improperly sealed microplate	Check the microplate pouch for proper sealing. Check that the microplate pouch has no punctures. Check that three desiccants are inside the microplate pouch prior to sealing

Problem	Cause	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
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 ELISA kit	Store the reconstituted protein at -80°C, all other assay components 4°C. Keep substrate solution protected from light.

20. Notes

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