Version 9a, Last updated 29 May 2025

ab111749 Myeloperoxidase (MPO) Activity Assay Kit (Fluorometric)

For the rapid, sensitive and accurate measurement of myeloperoxidase activity in various samples.

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

Table of Contents

1. Overview 1
2. Protocol Summary 2
3. Precautions 3
4. Storage and Stability 3
5. Limitations 4
6. Materials Supplied 4
7. Materials Required, Not Supplied 5
8. Technical Hints 6
9. Reagent Preparation 7
10. Standard Preparation 8
11. Sample Preparation 9
12. Assay Procedure 11
13. Calculations 13
14. Typical data 15
15. Quick Assay Procedure 16
16. Troubleshooting 17
17. Interferences 19
18. FAQs 19
19. Notes 22

1. Overview

Myeloperoxidase (MPO) Assay Kit (Fluorometric) (ab111749) provides a rapid, simple, sensitive, and reliable method to study MPO activity. MPO catalyzes the production of sodium hypochlorite (NaClO) from hydrogen peroxide (H_2O_2) and sodium chloride (NaCl). Subsequently, NaClO will react stoichiometrically with the free radical sensor aminophenyl fluorescein (APF) to generate fluorescein, which can be detected at Ex/Em = 485/525 nm.

This kit can be used to detect MPO activity as low as 0.5 µU per well.

Myeloperoxidase (MPO, EC 1.11.1.7) is a peroxidase enzyme most abundantly present in neutrophil granulocytes. It is a green hemoprotein found in neutrophils and monocytes that catalyzes the reaction of hydrogen peroxide and halide ions to form cytotoxic acids and other intermediates that play a role in the oxygen-dependent killing of tumor cells and microorganisms. Its heme pigment causes the green color in secretions rich in neutrophils, such as pus and some forms of mucus. Furthermore, it can oxidize tyrosine to a tyrosyl radical using hydrogen peroxide as an oxidizing agent.

2. Protocol Summary

Standard curve preparation

Sample preparation

Add reaction mix

Incubate for 30 minutes at RT and measure fluorescence (Ex/Em = 485/525 nm) in kinetic mode

**For kinetic mode detection, incubation time given in this summary is for guidance only*

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 in the dark immediately upon receipt. 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.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

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 (Before prep)	Storage Condition (After prep)
Assay Buffer 54	25 mL	-20°C	-20°C
Hydrogen Peroxide Solution	50 µL	-20°C	-20°C
MPO Chlorination Probe	200 µL	-20°C	-20°C
Fluorescence Standard IV	50 µL	-20°C	-20°C
MPO Positive Control	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 54 was previously labelled as Assay Buffer LVIV and MPO Assay Buffer, and Hydrogen Peroxide Solution as Hydrogen Peroxide Solution II and MPO Substrate Stock. The composition has not changed.

7. Materials Required, Not Supplied

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 485/525 nm
- MilliQ water or other type of double distilled water (ddH_2O)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96 well plate with clear flat bottom, preferably black
- Dounce homogenizer or pestle (if using tissue)

For white blood cells (WBC):

- Red Blood Cell (RBC) Lysis Buffer (ab204733)

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.
- 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.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples generating values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer 54:

Ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

9.2 Hydrogen Peroxide Solution:

Dilute 4 µL of Hydrogen Peroxide Solution in 700 µL of Assay Buffer 54 to create **MPO Substrate Solution**. Keep on ice while in use. Do not store diluted solution for further use.

Aliquot remaining Hydrogen Peroxide Solution so that you have enough volume to perform the desired number of assays. Store at -20°C protected from light.

9.3 MPO Chlorination Probe:

Ready to use as supplied. Aliquot probe so that you have enough volume to perform the desired number of assays, Store at -20°C protected from light. Once the probe is thawed, use within two months.

9.4 Fluorescence Standard IV:

Ready to use as supplied. Aliquot standard so that you have enough volume to perform the desired number of assays, Store at -20°C protected from light.

9.5 MPO Positive Control:

Reconstitute positive control in 50 µL Assay Buffer 54. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -20°C.

Avoid freeze/thaw cycles. Use within 1 month.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Prepare Fluorescence Standard IV immediately prior the assay as it needs to be read quickly after preparation.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare 1 mL of Fluorescence Standard IV solution by diluting 5 µL of the Fluorescence Standard IV (see Section 9.4) in 995 µL of Assay Buffer 54

10.2 Using 5 µM standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Fluorescence Standard IV (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount Fluorescein in well (pmol/well)
1	0	300	100	0
2	6	294	100	10
3	12	288	100	20
4	18	282	100	30
5	24	276	100	40
6	30	270	100	50

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 µL).

11. Sample Preparation

General sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you snap freeze your samples in liquid nitrogen upon extraction and store them immediately at -80°C. When you are ready to test your samples, thaw them on ice and proceed with the Sample Preparation step. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).
11.1.2 Wash cells with cold PBS.
11.1.3 Resuspend cells in 100 µL of ice cold Assay Buffer 54.
11.1.4 Homogenize cells quickly by pipetting up and down a few times.
11.1.5 Incubate sample on ice for 10 minutes.
11.1.6 Centrifuge for 5 minutes at top speed using a cold microcentrifuge to remove any insoluble material.
11.1.7 Collect supernatant.
11.1.8 Transfer to a new tube.
11.1.9 Keep on ice.

11.2 Tissue samples:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation = 10 mg tissue).
11.2.2 Wash cells with cold PBS.
11.2.3 Resuspend tissue in 100 µL ice cold Assay Buffer 54.
11.2.4 tissue with a Dounce homogenizer or pestle sitting on ice, with 10 – 15 passes.
11.2.5 Incubate sample on ice for 10 minutes.
11.2.6 Centrifuge for 2 - 5 minutes at top speed using a cold microcentrifuge to remove any insoluble material.
11.2.7 Collect supernatant and transfer to a new tube.
11.2.8 Keep on ice.

11.3 White blood cells:

11.3.1 Take 2 mL blood.
11.3.2 Lyse RBC (red blood cells) using Red Blood Cell (RBC) Lysis Buffer (ab204733).
11.3.3 Incubate for 10 minutes at room temperature.
11.3.4 Centrifuge at 400 x g for 5 minutes.
11.3.5 Remove supernatant carefully.
11.3.6 Wash pellet with 1 mL PBS.
11.3.7 Centrifuge at 400 x g for 5 minutes and remove supernatant carefully.
11.3.8 Lyse pellet using 200 µL Assay Buffer 54.
11.3.9 Keep on ice for 10 minutes.
11.3.10 Centrifuge at 10,000 x g for 10 minutes to remove insoluble material. Collect supernatant.
11.3.11 Dilute supernatant 10 times with Assay Buffer 54.
11.3.12 Use 1 – 10 µL of diluted WBC lysate for the assay.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. 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.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

12.1 Fluorescence Standard IV:

12.1.1 Prepare Standard curve as directed in Section 10.
12.1.2 Incubate for 5 minutes at room temperature.
12.1.3 Measure Standard curve fluorescence on a microplate reader at Ex/Em = 485/525 nm in end point mode.

12.2 Plate Loading:

- Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with Assay Buffer 54).
- Optional: MPO Positive control = 10 µL MPO positive control + 40 µL Assay Buffer 54

12.3 Assay Reaction:

12.3.1 Prepare 50 µL of MPO Reaction Mix for each reaction. Mix enough reagents for the number of assays (samples and positive control) to be performed. Prepare a master mix of the Reaction mix to ensure consistency.

Component	Reaction Mix (µL)
Assay Buffer 54	46
MPO Substrate Solution	2
MPO Chlorination Probe	2

12.3.2 Add 50 µL of Reaction Mix into each sample and positive control. Mix well.

12.4 Measurement:

12.4.1 Measure output at Ex/Em = 484/525 nm on a microplate reader in kinetic mode, every 2 – 3 minutes, for at least 30 minutes at room temperature protected from light.

Δ Note: Incubation time depends on the MPO activity in the samples. Longer incubation time may be required if activity in the sample is low. We recommend measuring fluorescence in a kinetic mode, and choosing two time points (T1 and T2) to calculate the MPO activity of the samples.

13. Calculations

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:

13.1.1 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.1.2 Average the duplicate reading for each standard.

13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of MPO in the sample:

13.2.1 For all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding RFU values at those points (RFU1 and RFU2).

13.2.2 Calculate ΔRFU for sample as follows:

$\Delta RFU = RFU2 - RFU1$

13.3 MPO activity (pmol/min/mL or µU/mL) in the test samples is calculated as:

$$MPO\ Activity = \left(\frac{B}{\Delta T x\ V}\right) * D$$

Where:

B = amount of Fluorescein in sample well calculated from standard curve (pmol).

ΔT = linear phase reaction time $T_2 - T_1$ (minutes).

V = original sample volume added into the reaction well (mL).

D = sample dilution factor.

Unit definition:

1 Unit MPO activity = amount of MPO that oxidizes the APF substrate to generate 1.0 µmol of fluorescein per minute at 25°C.

14. Typical data

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

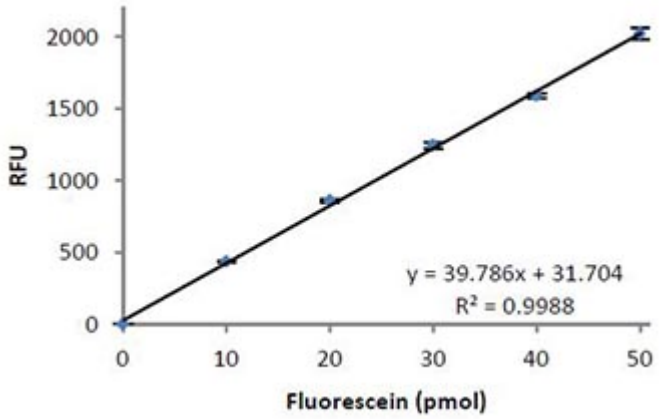

Figure 1. Typical standard calibration curve.

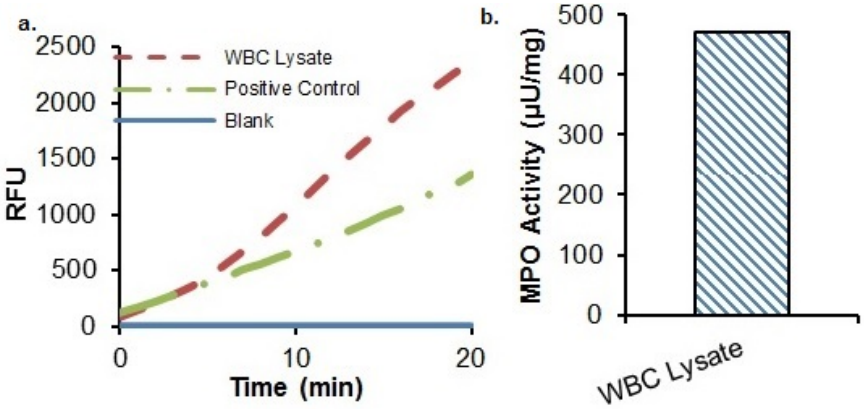

Figure 2. a) Measurement of MPO activity in white blood cells (WBC) lysate (0.1 µg) and MPO positive control (included in the kit); b) MPO specific activity in WBC lysate.

15. Quick Assay Procedure

Δ Note: this procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Prepare MPO standard, substrate stock, positive control and probe (aliquot if necessary); get equipment ready.
- Prepare MPO standard dilution [10 - 50 pmol/well].
- Set up plate in duplicate for standard (100 µL). Incubate MPO standard for 5 minutes at RT. Measure fluorescence on a microplate reader at Ex/Em = 485/525 nm in end point mode.
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for samples (50 µL) and (if using) positive control wells (50 µL).
- Prepare a master mix for MPO Reaction Mix:

Component	Reaction Mix (µL)
Assay Buffer 54	46
MPO Substrate Solution	2
MPO Chlorination Probe	2

- Add 50 µL Reaction to sample and positive wells.
- Measure plate at Ex/Em= 485/525 nm on a microplate reader in a kinetic mode, every 2 – 3 minutes, at RT for 30 – 60 minutes protected from light.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at - 80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

Problem	Reason	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 µL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. Interferences

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA Buffer: contains SDS which can destroy/decrease the activity of the enzyme.
- EDTA: MPO is an enzyme and needs some ions/cofactors to function. As EDTA is a chelator, it might interfere with the enzymatic activity and make MPO non-functional.

18. FAQs

Q. Can this kit be used with frozen cells/tissue?

A. We always prefer fresh samples for the most accurate results. It is possible to use frozen cells/tissue samples as long as they have been stored with minimum delay after collection and have been stored at -80°C without freeze-thaw. However, the MPO enzyme is quite vulnerable to losing activity during storage. The functional enzyme contains disulphide bonds and if reduced, the enzyme can lose activity. It also contains heme which can get oxidized by the peroxide generated by MPO activity and the result is that the enzyme loses activity by auto-oxidation.

Q. How much cell/tissue sample is needed for this assay?

A. There is no set amount of cells/tissue needed for this assay. The amount depends on how much active MPO is present in the samples. Typically, 1 – 2 x 10^6 cells and 10 – 100 mg tissue can be used per assay.

Q. What is the activity of the positive control? How can the value be higher to compare with samples?

A. The positive control is only a benchmark sample. As long as the values are within the range of the standard curve this is fine. The positive control is not to be used to compare values with the samples. The positive control is provided to validate that the assay

components are all working. If the values are low, the customer can add more volume to get higher values but this is not necessary as long as the values are within the std. curve range. MPO is a very vulnerable enzyme to freeze-thaw and can lose activity with storage over time.

Q. What is the dilution factor used for?

A. If a certain volume of neat sample is added to the well and volume is made up with the assay buffer up to 50 µL, then dilution factor does not apply. If the sample is pre-diluted before adding to the well, then the dilution factor is used. For example, if 10 µL of a 5X diluted sample is used, then V=0.01 mL and Dilution factor =5.

Q. I have used this product (ab111749) and its colorimetric equivalent (ab105136) but got very different raw data for the increasing dilutions of the samples. Why?

A. It is very important to be able to distinguish the two kits by principle. For MPO Activity Colorimetric (ab105136), the lower the OD, the higher the MPO activity. If you add too much sample, the OD will be too low and it will probably be below the detection limit of absorbance instruments. For MPO Activity Fluorometric (ab111749), the higher the RFU, the higher the MPO activity. So, for this kit, adding too much sample can saturate the detector and the substrate can be limiting. This will result in discrepant differences between dilutions.

Q. How does the sensitivity compare between MPO Activity Assay Kit (Colorimetric) (ab105136) and this product (ab111749)?

A. This kit (ab111749) is a fluorometric assay and is at least 10 times more sensitive than ab105136. The standard curve range for ab105136 is 1 – 50 nmoles of TNB formed, whereas the standard curve range for ab111749 is between 1 – 50 pmoles of Fluorescein.

Q. Can RIPA buffer be used to prepare samples for this kit?

A. For any enzyme assay, we do not recommend RIPA buffer since it contains SDS and this can denature proteins and affect enzyme activity. We have tested and recommend using the assay buffer provided in the kit for best results.

19. Notes

Technical Support

For all technical or commercial enquiries please go to:
www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)