ab273334 – Myeloperoxidase (MPO) Peroxidation Activity Assay Kit (Fluorometric)

For the determination of Myeloperoxidase (MPO) Peroxidation activity in adherent/suspension cells and tissue.
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab273334 (use http://www.abcam.cn/ab273334 for China, or http://www.abcam.co.jp/ab273334 for Japan)

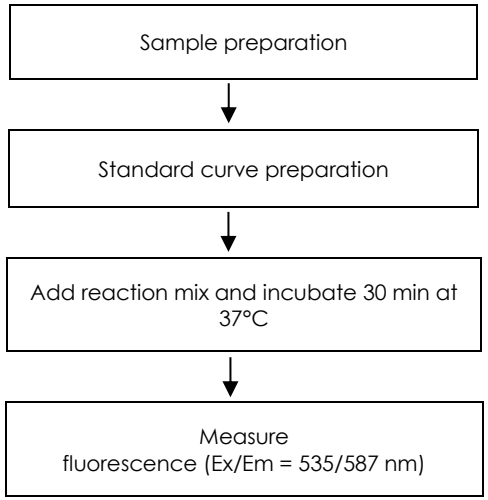
Background:

Myeloperoxidase (MPO) Peroxidation Activity Assay Kit (ab273334) is a rapid, simple, sensitive, and reliable fluorometric assay to measure MPO-mediated peroxidation, in various samples, such as tissue and cell lysates.

In the MPO assay, a substrate is oxidized to generate fluorescence (Ex/Em = 535/587 nm), which is directly proportional to total peroxidase activity in the sample. A specific MPO inhibitor is provided, which suppresses peroxidase activity due to MPO. This permits differentiation of MPO-mediated peroxidation from the activity of other peroxidases which may be present in the sample. This kit provides a sensitive method for detecting MPO activity and as low as 2 µU of MPO activity.

Assay Summary:

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



QUICK ASSAY PROCEDURE

- Bring plate reader to 37°C
- Solubilize MPO Positive Control. Thaw Assay Buffer LVI, OxiRed™ Probe, Hydrogen Peroxide Solution, Resorufin Standard and MPO Inhibitor Control (aliquot if necessary); get equipment ready
- Prepare standard curve.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Set up plate for standard (50 µL) and samples (50 µL).
- Prepare Reaction Mix.
- Add 40 µL Reaction Mix to each well.

Precautions & Limitations:

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.

- Modifications to the kit components or procedures may result in loss of performance.
- 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.

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. Reconstituted components are stable for 2 months.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Hydrogen Peroxide Solution	50 µL	-20°C	-20°C
Assay Buffer LVI	50 mL	-20°C	+4°C
MPO Inhibitor Control	100 µL	-20°C	-20°C
OxiRed™ Probe	0.2 mL	-20°C	-20°C
MPO Positive Control	1 Each	-20°C	-70°C
Resorufin Standard	50 µL	-20°C	-20°C

PLEASE NOTE: Hydrogen Peroxide Solution was previously labeled as Hydrogen Peroxide Solution II and Hydrogen Peroxide, and OxiRed™ Probe as OxiRed Probe and MPO Peroxidation Substrate. The composition has not changed.

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96 well black plate with flat bottom (fluorometric assay)
- Microcentrifuge
- Dounce homogenizer (if using tissue)
- 1x PBS, pH 7.4
- MilliQ water or other type of double distilled/deionized water (ddH_2O)

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Assay Buffer LVI is ready to use as supplied. Equilibrate to room temperature before use. Store at +4°C.

MPO Inhibitor Control and **Resorufin Standard** are ready to use as supplied. Equilibrate to room temperature before use. Store at -20°C.

OxiRed™ Probe: Ready to use as supplied. Warm by placing in a 37°C bath for 1 – 5 minutes to thaw the DMSO solution before use. Keep at room temperature during the assay. Store at -20°C and protect from light and moisture. Once the probe is opened and thawed, it is stable for at least 3 additional freeze/thaw cycles but should be used within two months. After use, promptly retighten the cap to minimize adsorption of airborne moisture.

MPO Positive Control: Reconstitute each with 200 µL Assay Buffer LVI. Keep on ice during the assay. Aliquot and store at -70°C. Use within two months. Avoid freeze and thaw.

Hydrogen Peroxide Solution: Prepare 5 mM working Hydrogen Peroxide solution by adding 4 µl Hydrogen Peroxide Solution (0.88 M) to 700 µl Assay Buffer LVI. Always prepare fresh.

Sample Preparation:

- 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 complete the Sample Preparation step before storing the samples. Alternatively, 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. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.
- We recommend adding a protease inhibitor cocktail to Assay Buffer LVI at 1:1000 ratio to preserve MPO activity, while preparing the cell/tissue samples.

Cells (adherent or suspension) samples

1. Harvest 6 x10^6 cells by centrifugation at 1,000 x *g* for 5 mins at 4°C.
2. Wash cells in cold PBS.
3. Resuspend cell pellet in 500 µl Assay Buffer LVI, mix well and incubate on ice for 10 mins.
4. Homogenize cells with a Dounce homogenizer sitting on ice.
5. Centrifuge at top speed for 10 mins at 4°C.
6. Collect the supernatant and transfer to a clean tube.
7. Keep on ice.

Tissue Samples:

1. Use perfused tissue (after removing intravascular blood) samples.
2. Wash tissues (~20 mg) with cold PBS,
3. Resuspend in 500 µl of Assay Buffer LVI. A protease inhibitor cocktail should be added at 1:1000 ratio to preserve MPO activity.
4. Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
5. Centrifuge at top speed for 10 mins at 4°C.
6. Collect the supernatant and transfer to a clean tube.
7. Keep on Ice.

For Positive Control, dilute 1:10 MPO Positive Control by taking 10 µl of MPO Positive Control to 90 µl Assay Buffer LVI, and mix well.

For MPO Inhibitor Control, dilute inhibitor 10 times by adding 50 µl of MPO Inhibitor Control to 450 µl Assay Buffer LVI and mix well.

Standard Preparation:

- Always prepare a fresh set of standards for every use and keep on ice.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).

1. Prepare serial dilution of Resofurin Standard as follows:
   - 10 pmoles/µL dilution: Add 2 µL of undiluted Resofurin Standard to 998 µl Assay Buffer LVI. Gently pipette up and down a few times to ensure all standard is removed from tip. Mix well by inversion.
   - 1 pmoles/µL dilution: Transfer 50 µL of 10 pmol/µL dilution to 450 µl Assay Buffer LVI. Gently pipette up and down and then mix well by inversion.
2. Using 1 pmoles/µL Resorufin standard, add 0, 4, 8, 12, 16, 20 µL Resorufin Standard into a series of wells, generating 0, 4, 8, 12, 16, 20 pmol/well of Resorufin Standard. Adjust the volume to 60 µl/well with Assay Buffer LVI. Or prepare duplicate standard curve dilutions as described below.

Standard #	Volume of 1 pmol/µl Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End Resofurin Amount (pmoles/well)
1	0	150	60	0
2	10	140	60	4
3	20	130	60	8
4	30	120	60	12
5	40	110	60	16
6	50	100	60	20

*Note: If desired, a linear Standard curve can be generated up to 100 pmoles of Resorufin depending on the sample MPO activity.

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

1. Set up Reaction wells:
   - Standard wells = 60 µL standard dilutions.
   - Sample wells = 2 – 50 µL samples or 2-20 µl of diluted MPO Positive Control per well. Adjust volume to 60 µL/well with Assay Buffer LVI.
   - Sample Inhibitor wells = 2 – 50 µL samples or 2-20 µl of diluted MPO Positive Control per well. Add 10 µl of diluted MPO Inhibitor Control and adjust volume to 60 µL/well with Assay Buffer LVI.

   Each sample well (and duplicate) must have a corresponding sample inhibitor well (and duplicate) containing the same amount of sample. The sample wells which do not contain MPO Inhibitor Control will show total Peroxidase Activity. The sample wells containing MPO Inhibitor Control will suppress MPO and show the level of non-MPO activity from other peroxidases.

2. Each well (standards, samples, and controls) requires 40 µL of Reaction Mix as shown in the table below. To ensure consistency, use the table below to prepare a Master Mix of the appropriate Reaction Mix for your assay using the following calculation:

   X µL component x (Number reactions +1).

Component	Fluorometric Assay Reaction Mix (µL)
Assay Buffer LVI	37
OxiRed™ Probe	1
Hydrogen Peroxide Solution	2

3. Mix Master Reaction Mix by inversion. Add 40 µL of the Master Reaction Mix to each well. Use a clean tip for each well.
4. Mix well and measure the kinetic fluorescence at Ex/Em= 535/587 nm for 5-20 mins at 37°C.

   Note: Incubation time depends on the myeloperoxidase activity in the samples. We recommend measuring the RFU in the sample (RFU_S) and sample with inhibitor (RFU_I) in a kinetic mode and choose two time points (T_1 and T_2) in the linear range to calculate the rate of the reaction. The Resorufin standard curve can be read in Endpoint mode (i.e., at the end of the sample incubation time).

Calculations:

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and re-perform assay. Remember to multiply the determined concentration by the sample dilution factor.

1. Average the duplicate reading for each standard, sample and inhibitor wells.
2. Subtract the mean RFU value of the blank (Standard #1) from all standard and sample readings. This is the corrected RFU.
3. Plot the corrected RFU values for each standard as a function of the final concentration of Resorufin and determine the slope of the standard curve using a linear regression.
4. Calculate MPO-mediated peroxidase activity of the test sample:

$$\Delta RFU = (RFU_S(T_2) - RFU_S(T_1)) - (RFU_I(T_2) - RFU_I(T_1))$$

5. Apply the ΔRFU to the Resorufin Standard Curve to get B pmoles of Resorufin generated by Myeloperoxidase during the reaction time ($\Delta T = T_2 - T_1$).

$$MPO\ activity = \frac{B}{(\Delta T \times V)} \times D = pmol/(min \times ml) = \mu U/ml$$

Where:
B = Resorufin amount from the Standard Curve (in pmoles).
ΔT = Reaction time ($T_2 - T_1$) (in minutes)
V = Sample volume added to the reaction well (in mL).
D = sample dilution factor (prior to addition to the well).

MPO activity can also be expressed in µU/mg of total protein in the sample.

Unit Definition: A unit of MPO is the amount of enzyme that generates 1.0 µmol of resorufin per min at pH 7.0 at 37°C.

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For all technical or commercial enquiries please go to:
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