ab105136 Myeloperoxidase Activity Assay Kit (Colorimetric)

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.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab105136 (use abcam.cn/ab105136for China, or abcam.co.jp/ab105136 for Japan)

Materials Supplied and Storage

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. Observe the storage conditions for individual prepared components below. Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
MPO Assay Buffer II/MPO Assay Buffer	25 mL	-20°C	-20°C
DTNB Probe (100 mM)	50 µL	-20°C	-20°C
TCEP (50mM)	1 vial	-20°C	-20°C
Hydrogen Peroxide Solution II/MPO Substrate	50 µL	-20°C	-20°C
Stop Mix (Lyophilized)	20 µL	-20°C	-20°C
MPO Positive Control (Lyophilized)	1 vial	-20°C	-20°C

Materials Required, Not Supplied

- MilliQ water or other type of double distilled water (ddH_2O)
- PBS
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD412 nm
- 96 well plate (clear-bottom plate)
- Orbital shaker
- Dounce homogenizer

1.Reagent Preparation:

Briefly centrifuge vials at low speed prior to opening. Store all at -20°C.

1.1 **MPO Assay Buffer II/MPO Assay Buffer:** Ready to use as supplied. Equilibrate to room temperature before use.

1.2 **DTNB Probe:** Ready to use as supplied. Aliquot probe so that you have enough to perform the desired number of arrays.

1.3 **TCEP:** Reconstitute with 75 µL dH_2O. Aliquot TCEP so that you have enough to perform the desired number of assays.

1.4 **Hydrogen Peroxide Solution II/MPO Substrate:** Aliquot and store at -20°C. Stable for 2 months. Working solution: Add 5µL MPO Substrate to 300 µL dH_2O. Make fresh and discard unused portion.

1.5 **Stop Mix:** Reconstitute with 200 µL dH_2O. Aliquot Stop Mix so that you have enough to perform the desired number of assays. Use within two months.

1.6 **MPO Positive Control:** Reconstitute with 100 µL MPO Assay Buffer. Aliquot positive control so that you have enough to perform the desired number of assays. Use within two months.

2.Standard Preparation:

Prepare fresh standards for every use. TNB is easily oxidizable so prepare from DTNB Probe as needed. Use the same day as prepared, discard any unused TNB reagent/standard.

2.1 Prepare TNB Reagent /standard: Each sample, standard and background control well requires a total volume of 50 µL of TNB Reagent /Standard Mix, as per the following:

Reagent	Volume (µL)
DNTB probe	0.5
TCEP	0.5
MPO Assay Buffer/ Assay Buffer	49

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the TNB Reagent/Standard to ensure consistency. We recommend this calculation: **X µL component x (Number samples + standards +1)**

NOTE: Do not add the reagent Mix to the plate at this stage.

2.2 Add only the MPO Assay Buffer II/MPO Assay Buffer to the plate at this stage, as per the following table:

Standard #	Volume of TNB Standard (µL)*	Assay Buffer (µL)	End [TNB] nmol in well
1	See step 4.7	150	0
2		140	10
3		130	20
4		120	30
5		110	40
6		100	50

3. Sample Preparation

Perform several dilutions of your sample to ensure the readings are within the standard value range. 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, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples 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.

3.1 Cell (adherent or suspension) samples:

3.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 2 x 10^6 cells).

3.1.2 Wash cells with cold PBS.

3.1.3 Homogenize and resuspend cells in 4 volumes of MPO Assay Buffer II/MPO Assay buffer.
3.1.4 Centrifuge sample at 13,000g for 10 minutes to remove any insoluble material.
3.1.5 Collect supernatant and transfer to a clean tube.
3.1.6 Keep on ice.

3.2 Tissue samples:

3.2.1 Harvest amount of tissue necessary for each assay (initial recommendation=10 mg).
3.2.2 Wash tissue in cold PBS.
3.2.3 Homogenize and resuspend tissue in 4 volumes of MPO Assay buffer II/MPO Assay buffer.
3.2.4 Centrifuge sample at 13,000g for 10 minutes to remove any insoluble material.
3.2.5 Collect supernatant and transfer to a clean tube.
3.2.6 Keep on ice.

3.3 Serum samples:

3.3.1 Serum samples can be directly diluted in the MPO Assay buffer II/MPO Assay Buffer.

3.4 White blood cells:

3.4.1 For white blood cells, take 2 mL of blood and lyse RBC using RBC Lysis Buffer.
3.4.2 Incubate for 10 minutes at room temperature.
3.4.3 Centrifuge at 400 x g for 5 min. and remove the supernatant carefully.
3.4.4 Wash the pellet with 1 mL 1X PBS.
3.4.5 Centrifuge at 400 x g for 5 min, and remove the supernatant carefully.
3.4.6 Lyse the pellet using 200 µL MPO Assay buffer II/MPO Assay Buffer.
3.4.7 Keep on ice for 10 minutes.
3.4.8 Centrifuge at 10,000 x g for 10 min. to remove insoluble material.
3.4.9 Collect the supernatant.
3.4.10 Use 1-10 µL of the WBC lysate into a 96-well plate.
3.4.11 Prepare parallel sample well(s) as background control.

NOTE: *Use different volumes of sample to ensure readings are within the Standard Curve range*

4. Assay Procedure

Equilibrate all materials and prepared reagents to RT prior to use. Assay in duplicate.

4.1 Set up Reaction wells:

Standard wells: Set up MPO Assay Buffer in series of wells as per step 2.2.
Sample wells = 1 – 50 µL samples (adjust volume to 50 µL/well with MPO Assay Buffer).
Positive control wells = 5 -10 µL MPO Positive Control (adjust to 50 µL/well with MPO Assay Buffer).
Sample Background wells = 1 – 50 µL samples (Adjust volume to 50 µL/well with MPO Assay Buffer). These wells are optional if one is only comparing relative absorbances of samples, but are necessary for calculation of MPO activity.

4.2 Reaction Mix:

Component	Reaction Mix Samples (µL)	Background Control Mix (µL)
MPO Assay buffer II/MPO Assay Buffer	40	40
Hydrogen Peroxide Solution II/MPO Substrate	10	0
dH_2O	0	10

Mix enough reagents for the number of assays (samples and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation: **X µL component x (Number samples/background control+1)**

4.2 Add 50 µL of the Reaction Mix to each well containing the positive controls and samples.
4.3 Add 50 µL of the Background Control Mix to the background sample wells.
NOTE: *Do not add Reaction Mix to standard wells.*
4.4 Mix and incubate at 25°C for 30 minutes to 2 hours. Record this time as T.
NOTE: *It is suggested to run samples for 30 min, 1 hr and 2 hr, followed by the Stop Mix and TNB Reagent additions at each time point to ensure values will fall within the linear range of the Standard Curve.*
4.5 Add 2 µL Stop Mix to all sample, standard wells, background sample and positive control wells. Mix and incubate at RT for 10 minutes.
4.6 Add 50 µL TNB Reagent/Standard (from step 2.1) to each of the sample, background sample and positive control wells.
4.7 At this time, add TNB Reagent/Standard to the Standard wells (which already contain MPO Assay Buffer from step 2.2) as per the following table:

Standard #	Volume of Standard (µL)*	End [TNB] nmol in well
1	0	0
2	10	10
3	20	20
4	30	30
5	40	40
6	50	50

4.8 Mix and incubate at room temperature for 5 - 10 minutes.
4.9 Measure output (OD412 nm) on a microplate reader.
The positive controls and samples will show decreased color proportional to the amount of enzyme present, calculated as: **ΔA412nm = A sample background – A sample**
It is recommended to use the ΔA values which are in the linear range of the Standard Curve.

5. Calculations

Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor. For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

5.1 Average the duplicate reading for each standard and sample.
5.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
5.3 Plot the corrected absorbance values for each standard as a function of the final concentration of TNB.
5.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

5.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$B = \left(\frac{Corrected\ absorbance - (y - intercept)}{Slope}\right)$$

5.6 MPO activity in the test samples is calculated as:

$$MPO\ Activity = \frac{B}{\Delta T\ x\ V}\ x\ Sample\ Dilution\ Factor$$

Where:

B = TNB amount calculated from the ΔA412nm (= absorbance of sample background – absorbance of sample) (in nmol).
T = time of the first incubation (in min) i.e. pre-Stop Mix
V = pre-adjusted sample volume (mL) added into the reaction well.
Unit Definition: One unit of MPO is the amount of MPO which hydrolyzes the substrate and generates taurine chloramine to consume 1.0 µmol of TNB per minute at 25°C.

FAQs:

How do you recommend standardizing the amount of sample used for all the wells? Start with the same weight of tissue and homogenize in 2-3 volumes of the assay buffer. Then a total protein quantitation assay can be done and the same total protein can be added to each well for all samples. If a protein assay is not done, the same sample volume should be used or all samples.

We have used MPO Activity Assay kit (colorimetric) (ab105136) and MPO Activity Assay kit (fluorometric) (ab111749) but got very different raw data for the increasing dilutions of the samples. Why? It is very important to be able to distinguish the two kits by principle. For the colorimetric assay, the lower the OD, the higher the MPO activity. If you add too much sample, the OD will be so low; it could be below the detection limit of absorbance instruments. For the fluorometric assay, 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.

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

What is the dilution factor used for? 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.

Can one background control be used for all samples? Ideally, it is recommended to have parallel controls for each sample so that corresponding background can be subtracted for each sample correctly.

Technical Support

For all technical or commercial enquiries please go to:
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