# ab105136 – Myeloperoxidase (MPO) Peroxidation Activity Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement 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/ab105136** (use http://www.abcam.cn/ab105136 for China, or http://www.abcam.co.jp/ab105136 for Japan)

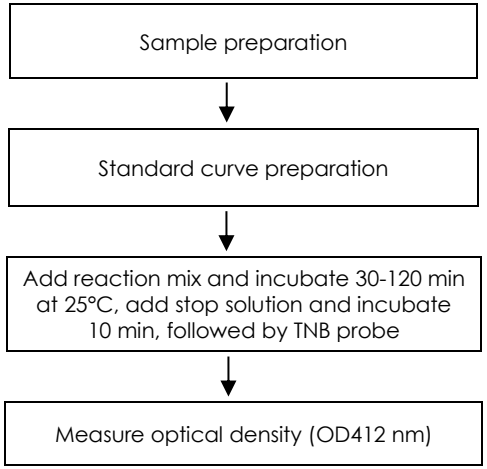
**Background:**

Myeloperoxidase (MPO) Activity Assay Kit ab105136 is a rapid, simple, sensitive, and reliable colorimetric assay suitable for use as a high throughput MPO activity assay, in various samples, such as tissue and cell lysates, serum, urine (UTI) and cell culture supernatant.

In the MPO assay, myeloperoxidase produces HClO from $H_2O_2$ and $Cl^-$. The HClO reacts with taurine to generate the taurine chloramine, which subsequently reacts with the DTNB probe to eliminate color (412 nm). The absorbance is inversely proportional to the amount of MPO enzyme. This MPO assay kit can be used to detect myeloperoxidase as low as 0.05 mU per well.

**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

- Set plate reader to room temperature
- Solubilize MPO Positive Control, TCEP and Stop Mix, Thaw MPO Assay Buffer, DTNB probe, and Hydrogen Peroxide Solution (aliquot if necessary); get equipment ready.
- Prepare TNB probe.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings).
- Prepare Reaction Mix (Number samples + standards + 1).
- Add 50 µL Reaction Mix to corresponding wells.
- Incubate plate at 25°C for 30-120 mins.
- Add 2 µL Stop mix to all wells and incubate plate at 25°C for 10 min.
- Add 50 µL TNB probe to corresponding wells.
- Prepare standard curve, using TNB probe.
- Measure plate at OD 412 nm .

**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. Do not use kit or components if they have exceeded the expiry date.

**Materials Supplied:**

| Item | Quantity | Storage Temperature (on receipt) | Storage temperature (reconstituted) |
|---|---|---|---|
| MPO Assay Buffer | 25 mL | -20°C | -20°C |
| DTNB Probe | 50 µL | -20°C | -20°C |
| TCEP | 1 Each | -20°C | -20°C |
| Hydrogen Peroxide Solution | 50 µL | -20°C | -20°C |
| Stop Mix | 1 Each | -20°C | -20°C |
| MPO Positive Control | 1 Each | -20°C | -80°C |

PLEASE NOTE: MPO Assay Buffer was previously labeled as MPO Assay Buffer II, and Hydrogen Peroxide Solution as Hydrogen Peroxide Solution II and MPO 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 absorbance at OD = 412 nm
- 96 well clear plate with flat bottom
- Microcentrifuge
- Orbital shaker
- Dounce homogenizer (if using tissue)
- 1x PBS, pH 7.4
- MilliQ water or other type of double distilled/deionized water (dd$H_2O$)

**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.

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

**DTNB Probe:** Ready to use as supplied. Equilibrate to room temperature before use. Aliquot probe so that you have enough to perform the desired number of assays.

**TCEP:** Reconstitute with 75 µL dH2O. Aliquot TCEP so that you have enough to perform the desired number of assays.

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

**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.

**MPO Positive Control:** Reconstitute with 100 µL MPO Assay Buffer. Aliquot and store at -20°C. Use within two months.

**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).

Prepare TNB Reagent/Standard as follows:

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

| TNB Reagent/Standard | Volume (µL) |
|---|---|
| DNTB probe | 0.5 |
| TCEP | 0.5 |
| MPO Assay Buffer | 49 |

2. Prior to performing the assay prepare enough TNB Reagent/Standard 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 using this calculation:

**X µL component x (Number samples + standards +1)**

3. Do not add the TNB Reagent/Standard to the plate at this stage. Keep on Ice until required (see Assay Procedure, step 9).
4. Do add the MPO Assay Buffer to the standard wells on the plate, as per the following table:

| Standard # | Volume of TNB Reagent/Standard (µL) | Assay Buffer (µL) | Final Amount of TNB in well (nmoles) |
|---|---|---|---|
| 1 | 0 | 150 | 0 |
| 2 | 10 | 140 | 10 |
| 3 | 20 | 130 | 20 |
| 4 | 30 | 120 | 30 |
| 5 | 40 | 110 | 40 |
| 6 | 50 | 100 | 50 |

**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 MPO Assay Buffer at 1:1000 ratio to preserve MPO activity, while preparing the cell/tissue samples.

**Cells (adherent or suspension) samples**

1. Harvest 2 x$10^6$ 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 4x volumes of MPO Assay Buffer,
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 (~10 mg) with cold PBS.
3. Resuspend in 500 µl of MPO Assay Buffer.
4. Homogenize tissue 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.

**Serum samples:** Serum samples can be directly diluted in the MPO Assay buffer.

**White blood cells:**

1. For white blood cells, take 2 mL of blood and lyse RBC using RBC Lysis Buffer (ab204733).
2. Incubate for 10 minutes at room temperature.
3. Centrifuge at 400 x g for 5 min. and remove the supernatant carefully.
4. Wash the pellet with 1 mL 1x PBS.
5. Centrifuge at 400 x g for 5 min and remove the supernatant carefully.
6. Lyse the pellet using 200 µL MPO Assay buffer.
7. Keep on ice for 10 minutes.
8. Centrifuge at top speed for 10 min. to remove insoluble material.
9. Collect supernatant and transfer to a clean tube.
10. Keep on ice.

**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 = MPO Assay Buffer (Standard Preparation, step 4).
   - 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 volume to 50 µL/well with MPO Assay Buffer.
   - Sample background well= 1 – 50 µL samples Adjust volume to 50 µL/well with MPO Assay Buffer. These wells are optional if you are only interested in comparing the relative absorbances of samples but are required for calculation of MPO activity.

2. Each well (samples and controls) but **NOT Standards** requires 50 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 | Positive Control/Sample Reaction Mix (µL) | Background Control Reaction Mix (µL) |
|---|---|---|
| MPO Assay buffer | 40 | 40 |
| Working Hydrogen Peroxide Solution | 10 | 0 |
| dH2O | 0 | 10 |

3. Add 50 µL of the Positive Control/Sample Reaction Mix to each well containing either positive controls or samples.
4. Add 50 µL of the Background Control Mix to the background sample wells.
5. Do not add any reaction mix to the standard wells. At this stage, the standard wells should only contain MPO Assay Buffer (Standard Preparation, step 4)
6. Mix well 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*
7. Add 2 µL Stop Mix to all sample, standard wells, background sample and positive control wells. Mix well and incubate at room temperature for 10 minutes.
8. Add 50 µL TNB Reagent/Standard (Standard Preparation, Step 1-2) to each of the sample, background sample and positive control wells.
9. You should now add TNB Reagent/Standard to the Standard wells. The required volume is shown in the table under Standard Preparation.
10. Mix well and incubate at room temperature for 5 - 10 minutes.
11. Mix well and measure the absorbance at OD 412 nm on a microplate reader.

The positive controls and samples will show decreased color proportional to the amount of enzyme present, calculated as:

**$\Delta A_{412}$ = Absorbance (Sample Background) – Absorbance (Sample)**

You should use the ΔAbsorbance values that are in the linear range of the standard curve.

**Calculations:**

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.

1. Average the duplicate reading for each standard and sample.
2. Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.
3. Plot the corrected absorbance values for each standard as a function of the final concentration of TNB and determine the slope of the standard curve using a linear regression.
4. Interpolate the amount of TNB in the sample wells by using the linear equation, using the sample corrected readings.
5. MPO activity in the test samples is calculated as:

$$MPO\ activity\ = \frac{B}{(\Delta \mathrm{T}\ \times\ \mathrm{V})}\ \times\ D = \frac{nmoles}{min \times ml} = mU/ml$$

Where:
B = TNB amount calculated from the Δ A412 nm
ΔT = Reaction time (in mins). This is the first incubation step prior to adding the stop mix.
V = pre-adjusted sample volume added to the reaction well (in mL).
D = sample dilution factor (prior to adding to the plate).

**Unit Definition:** One unit of MPO is the amount of MPO which hydrolyzes the substrate and generates taurine chloramine to consume 1.0 µmole of TNB per minute at 25°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**



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