MPO/Myeloperoxidase Activity Assay Kit (Colorimetric)

MPO/Myeloperoxidase Activity Assay Kit (Colorimetric) can be used to measure Myeloperoxidase activity in whole neutrophils, neutrophil lysates, tissue homogenates and plasma (EDTA).

Catalog number: ARG82770

Package: 200 assays

For research use only. Not for use in diagnostic procedures.

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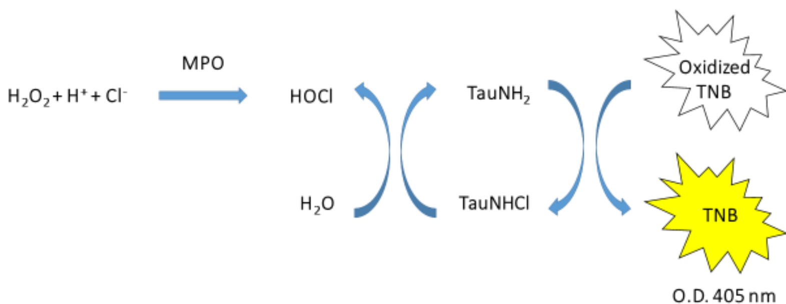
INTRODUCTION

Myeloperoxidase (MPO) is a peroxidase enzyme that in humans is encoded by the MPO gene on chromosome 17. MPO is most abundantly expressed in neutrophil granulocytes (a subtype of white blood cells), and produces hypohalous acids to carry out their antimicrobial activity, including hypochlorous acid, the sodium salt of which is the chemical in bleach. It is a lysosomal protein stored in azurophilic granules of the neutrophil and released into the extracellular space during degranulation. Neutrophil myeloperoxidase has a heme pigment, which causes its green color in secretions rich in neutrophils, such as mucus and sputum. The green color contributed to its outdated name verdoperoxidase. [Provide by Wikipedia: Myeloperoxidase]

PRINCIPLE OF THE ASSAY

This MPO/Myeloperoxidase Activity Assay Kit (Colorimetric) is a simple colorimetric assay that measures the amount of MPO present in whole neutrophils, neutrophil lysates, tissue homogenates and plasma (EDTA). The MPO enzyme catalyzes the reaction of hydrogen peroxide (H2O2) with chloride ions to create hypochlorous acid (HOCl), which rapidly reacts with taurine to produce a stable taurine chloramine product. This step readily neutralizes the HOCl, which would otherwise accumulate and inactivate MPO. A catalase-containing stop solution is added to stop MPO catalysis by eliminating hydrogen peroxide. Finally, taurine chloramine reacts with the yellow TNB chromogen probe, with a decrease in color indicating higher MPO activity. The concentration of MPO in the samples is then determined by comparing the O.D. 405-412 nm absorbance of samples to the standard curve. (See the Figure below)



MATERIALS PROVIDED & STORAGE INFORMATION

Upon receipt, store the HTAB Extraction Reagent at Room Temperature. Store all other kit components at 4°C.

Component	Quantity	Storage information
5X Assay Buffer	25 mL	4°C
Hydrogen Peroxide (8.82M)	100 µL	4°C (Protect from light)
100X Colorimetric Probe	100 µL	4°C (Protect from light)
100X TCEP Reagent	100 µL	4°C
500X Stop Solution	25 µL	4°C (Protect from light)
HTAB Extraction Reagent	20 mL	Room temperature

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading in the 405-412 nm range
- Sonicator or tissue homogenizer
- Standard 96-well microplate
- Microcentrifuge tubes
- Deionized or Distilled water
- 100 mM Phosphate Buffer, pH 6.0
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Conical tubes, microcentrifuge tubes and bottles

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon receipt, store the HTAB Extraction Reagent at Room Temperature. Store all other kit components at 4°C.
- The TNB chromogen standards should be prepared immediately prior to running the assay.
- The concentrations can vary over a wide range depending on the different samples. For unknown samples, we recommend doing a pilot experiment & testing several doses to ensure the readings are within the standard curve range.
- All reagents should be mixed by gentle inversion or swirling prior to use. Do not induce foaming.
- Before using the kit, spin tubes and bring down all components to the bottom of tubes.
- It is highly recommended assaying the Standards and samples in duplicates.
- Change pipette tips between the addition of different reagent or samples.

SAMPLE COLLECTION & STORAGE INFORMATION

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Tissue Lysates: Wash or perfuse tissues with cold 100 mM Phosphate Buffer, pH 6.0 prior to homogenization to eliminate MPO from blood. Homogenize approximately 100 mg of tissue in 1-2 mL cold 100 mM Phosphate Buffer, pH 6.0 containing 0.5% HTAB. Centrifuge the homogenate at 10,000 x g for 10 minutes at 4°C. Collect the supernatant. Store on ice if using immediately.

Plasma: Collect blood with EDTA and centrifuge at 2,000 x g and 4°C for 10 minutes. The supernatant should be assayed directly or diluted as necessary in 1X Assay Buffer.

Whole Neutrophils: Collect whole blood in EDTA blood collection tubes. Isolate neutrophils using density-gradient centrifugation. Re-suspend the neutrophil pellet in HBSS or DPBS (free of calcium and magnesium) at 1-2 x 10^6 cells/mL for use in the assay. Stimulate myeloperoxidase activity with PMA.

Neutrophil lysates: Collect whole blood in EDTA blood collection tubes. Isolate neutrophils using density-gradient centrifugation. Stimulate myeloperoxidase activity with PMA. Re-suspend the neutrophil pellet in cold 100 mM Phosphate Buffer, pH 6.0, 1 mM EDTA containing 0.5% HTAB at 1-2 x 10^7 cells/mL. Lyse cells by sonication or multiple freeze-thaw cycles. Collect neutrophil extract in the supernatant following centrifugation at 10,000 x g for 15 minutes at 4°C.

Note:

1. Do not use haemolytic, icteric or lipaemic specimens.
2. Avoid disturbing the white buffy layer when collection serum / plasma sample.

3. EDTA-plasma is recommended for MPO measurement in blood samples. Heparin-plasma, citrate-plasma and serum samples are not recommended due to ex vivo release of MPO from neutrophils.
4. All samples should be assayed immediately or stored at-80°C for up to 2 months. Run proper controls as necessary. Optimal experimental conditions for samples must be determined by the investigator. Always run a standard curve with samples.
5. High levels of interfering substances may cause variations in results.

REAGENT PREPARATION

- **1X Assay Buffer:** Dilute the 5X Assay Buffer into deionized water to yield 1X Assay Buffer. (E.g., add 15 mL of 5X Assay Buffer into 60 mL of deionized water to a final volume of 75 mL) Mix to homogeneity. Use this buffer for preparing kit reagents. Store the 1X Assay Buffer at 4°C.
- **1 mM Chromogen Working Solution:** Prepare this solution before use and prepare only enough for immediate applications since it is easily oxidized. The 1 mM Chromogen Working Solution is used to detect the MPO activity in samples as well as to prepare the TNB chromogen standards. Prepare by diluting both the 100X Colorimetric Probe and 100X TCEP Reagent 1:100 in 1X Assay Buffer (E.g., add 50 µL of Colorimetric Probe and 50 µL of TCEP Reagent into 4.90 mL of 1X Assay Buffer to a final volume of 5 mL). Vortex thoroughly. The solution should appear bright yellow. Protect from light until needed and store at 4°C.
- **1 mM Hydrogen Peroxide Solution:** Prepare by performing a two-step dilution. First, dilute the provided Hydrogen Peroxide 1:1000 in deionized water (E.g., add 5 µL of Hydrogen Peroxide stock into 4.995 mL of deionized water to a final volume of 5 mL). Further dilute this preparation by adding 800 µL to 6.2 mL of 1X Assay Buffer to yield 7 mL at a final 1 mM concentration. Vortex thoroughly. Prepare only what is needed for immediate applications and do not store any of the diluted solutions.
- **1X Stop Solution:** Immediately prior to use, vortex Stop Solution suspension and prepare a 1:500 dilution in 1X Assay Buffer (E.g., Add 10 µL into 4.990 mL of 1X Assay Buffer to a final volume of 5 mL). Vortex thoroughly. Prepare only what is needed for immediate applications and

do not store diluted Stop Solution.

- **Standards:** Prepare TNB chromogen standards with the prepared 1 mM Chromogen Working Solution. Use microcentrifuge tubes to prepare a series of standards according to the table below. Prepare standards immediately prior to each assay performed. Vortex tubes thoroughly. Do not store or reuse the chromogen standard preparations.

Standard tube	Final TNB Chromogen conc. (µM)	Final TNB Chromogen (nmol/well)*	Volume of 1X Assay Buffer (µL)	Volume of 1 mM Chromogen Working Solution (µL)
S1	333	50	500	250
S2	267	40	550	200
S3	200	30	600	150
S4	133	20	650	100
S5	67	10	700	50
S6	33.3	5	725	25
S7	16.7	2.5	737.5	12.5
S8	8.3	1.25	743.7	6.25
S9	4.2	0.63	746.8	3.13
S0	0	0	750	0

Note: The TNB chromogen standards should be prepared immediately prior to running the assay. *: Based on 150 µL volume/well.

ASSAY PROCEDURE

Each Standard and sample should be assayed in duplicate or triplicate. A freshly prepared standard curve should be used each time the assay is performed.

Note: In order to ensure the values of samples fall within the linear range of the standard curve, the plate may be read at multiple time points (optional). If this is desired, duplicates of each unknown sample should be added to the plate for each time point (E.g., 6 replicates for 3 time points). Suggested time points are 30, 60, and 120 minutes.

1. Add **25 µL** of **samples** or **sample buffer control** into 96-well microplate.

   Note: A sample buffer control must be run for each sample type being tested. When testing whole neutrophils, for example, include an additional well of 25 µL of the buffer used to re-suspend the neutrophil pellets. If testing plasma samples, 25 µL of 1X PBS may be used as the sample buffer control.

2. Add **25 µL** of **1 mM Hydrogen Peroxide solution** to each well and mix thoroughly. Cover plate to protect from light and incubate for **30-120 minutes** at **room temperature**.

   Note: If only one time point is desired, 60 minutes should be sufficient for most samples.

3. Vortex the **1X Stop Solution** and add **50 µL** to each sample well to be read at the current time point. Mix briefly. Incubate for **10-15 minutes** at **room temperature**.

4. Add **50 µL** of the **1 mM Chromogen Working Solution** to each well which **1X Stop Solution** was added in **step 3**, and mix briefly. Cover plate to protect from light and incubate for **10-15 minutes** at **room temperature**.

5. Read absorbance of each well on a microplate reader at **405-412 nm**.
6. For time course studies cover the plate and incubate until the next time point, and then **repeat steps 3 through 6**. Proceed immediately to Standard Curve Generation below.

Standard Curve Generation

Note: TNB chromogen standards should be prepared immediately prior to the assay.

1. Add **150 µL** of each freshly prepared **Standards** to a 96-well plate. Each standard should be assayed in duplicate or triplicate.
2. Read absorbance of each well on a microplate reader at **405-412 nm.**

CALCULATION OF RESULTS

1. Calculate the average absorbance value for each set of Standards, Control and samples. Subtract the average Standard 0 value from itself and all standard and sample values. This is the corrected absorbance value.
2. Using log-log, semi-log or linear graph paper, construct a standard curve by plotting the mean absorbance value obtained from each standard against its concentration with absorbance value on the vertical (Y) axis and concentration on the horizontal (X) axis.
3. Subtract the sample buffer control well values from the sample well values to obtain the difference. The absorbance difference is due to the MPO activity:

   $\Delta OD = (OD_{\text{sample well}}) - (OD_{\text{sample buffer control well}})$
4. Compare the change in absorbance (ΔOD) of each sample to the chromogen standard curve to determine the amount of chromogen consumed within the assay. Only use values within the range of the standard curve.
5. Determine the myeloperoxidase activity in milliunits/mL (mU/mL) of a sample using the equation:

MPO Activity (mU/mL) = Q / (T x 0.025 mL*)

***:** 25 µL sample volume. Be sure to account for any dilution factors made on unknown samples prior to the assay.

Q = Quantity (in nmoles/well) of chromogen consumed as determined from graph

T = Reaction time (in minutes) determined from the time the Stop Solution was added

Example Calculation

MPO Sample OD = 0.175

MPO Sample Buffer Control OD = 1.275

Zero Standard = 0.025

Time = 30 minutes

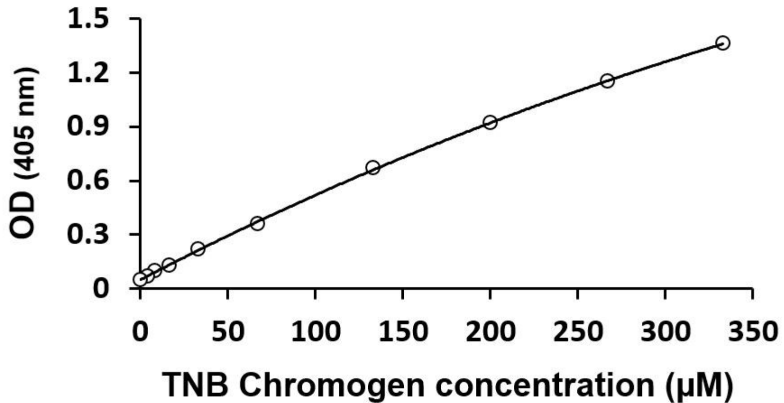
Sample Volume = 0.025 mL

1. Subtract the zero standard OD from MPO sample OD: 0.175 – 0.025 = 0.150
2. Subtract the zero standard OD from MPO sample buffer control OD: 1.275 – 0.025 = 1.250
3. Calculate the change in absorbance (ΔA): 1.250 - 0.150 = 1.100
4. Using your graphed standard curve, extrapolate the amount of nmoles consumed. (E.g., Typical Standard Curve indicates Q = 38 nmoles)
5. Solve for MPO activity:

   38 nmoles / (30 minutes x 0.025 mL) = 50.7 mU/mL

EXAMPLE OF TYPICAL STANDARD CURVE

The following figures demonstrate typical results with the MPO/Myeloperoxidase Activity Assay Kit (Colorimetric). One should use the data below for reference only. This data should not be used to interpret actual results.



QUALITY ASSURANCE

Sensitivity

4.2 μM