Product Manual

OxiSelect™ Myeloperoxidase Chlorination Activity Assay Kit (Colorimetric)

Catalog Number

STA-803 **200 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Myeloperoxidase (MPO) is a heme-based peroxidase enzyme responsible for antimicrobial activity against a wide range of organisms. MPO is copiously expressed in stimulated neutrophil granulocytes, where it catalyzes the production of hypoalous acids, such as hypochlorous acid (HOCl), from hydrogen peroxide (H_2O_2) and chloride ion (Cl^-), or other halides. The characteristic heme green pigment of MPO can be seen in secretions containing neutrophils such as pus and mucus.

Myeloperoxidase has been implicated in many disease states including arthritis, cancer, renal dysfunction, and cystic fibrosis. While myeloperoxidase deficiency is a hereditary disease that results in immune deficiency, antibodies against MPO have been linked to various types of vasculitis. The reactive species it creates may also damage normal tissues, thereby contributing to inflammation. An association of elevated MPO levels and coronary artery disease has been demonstrated and it has been reported that the enzyme could serve as a predictor of myocardial infarction in certain patients. The numerous roles MPO plays in the innate immune response and disease states make it a target for potential therapies.

Cell Biolabs' OxiSelect™ Myeloperoxidase Chlorination Activity Assay Kit is a quantitative assay for measuring myeloperoxidase activity within plasma, tissues, cell extracts, and purified samples. The assay is not species specific and should be well-suited to active MPO enzyme systems. Myeloperoxidase activity is defined as follows: 1 unit of enzyme reduces 1 µmole hydrogen peroxide (H_2O_2) per minute at pH 6.0 and 25ºC, and generates taurine chloramine to consume 1 µmole of chromophore. Each kit provides sufficient reagents to perform up to 200 assays, including standard curve and unknown samples.

Assay Principle

The OxiSelect™ Myeloperoxidase Chlorination Activity Assay Kit is a quantitative assay for measuring the myeloperoxidase activity within a sample. The MPO enzyme catalyzes the reaction of hydrogen peroxide (H_2O_2) 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 (Figure 1). Absorbance is measured at 405-412 nm. The myeloperoxidase activity in unknown samples is determined by comparison with the predetermined TNB chromogen standard curve. The rate of chromophore reduction is proportional to the concentration of myeloperoxidase activity within the sample.

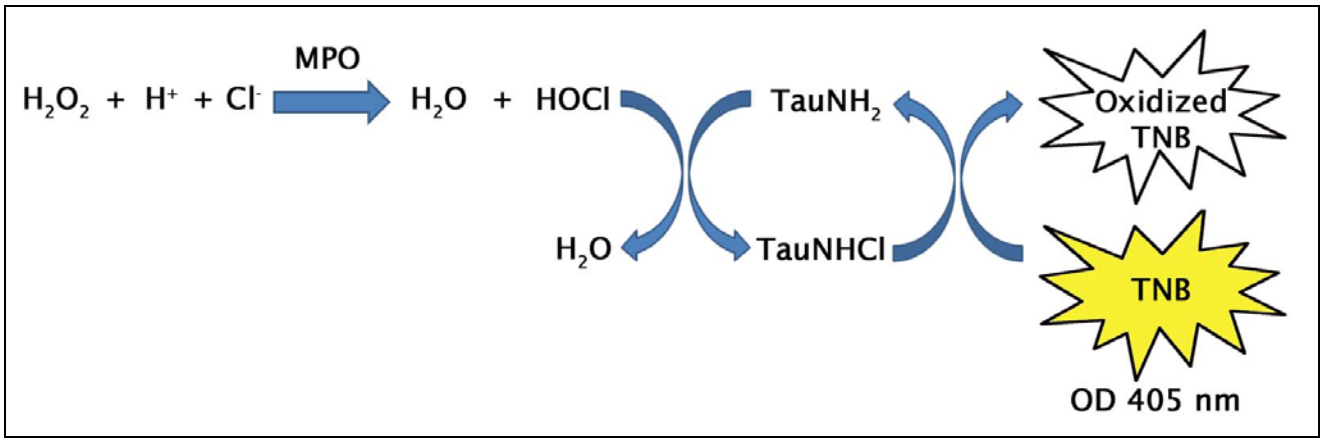

Figure 1. Assay Principle

Related Products

1. STA-312: OxiSelect™ Total Glutathione Assay Kit
2. STA-330: OxiSelect™ TBARS Assay Kit (MDA Quantitation)
3. STA-340: OxiSelect™ Superoxide Dismutase Activity Assay
4. STA-341: OxiSelect™ Catalase Activity Assay Kit
5. STA-342: OxiSelect™ Intracellular ROS Assay Kit (Green Fluorescence)
6. STA-347: OxiSelect™ *In Vitro* ROS/RNS Assay Kit (Green Fluorescence)
7. STA-345: OxiSelect™ ORAC Activity Assay
8. STA-802: OxiSelect™ *In Vitro* Nitric Oxide (Nitrite / Nitrate) Assay kit
9. STA-832: OxiSelect™ MDA Competitive ELISA Kit
10. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

1. Hydrogen Peroxide (Part No. 234102): One 100 µL amber vial of an 8.82 M solution
2. Chromogen Probe (100X) (Part No. 280301): One 100 µL amber tube
3. Assay Buffer (5X) (Part No. 280302): One 25 mL bottle
4. TCEP Reagent (100X) (Part No. 280303): One 100 µL tube
5. Stop Solution (500X) (Part No. 280304): One 25 µL amber tube
6. HTAB Extraction Reagent (Part No. 280305): One 20 mL bottle containing 5% hexadecyltrimethylammonium bromide in PBS

Materials Not Supplied

1. 96-well microtiter plate
2. Distilled or deionized purified water
3. 100 mM Phosphate Buffer, pH 6.0

4. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
5. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
6. Conical tubes, microcentrifuge tubes, and bottles for sample and buffer preparation
7. Centrifuge and/or microfuge
8. Sonicator or tissue homogenizer
9. Multichannel micropipette reservoirs
10. Microplate reader capable of reading 405-412 nm

Storage

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

Preparation of Reagents

- 1X Assay Buffer: Prepare 1X Assay Buffer by adding deionized water to the 5X Assay Buffer (e.g. add 10 mL of 5X Assay Buffer to 40 mL of deionized water). Mix thoroughly until homogeneous. Use this buffer for preparing kit reagents. Store at 4ºC when not in use.
- 1 mM Chromogen Working Solution: Prepare this solution just 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 Chromogen Probe and 100X TCEP Reagent 1:100 in 1X Assay Buffer (e.g. add 50 µL Chromogen Probe and 50 µL TCEP Reagent to 4.90 mL 1X Assay Buffer). Vortex thoroughly. The solution should appear bright yellow. Protect from light until needed and store at 4ºC.
- 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 to 4.995 mL deionized water). Further dilute this preparation by adding 800 µL to 6.2 mL 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 to 4.990 mL of 1X Assay Buffer). Vortex thoroughly. Prepare only what is needed for immediate applications and do not store diluted Stop Solution.

Preparation of Samples

These preparation protocols are intended as a guide for preparing unknown samples. The user may need to adjust the sample treatment accordingly. All samples should be assayed immediately or stored for up to 2 months at -80°C. A trial assay with a representative test sample should be performed to determine the sample compatibility with the dynamic range of the standard curve. High levels of interfering substances may cause variations in results. Samples may be diluted in 1X Assay Buffer as necessary before testing. Run proper controls as necessary. Always run a standard curve with samples.

- Plasma: Collect blood sample and add to a blood collection tube containing EDTA as the anticoagulant. Centrifuge at 3,000 rpm for 10-15 minutes at 4ºC. Remove the upper yellow plasma supernatant layer without disturbing the white buffy coat (leukocytes).

Note: EDTA-plasma is recommended for MPO measurement in blood samples. Heparin-plasma, citrate-plasma and serum samples are <u>not</u> recommended due to ex vivo release of MPO from neutrophils.

- Whole Neutrophils: Collect whole blood in EDTA blood collection tubes. Isolate neutrophils using density-gradient centrifugation. Resuspend 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 or other stimulants (ref. 3).
- Neutrophil Lysates: Collect whole blood in EDTA blood collection tubes. Isolate neutrophils using density-gradient centrifugation. Stimulate myeloperoxidase activity with PMA or other stimulants (ref. 3). Resuspend 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 12,000 rpm for 15 minutes at 4ºC.
- Tissue Lysates/Homogenates: Wash or perfuse tissues with cold sterile saline or 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 12,000 rpm for 15 minutes at 4ºC. Collect the supernatant. Store on ice if using immediately or freeze at -80ºC for up to 1-2 months.

Preparation of Standard Curve

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

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

Standard Tubes	1 mM Chromogen working solution (µL)	1X Assay Buffer (µL)	Final TNB Concentration (µM)	Final TNB Chromogen (nmol/well)*
1	250	500	333	50
2	200	550	267	40
3	150	600	200	30
4	100	650	133	20
5	50	700	67	10
6	25	725	33.3	5
7	12.5	737.5	16.7	2.5
8	6.25	743.7	8.3	1.25
9	3.13	746.8	4.2	0.63
10	0	750	0	0

Table 1. Preparation of TNB chromogen standards.

**Based on 150 µL volume/well.*

Assay Protocol

I. Taurine Chloramine Formation and Chromogen Reduction

1. Prepare and mix all reagents thoroughly before use. Each sample and control should be assayed in duplicate or triplicate.

   Note: In order to ensure the values of unknowns 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 <u>each</u> time point (e.g. 6 replicates for 3 time points). Suggested time points are 30, 60, and 120 minutes.
2. Add 25 µL of each unknown sample or sample buffer control to a 96-well plate.
3. Add 25 µL of the 1 mM Hydrogen Peroxide solution to each well and mix thoroughly on a horizontal shaker or by pipetting. Cover plate to protect from light and incubate at room temperature for 30-120 minutes.

   Note: If only one time point is desired, 60 minutes should be sufficient for most samples.
4. Vortex the 1X Stop Solution and add 50 µL to each sample well to be read at the current time point. Mix briefly. Incubate at room temperature for 10-15 minutes.
5. Add 50 µL of the 1 mM Chromogen working solution to each well to which 1X Stop Solution was added in step 4, and mix briefly. Cover plate to protect from light and incubate at room temperature for 10-15 minutes.
6. Read absorbance of each microwell on a spectrophotometer using 405-412 nm as the primary wave length.
7. For time course studies cover the plate and incubate until the next time point, and then repeat steps 4 through 7. Proceed immediately to section II below.

II. Standard Curve Generation

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

1. Add 150 µL of each freshly prepared TNB chromogen standard to a 96-well plate. Each standard should be assayed in duplicate or triplicate.
2. Read absorbance of each microwell on a spectrophotometer using 405-412 nm as the primary wave length.

Calculation of Results

Determine the average absorbance values for every myeloperoxidase sample, sample buffer control, and TNB chromogen standard. Subtract the average zero standard value from itself and all standard and sample values. This is the background correction. Graph the standard curve (see Figure 2).

1. Calculate the change in OD absorbance value (ΔA) between each sample buffer control (A_{blank}) and the sample itself (A_{sample}):

$$(\Delta A) = (A_{blank}) - (A_{sample})$$

2. Compare the change in absorbance (ΔA) 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.

3. Determine the myeloperoxidase activity in milliunits/mL (mU/mL) of a sample using the equation:

   Q = Quantity (in nmole/well) of chromogen consumed as determined from graph
   T = Reaction time (in minutes) determined from the time the Stop Solution was added

$$\text{MPO Activity (mU/mL)} = \left[\frac{Q}{T \times 0.025 \text{ mL}^*} \right]$$

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

MPO activity is quantified as nmole/min/mL = milliunit/mL (mU/mL)

Example Calculation
MPO Sample OD = 0.175
MPO Sample Blank 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

   Subtract the zero standard OD from MPO sample blank OD: 1.275 – 0.025 = 1.250

   Calculate the change in absorbance (ΔA): 1.250 - 0.150 = 1.100

2. Using your graphed standard curve, extrapolate the amount of nmoles consumed. (E.g. Figure 2 indicates Q = 38 nmoles)

3. Solve for MPO activity: $\left[\frac{(38 \text{ nmoles})}{(30 \text{ minutes})(0.025 \text{ mL})} \right] = 50.7 \text{ mU/mL}$

Example of Results

The following figures demonstrate typical Myeloperoxidase Assay results at 405 nm. One should use the data below for reference only. This data should not be used to interpret actual results.

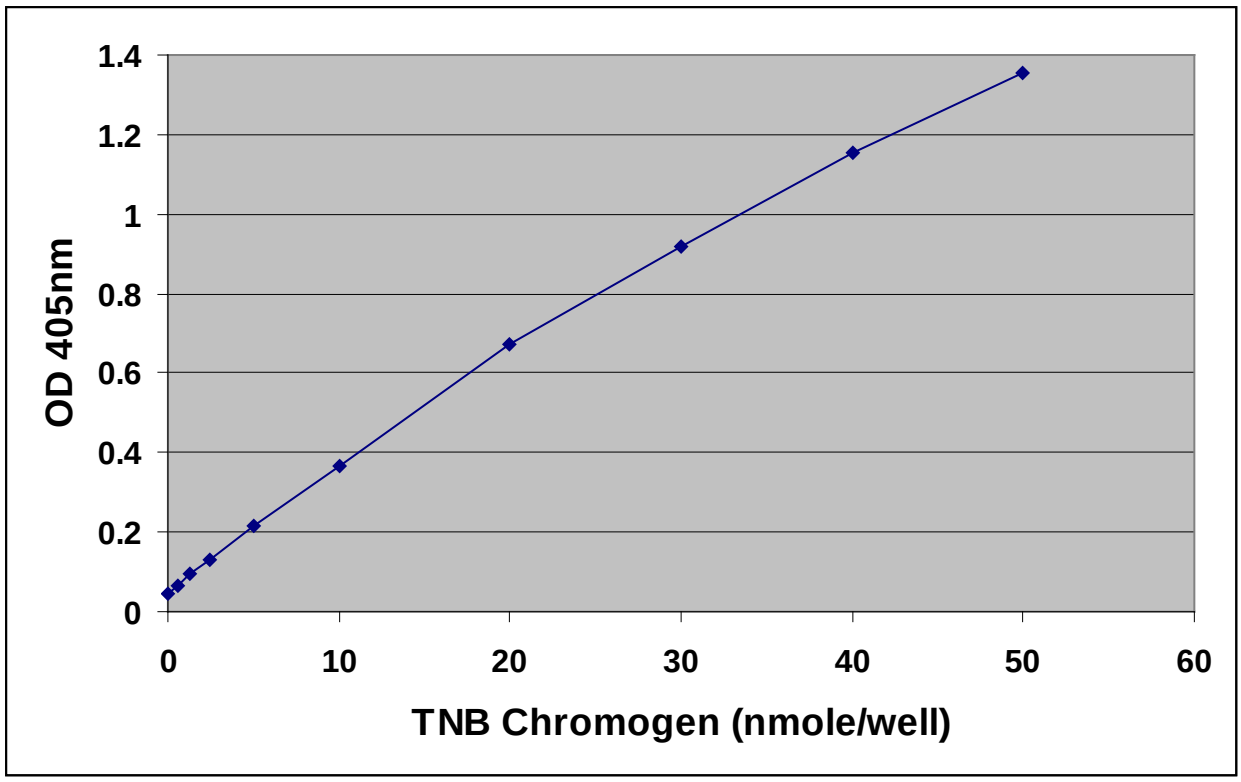

Figure 2. TNB Chromogen Standard Curve.

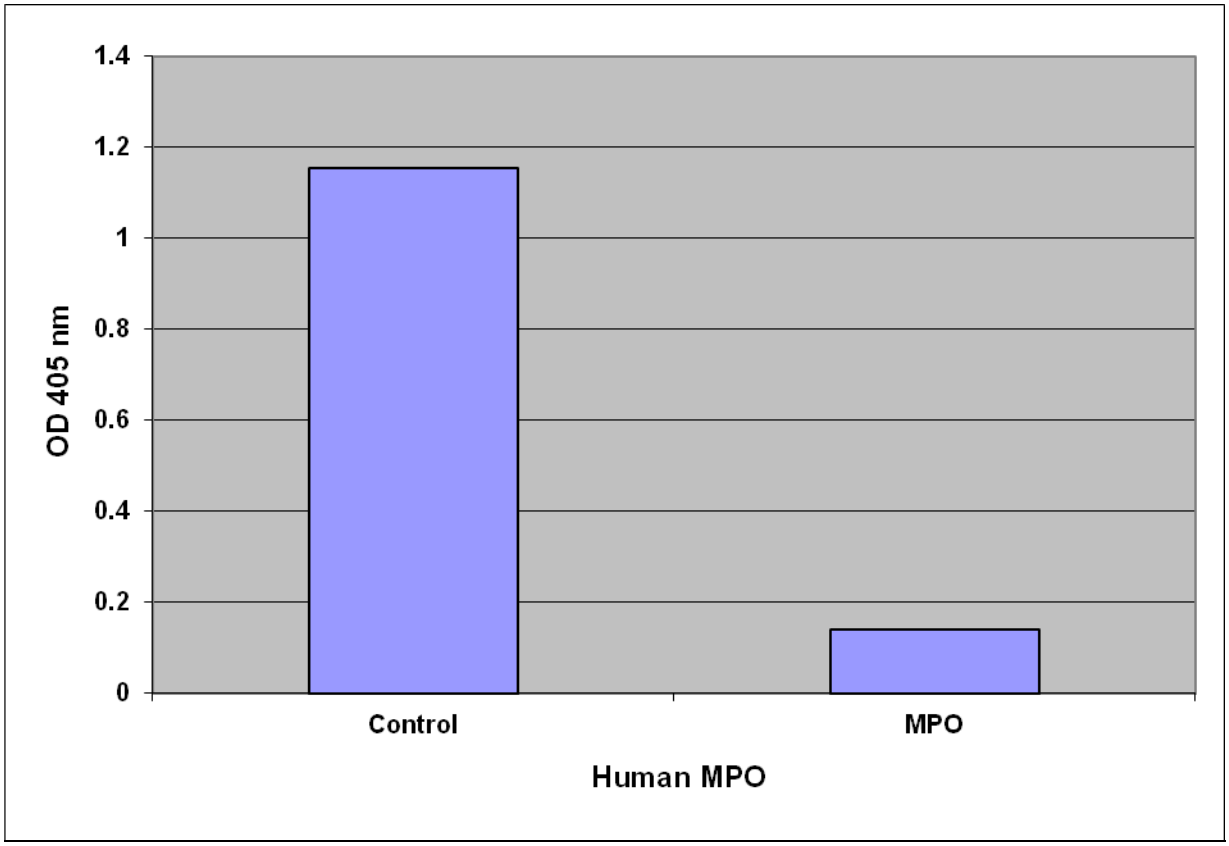

Figure 3: Purified Human MPO. 200 nM of purified myeloperoxidase was incubated 60 minutes at room temperature according to the assay protocol. OD values were measured in this sample (right) and a negative control (left). The activity was determined to be 24 mU/mL.

References

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Recent Product Citation

1. Sheng Cheng, H. (2017). The Ameliorative Effects of a Tocotrienol-Rich Fraction on the AGE-RAGE Axis and Hypertension in High-Fat-Diet-Fed Rats with Metabolic Syndrome. *Nutrients*. **9**(9): 984. doi: 10.3390/nu9090984
2. Goswami, S. K. et al. (2016). Anti-ulcer efficacy of soluble epoxide hydrolase inhibitor TPPU on diclofenac sodium induced intestinal ulcers. *J Pharmacol Exp Ther*. doi:10.1124/jpet.116.232108.

Warranty

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Contact Information

Cell Biolabs, Inc.
7758 Arjons Drive
San Diego, CA 92126
Worldwide: +1 858-271-6500
USA Toll-Free: 1-888-CBL-0505
E-mail: tech@cellbiolabs.com
www.cellbiolabs.com