
Product Manual

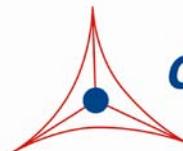
OxiSelect™ Myeloperoxidase Chlorination Activity Assay Kit (Fluorometric)

Catalog Number

STA-804

192 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 found in neutrophils, monocytes, and some soft tissue macrophages. MPO is copiously expressed in stimulated neutrophil granulocytes, where it catalyzes the production of hypochlorous 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. MPO exhibits both chlorination and peroxidation activities (see Figure 1).

Cell Biolabs' OxiSelect™ Myeloperoxidase Chlorination Activity Assay Kit is a quantitative fluorescence assay for measuring myeloperoxidase activity within cell lysates and purified enzyme samples. The assay is not applicable for use in serum samples. Myeloperoxidase activity is defined as follows: 1 unit of MPO enzyme is the amount of enzyme that will oxidize the MPO substrate to generate 1 μ mole of fluorescein per minute at 25°C. Each kit provides sufficient reagents to perform up to 192 assays, including standard curve and unknown samples.

Assay Principle

The OxiSelect™ Myeloperoxidase Chlorination Activity Assay Kit is a quantitative fluorescence-based assay for measuring the myeloperoxidase chlorination activity within a sample. Ferric, or native, MPO enzyme reacts with hydrogen peroxide (H_2O_2) to form the active redox intermediate MPO-I. The MPO-I then oxidizes chloride ions (Cl^-) to create hypochlorous acid (HOCl). This reaction encompasses the chlorination cycle. Hypochlorite ion (OCl^-) then cleaves the non-fluorescent aminophenyl fluorescein (APF) to generate fluorescein ($\lambda_{ex} = 485nm/\lambda_{em} = 525nm$). The myeloperoxidase activity in unknown samples is determined by comparison with the predetermined fluorescein standard curve.

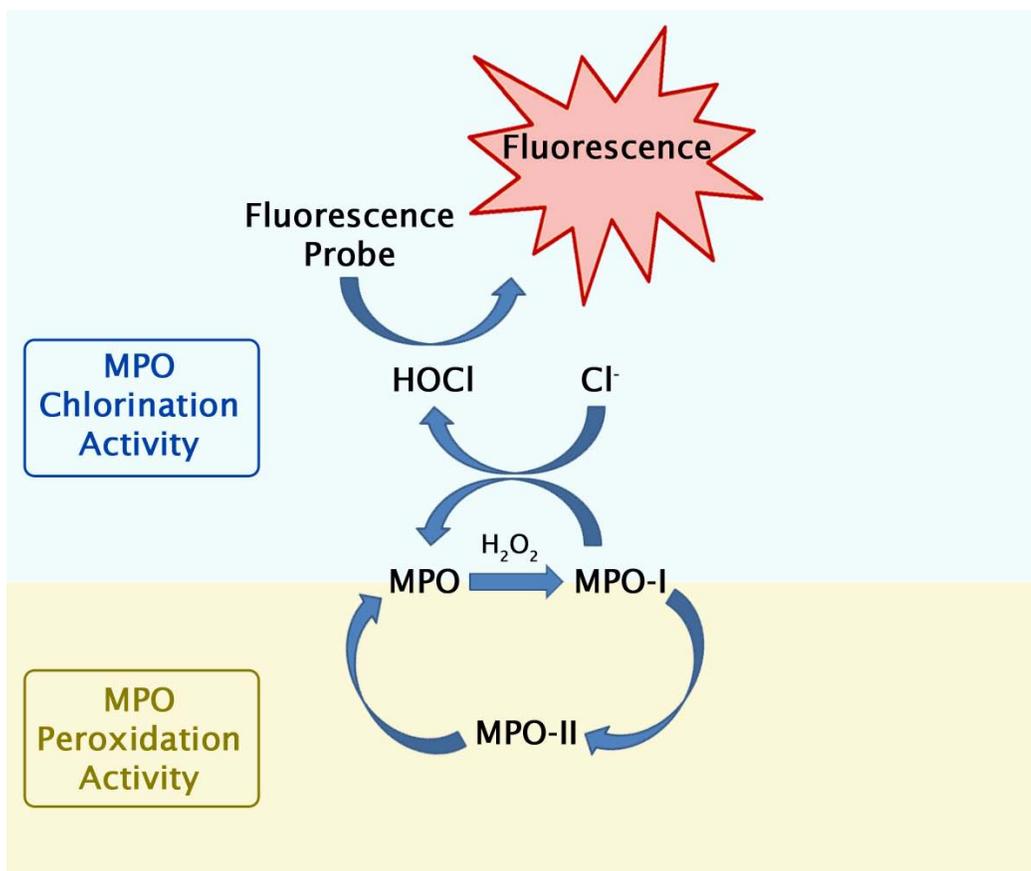


Figure 1. Chlorination and Peroxidation Activities of Myeloperoxidase (MPO). To measure the peroxidation activity of myeloperoxidase, use the OxiSelect™ Myeloperoxidase Peroxidation Activity Assay (Cat. #STA-805).

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-802: OxiSelect™ *In Vitro* Nitric Oxide (Nitrite / Nitrate) Assay kit
8. STA-803: OxiSelect™ Myeloperoxidase Activity Assay Kit (Colorimetric)
9. STA-832: OxiSelect™ MDA Competitive ELISA Kit
10. STA-838: OxiSelect™ HNE Adduct Competitive ELISA Kit

Kit Components

1. 96-well Microtiter Plate (Part No. 234501): Two 96-well clear bottom black plates
2. Hydrogen Peroxide (Part No. 234102): One 100 µL amber vial of an 8.82 M solution

3. Fluorescence Probe (500X) (Part No. 280401): One 20 μL amber tube of a 5 mM solution in DMF
4. Assay Buffer (10X) (Part No. 280402): One 25 mL bottle
5. Fluorescein Standard (Part No. 280403): One 100 μL amber tube of a 1 mM solution

Materials Not Supplied

1. Distilled or deionized purified water
2. 1X PBS
3. 10 μL to 1000 μL adjustable single channel micropipettes with disposable tips
4. 50 μL to 300 μL adjustable multichannel micropipette with disposable tips
5. Conical tubes, microcentrifuge tubes, and bottles for sample and buffer preparation
6. Centrifuge and/or microfuge
7. Sonicator or tissue homogenizer
8. Multichannel micropipette reservoirs
9. Chlorination inhibitor such as 4-aminobenzhydrazide (optional)
10. Fluorescence microplate reader equipped with a 480-490 nm excitation filter and 515-530 nm emission filter

Storage

Upon receipt, store the Fluorescence Probe and Fluorescein Standard at -20°C . Store the remaining kit components at 4°C .

Preparation of Reagents

- 1X Assay Buffer: Prepare 1X Assay Buffer by adding deionized water to the 10X Assay Buffer (e.g. add 25 mL of 10X Assay Buffer to 225 mL of deionized water). Mix thoroughly until homogeneous. Use this buffer for preparing kit reagents. Store at 4°C when not in use.
- 5 mM Hydrogen Peroxide Solution: Immediately prior to use, prepare by diluting the provided 8.82 M Hydrogen Peroxide in 1X Assay Buffer to 5 mM. (e.g. add 4 μL of Hydrogen Peroxide stock to 7 mL 1X Assay Buffer). Vortex thoroughly. Prepare only what is needed for immediate applications and do not store any of the diluted solutions.
- Working Solution: Prepare this solution just before use and prepare only enough for immediate applications. The Working Solution is used to prepare the MPO Chlorination Solution as well as to prepare the fluorescein standards. Prepare by diluting the 5 mM Hydrogen Peroxide Solution 1:250 in 1X Assay Buffer for a 20 μM final concentration. (e.g. add 40 μL of 5 mM Hydrogen Peroxide Solution to 9.960 mL 1X Assay Buffer). Vortex thoroughly.
- MPO Chlorination Solution: Immediately prior to use, prepare by diluting the Fluorescence Probe 1:500 in Working Solution (e.g. Add 5 μL to 2.495 mL of Working Solution for 50 assays). Vortex thoroughly. Prepare only what is needed for immediate applications and do not store diluted solutions. The solution should appear bright yellow. Protect from light until needed.

Preparation of Samples

Note: 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.

Samples may be diluted in 1X Assay Buffer or 1X PBS, pH 7.4, or concentrated with a centrifugal concentrator with a molecular weight cut-off of 30,000 as necessary before testing. High levels of interfering substances may cause variations in results. Run proper controls as necessary. Always run a standard curve with samples.

- Cell Lysates: Collect cells (~5-9 x 10⁶) by centrifugation at 1000-2000 x g for 10 minutes at 4°C. For adherent cells, do not use proteolytic enzymes; instead use a rubber policeman. Sonicate cell pellet in 0.5-1 mL ice-cold 1X PBS, pH 7.4. Centrifuge at 13,000 x g for 10 minutes at 4°C. Remove the supernatant and store on ice if testing immediately or freeze at -80°C for up to 1-2 months.
- Tissue Lysates: Homogenize 10 mg of tissue in 4 volumes of ice-cold 1X PBS, pH 7.4 or 1X Assay Buffer. Centrifuge the samples at 13,000 x g for 10 minutes at 4°C. Remove the supernatant and store on ice if testing immediately or freeze at -80°C for up to 1-2 months.

Note: This assay is not applicable for use with serum samples.

Preparation of Standard Curve

Prepare fluorescein standards by first diluting the 1 mM Fluorescein Standard 1:1000 in 1X Assay Buffer for a 1 µM (1000 nM) 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 fluorescein standard preparations.

Standard Tubes	1000 nM Fluorescein Standard (µL)	1X Assay Buffer (µL)	Final Fluorescein Concentration (nM)	Final Fluorescein (pmol/well)*
1	200	300	400	20
2	250 of Tube #1	250	200	10
3	250 of Tube #2	250	100	5
4	250 of Tube #3	250	50	2.5
5	250 of Tube #4	250	25	1.25
6	250 of Tube #5	250	12.5	0.625
7	250 of Tube #6	250	6.25	0.313
8	250 of Tube #7	250	3.13	0.156
9	250 of Tube #8	250	1.56	0.078
10	0	250	0	0

Table 1. Preparation of fluorescein standards.

**Based on 50 µL volume/well.*

Assay Protocol

I. Standard Curve Generation

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

1. Prepare and mix all reagents thoroughly before use. Standards should be assayed in duplicate or triplicate.
2. Add 50 μL of the fluorescein standards to unused wells within the 96-well microtiter plate.
3. Add 50 μL of Working Solution to each standard well and mix briefly.
4. Read absorbance of each microwell on a fluorometer using 480-490 nm excitation filter and 515-530 nm emission filter

II. Myeloperoxidase Activity

Note: The assay is continuous, thereby allowing for readings at multiple time points. This may be necessary in order to ensure the values of unknowns fall within the linear range of the standard curve.

1. Add 50 μL of unknown samples or controls to the microtiter plate. Samples and controls should be assayed in duplicate or triplicate.
2. Add 50 μL of MPO Chlorination Solution to each sample well to be read. Mix briefly.
3. Immediately read the fluorescence of each sample microwell on a fluorometer using 480-490 nm excitation filter and 515-530 nm emission filter. This is the initial time point plate reading (T_{initial}). Cover plate to protect from light and continue to incubate at room temperature for up to 30-60 minutes.

Note: If measuring multiple time points, begin reading samples after adding the MPO Chlorination Solution and every set time point (eg. 5 minutes) until complete. Samples with high MPO concentrations may need to be diluted to ensure measurements are with the standard curve range. Continue taking measurements until the value of the most active sample is greater than the standard curve values. At this point, the most active sample value exceeds the end of the linear range of the standard curve. The final fluorescence value prior to exceeding the highest standard curve value within the linear range of the curve is the penultimate reading, and is used to determine MPO activity. It is important that the final measured value fall within the linear range of the standard curve.

4. Once the assay is complete, read the fluorescence of each microwell on a fluorometer using 480-490 nm excitation filter and 515-530 nm emission filter. This is the final time point plate reading (T_{final}).

Calculation of Results

Determine the average fluorescence values for every myeloperoxidase sample, control, and fluorescein 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 sample fluorescence values (ΔF) between the initial fluorescence (F_{initial}) and the final fluorescence (F_{final}):

$$(\Delta F) = (F_{\text{final}}) - (F_{\text{initial}})$$

- Compare the change in fluorescence (ΔF) of each sample to the fluorescein standard curve to determine the amount of fluorescein produced within the assay. Only use values within the linear range of the standard curve.
- Determine the myeloperoxidase activity in microunits/mL ($\mu\text{U/mL}$) of a sample using the equation:

Q = Quantity (in pmole/well) of fluorescein produced as determined from graph

T = Reaction time (in minutes) determined by $T_{\text{final}} - T_{\text{initial}}$

$$\text{MPO Activity } (\mu\text{U/mL}) = \left[\frac{Q}{T \times 0.050 \text{ mL}^*} \right]$$

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

MPO activity is quantified as pmole/min/mL = microunit/mL ($\mu\text{U/mL}$)

Example Calculation

MPO Sample $F_{\text{initial}} = 40$

MPO Sample $F_{\text{final}} = 240$

Zero Fluorescein Standard $F = 10$

Time = 30 minutes

Sample Volume = 0.050 mL

- Subtract the zero standard fluorescence from MPO sample fluorescence:

$$\text{MPO Sample } F_{\text{initial}} = 40 - 10 = 30$$

$$\text{MPO Sample } F_{\text{final}} = 240 - 10 = 230$$

$$\text{Calculate the change in fluorescence } (\Delta F): \quad 230 - 30 = 200$$

- Using the equation of the trendline of the graphed standard curve, extrapolate the amount of pmoles consumed.

(E.g. The calculated value using the standard curve in Figure 2 is $Q = 17$ pmoles)

- Solve for MPO activity:

$$\left[\frac{(17 \text{ pmoles})}{(30 \text{ minutes})(0.050 \text{ mL})} \right] = 11.33 \mu\text{U/mL}$$

Example of Results

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

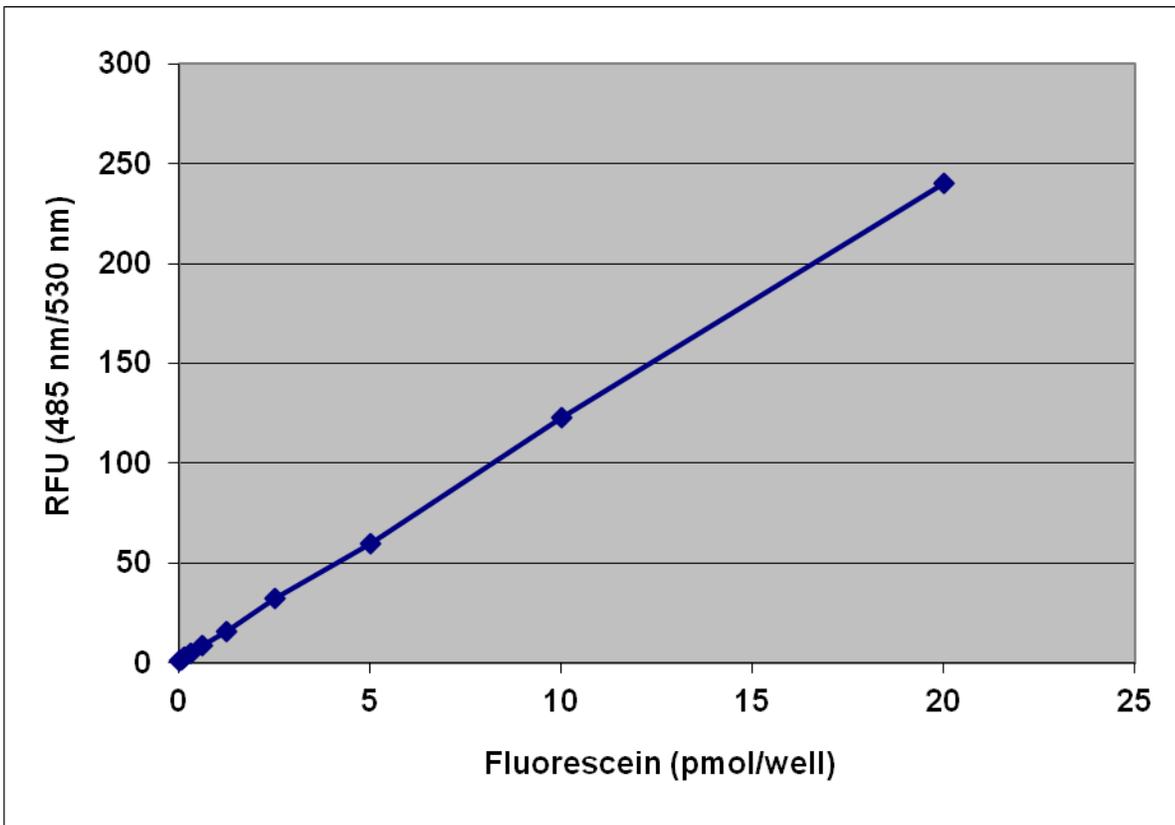


Figure 2. Fluorescein Standard Curve.

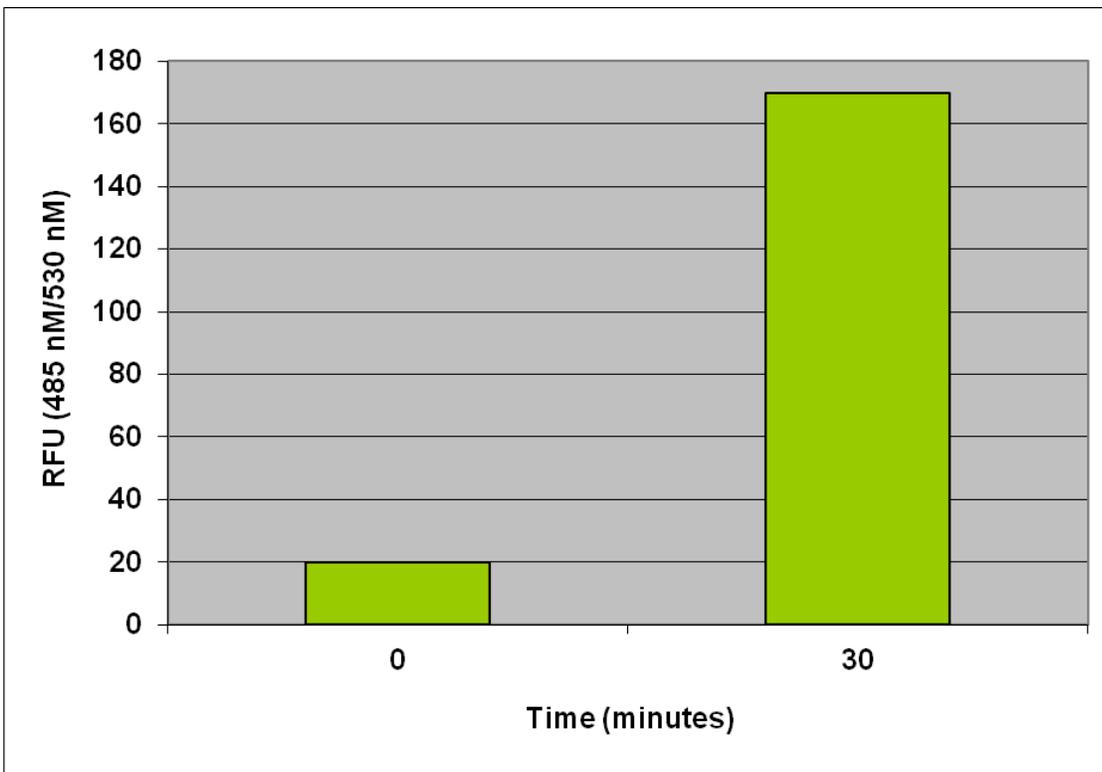


Figure 3: Purified Human MPO. 300 pM of purified human myeloperoxidase was incubated 30 minutes at room temperature according to the assay protocol. The activity was determined to be 8.7 μ U/mL.

References

1. Kettle, A.J., et al. *J. Clin. Invest.* (1994) 233: 502-512.
2. Malle, E., et al. *Br. J. Pharmacol.* (2007) 152: 838-854.
3. Marquez, L. A., et al. *J. Biol. Chem.* (1994) 269: 7950-7956.

Warranty

These products are warranted to perform as described in their labeling and in Cell Biolabs literature when used in accordance with their instructions. THERE ARE NO WARRANTIES THAT EXTEND BEYOND THIS EXPRESSED WARRANTY AND CELL BIOLABS DISCLAIMS ANY IMPLIED WARRANTY OF MERCHANTABILITY OR WARRANTY OF FITNESS FOR PARTICULAR PURPOSE. CELL BIOLABS' sole obligation and purchaser's exclusive remedy for breach of this warranty shall be, at the option of CELL BIOLABS, to repair or replace the products. In no event shall CELL BIOLABS be liable for any proximate, incidental or consequential damages in connection with the products.

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