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NWLSS™ Myeloperoxidase Activity

Colorimetric Assay for Myeloperoxidase Activity

Product NWK-MPO03
For Research Use Only

Simple assay kit for quantitative measurement of myeloperoxidase enzyme activity in biological samples such as plasma, tissue homogenates & cell lysates.

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Introduction:

Myeloperoxiase (MPO), the most abundant protein in neutrophils plays a major role in many inflammatory pathologies. Much recent research has focused on MPO as a potentially valuable biomarker for human cardiovascular risk.

The ability of MPO to catalyze the reaction between chloride and hydrogen peroxide (H_2O_2) to form hypochlorous acid (HOCl) is unique among mammalian enzymes and is considered to be the dominant function of MPO *in vivo*. HOCl is a powerful antimicrobial agent, and is extremely reactive with biological molecules resulting in neutrophil mediated tissue damage that is characteristic of inflammatory disease.

MPO also exhibits peroxidase activity; catalyzing the H_2O_2 mediated oxidation of multiple substrates. This activity can and has been used to assess the activity of MPO enzyme. However, the specificity of this method is not adequate for most biological samples due to of presence of other peroxidases. In contrast to MPO however, other peroxidases, generally do not produce HOCl. The only exception is eosinophil peroxidase which can produce HOCl at pH below 5. Since the chlorination activity of MPO has an optimum pH of near neutral (7.0), assay conditions can be set such that MPO activity can be measured as a function of HOCl production.

Intended Use:

The NWLSS™ Myeloperoxidase Activity Assay is intended for use as a simple colorimetric assay for the study of MPO activity in various biological and purified samples. The assay is not species specific and should be compatible in all model systems where active MPO enzyme is thought to exist.

Test Principle:

The NWLSS™ Myeloperoxidase Activity Assay method has been described by Weiss and coworkers (1982). Briefly, hypochlorous acid (HOCl) is formed from the MPO catalyzed reaction between chloride and hydrogen peroxide.

$$H_2O_2 + Cl^- + H^+ \longrightarrow HOCl + H_2O_2$$

HOCl is rapidly trapped by β-amino acid taurine to form the stable oxidant taurine chloramine. This action prevents accumulation of HOCl that could otherwise deactivate MPO and the Taurine reagent does not react with MPO enzyme intermediate and thus does not interfere with MPO catalysis.

$$HOCl + TauNH_2 \longrightarrow TauNHCl + H_2O$$

Test Principle (continued):
After incubation for specific time, the MPO catalyzed reaction is stopped by adding catalase to eliminate hydrogen peroxide. Taurine chloramine is then allowed to react with 5-thio-2-nitrobenzoic acid (TNB), a yellow complex with maximal absorbance at 412 nm.

$$TauNHCl + 2TNB \longrightarrow DTNB + Cl^- + TauNH_2$$
(Baseline Abs_{412} Is Decreased)

The products of the reaction above are colorless resulting in a reduction of measurable absorbance at 412 nm with increased production of TauNHCL. Reduction in Abs_{412} is quantified by comparing sample values with TNB baseline absorbance (e.g. Abs_{412} measured when no MPO is present and thus no HOCL produced). The HOCL and thus MPO dependant reduction in Abs_{412} forms the basis of determining MPO enzyme activity. One unit MPO activity is defined as the amount of enzyme that can catalyze sufficient HOCL production resulting in formation of 1 nmole TauNHCl at pH 6.5, 25°C over 30 minutes in the presence of 100 mM chloride and 100 µM H_2O_2.

General Specifications:

Format:	96 well microplate or 30 Cuvette assays
Specificity:	Myeloperoxidase Enzyme Activity
Sensitivity:	LLD = 10U/mL in sample assayed

Kit Contents:
Materials provided for 1 X 96 microplate or 30 test tube tests:

• Assay Buffer (Containing Taurine)	2 X 30 mL
• H_2O_2 Reagent	1 X 1.0 mL
• Catalase Reagent	1 Vial
• TNB Reagent	1 X 0.7 mL
• Concentrated Taurine Solution	1 Vial

Required Materials Not Provided:
Spectrophotometer or microplate reader
Disposable semi-micro cuvettes (1.0 mL), or microplate
Centrifuge
Microcentrifuge tubes
Plastic or glass bottles
Adjustable pipettors 0.0 – 1.0 mL
Multichannel Pipettor can be useful
Disposable pipette tips

Required Instrumentation:
Microplate reader with near 412 nm capability or spectrophotometer.

Warnings, Limitations, Precautions:
Let kit warm up to room temperature (~ 2 hours) before using.

For best results set-up the assays in duplicate or triplicate.

Storage Instructions:
The kit should be stored complete in the original box in a refrigerator at 4°C Stored in this manner the kit is stable until the expiration date printed on the product label.

Instrument Preparation:
Plate Reader Setup

Wavelength:	412 nm
Mode:	Endpoint
Shaker:	On

Reagent Preparation:
Let kit warm up to room temperature (~ 2 hours). While waiting, make up the Working H_2O_2 Reagent as shown below.

Assay Buffer:
Supplied ready to use with the appropriate concentration of Taurine reagent already added for the necessary reaction with HOCl. In the case of the enzyme protocol for tissue homogenates or cell extracts no additional Taurine reagent is required.

H_2O_2 Reagent:
Mix 12 µL of solution from the Hydrogen Peroxide Vial into 4988 µL of Assay Buffer. Label as **Working H_2O_2 Solution** mix and incubate for 60 minutes at room temperature before use. The reconstituted soTaurine lution must be used within 3 hours.

Catalase Reagent:
Reconstitute the Catalase Reagent with 2.0 mL of Assay Buffer just before using. Mix and label as **Working Catalase Solution.**

TNB Reagent:
Add 2.8 mL Assay Buffer to the TNB vial. Mix and label as **Working TNB Solution.** Let stand at room temp for at least 5 minutes before using.

Note: Working H_2O_2 , Catalase and TNB Solutions are stable for 3 hours after dilution and must be used within that time.

Sample Handling/Preparation:

The multi-disciplinary interest in measuring MPO enzyme activity has resulted in a myriad of sample types and experimental conditions and is beyond the scope of this product insert to describe sample processing in detail for each case. However, general guidelines are provided below for representative sample types.

Tissue

Homogenize tissue in 50 mM PBS or other buffers (perfuse the tissue before homogenization to eliminate MPO activity from blood). Store the homogenized tissue at –80°C to lyse the cells. After thawing, centrifuge the sample for 30 minutes at >10,000x g at 4°C. Take the supernatant for assay. For mice infected by bacteria, unperfused lung (homogenized in 1.0 mL PBS buffer) showed ca. 900 U/mL myeloperoxidase chlorination activity and had to be diluted 1/10x before the assay. Myeloperoxidase level in some tissues from unstimulated animals may be below the detection limit of our assay kit.

Neutrophil extract (blood samples):

Remove red blood cells by Dextran sedimentation, followed by hypotonic lysis. Purify using Ficoll-Hypaque (Histopaque). Resuspend the neutrophil pellet with Extraction Buffer at a concentration of ~$2x10^7$ neutrophils/mL. Extraction Buffer (not provided) contains 20 mM acetate buffer (pH 4.7) containing 0.2 M NaCl, 0.5% cetyltrimethylammonium bromide (CETAB), 10 µg/mL of phenylmethylsulfonyl fluoride, 10 µg/mL L-1-tosylamide-2-phenylethylchloromethyl ketone and 1 mM EDTA. Neutrophil extract (supernatant) is obtained after ultracentrifugation. Myeloperoxidase activity level in samples from unstimulated animals may be below the detection limit of our assay kit.

Intact neutrophil (blood samples): Remove red blood cells by Dextran sedimentation, followed by hypotonic lysis. Purify using Ficoll-Hypaque (Histopaque). Resuspend the neutrophil pellet with HBSS Buffer (not provided) at a concentration of ~$2x10^6$ neutrophils/mL. Myeloperoxidase activity in intact neutrophils is typically measured in the presence of PMA or other stimulants (not provided).

Assay Protocols:
A "Zero MPO Standard" is created by substituting Assay Buffer for sample in Step 4 and must be measured in order to establish the baseline for TNB absorbance A_{base} used later when analyzing the data.

For samples exhibiting background absorbance at 412 nm, it may also be necessary to measure a sample blank by substituting Assay Buffer for TNB at Step 5.

Tissue Homogenate or Cell Extract (Enzyme Protocol for Cuvettes):
1. Set temperature of water bath or heating block to 25°C.

2. Label microcentrifuge tubes appropriately (be sure to include the "Zero MPO Standard") and add 880 µL Assay Buffer to each tube. (Note that the Assay Buffer already contains the necessary Taurine concentration).

3. Place tubes in 25°C apparatus for assay.

4. Add 80 µL Assay Buffer to Zero MPO Standard tube or 80 µL sample to each tube according to assay template.

5. Incubate 5 minutes.

6. Add 40 µL **Working H_2O_2 Solution** to each tube and mix quickly by repeated pipetting (~5 times) with the same pipette tip, or by capping/ inversion. Keep the same pace of pippetting/mixing throughout the whole experiment.

7. Incubate 30 minutes.

8. Using the same pace of pipetting/mixing, add 40 µL **Working Catalase Solution** to each tube.

8. Incubate 5 minutes.

9. Add 100 µL **Working TNB Solution** to each tube (or 100 µL **Assay Buffer** for sample blanks) and mix well.

10. Incubate for 20 minutes.

11. Measure absorbance at 412 nm.
Note: If the absorbance at 412 nm is less than 0.06, dilute the sample and assay it again with a zero MPO sample.

Tissue Homogenate or Cell Extract (Enzyme Protocol for 96 well plate):
The assay may be run in a microplate by scaling down the volumes shown in the above protocol above by 1/4.

Assay Protocols (continued):

Intact Neutrophil Protocol (Whole Cell Protocol)

1. Set temperature of water bath or heating block to 25 °C.

2. Label microcentrifuge tubes appropriately (be sure to include the "Zero MPO Standard"). Add 920 µL of ~2x10^6 neutrophil/mL cell suspension or Assay Buffer only in the case of the Zero MPO Standard tube.

3. Add 40.0 µL of Concentrated Taurine Solution.

4. Incubate 5 minutes.

3. To each tube, add 40 µL of appropriate concentration of PMA (~100 ng/mL final concentration) or other stimulants. Mix. Keep the same pace of pippetting/mixing throughout the whole experiment. Incubate for 30 minutes, rotate microcentrifuge tubes gently every 5 minutes to ensure that cells are kept in suspension and are fully aerated.
4. Using the same pace of pipetting/mixing, add 40 µL of Catalase Solution to each tube. Centrifuge at 13,000g for 5 minutes. Transfer supernatants to clean cuvettes.

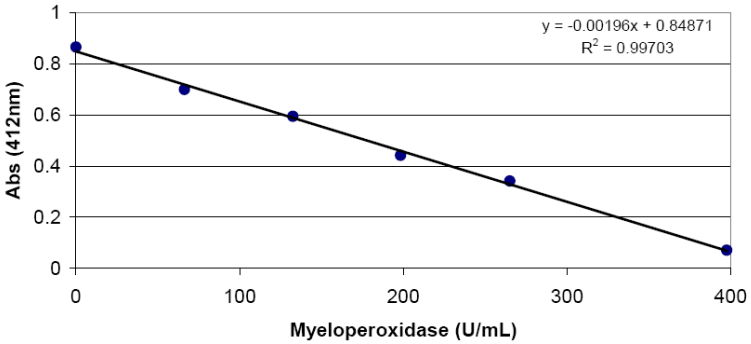
5. Add 100 µL of TNB Working Solution to each cuvette (or 100 µL of Assay Buffer for sample blanks). Mix and incubate for 20 minutes.

6. Record absorbance at 412 nm.

Note: If the absorbance at 412 nm is less than 0.06, dilute the sample and assay it again along with a zero MPO sample.

Data Analysis:

The figure below shows the correlation between myeloperoxidase activity and absorbance at 412 nm.



1. Calculate the average absorbance at 412 nm for zero MPO sample (A_0).

2. Calculate the average absorbance at 412 nm for sample blank where applicable (A_{blank}).

3. Using an extinction coefficient for TNB of 14,100 M-1•cm-1, calculate MPO activity in original sample using following formula:

Enzyme Protocol

Units MPO/mL $= [(A_{base} + A_{blank} - A_{Sample})/(0.0141*2)]*(1140/80)$
$= 505.3*(A_{base} + A_{blank} - A_{Sample})$

where A_{Sample} is the average absorbance at 412 nm for the sample replicate.

Whole Cell Protocol:

Units MPO/mL $= [(A_{base} + A_{blank} - A_{Sample})/(0.0141*2)]*(1140/920)$
$= 43.94*(A_{base} + A_{blank} - A_{Sample})$

where A_{Sample} is the average absorbance at 412 nm for the sample replicate.

4) Calculate the average value of MPO activity of sample. Multiply dilution factor if the sample is diluted for assay.

Assay Performance:

Lower limit of detection (LLD):	8 U MPO/mL original sample
Linearity:	Linearity is maintained up to 400 U MPO/mL sample
Precision:	Intra-assay coefficient of variation 2.1% at 130 U MPO/mL (n=5)

References:

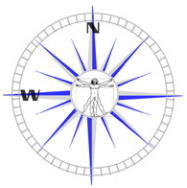
1. Weiss S. J., Klein R., Slivka A. & Wei M., "Chlorination of Taurine by Human Neutrophil", *J. Clin. Invest.* (1982), *70*:598-607

2. Kettle A. J., Winterbourn C. C., "Assays for the Chlorination Activity of Myeloperoxidase", *Methods in Enzymology* (1994), *233*:502-512

Statement of Limited Warranty:

Northwest Life Science Specialties, LLC (NWLSS) makes no guarantee of any kind, expressed or implied, that extends beyond the description of the material in this kit, except that they will meet our specifications at the time of delivery. Customer's remedy and NWLSS' sole liability is limited to, at NWLSS' option, refund of the purchase price, or the replacement of material not meeting our specification. By acceptance of our product, customer assumes all liability and will indemnify and hold NWLSS harmless for the consequence of this product's use or misuse by the customer, its employees, or others. Refund or replacement is conditioned of customer notifying NWLSS within twenty-one (21) days of the receipt of product. Failure to give notice within 21 days shall constitute a waiver by the customer of all claims hereunder with respect to said product.

Notes:



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