

NWLSS™ ***Myeloperoxidase ELISA***

Product NWK-MP002

For Research Use Only



Simple ELISA kit for quantification of myeloperoxidase in biological samples.

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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:

Procedure Checklist

- Create an assay template
- Equilibrate reagents to room temperature.
- Set-up required number of strips in frame supplied.
- Prepare reagents
 - Reconstitute lyophilized components
 - Dilute Reagent Vials 1, 2, 3, 5 and 6 as required.
- Perform a 2/5 serial dilution of the 250 ng/mL standard
- Prepare samples, making any necessary dilutions.
- Add 100 μ L **standard, sample or control** to each replicate well according to assay template.
- Apply Adhesive Cover and Incubate 1 Hour at room temperature.
- Wash wells 3 times with 200 μ L **Working Wash Buffer**
- Add 100 μ L **Working Tracer** to each well.
- Apply Adhesive Cover and Incubate 1 Hour at room temperature.
- Add 100 μ L of **Working SAP Conjugate** to each well.
- Apply Adhesive Cover and Incubate 1 Hour at room temperature.
- Add 100 μ L **TMB Substrate** to each well.
- Apply Adhesive Cover and incubate 20-30 minutes at room temperature in the dark.
- Stop the reaction by adding 100 μ L **Stop Solution**.
- Measure absorbance at 450 nm
- Analyze data using standard curve plot.

Introduction:

Myeloperoxidase (MPO) is an iron containing protein tetramer expressed in all cells of the myeloid lineage. MPO is abundantly present in azurophilic granules of polymorphonuclear neutrophils. It is an important enzyme used during phagocytic lysis of engulfed foreign particles which takes part in the defense of the organism through production of hypochlorous acid (HOCl), a potent oxidant. MPO is rapidly released by activated neutrophils. Involvement of MPO has been described in numerous diseases such as atherosclerosis, lung cancer, Alzheimer's disease and multiple sclerosis. Autoimmune antibodies to MPO are involved in Wegener's disease. Since the discovery of MPO deficiency, initially regarded as rare and restricted to patients suffering from severe infections, MPO has attracted more clinical attention. Today it is regarded as one of the leading candidates for prognosis of cardiac disease. The classical MPO assay is an enzymatic assay for activity which can be hampered by the presence of inhibitory compounds in many tissue homogenates. The NWLSS™ MPO assay utilizes a unique buffer system to reduce the possible effects of loss of enzyme activity on enzyme detection.

Intended Use:

The NWLSS™ Myeloperoxidase ELISA kit is intended for the quantitative measurement of human MPO in plasma, cell culture medium and other biological fluids where MPO may be present.

Test Principle:

The NWLSS™ Myeloperoxidase Assay is a simple "sandwich" ELISA using a plate bound capture antibody to human MPO and a biotinylated secondary tracer antibody. Addition of streptavidin-peroxidase followed by tetramethylbenzidine (TMB) facilitates color development directly proportional to the myeloperoxidase present in the sample. The reaction is stopped using a citric acid solution and the assay is read on a plate reader at **450 nm**.

Specifications:

Format::	2 X 96 well ELISA	
Number of tests:	Triplicate =	48
	Duplicate =	80
Specificity:	Human myeloperoxidase	
Sensitivity:	0.4 ng/mL	
Range:	0.4–100 ng/mL	

Kit Contents:

20X Concentrated Wash Buffer: 2 X 20 mL
Tris buffered saline containing Tween-20.

5X Concentrated Dilution Buffer A: 1 X 20 mL
Tris buffered saline containing Tween-20 with 2-chloroacetamide preservative.

10X Concentrated Dilution Buffer B: 2 X 5 mL
Protein solution containing 2-chloroacetamide preservative:

Myeloperoxidase Standard: 2 Vials
Lyophilized. Concentration is lot specific and indicated on label.

Tracer: 2 Vials
Lyophilized Biotinylated anti-myeloperoxidase in protein stabilized buffer with 2-chloroacetamide preservative.

Streptavidin-peroxidase Conjugate: 1 Vial
Lyophilized; concentrated with 2-chloroacetamide preservative.

TMB Substrate: 1 X 22 mL

2.0 M Citric Acid Stop Solution: 1 X 22 mL

Microplates 2 each
12 X 8 well strips pre-coated with anti myeloperoxidase antibody.

Microwell Strip Frame: 1 each

Adhesive plate covers: 4 each

Required Materials Not Provided:

Adjustable micropipettes with disposable tips (50-1000 μ L). Multi-channel pipettes are useful and help to reduce intra-sample variability.

Serological pipettes.

Deionized water.

Polypropylene tubes

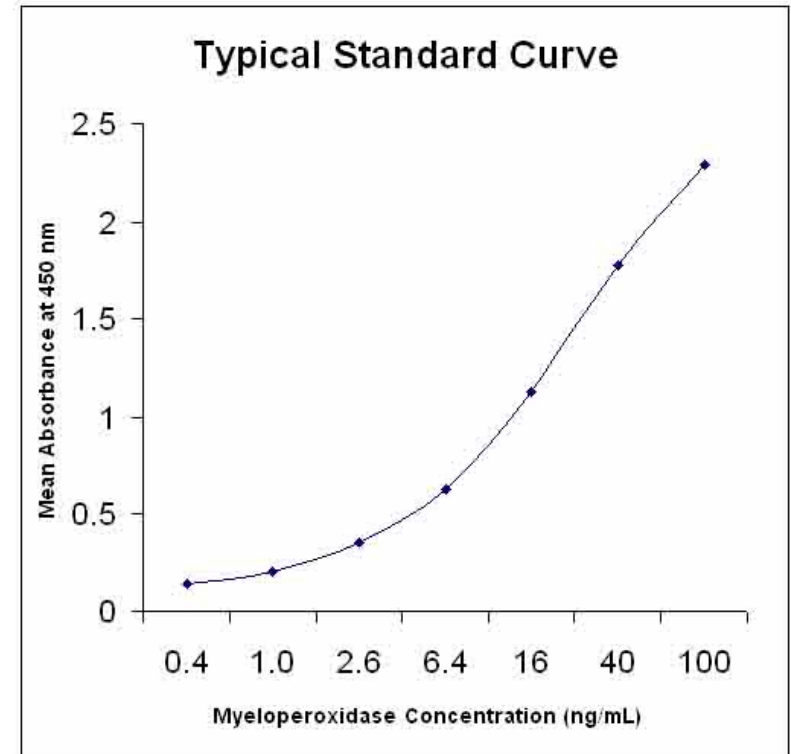
Automatic plate washer or other aspiration devices are optional.

Required Instrumentation:

Plate reader with 450 nm capability.

Data Analysis:

1. Plot the mean absorbance at 450 nm for each standard replicate versus the nitrotyrosine concentrations. This can typically be done using the software provided with most modern plate readers. An example curve is shown below.



2. Unknown myeloperoxidase concentrations are determined by comparing their absorbance measurements at 450 with those of the standard curve.

Assay Protocol:

Allow approximately 4.5 hours for procedure.

1. Add 100 μL standard, sample or control to each well according to the assay template created earlier.
2. Cover the wells with adhesive tape and incubate for 1 hour at room temperature (18-25°C).
3. Carefully remove the adhesive cover. Empty the plate by inverting and shaking contents over sink. Keep inverted and tap dry on a thick layer of tissues or paper towels. Wells may also be aspirated using a multi-channel pipette or plate washer. In either case, wash the plate 3 times with **200 μL Working Wash Buffer** waiting 20 seconds before aspirating.
5. Add **100 μL of Working Tracer** to each well keeping the same sequence as used in step 1. Avoid touching the side or top of the wells.
6. Cover the tray with adhesive tape and incubate for 1 hour at room temperature.
7. Repeat washing procedure as above.
8. Add **100 μL Working SAP Conjugate** to each well keeping the same sequence as used earlier.
9. Cover the tray with adhesive tape and incubate for 1 hour at room temperature.
10. Repeat washing procedure as above.
11. Add **100 μL TMB Substrate** solution (Vial 6) to each well, keeping the same sequence as used in earlier steps.
12. Cover the tray with adhesive tape and **incubate in the dark for 20-30 minutes at room temperature.**
13. Stop the reaction by adding **100 μL Stop Solution** (Vial 7) taking care to use the same sequence and timing as previous steps.
14. Measure the absorbance at 450 nm.

Warnings, Limitations, Precautions:

Do not add sodium azide as preservative to any component since its presence will inactivate the peroxidase conjugate.

Components containing 2-chloroacetamide and citric acid may be hazardous if in direct contact with skin, eyes, etc. Contact should be minimized through the use of gloves and standard good laboratory practices. If contact occurs, rinse the site immediately with water.

Storage Instructions:

Upon receipt, store this product at 2-8 °C...**DO NOT FREEZE.** Lyophilized components are stable for 1 month after reconstitution if stored at 2-8°C.

All reagents should be brought to room temperature (18-25°C) prior to use and stored at 2-8°C immediately after use.

Prolonged exposure of kit components to light should be avoided.

Coated microwell strips may be used until the product expiration date as long as they are returned to pouch and stored dry at 2-8°C.

Assay Preparation

1. Determine the number of wells required to assay standards, samples and controls for the appropriate replicate. It is recommended that testing be performed in duplicate or triplicate if possible.
2. Create an assay template showing positioning of standards, controls and samples.
3. Bring all samples and reagents to room temperature before use.
4. To avoid condensation, do not open foil pouches containing the microtiter strips until after they have reached room temperature. Next remove the required number of strips and place in the frame supplied.

Note: The bottom of some wells may be slightly white due to the preservation treatment. This does not influence assay performance.

Return unused wells to the storage bag with desiccant, seal and store at 2-8°C.

Reagent Preparation:**1. Vial 1, 20X Wash Buffer:**

For each 96 well plate to be assayed, dilute 20 mL of 20X Wash Buffer (1 bottle) with 380 mL de-ionized water. If fewer than 96 tests are to be performed, make up only the required volume. Label as **Working Wash Buffer**

2. Vial 2, 5X Dilution Buffer A & Vial 3, 10X Dilution Buffer B:

For each 96 well plate to be assayed dilute 10 mL of 5X Dilution Buffer A with 15 mL de-ionized water and dilute 5 mL of 10X Dilution Buffer B with 20 mL de-ionized water; mix both solutions 1:1 to yield 50 mL of **Working Dilution Buffer** sufficient for 1 plate. If fewer than 96 tests are to be performed, make up only the required volume adhering to the dilution ratios described above.

Note: Lyophilized components are under vacuum. In order to avoid product loss, allow pressure to equalize slowly before opening fully.

3. Vial 4: Lyophilized Standard:

Reconstitute the standard with deionized water according to the directions on the vial label to create a **500 ng/mL Stock Standard Solution**.

4: Vial 5A & 5B, Lyophilized, Biotinylated anti-myeloperoxidase Tracer:

Reconstitute 1 vial with 1 mL deionized water per 96 well plate assayed. Next dilute the 1 mL reconstituted Tracer with 11 mL *Working Dilution Buffer*. If fewer than 96 tests are to be performed, make up only the required volume adhering to the dilution ratios described above. Label as **Working Tracer**.

5. Vial 6, Lyophilized, Streptavidin-Peroxidase (SAP) Conjugate:

Reconstitute the vial with 1 mL deionized water. For each 96 well plate to be assayed dilute 0.5 mL reconstituted SAP Conjugate with 11.5 mL *Working Dilution Buffer*. If fewer than 96 tests are to be performed, make up only the required volume adhering to the dilution ratios described above. Label as **Working SAP Conjugate**.

6. Vial 6, TMB Substrate and Vial 7, Stop Solution are supplied ready to use.**Standard Curve Preparation::**

1. Label microtubes 1-8. Dilute Stock standard 1:4 (150 µL:600 µL) with *Working Dilution Buffer* in tube 1 creating the 100 ng/mL standard. Add 390 µL *Working Dilution Buffer* to remaining tubes 2-8.

2. Transfer 260 µL of 100 ng/mL standard to tube 2, mix well and continue 2/5 (2:3) serial dilutions across remaining tubes 3-7 to create standards of 100 - 0.4 ng/mL. Leave tube 8 as a buffer only zero control.

Std Tube # :	<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>
Conc. (ng/mL):	100	40	16	6.4	2.6	1.0	0.40	zero control

Standards may be stored for 1 month at 2-8°C or longer at -80°C.

Sample Handling/Preparation

1. Before performing the assay, all samples should be brought to room temperature.

2. Samples should be diluted if necessary with Working Dilution Buffer and mixed gently taking care to avoid foaming.

Note that 100 µL sample is required per replicate well.

Plasma samples:

Note that serum is not recommended as the clotting process can result in the release of MPO into the serum resulting in overestimation of [MPO].

1. Heparin anticoagulant is recommended.

2. Blood samples should be stored on ice prior to separation.

3. Samples frozen long term at -80°C are suitable for assay.

4. Avoid multiple freeze thaw cycles. In the case of frozen samples, use thawed sample within 24 hours.

5. It is recommended that plasma samples be diluted at least 2X, preferably 10X prior to assay.

Tissue and/or cell samples:

1. We recommend preparing lysis buffer as follows:
200 mM NaCl, 5 mM EDTA, 10 mM Tris, 10% Glycine, 1 mM PMSF, 1 µg/ml Leupeptine and 28 µg/ml Aprotinine (pH 7.4)

2. Add 200 µL lysis buffer to 10 mg tissue.

3. Homogenize the tissue using dounce homogenizer or similar.

4. Centrifuge the homogenates at 1500 g at 4°C for 15 minutes.

5. Harvest supernatant to fresh tubes.

6. Centrifuge supernatants again at 1500 g at 4°C for 15 minutes to additionally clarify.

7. Aliquot appropriate quantities for immediate assay else store at -70°C.