MA-0135

Myeloperoxidase Activity Assay

(100 wells, Colorimetric, OD 412 nm, Store at -20°C)

Introduction:

Myeloperoxidase, an oxidative enzyme, is abundantly expressed in neutrophils. Upon neutrophil activation, MPO is released into the extracellular space where it catalyzes the formation of HOCl from hydrogen peroxide and chloride ion. The HOCl, a very strong oxidant, kills invading bacteria, viruses and fungi. MPO activity has metabolic implications beyond host defense, since it can also oxidize lipids, proteins and DNA, leading to oxidative damage in chronic inflammatory conditions such as atherosclerosis, COPD and neurodegenerative disease. AkrivisBio's MPO Assay is a simple sensitive means of measuring enzyme activity below 0.05 mU per well.

Assay Principle:

1 – Myeloperoxidase produces HOCl (hypochlorous acid) from hydrogen peroxide and chloride
2 – HOCl reacts with taurine forming taurine chloramine
3 – Taurine chloramine reacts with TNB, the reduced form of DTNB, Ellman's Reagent, bleaching out the color causing a decrease of absorbance.

Assay Components:

Assay Buffer	25 ml	WM	MA-0135-A
DTNB	50 µl	Red	MA-0135-B
TCEP	50 µl	Clear	MA-0135-C
Hydrogen Peroxide	50 µl	Blue	MA-0135-D
Stop Reagent (Catalase)	lyoph	Green	MA-0135-E
MPO Positive Control	lyoph	Purple	MA-0135-F

Storage and Handling:

Store kit at -20°C prior to use. Allow Assay Buffer to warm to room temperature before use. Centrifuge small vials briefly before opening.
Assay Buffer: Ready to use as supplied. Store at 4°C.
DTNB Reagent: Ready to use as supplied. Store at 4°C
TCEP: Ready to use as supplied. Store at 4°C
Hydrogen Peroxide: Ready to use as supplied. Store at 4°C.
Stop Reagent (Catalase): Reconstitute with 200 µl of DI H_2O. Store at -20°C.
MPO Positive Control: Reconstitute with 100 µl Assay Buffer. Store at -20°C.
Note: If the assay is going to be used several times over a period of time, Aliquot the enzymes (Stop Reagent and Positive Control) into convenient portions and store at -20°C to avoid repeated freeze-thaw cycles.

Assay Protocol:

1. **Working Solutions:**
   TNB Reagent: Make TNB Reagent for the standard wells. TNB is readily oxidizable to DTNB, so prepare it as needed, on the day it is to be used. Take 2 µl of DTNB, 2 µl of TCEP and 196 µl of Assay Buffer, mix well and put on ice. Discard any unused TNB reagent.
   Hydrogen Peroxide: To prepare a working solution, transfer 5 µl of hydrogen peroxide to 300 µl of DI H_2O. Make working solution immediately before use. Discard any unused portion.
2. **Standard Curve:** Transfer 0 – 10 – 20 – 30 – 40 – 50 µl TNB Reagent to a series of wells on a 96 well plate. Bring all well volumes to 150 µl by adding 150, 140, 130, 120, 110 and 100 µl of Assay Buffer to the wells respectively, giving 0 – 10 – 20 – 30 – 40 – 50 nmoles of TNB.
3. **Read the Standard Curve** values at 412 nm.
4. **Sample Preparation:**

- Homogenize tissue (10 mg) or cells (10^6) in 100 µl of Assay Buffer, centrifuge at 16,000 X g, 5 min) to remove particulates/cell debris. Transfer the clear supernatant to a fresh tube. Transfer 5-50 µl to a 96 well plate. Dilute serum with Assay Buffer and transfer to wells. For neutrophils: Take 2 ml of blood and lyse using 10 volumes of RBC Lysis Buffer (150 mM ammonium chloride, 10 mM sodium bicarbonate, 0.1 mM EDTA); incubate for 10 min. at RT; centrifuge at 400 x g for 5 min; remove the supernatant carefully. Wash pellet with 1 ml 1X PBS and repellet at 400 x g for 5 min, and remove the supernatant carefully. Lyse the pellet using 200 µl Assay Buffer; keep on ice for 10 min. then centrifuge at 16,000 x g for 5 min. to remove particulates. Transfer clear supernatant to a fresh tube. Transfer up to10 µl of the lysate to a 96-well plate in duplicate, using one well as a background control. Adjust all sample and background well volumes to 50 µl with Assay Buffer.

5. **Positive Control:** Transfer 5 µl of reconstituted MPO Positive Control to an optional Positive Control well. Adjust the well volume to 50 µl with Assay Buffer.
6. **Initiate Reaction:** Each well will require 50 µl of Reaction Mix. Prepare a sufficient amount for the number of sample and positive control wells to be run:

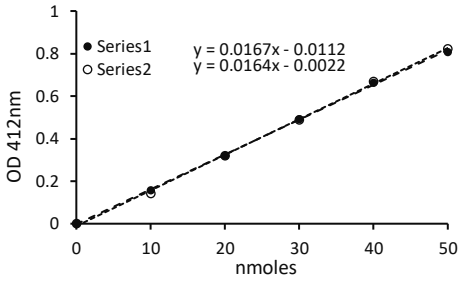
	Reaction Mix	Background Control Mix
MPO Assay Buffer	40 µl	40 µl
Hydrogen Peroxide	10 µl	-------
H_2O	-------	10 µl

Add 50 µl of Reaction Mix to each Sample and Positive Control well. Add 50 µl of the Background Control Mix to background control wells. Mix well. **Do not add reaction mix to the standard wells.**

7. **Incubate** the plate at 25°C for 30 min. then add 2 µl Stop Reagent to all Sample, Standard, Background Control and Positive Control wells. Mix and incubate the plate 10 min to stop the reaction. Ideally, samples will give a signal at the end of the assay of 50-90% of the range of the standard curve. If the signal is too low after 30 minutes, repeat the assay for a longer time. Iif signal is greater than 90% of the standard curve range, rerun the assay with less or diluted sample. Run times of 2 or 3 hours area acceptable if the enzyme activity is low. **During this incubation phase**, prepare TNB Reagent and keep it on ice. Prepare enough for the total number of wells run including samples, background controls and positive controls. Each well will require 50 µl. Add 50 µl TNB Reagent to each of the Sample, Background Control and Positive Control wells.
8. **Measurement:** After adding TNB reagent, wait 5-10 minutes for the TNB to be bleached then read at 412 nm. Positive Control and samples will show decreased color proportional to the amount of enzyme present. This is simply calculated as OD (412 nm) background - OD (412nm) sample. Values approaching the high end of the standard range are suspect and those samples should be diluted and rerun.

9. Typical Results:

nmol Standard	Standard Raw Values OD		Background Corrected Values OD	
0	0.0888	0.0813	0	0
10	0.2458	0.2242	0.1570	0.1429
20	0.4101	0.4024	0.3213	0.3211
30	0.5813	0.5703	0.4925	0.4890
40	0.7534	0.7502	0.6646	0.6689
50	0.8962	0.9043	0.8074	0.8230



10. Calculation: Subtract 0 Standard reading from all Standard readings. Plot the TNB Standard Curve. Determine the slope of the standard curve. This defines the OD/nmole TNB in solution. Subtract background control well values from the paired sample absorbance values. This difference corresponds to TNB consumed during the reaction. Divide this background corrected absorbance by the slope of the standard curve to get nmoles TNB consumed in the well. Divide by reaction time to get nmoles/minute (mU enzyme activity in the well). To convert back to enzyme activity in the original sample:

A. Divide mU in well by µl sample added to well = mU/ µl sample
B. Multiply mU/ µl sample X total volume of supernatant recovered = total mU enzyme activity in sample
C. Divide total mU enzyme activity in sample by mg original sample processed (or # of cells or ml of blood, etc.)

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