3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Myeloperoxidase Colorimetric Activity Assay Kit

Catalog Number **MAK068**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Myeloperoxidase (MPO) is a heme-containing enzyme that catalyzes the hydrogen peroxidase-mediated oxidation of halide ions to hypohalous acid. MPO is a lysosomal protein, highly expressed in neutrophils, that plays a role in the antimicrobial actions that occur as a result of neutrophil stimulation. The actions of MPO may also contribute to the initiation and pathogenesis of cardiovascular disease.

In this assay, MPO catalyzes the formation of hypochlorous acid, which reacts with taurine to form taurine chloroamine. Taurine chloroamine reacts with the chromophore TNB, resulting in the formation of the colorless product DTNB. One unit of MPO activity is defined as the amount of enzyme that hydrolyzes the substrate and generates taurine chloramine to consume 1.0 μmole of TNB per minute at 25 °C.

Components

The kit is sufficient for 100 assays in 96 well plates.

MPO Assay Buffer Catalog Number MAK068A	25 mL
MPO Substrate Stock Catalog Number MAK068C	50 μL
Stop Mix Catalog Number MAK068D	20 μL
DTNB Probe, 100 mM Catalog Number MAK068E	50 μL
MPO Positive Control Catalog Number MAK068F	1 vl
TCEP, 50 mM Catalog Number MAK068G	50 μL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use clear plates for colorimetric assays.
- Spectrophotometric multiwell plate reader.
- Phosphate buffered saline (Catalog Number P3813 or equivalent)

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

MPO Assay Buffer – Allow buffer to come to room temperature before use.

MPO Substrate – Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. To create a working solution, dilute 5 μL of MPO substrate stock with 300 μL of water. The working solution is stable for one week at –20 °C**.**

Stop Mix – Add 200 μL of water to the vial containing the 20 μL of Stop Mix. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

MPO Positive Control – Reconstitute in 100 μL of MPO Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 2 months of reconstitution.

DTNB Probe– To create the TNB Reagent/Standard, prepare fresh on the day of use from the DTNB probe as TNB is easily oxidized. For each sample well to be assayed, mix 0.5 µL of DTNB Probe with 0.5 µL of TCEP and 49 µL of MPO Assay Buffer to create a 1 mM (1 nmole/µL) TNB Reagent/ Standard. For the standard curve wells, prepare TNB Reagent/Standard solution as for the sample wells and add the indicated amount of solution to each well. Keep vials tightly closed when not in use.

Storage/Stability
The kit is shipped on wet ice. Storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

TNB Standards for Colorimetric Detection
Add 150, 140, 130, 120, 110, and 100 µL of the MPO Assay Buffer in duplicate into a 96 well plate. The TNB Standard will be added to the wells (0 (blank), 10, 20, 30, 40, and 50 µL/well (1 nmole/well)) at the end of the sample incubation period.

Sample Preparation
Tissue or cells should be rapidly homogenized in 4 volumes of MPO Assay Buffer. Centrifuge at 13,000 × *g* for 10 minutes at 4 °C to remove insoluble material.

Serum samples may be assayed directly or diluted in MPO Assay Buffer.

Add 1–50 µL samples into duplicate wells of a 96 well plate for each of the time points to be measured (30, 60, and 120 minutes). Bring samples to a final volume of 50 µL with MPO Assay Buffer.

For unknown samples, it is suggested to test several sample dilutions to ensure the readings are within the linear range of the standard curve.

Include a blank sample for each sample by omitting the MPO Substrate in the Reaction Mix.

For the positive control (optional), add 5–10 µL of the MPO Positive Control to wells and adjust well volume to 50 µL with MPO Assay Buffer.

Assay Reaction
1. Set up the Master Reaction Mixes according to the scheme in Table 1. 50 µL of the appropriate Reaction Mix is required for each reaction (well).

Table 1.
Reaction Mixes

Reagent	**Sample Blank**	**Samples & Positive Control**
MPO Assay Buffer	40 µL	40 µL
MPO Substrate	–	10 µL
Water	10 µL	–

2. Add 50 µL of the appropriate Reaction Mix to each of the positive control, sample, and sample blank wells. Do not add Reaction Mix to the standard wells. Mix well using a horizontal shaker or by pipetting. Protect the plate from light during the incubation. Incubate plates at room temperature.

3. In order to ensure the values are in the linear range of the standard curve, it is recommended to read the assay at 3 time points, 30 minutes, 60 minutes, and 120 minutes. At each time point (30, 60, and 120 minutes), add 2 µL of Stop Mix to the appropriate wells and mix well. Incubate for 10 minutes to stop the reaction and then add 50 µL of TNB Reagent/Standard to each well with the just added Stop Mix. Do not add the Stop Mix or TNB Reagent/Standard to the TNB Standard wells. Color development should be stable and all wells can be read together after the final time point is completed.

4. Add 0, 10, 20, 30, 40, and 50 µL of the 1 mM TNB Reagent/Standard to the 150, 140, 130, 120, 110, and 100 µL Assay Buffer-containing standard wells and incubate the plate for an additional 10 minutes.

5. Measure the absorbance at 412 nm (A_{412}).

Results

Calculations

The background of the assay is the value obtained for the 0 (blank) TNB standard. Correct for the background by subtracting the blank value from all readings. Background values can be significant and must be subtracted from all readings. Plot the TNB standard curve.

Note: A new standard curve must be set up each time the assay is run.

Calculate the change in measurement between each sample blank and its corresponding sample (ΔA_{412}). Use only values that are within the linear range of the TNB standard curve. This will give the change in absorbance due to consumption of the TNB Reagent/Standard by MPO-generated taurine chloramine.

$$\Delta A_{412} = (A_{412})_{\text{sample blank}} - (A_{412})_{\text{sample}}$$

Compare the ΔA_{412} of each sample to the standard curve to determine the amount of TNB consumed by the enzyme assay.

The MPO activity of a sample may be determined by the following equation:

$$\text{MPO Activity} = \frac{B \times \text{Sample Dilution Factor}}{(\text{Reaction Time}) \times V}$$

B = Amount (nmole) of TNB consumed
Reaction Time = (in minutes, at point Stop Mix was added)
V = sample volume (mL) added to well

MPO activity is reported as nmole/min/mL = milliunit/mL. One unit of MPO activity is defined as the amount of enzyme that hydrolyzes the substrate and generates taurine chloramine to consume 1.0 μmole of TNB per minute at 25 °C.

Example:
Amount of TNB consumed (B) = 5.84 nmole
Assay time (T) = 30 minutes
Sample volume (V) = 0.005 mL
Sample dilution is 1

MPO activity is:

$$\frac{5.84 \times 1}{(30) \times 0.005} = 38.93 \text{ milliunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	Ice Cold Assay Buffer	Assay Buffer must be at room temperature
	Omission of step in procedure	Refer and follow Technical Bulletin precisely
	Plate reader at incorrect wavelength	Check filter settings of instrument
	Type of 96 well plate used	For colorimetric assays, use clear plates
Samples with erratic readings	Samples prepared in different buffer	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
	Cell/Tissue culture samples were incompletely homogenized	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Samples used after multiple freeze-thaw cycles	Aliquot and freeze samples if samples will be used multiple times
	Presence of interfering substance in the sample	If possible, dilute sample further
	Use of old or inappropriately stored samples	Use fresh samples and store correctly until use
Lower/higher readings in samples and standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Use of expired kit or improperly stored reagents	Check the expiration date and store the components appropriately
	Allowing the reagents to sit for extended times on ice	Prepare fresh Master Reaction Mix before each use
	Incorrect incubation times or temperatures	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect volumes used	Use calibrated pipettes and aliquot correctly
Non-linear standard curve	Use of partially thawed components	Thaw and resuspend all components before preparing the reaction mix
	Pipetting errors in preparation of standards	Avoid pipetting small volumes
	Pipetting errors in the Reaction Mix	Prepare a Master Reaction Mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Refer to the standard dilution instructions in the Technical Bulletin
	Calculation errors	Recheck calculations after referring to Technical Bulletin
	Substituting reagents from older kits/lots	Use fresh components from the same kit
Unanticipated results	Samples measured at incorrect wavelength	Check the equipment and filter settings
	Samples contain interfering substances	If possible, dilute sample further
	Sample readings above/below the linear range	Concentrate or dilute samples so readings are in the linear range

SS,KVG,MJK,LS,MAM 12/19-1

©2019 Sigma-Aldrich Co. LLC. All rights reserved. SIGMA-ALDRICH is a trademark of Sigma-Aldrich Co. LLC, registered in the US and other countries. Sigma brand products are sold through Sigma-Aldrich, Inc. Purchaser must determine the suitability of the product(s) for their particular use. Additional terms and conditions may apply. Please see product information on the Sigma-Aldrich website at www.sigmaaldrich.com and/or on the reverse side of the invoice or packing slip.