Technical Bulletin

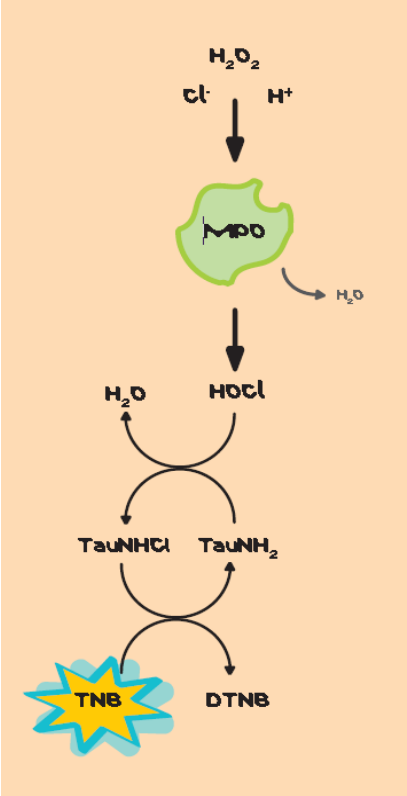
MPO Colorimetric Activity Assay Kit

Catalogue number MAK563

Product Description

Myeloperoxidase (MPO) is a lysosomal protein primarily found in neutrophils, and it plays a crucial role in the antimicrobial responses triggered by neutrophil activation. Additionally, MPO has been implicated in the development and progression of cardiovascular disease.

This assay utilizes MPO to catalyze the production of hypochlorous acid, which then reacts with taurine to generate taurine chloramine. Taurine chloramine further reacts with the chromophore TNB, resulting in the formation of a colorless product called DTNB.



The activity of MPO is measured by determining the rate at which it hydrolyzes the substrate and generates taurine chloramine, leading to the consumption of 1.0 µmole of TNB per minute at a temperature of 25 °C. One unit of MPO activity corresponds to this defined enzymatic reaction rate.

Components

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

- MPO Assay Buffer 25 mL
  Catalogue Number MAK563A
- MPO Substrate Stock 50 µL
  Catalogue Number MAK563B
- Stop Mix 200 µL
  Catalogue Number MAK563C
- DTNB Probe, 100 mM 50 µL
  Catalogue Number MAK563D
- MPO Positive Control 50 µL
  Catalogue Number MAK563E
- TCEP, 50 mM 50 µL
  Catalogue Number MAK563F

Reagents and Equipment Required but Not Provided

- 96-well flat-bottom plate.
  - Clear plates for colorimetric assays (Catalogue number M2936 or equivalent)
  - Cell culture or tissue culture treated plates are not recommended.
- Plate reader that is capable to read absorbance at 412 nm.
- Pipettors and Pipettes
- Horizontal shaker
- Cetyltrimethylammonium bromide (CTAB)

- Phorbol 12-myristate 13-acetate (PMA) or other stimulants

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.

Storage/Stability

The product is shipped on wet ice. Store at -20 °C upon receipt, protected from light.

Preparation Instructions

Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. Avoid repeated freeze/thaw cycles.

MPO Assay Buffer

Allow buffer to come to room temperature before use. Aliquot and store at –20 °C.

Working Solution

Mix well by pipetting, then aliquot and store at –20 °C, protected from light.

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

Store at -20°C, protected from light. Keep on ice while in use.

TNB Probe/Standard

To create the assay standard and probe, combine the DTNB and TCEP components supplied in the kit. This mixture results in the formation of the TNB probe, recognizable by its yellow color.

Prepare fresh on the day of use and keep away from light.

To ensure an adequate supply of the probe in this kit, it is important to prepare a sufficient amount for your experiment. You will need 50 µL of the probe for one well/sample, or blank.

Additionally, for the calibration curve of the standard, you will need 150 µL of the probe solution.

For the preparation of a 100 µL probe or standard, carefully transfer 1 µL each of DTNB and TCEP into 98 µL of assay buffer. This will result in a 1 mM (1 nmol/µL) TNB solution.

It is recommended to use the customer calculator available on the product webpage to calculate the precise quantity of probe required for both the samples and the calibration curves. This will ensure sufficient preparation for the entire experiment and accurate measurements throughout.

MPO Positive Control

Store at -20 °C, protected from light. Keep on ice while in use.

Procedure

All samples and standards should be run in technical duplicates or triplicates.

Tips for Success

1. Ensure that all reagents, except for the stop mix and MPO Positive, are brought to room temperature before use.
2. Keep the stop mix and MPO Positive Control on ice while they are being used.
3. Take precautions to protect TNB from prolonged exposure to light, as it is light sensitive.
4. Utilize the " customer calculator " to calculate the necessary amounts of reagents and to determine the test results.
5. Maintain a consistent pace of pipetting and mixing throughout the entire experiment.

Sample Preparation

The wide range of applications in measuring MPO enzyme activity encompasses various sample types and experimental conditions. While sample-specific processing details are beyond the scope of this product. However, general guidelines are provided below to assist with sample preparation.

Tissue or cells should be rapidly homogenized using 4 volumes of MPO Assay Buffer. If you wish to extract the sample, include 0.5% cetyltrimethylammonium bromide (CTAB) in the MPO Assay Buffer. Please note that CETAB is not provided with the kit. This will enable the extraction process. However, if you intend to test a non-extracted sample, you can use the assay buffer without adding CTAB.

Centrifuge the homogenized mixture at 13,000 x g for 10 minutes at 4 °C to remove insoluble materials.

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

Note: To ensure optimal results, it is recommended to stimulate the cells or tissue with phorbol 12-myristate 13-acetate (PMA) or another stimulant.

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

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

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

For the positive control (optional), add 2–5µL of the MPO Positive Control to wells and adjust well volume to 50 µL with MPO Assay Buffer

Preparation of TNB Standards

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

Assay Reaction

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

Table 1.
Master Reaction Mix Preparation

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.
3. Mix well by pipetting. Incubate plates at RT, using a horizontal shaker.
   Note: Time the reaction for activity calculation.
4. 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.
5. At each time point (30, 60, and 120 minutes) add 2 µL of Stop Mix to the appropriate wells (Positive control, Blank and Sample) and mix well by pipetting. Incubate using horizontal shaker at RT for at least 10 minutes to stop the reaction.
6. Add 50 µL of TNB Reagent/Standard to each well with the just added Stop Mix. Protect the plate from light and incubate using horizontal for at least 10 minutes. 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.
7. 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. Protect the plate from light and incubate using horizontal for at least 10 minutes.

8. Measure the absorbance at 412 nm (A412).

Results

Calculations

For your convenience, an online Excel-based calculator is available on the product's webpage, at www.sigmaladrich.com/MAK563.

Simply copy and paste the raw data obtained from the plate reader according to the instructions.

In the assay, the background is represented by the value obtained for the 0 (blank) TNB standard. To correct for this background, it is necessary to subtract the blank value from all subsequent readings. Background values can be substantial and must be subtracted from all readings during the analysis process. Additionally, it is recommended to plot the TNB standard curve to visualize and interpret the results effectively.

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

Calculate the change in absorbance measurement from T initial to T final for the samples.

Calculate the change in measurement between each sample blank and its corresponding sample (Δ A412). 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.

ΔA_{412} = (A_{412}) sample blank – (A_{412}) sample

Compare the ΔA412 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:

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

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

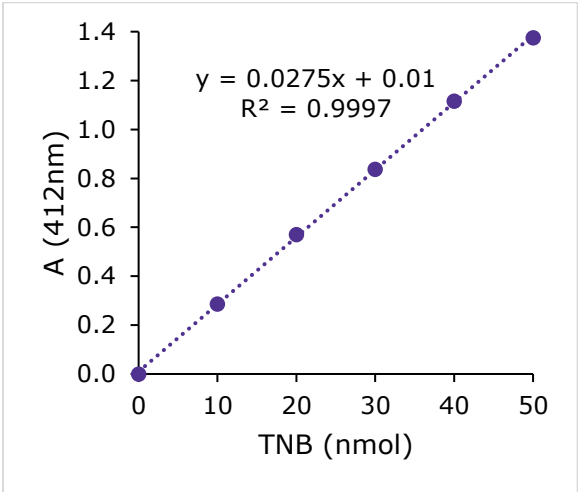
Assay time (T) = 30 minutes

Sample volume (V) = 0.005 mL

$$\text{MPO Activity} = \frac{5.84\ \text{nmol} \times 1}{(30\text{min}) \times 0.005\ \text{mL}} = 38.93\ \text{mU}$$

Figure 1:

An exemplary standard curve.



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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	Use the Assay Buffer provided or refer to Technical Bulletin for instructions
Samples with erratic readings	Samples prepared in different buffer	Repeat the sample homogenization, increasing the length and extent of homogenization step.
	Cell/Tissue culture samples were incompletely homogenized	Aliquot and freeze samples if samples will be used multiple times
	Samples used after multiple freeze-thaw cycles	If possible, dilute sample further
	Presence of interfering substance in the sample	Use fresh samples and store correctly until use
	Use of old or inappropriately stored samples	Thaw all components completely and mix gently before use
Lower/higher readings in samples and standards	Improperly thawed components	Check the expiration date and store the components appropriately
	Use of expired kit or improperly stored reagents	Prepare fresh Master Reaction Mix before each use
	Allowing the reagents to sit for extended times on ice	Refer to Technical Bulletin and verify correct incubation times and temperatures
	Incorrect incubation times or temperatures	Use calibrated pipettes and aliquot correctly
	Incorrect volumes used	Thaw and resuspend all components before preparing the reaction mix
Non-linear standard curve	Use of partially thawed components	Avoid pipetting small volumes

	Pipetting errors in preparation of standards	Prepare a Master Reaction Mix whenever possible
	Pipetting errors in the Reaction Mix	Pipette gently against the wall of the tubes
	Air bubbles formed in well	Refer to the standard dilution instructions in the Technical Bulletin
	Standard stock is at incorrect concentration	Recheck calculations after referring to Technical Bulletin
	Calculation errors	Use fresh components from the same kit
	Substituting reagents from older kits/lots	Check the equipment and filter settings
Unanticipated results	Samples measured at incorrect wavelength	If possible, dilute sample further
	Samples contain interfering substances	Concentrate or dilute samples so readings are in the linear range
	Sample readings above/below the linear range	Use the Assay Buffer provided or refer to Technical Bulletin for instructions

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