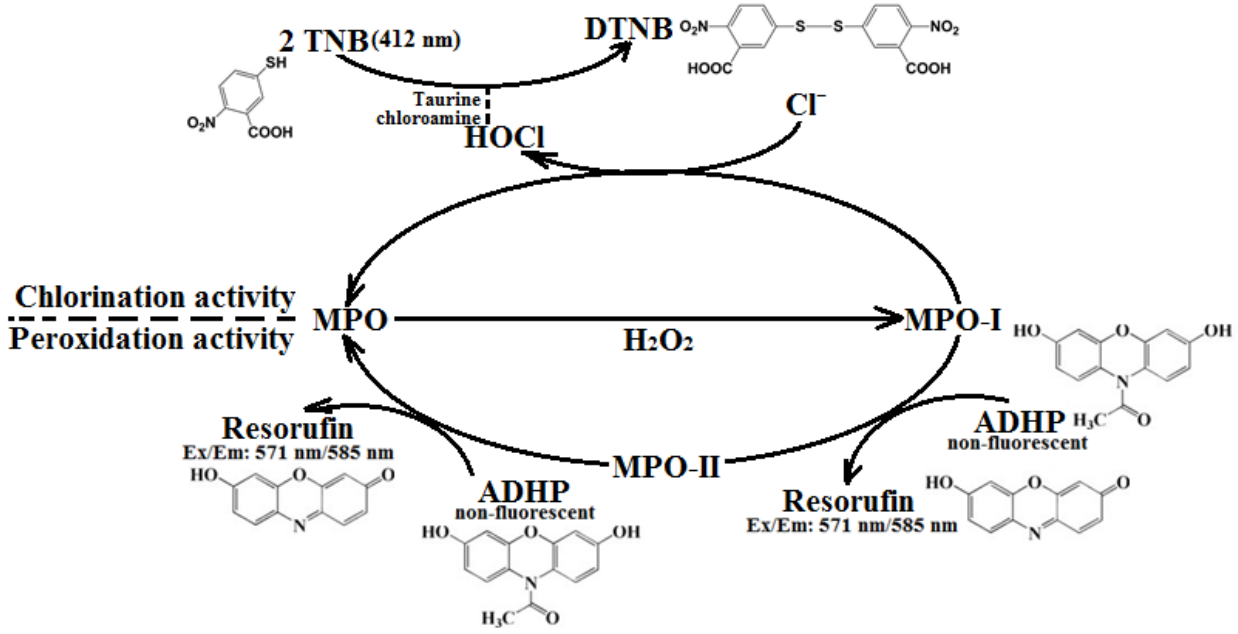
Myeloperoxidase Colorimetric Activity Assay Kit

Cat. No. CTG-BK032C 1 kit

Product Description

Myeloperoxidase (MPO) Colorimetric Activity Assay Kit is a rapid, simple, sensitive, and reliable colorimetric assay suitable for use as a high throughput MPO activity assay. In the MPO assay protocol, myeloperoxidase produces HClO from H_2O_2 and Cl^-. Myeloperoxidase (MPO) is a peroxidase enzyme (EC 1.11.1.7) most abundantly present in neutrophil granulocytes. It is a green hemoprotein found in neutrophils and monocytes that catalyzes the reaction of hydrogen peroxide and halide ions to form cytotoxic acids and other intermediates that play a role in the oxygen-dependent killing of tumor cells and microorganisms. Its heme pigment causes the green color in secretions rich in neutrophils, such as some forms of mucus. Furthermore, it can oxidize tyrosine to a tyrosyl radical using hydrogen peroxide as an oxidizing agent. In Celltechgen's MPO colorimetric Assay Kit, 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. Myeloperoxidase peroxidation activity, please check Celltechgen's Myeloperoxidase Peroxidation Activity Assay Kit (Cat. No. CTG-BK032P)



Application

MPO Colorimetric Activity Assay Kit has been used to determine the activity of myeloperoxidase in samples.

Kit Size
100 rxns

Kit Components

MPO Assay Buffer	25 ml
MPO Substrate Stock	50 µl
DTNB Probe	50 µl
Stop Mix	20 µl
MPO Positive Control	1 vial (MPO)
TCEP, 50 mM	50 µL

Kit storage/stability
Store kit at -20°C protected from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge small vials before opening.

Usage
Celltechgen provides high-quality reagents and materials for research use only. For proper handling of potentially hazardous chemicals, please request the Safety Data Sheet (SDS) provided for the product.

Protocol

1. Prepare solutions

1) Briefly centrifuge vials before opening. Use ultrapure water for the preparation of reagents. To maintain reagent integrity, avoid repeated freeze/thaw cycles.
2) MPO Assay Buffer – Allow buffer to come to room temperature before use.
3) MPO Substrate – Mix well by pipetting, then aliquot and store, protected from light, at –20 ^{o}C. To create a working solution, dilute 5 µL of MPO substrate stock with 870 µL of water.
4) 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 ^{o}C. Use within 2 months of reconstitution.
5) MPO Positive Control – Reconstitute in 100 µL of MPO Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 ^{o}C. Use within 2 months of reconstitution.
6) 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.

2. 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.

3. Sample Preparation

1) Tissue or cells should be rapidly homogenized in 4 volumes of MPO Assay Buffer. Centrifuge at 13,000 X g for 10 minutes at 4 ^{o}C to remove insoluble material. Serum samples may be assayed directly or diluted in MPO Assay Buffer.
2) 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.
3) Include a blank sample for each sample by omitting the MPO Substrate in the Reaction Mix.
4) 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.

3. Chlorination activity assay

1) Set up the Master Reaction Mixes by mixing 2µl MPO Substrate with 48µl MPO Assay Buffer, 50 µL of the appropriate Reaction Mix is required for each reaction (well). For blank sample, use MPO assay buffer only.
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, 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, incubate the plate for an additional 10 minutes. Color development should be stable and all wells can be read together after the final time point is completed.
4) Measure the absorbance at 412 nm (A412nm).

4. Activity 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 (ΔA412). 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.

$\Delta A412 = (A_{412nm})$sample blank $– (A_{412nm})$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:
MPO Activity = B × Sample Dilution Factor/ ((Reaction Time) × 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.