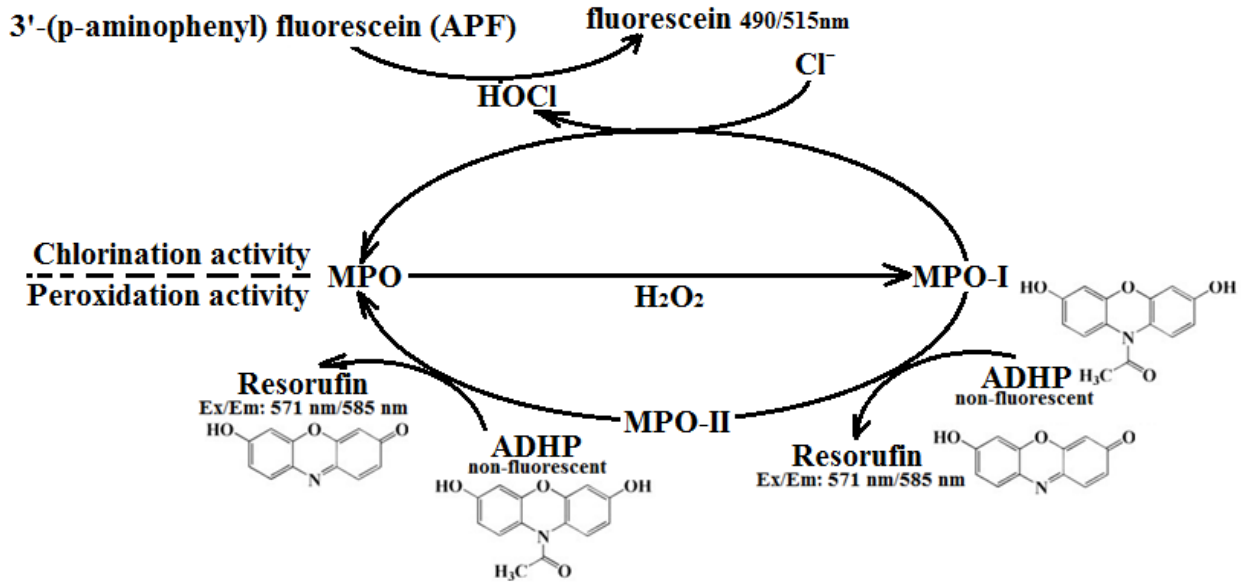
Myeloperoxidase Fluorescenic Activity Assay Kit

Cat. No. CTG-BK032F 1 kit

Product Description

Myeloperoxidase (MPO) Fluorescenic 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 fluorescenic Assay Kit, MPO catalyzes the production of NaClO from H_2O_2 and NaCl. Subsequently, NaClO will react stoichiometrically with Aminophenyl fluorescein (APF) to generate fluorescein, which has a strong fluorescence and can be detected at Ex/Em = 490/515 nm. The kit provides a rapid, simple, sensitive, and reliable test suitable as a high throughput assay of MPO activity. This kit can be used to detect MPO activity as low as 0.5 µU per well. Myeloperoxidase peroxidation activity, please check Celltechgen's Myeloperoxidase Peroxidation Activity Assay Kit (Cat. No. CTG-BK032P)



Application

MPO Fluorescenic 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
MPO Probe	200 µl
Stop Mix	100 µl
MPO Positive Control	1 vial (MPO)
Fluorescein Standard (1 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.

Experiment 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.

2. FITC Standards for Fluorecenuc Detection
Standard Curve Preparation: Mix 5 µl of 1 mM Fluorescein Standard with 995 µl of Assay Buffer to prepare a 5 µM Fluorescein Standard solution. Add 0, 2, 4, 6, 8, 10 µl of 5 µM Fluorescein Standard solution into a series of wells. Adjust volume to 100 µl/well with Assay Buffer to generate 0, 10, 20, 30, 40, 50 pmol/well of Fluorescein Standard. Mix well. Read the Standard Curve at Ex/Em = 490/515 nm after 5 min.

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 diluting MPO Substrate five-fold using 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. Color development should be stable and all wells can be read together after the final time point is completed.
4) Measure the fluorescence intensity at Ex/Em = 490/515 nm.

4. Activity Calculations

Read Ex/Em = 485/525 nm R1 at T1. Read R2 again at T2 after incubating the reaction at room temperature for 30 min (or longer time if the Sample's activity is low); protect from light. The amount of RFU generated in the time interval T2 - T1 is ΔRFU = R2 - R1. It is recommended to read kinetically to choose the R1 and R2 within the linear range. Plot the Fluorescein Standard Curve and apply the ΔRFU to the Standard Curve to get B pmol of fluorescein (amount of fluorescein generated between T1 and T2 in the reaction wells).

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 FITC
- 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 produce 1.0 µmole of FITC per minute at 25 °C.