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Product Information

Myeloperoxidase (MPO) Fluorometric Activity Assay Kit

Catalog Number **MAK069**
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.

The MPO Activity Flurometric Assay kit provides a simple and direct procedure for measuring MPO activity in a variety of samples. In this assay, MPO catalyzes the formation of hypochlorous acid with reacts with the substrate, aminophenyl fluorescein, to generate fluorescein (λ_{ex} = 485/λ_{em} = 525 nm). One unit of MPO is the amount of enzyme that will oxidize the MPO substrate to yield 1.0 μmole of fluorescein per minute at room temperature.

Components

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

MPO Assay Buffer Catalog Number MAK069A	25 mL
MPO Substrate Stock Catalog Number MAK069B	50 μL
MPO Probe Catalog Number MAK069C	0.2 mL
Fluoroscein Standard, 1 mM Catalog Number MAK069D	50 μL
MPO Positive Control Catalog Number MAK069E	1 vl

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates with clear bottoms for fluorescence assays.
- Fluorescence multiwell plate reader

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge vials before opening. To maintain reagent integrity, avoid repeated freeze/thaw cycles.

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

MPO Substrate – Dilute 4 μL of MPO Substrate Stock with 700 μL of MPO Assay Buffer Assay Buffer. Mix well by pipetting. Prepare fresh each time assay is run.

MPO Positive Control – Reconstitute with 50 μL of MPO Assay Buffer. Mix well by pipetting, then aliquot and store, protected from light, at –20 °C. Use within 1 month of reconstitution.

Storage/Stability

The kit is shipped on wet ice and storage at –20 °C, protected from light, is recommended.

Procedure
All samples and standards should be run in duplicate.

Fluoroscein Standards for Fluorometric Detection
Dilute 5 µL of the 1 mM Fluoroscein Standard solution with 995 µl of the MPO Assay Buffer to prepare a 5 µM standard solution. Add 0, 2, 4, 6, 8, and 10 µL of the 5 µM standard solution into a 96 well plate, generating 0 (blank), 10, 20, 30, 40, and 50 pmole/well standards. Add MPO Assay Buffer to each well to bring the volume to 100 µL. After 5 minutes, measure the fluorescence intensity (FLU, λ_{ex} = 485/λ_{em} = 525 nm).

Sample Preparation
Tissue (10 mg) or cells (2×10^6) can be homogenized in 4 volumes of ice-cold MPO Assay Buffer. Centrifuge the samples at 13,000 × *g* for 10 minutes to remove insoluble material.

Serum samples can be directly added to the wells.

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

Bring test samples to a final volume of 50 µL with MPO Assay Buffer.

For the positive control, add 10 µL of the MPO positive control solution to wells and adjust to 50 µL with the MPO Assay Buffer.

Assay Reaction
1. Set up the Master Reaction Mix according to the scheme in Table 1. 50 µL of the Master Reaction Mix is required for each sample and positive control well. Do not add the Master Reaction Mix to the Standard Curve wells.

Table 1.
Master Reaction Mix

Reagent	Volume
MPO Assay Buffer	46 µL
MPO Substrate	2 µL
MPO Probe	2 µL

2. Add 50 µL of the Master Reaction Mix to each of the sample and positive control wells. Mix well using a horizontal shaker or by pipetting. Cover the plate and protect from light during the incubation.

3. Incubate the plate at room temperature. After 2 minutes, take the initial measurement ($T_{initial}$). Measure the fluorescence intensity ($FLU_{initial}$, λ_{ex} = 485/λ_{em} = 525 nm).
   Note: It is essential $(FLU)_{initial}$ is in the linear range of the standard curve.

4. Continue to incubate the plate at room temperature taking measurements (FLU) every 5 minutes. Protect the plate from light during the incubation.

5. Continue taking measurements until the value of the most active sample is greater than the value of the highest standard (50 pmole/well). At this time the most active sample is near or exceeds the end of the linear range of the standard curve.

6. The final measurement [(FLU_{final}] for calculating the enzyme activity would be penultimate reading or the value before the most active sample is near or exceeds the end of the linear range of the standard curve, see step 5. The time of the penultimate reading is T_{final}.
   Note: It is essential the final measurement falls within the linear range of the standard curve.

Calculations

Plot the fluoroscein standard curve.

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

Calculate the change in FLU from $T_{initial}$ to T_{final} for the samples.

$$\Delta FLU = FLU_{final} - FLU_{initial}$$

Compare the ΔFLU of each sample to the standard curve to determine the amount of Fluoroscein generated by the MPO assay between $T_{initial}$ and T_{final} (B).

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 (pmole) of fluorescein released between $T_{initial}$ and T_{final}.

Reaction Time = $T_{final} - T_{initial}$ (minutes)

V = sample volume (mL) added to well

MPO activity is reported as pmole/min/mL (microunit/mL)

One unit of MPO is the amount of enzyme that will oxidize the MPO substrate to yield 1.0 μmole of fluoroscein per minute at room temperature.

Example:

Fluroescein amount (B) = 38 pmole

First reading ($T_{initial}$) = 2 minutes

Second reading (T_{final}) = 35 minutes

Sample volume (V) = 0.05 mL

Sample dilution is 1

MPO activity is:

$$\frac{38 \times 1}{(35-2) \times 0.05} = 23.03 \text{ microunits/mL}$$

Troubleshooting Guide

Problem	Possible Cause	Suggested Solution
Assay not working	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 fluorometric assays, use black plates with clear bottoms
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 needed to use 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 plate well
	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

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