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 Fluorometric 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 (\( \lambda_{\text{ex}} = 485/\lambda_{\text{em}} = 525 \text{ nm} \)). 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.

**Fluorescein 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<sub>ex</sub> = 485/λ<sub>em</sub> = 525 nm).

**Sample Preparation**

Tissue (10 mg) or cells (2 × 10⁶) 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.**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO Assay Buffer</td>
<td>46 µL</td>
</tr>
<tr>
<td>MPO Substrate</td>
<td>2 µL</td>
</tr>
<tr>
<td>MPO Probe</td>
<td>2 µL</td>
</tr>
</tbody>
</table>

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<sub>initial</sub>). Measure the fluorescence intensity (FLU<sub>initial</sub>, λ<sub>ex</sub> = 485/λ<sub>em</sub> = 525 nm).

**Note:** It is essential (FLU<sub>initial</sub>) 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<sub>final</sub>]) 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<sub>final</sub>.

**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 $T_{\text{initial}}$ to $T_{\text{final}}$ for the samples.

$$\Delta \text{FLU} = \text{FLU}_{\text{final}} - \text{FLU}_{\text{initial}}$$

Compare the $\Delta \text{FLU}$ of each sample to the standard curve to determine the amount of Fluoroscein generated by the MPO assay between $T_{\text{initial}}$ and $T_{\text{final}}$ (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 $T_{\text{initial}}$ and $T_{\text{final}}$.

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

$V =$ sample volume (mL) added to well

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

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.

Example:

Fluorescein amount (B) = 38 pmole
First reading ($T_{\text{initial}}$) = 2 minutes
Second reading ($T_{\text{final}}$) = 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

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