3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

MPO Inhibitor Screening Kit (Fluorometric)

Catalog Number **MAK283**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Myeloperoxidase (MPO, EC 1.11.2.2) is a peroxidase abundantly expressed in neutrophil granulocytes. It catalyzes the hydrogen peroxide dependent oxidation of halide ions to generate hypochlorite (HClO), the reaction by which MPO exhibits cytotoxic activity against tumor cells and microorganisms. MPO also oxidizes various substances such as phenol and anilines. MPO undergoes the chlorination or peroxidation reaction depending upon the relative concentrations of chloride and the reducing substrates. Recent studies suggest that increased levels of MPO are associated with an increased risk for cardiovascular disease and myocardial infarction, thus development of novel and specific inhibitors of MPO is critical for therapeutic purposes.

This MPO Inhibitor Screening Kit provides screening assays for both MPO chlorination and peroxidation activities. In the chlorination assay (specific for MPO), MPO converts hydrogen peroxide and sodium chloride to sodium hypochlorite, which reacts with the Chlorination Substrate to give an intensely fluorescent product, λ_{ex} = 480 nm/λ_{em} = 520. In the Peroxidation Assay, MPO oxidizes Peroxidation Substrate to generate fluorescence λ_{ex} = 535 nm/λ_{em} = 587. The fluorescence generated is directly proportional to any peroxidase activity present. In the presence of MPO inhibitor, reactions are impeded, thus decreasing the rate and/or extent of generation of MPO-dependent fluorescence.

This kit provides a sensitive, quick, and easy method for screening potential inhibitors of MPO, and identifying whether one or both activities are inhibited. The MPO Inhibitor Control is included to compare the efficacy of test inhibitors. The assay is high-throughput adaptable and can be performed in less than 20 minutes.

MPO Chlorination Assay:

$$H_2O_2 + NaCl \xrightarrow{MPO} NaClO + \text{Chlorination Substrate} \rightarrow \text{Fluorescence}$$

λ_{ex} = 480 nm/λ_{em} = 520

MPO Peroxidation Assay:

$$H_2O_2 + \text{Peroxidation Substrate} \xrightarrow{MPO} \text{Fluorescence}$$

λ_{ex} = 535 nm/λ_{em} = 587

Components

The kit is sufficient for 200 assays in 96 well plates.

MPO Assay Buffer Catalog Number MAK283A	50 mL
MPO Chlorination Substrate (in DMSO) Catalog Number MAK283B	200 µL
MPO Peroxidation Substrate (in DMSO) Catalog Number MAK283C	200 µL
MPO Enzyme Catalog Number MAK283D	1 vial
Inhibitor Control (4-Aminobenzonic Hydrazide, in DMSO) Catalog Number MAK283E	100 µL
Hydrogen Peroxide Catalog Number MAK283F	50 µL

Reagents and Equipment Required but Not Provided.

- 96 well flat-bottom plate – It is recommended to use black plates for this assay.
- 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 Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Briefly centrifuge small vials prior to opening.

MPO Assay Buffer – Bring to room temperature before use. Store at –20 °C or 4 °C.

MPO Chlorination Substrate and MPO Peroxidation Substrate – Aliquot and store at –20 °C. Bring to room temperature before use.

MPO Enzyme – Reconstitute with 1.1 mL of MPO Assay Buffer. Aliquot and store at –70 °C. Avoid freeze/thaw cycles. Keep on ice while in use. Use within two months.

Inhibitor Control – Bring to room temperature before use.

Storage/Stability
Store kit at –20 °C, protected from light. Briefly centrifuge small vials prior to opening.

Procedure
Read entire protocol before performing the assay.

MPO Chlorination Inhibitor Screening Assay – Screen Compounds, Inhibitor Control, and Enzyme Control Preparation
Dissolve candidate inhibitors into an appropriate solvent at 100× the highest final concentration to be tested. Dilute to 2× desired test concentration with MPO Assay Buffer. Add 45 µL of diluted candidate inhibitor or MPO Assay Buffer into desired wells, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor). For Inhibitor Control (IC), dilute Inhibitor Control 50× by adding 5 µL of Inhibitor Control to 245 µL of MPO Assay Buffer. Add 45 µL of diluted Inhibitor Control into desired well(s).

Note: Diluted Inhibitor Control is stable for 4 hours at 4 °C.

Enzyme Solution Preparation
Add 5 µL of MPO Enzyme into Sample, Enzyme Control, and Inhibitor Control wells.

Substrate Solution Preparation
Prepare 5 mM Hydrogen Peroxide solution by adding 1 µL of Hydrogen Peroxide to 175 µL of MPO Assay Buffer. Mix. Make enough reagents for the number of assays to be performed. For each well, prepare 50 µL of Substrate Solution, see Table 1.

Table1
Preparation of Substrate Solution

Reagent	Volume
MPO Assay Buffer	46 µL
MPO Chlorination Substrate	2 µL
Diluted Hydrogen Peroxide	2 µL

Mix and add 50 µL of Substrate Solution into each well. Mix well. Incubate for 10 minutes at room temperature with gentle shaking, protected from light.

Note: Don't store diluted Hydrogen Peroxide solution.

Measurement
Measure fluorescence (λ_{ex} = 480 nm/λ_{em} = 520).

MPO Peroxidation Inhibitor Screening Assay – Screen Compounds, Inhibitor Control, and Enzyme Control Preparation
Dissolve candidate inhibitors into an appropriate solvent at 100× the highest final concentration to be tested. Dilute to 2× desired test concentration with MPO Assay Buffer. Add 45 µL of diluted candidate inhibitor or MPO Assay Buffer into desired wells, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor). For Inhibitor Control (IC), dilute Inhibitor Control 50× by adding 5 µL of Inhibitor Control to 245 µL of MPO Assay Buffer. Add 45 µL of diluted Inhibitor Control to desired well(s).

Note: Diluted Inhibitor Control is stable for 4 hours at 4 °C.

Enzyme Solution Preparation
Mix enough reagents for the number of assays to be performed. For each well, prepare 5 µL of MPO Enzyme Solution, see Table 2.

Table2
Preparation of MPO Enzyme Solution

Reagent	Volume
MPO Assay Buffer	2.5 µL
MPO Enzyme	2.5 µL

Mix and add 5 µL of MPO Enzyme Solution into Sample, Enzyme Control, and Inhibitor Control wells.

Substrate Solution Preparation

Prepare 5 mM Hydrogen Peroxide solution by adding 1 μL of Hydrogen Peroxide to 175 μL of MPO Assay Buffer. Mix. Make enough reagents for the number of assays to be performed. For each well, prepare 50 μl of Substrate Solution, see Table 3.

Table 3

Preparation of Substrate Solution

Reagent	**Volume**
MPO Assay Buffer	46 μL
MPO Peroxidation Substrate	2 μL
Diluted Hydrogen Peroxide	2 μL

Mix and add 50 μL of Substrate Solution into each well. Mix well. Incubate for 5 minutes at room temperature with gentle shaking, protected from light.

Measurement

Measure fluorescence (λ_{ex} = 535 nm/λ_{em} = 587)

Results

Calculations

Set the RFU of Enzyme Control (EC) as 100%, and calculate the relative inhibition (%) of the candidate inhibitors as:

$$\% \text{ Relative Inhibition} = [RFU(EC) - RFU(S)]/RFU(EC) \times 100$$

RFU (EC) = the fluorescence reading of Enzyme Control

RFU (S) = the fluorescence reading of Sample Screen

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	It is recommended to use black plates for this assay.
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 samples will be used 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 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 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

KNV,SJ,MAM 08/17-1