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Myeloperoxidase (MPO) Inhibitor Screening Kit (Fluorometric)

rev. 08/15

(Catalog # K746-200; 200 assays; Store at -20°C)

I. Introduction:

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 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. BioVision's 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 Chlorination substrate to give an intensely fluorescent product (Ex/Em = 480/520 nm). In the Peroxidation Assay, MPO oxidizes peroxidation substrate to generate fluorescence (Ex/Em = 535/587 nm). 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. 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 min.

MPO Chlorination Assay:

$H_2O_2 + NaCl \xrightarrow{MPO} NaClO + \text{Chlorination Substrate} \longrightarrow \text{Fluorescence (Ex/Em = 480/520 nm)}$

MPO Peroxidation Assay:

$H_2O_2 + \text{Peroxidation Substrate} \xrightarrow{MPO} \text{Fluorescence (Ex/Em = 535/587 nm)}$

II. Application:

- Screening/characterizing/studying potential inhibitors of MPO

III. Kit Contents:

Components	K746-200	Cap Color	Part Number
MPO Assay Buffer	50 ml	NM	K746-200-1
MPO Chlorination Substrate (in DMSO)	200 µl	Red/White Dot	K746-200-2
MPO Peroxidation Substrate (in DMSO)	200 µl	Red	K746-200-3
MPO Enzyme	Lyophilized	Purple	K746-200-4
Inhibitor Control (4-Aminobenzonic Hydrazide, in DMSO)	100 µl	Orange	K746-200-5
Hydrogen Peroxide	50 µl	Blue	K746-200-6

IV. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (fluorescent plate reader)

V. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **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 MPO Assay Buffer. Aliquot and store at -70°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.
- **Inhibitor Control:** Bring to room temperature before use.

VI. MPO Inhibitor Screening Protocol:

A. MPO Chlorination Inhibitor Screening Assay:

1. **Screen Compounds, Inhibitor Control, and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent at 100X the highest final concentration to be tested. Dilute to 2X desired test concentration with MPO Assay Buffer. Add 45 µl 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 times by adding 5 µl Inhibitor Control to 245 µl MPO Assay Buffer. Add 45 µl of diluted Inhibitor Control into desired well(s).

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

2. **Enzyme Solution Preparation:** Add 5 µl MPO Enzyme into Sample, Enzyme Control and Inhibitor Control wells.
3. **Substrate Solution Preparation:** Prepare 5 mM Hydrogen Peroxide solution by adding 1 µl Hydrogen Peroxide to 175 µl MPO Assay Buffer. Mix. Make enough reagents for the number of assays to be performed. For each well, prepare 50 µl of Substrate solution containing:

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 min. at room temperature with gentle shaking, protected from light.

Note: Don't store diluted Hydrogen Peroxide solution.

4. **Measurement:** Measure fluorescence (Ex/Em = 480/520 nm).

B. MPO Peroxidation Inhibitor Screening Assay:

1. **Screen Compounds, Inhibitor Control and Enzyme Control Preparation:** Dissolve candidate inhibitors into an appropriate solvent at 100X the highest final concentration to be tested. Dilute to 2X desired test concentration with MPO Assay Buffer. Add 45 µl 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 times by adding 5 µl Inhibitor Control to 245 µl MPO Assay Buffer. Add 45 µl of diluted Inhibitor Control to desired well(s).

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

2. **Enzyme Solution Preparation**: Mix enough reagents for the number of assays to be performed. For each well, prepare 5 µl of MPO enzyme solution.

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.

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

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 min. at room temperature with gentle shaking, protected from light.

4. **Measurement:** Measure fluorescence (Ex/Em = 535/587 nm).

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

$$\textbf{Inhibitor}\ (\%) = \frac{\textbf{RFU(EC)} - \textbf{RFU(S)}}{\textbf{RFU (EC)}}\ \textbf{X 100}$$

Where: **RFU (EC)** is the fluorescence reading of Enzyme Control
RFU (S) is the fluorescence reading of Sample Screen

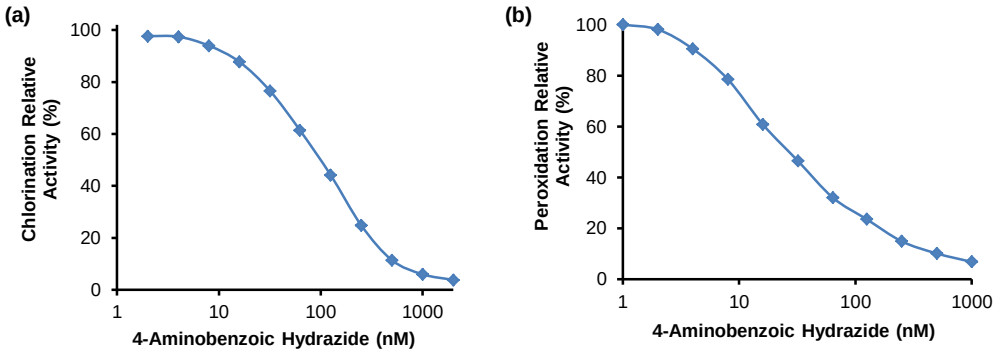

Figure: Inhibition of Chlorination activity (a) and Peroxidation activity (b) by 4-Aminobenzoic Hydrazide. Assays were performed following the kit protocol.

VIII. Related Products:

Myeloperoxidase (MPO) Activity Colorimetric Assay Kit (K744)
Myeloperoxidase (MPO) Activity Fluorometric Assay Kit (K745)
Hydrogen Peroxide Colorimetric/Fluorometric Assay Kit (K265)
Glutathione Reductase Activity Assay Kit (K761)
Glutathione Peroxidase Activity Assay Kit (K762)
Glutathione (GSH/GSSG/Total) Assay Kit (K264)
Glutathione Colorimetric Assay Kit (K261)
Glutathione Fluorometric Assay Kit (K251)
MPO Antibody (Clone 03D03) (5012)
MPO Blocking Peptide (3831BP)
MPO Antibody (Clone12D06) (5013)
Myeloperoxidase Inhibitor (1977)
Myeloperoxidase, human neutrophil (K4744)
Glutathione Peroxidase 1 Antibody (Clone2A10) (6214)
Glutathione Peroxidase 3 Antibody (Clone55A) (6215)
Glutathione Peroxidase 4 Antibody (Clone1H11) (6213)

FOR RESEARCH USE ONLY! Not to be used on humans.