ab283410 – Myeloperoxidase Inhibitor Screening Kit (Fluorometric)

For the screening of potential Myeloperoxidase inhibitors.
For research use only - not intended for diagnostic use.

PLEASE NOTE: With the acquisition of BioVision by Abcam, we have made some changes to component names and packaging to better align with our global standards as we work towards environmental-friendly and efficient growth. You are receiving the same high-quality products as always, with no changes to specifications or protocols.

For overview, typical data and additional information please visit:
http://www.abcam.com/ab283410

Storage and Stability

On receipt entire assay kit should be stored at -20°C, protected from light. Upon opening, use kit within 6 months.

Materials Supplied

Item	Quantity	Storage Condition
Assay Buffer LVI/Myeloperoxidase Assay Buffer	50 ml	-20°C
MPO Chlorination Probe/Myeloperoxidase Chlorination Substrate	200 µl	-20°C
OxiRed Probe/Myeloperoxidase Peroxidation Substrate	200 µl	-20°C
MPO Enzyme/Myeloperoxidase Enzyme	1 vial	-20°C
MPO Inhibitor Control/Inhibitor Control	100 µl	-20°C
Hydrogen Peroxide Solution II/Hydrogen Peroxide	50 µl	-20°C

Materials Required, Not Supplied

These materials are not included in the kit, but will be required to successfully utilize this assay:

- Multi-well spectrophotometer
- 96-well white plate with flat bottom

Reagent Preparation

- Before using the kit, spin the tubes prior to opening.

Assay Buffer LVI/Myeloperoxidase Assay Buffer: Bring the Assay Buffer LVI/Myeloperoxidase Assay Buffer to room temperature (RT) before use. Can store at -20°C or +4°C.

MPO Chlorination Probe/Myeloperoxidase Chlorination and OxiRed Probe/Peroxidation Substrate: Aliquot and store at -20°C. Bring to room temperature before use.

MPO Enzyme/Myeloperoxidase Enzyme: Reconstitute with 1.1 ml Assay Buffer LVI/MPO Assay Buffer. Aliquot and store at -70°C. Avoid freeze/thaw. Keep on ice while in use. Use within two months.

MPO Inhibitor Control/Inhibitor Control: Bring to RT before use.

Myeloperoxidase Chlorination Screening Protocol

Screening Compounds, Inhibitor Control, and Enzyme Control Preparation:

1. Dissolve candidate inhibitors into an appropriate solvent at 100X the highest final concentration to be tested.
2. Dilute to 2X desired test concentration with Assay Buffer LVI/MPO Assay Buffer.
3. Add 45 µl diluted candidate inhibitor or Assay Buffer LVI/MPO Assay Buffer into desired wells, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor).
4. For Inhibitor Control (IC), dilute MPO Inhibitor Control/Inhibitor Control 50 times by adding 5 µl MPO Inhibitor Control/Inhibitor Control to 245 µl Assay Buffer LVI/MPO Assay Buffer.
5. Add 45 µl of diluted MPO Inhibitor Control/Inhibitor Control into desired well(s).

Δ Note: *Diluted MPO Inhibitor Control/Inhibitor Control is stable for 4 hours at 4°C.*

Enzyme Solution Preparation:

1. Add 5 µl MPO Enzyme/Myeloperoxidase Enzyme into Sample, Enzyme Control and Inhibitor Control wells.

Substrate Solution Preparation:

1. Prepare 5 mM Hydrogen Peroxide solution by adding 1 µl Hydrogen Peroxide Solution II/Hydrogen Peroxide to 175 µl Assay Buffer LVI/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:

	Volume
Assay Buffer LVI/Myeloperoxidase Assay Buffer	46 µl
MPO Chlorination Probe/Myeloperoxidase Chlorination Substrate	2 µl
Diluted Hydrogen Peroxide Solution II/Hydrogen Peroxide	2 µl

2. 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.

Δ Note: *Do not store diluted Hydrogen Peroxide solution.*

Measurement:

1. Measure fluorescence (Ex/Em = 480/520 nm).

Myeloperoxidase Peroxidation Screening Protocol

Screening Compounds, Inhibitor Control, and Enzyme Control Preparation:

1. Dissolve candidate inhibitors into an appropriate solvent at 100X the highest final concentration to be tested.
2. Dilute to 2X desired test concentration with Assay Buffer LVI/MPO Assay Buffer.
3. Add 45 µl diluted candidate inhibitor or Assay Buffer LVI/MPO Assay Buffer into desired wells, as Sample Screen [S], or Enzyme Control [EC] (no inhibitor).
4. For Inhibitor Control (IC), dilute MPO Inhibitor Control/Inhibitor Control 50 times by adding 5 µl MPO Inhibitor Control/Inhibitor Control to 245 µl Assay Buffer LVI/MPO Assay Buffer.
5. Add 45 µl of diluted MPO Inhibitor Control/Inhibitor Control into desired well(s).

Δ Note: *Diluted MPO Inhibitor Control/Inhibitor Control is stable for 4 hours at 4°C.*

Enzyme Solution Preparation:

1. Mix enough reagents for the number of assays to be performed. For each well, prepare 5 µl of MPO enzyme solution5 µl MPO Enzyme/Myeloperoxidase Enzyme into Sample, Enzyme Control, and Inhibitor Control wells.

	Volume
Assay Buffer LVI/Myeloperoxidase Assay Buffer	2.5 µl
MPO Enzyme/Myeloperoxidase Enzyme	2.5 µl

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

Substrate Solution Preparation:

1. Prepare 5 mM Hydrogen Peroxide solution by adding 1 µl Hydrogen Peroxide Solution II/Hydrogen Peroxide to 175 µl Assay Buffer LVI/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:

	Volume
Assay Buffer LVI/Myeloperoxidase Assay Buffer	46 µl
OxiRed Probe/Myeloperoxidase Peroxidation Substrate	2 µl
Diluted Hydrogen Peroxide Solution II/Hydrogen Peroxide	2 µl

2. 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:

1. Measure fluorescence (Ex/Em = 535/587 nm).

Calculations:

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

$$\% \text{ Inhibitor} = \frac{RFU\ (EC) - RFU\ (S)}{RFU\ (EC)} \times 100$$

Where RFU (EC) is the fluorescence reading of Enzyme Control and RFU (S) is the fluorescence reading of Sample Screen.

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus
www.abcam.cn/contactus (China)
www.abcam.co.jp/contactus (Japan)