Neutrophil Myeloperoxidase Activity Assay Kit
Item No. 600620
Materials Supplied

Kit will arrive packaged as a -20°C kit. For best results, remove components and store as stated below.

<table>
<thead>
<tr>
<th>Item Number</th>
<th>Item</th>
<th>Quantity/Size</th>
<th>Storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>10009322</td>
<td>Cell-Based Assay Buffer Tablet</td>
<td>1 vial/1 tablet</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>600611</td>
<td>Cell-Based Assay Red Blood Cell Lysis Buffer</td>
<td>1 vial/100 ml</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>600612</td>
<td>Cell-Based Assay Neutrophil Isolation Histopaque®</td>
<td>1 vial/25 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>400145</td>
<td>Cell-Based PMA (1 mM)</td>
<td>1 vial/50 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>600622</td>
<td>Cell-Based Assay Myeloperoxidase Positive Control</td>
<td>1 vial/25 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>400074</td>
<td>TMB Substrate Solution</td>
<td>1 vial/12 ml</td>
<td>4°C</td>
</tr>
<tr>
<td>600621</td>
<td>Cell-Based Assay Myeloperoxidase Inhibitor</td>
<td>1 vial/50 µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>400014</td>
<td>96-Well Solid Plate (Colorimetric Assay)</td>
<td>2 plates</td>
<td>Room Temperature</td>
</tr>
<tr>
<td>400012</td>
<td>96-Well Cover Sheet</td>
<td>2 covers</td>
<td>Room Temperature</td>
</tr>
</tbody>
</table>

NOTE: Histopaque® is a product of Sigma-Aldrich Co.

If any of the items listed above are damaged or missing, please contact our Customer Service department at (800) 364-9897 or (734) 975-3999. We cannot accept any returns without prior authorization.

WARNING: This product is for laboratory research use only: not for administration to humans. Not for human or veterinary diagnostic or therapeutic use.
Precautions
Please read these instructions carefully before beginning this assay.
For research use only. Not for human or diagnostic use.

If You Have Problems

Technical Service Contact Information
Phone: 888-526-5351 (USA and Canada only) or 734-975-3888
Fax: 734-971-3641
Email: techserv@caymanchem.com
Hours: M-F 8:00 AM to 5:30 PM EST

In order for our staff to assist you quickly and efficiently, please be ready to supply the lot number of the kit (found on the outside of the box).

Storage and Stability
The kit should be stored at -20°C. Once it is opened, remove the Cell-Based PMA (1 mM) and Cell-Based Assay Myeloperoxidase Inhibitor from the kit and store at -20°C. Store the TMB Substrate Solution at 4°C. The rest of the components may be stored at room temperature. The kit will perform as specified if used before the expiration date indicated on the outside of the box.

Background
Myeloperoxidase (MPO) is a peroxidase enzyme that makes up no less than 1-2% of the dry weight of neutrophils, which are the most abundant type of leukocyte in human blood. The active enzyme is mainly stored within cytoplasmic azurophilic granules in the neutrophil until cell activation. Upon stimulation by pathogens or pharmacological agents such as phorbol myristate acetate (PMA), MPO may be released into the phagolysosome; or excreted into the extracellular region, either as free protein or associated with networks of extracellular traps (NETs). Together with other granule proteins released from activated neutrophils, neutrophil MPO plays a critical role in degrading invading pathogens and thus provides one of the earliest lines of defense in innate immunity. It catalyzes the hydrogen peroxide-mediated oxidation of halide ions to products such as hypochlorous acid (HOCl). These reactive products participate in a variety of secondary reactions which in turn lead to modifications of proteins and destruction of the extracellular matrix. In addition to its involvement in bacterial killing, MPO has been shown to play critical roles in a wide range of diseases, including cystic fibrosis, renal dysfunction, arthritis, and cancer. Because of its roles in the innate immunoresponse and various diseases, MPO is of interest as a potential therapeutic target.

About This Assay
Cayman’s Neutrophil Myeloperoxidase Activity Assay kit utilizes 3,3′,5,5′-tetramethylbenzidine (TMB) as a chromogenic substrate, which upon reacting with MPO, yields a blue color detectable by its absorbance at 650 nm. The color intensity is proportional to the amount of MPO in the sample. Reagents needed to isolate neutrophils from whole blood are included in the kit, as is PMA, which is known to stimulate MPO release from neutrophils. A specific inhibitor for MPO, 4-aminobenzhydrazide (ABH), is also included in the kit for verifying the specificity of the assay.
Reagent Preparation

1. **Cell-Based Assay Buffer**
   Dissolve one Cell-Based Assay Buffer Tablet (Item No. 10009322) in 100 ml of distilled water. Filter through a 0.2 µM filter before using to dilute the whole blood. Use the filtered Cell-Based Assay Buffer in a cell culture hood. This buffer should be stable for approximately one year at room temperature.

2. **Cell-Based Assay Red Blood Cell Lysis Buffer - (Item No. 600611)**
   Each vial contains 100 ml of sterile Red Blood Cell Lysis Buffer. It is ready to use to lyse red blood cells during the isolation of neutrophils. Use the sterile Red Blood Cell Lysis Buffer in a cell culture hood.

3. **Cell-Based Assay Neutrophil Isolation Histopaque® - (Item No. 600612)**
   The vial contains 25 ml of Histopaque®. It is ready to use for isolation of neutrophils from whole blood. Use the Cell-Based Assay Neutrophil Isolation Histopaque® in a cell culture hood.

4. **Cell-Based PMA (1 mM) - (Item No. 400145)**
   The vial contains 50 µl of 1 mM PMA in DMSO. For stimulating neutrophil MPO release, dilute this PMA 1:10,000 in the culture medium used for your cells.

5. **TMB Substrate Solution**
   The vial contains 12 ml TMB Substrate Solution. It is ready to use. The substrate is stable at 4°C for at least one year.
   **NOTE:** TMB is very sensitive to light. Avoid direct exposure to light.

6. **Cell-Based Assay Myeloperoxidase Inhibitor - (Item No. 600621)**
   The vial contains 50 µl of 25 mM 4-aminobenzhydrozide (4-ABH) in DMSO. Prior to use, dilute the Cell-Based Assay Myeloperoxidase Inhibitor 1:100 in Assay Buffer. The inhibitor is stable at -20°C for at least one year.

7. **Cell-Based Assay Myeloperoxidase Positive Control - (Item No. 600622)**
   This vial contains 25 µl human polymorphonuclear leukocyte myeloperoxidase at 0.1 mg/ml. To use the enzyme as a Positive Control, add 2 µl of the Cell-Based Assay Myeloperoxidase Positive Control (Item No. 600622) to 0.2 ml of the diluted Assay Buffer. Mix well. Add 50 µl of this diluted enzyme into corresponding wells in the assay plate.

Sample Preparation

**Neutrophil Isolation**

2. Transfer the blood to a 50 ml conical tube. Rinse the blood collection tube with 15 ml of filtered Cell-Based Assay Buffer prepared as described on page 6. Add the rinsed solution to the 50 ml conical tube.
3. Pipet 10 ml of Cell-Based Assay Neutrophil Isolation Histopaque to a different 50 ml conical tube. Slowly add 30 ml of the diluted blood on the top of Cell-Based Assay Neutrophil Isolation Histopaque.
4. Centrifuge at 500 x g for 20-30 minutes at 18-26°C.
5. Carefully aspirate the yellowish and clear top layers and leave the reddish pellet containing neutrophils and red blood cells in the tube.
6. Pipette 30 ml of the Cell-Based Assay Red Blood Cell Lysis Buffer into the tube. Vortex to ensure mixing of the cells with the Lysis Buffer. Rock the tube on a rocker for 10-15 minutes to lyse the red blood cells.
7. Centrifuge at 1,200 x rpm for 10 minutes to pellet the neutrophils.
8. Carefully aspirate the reddish supernatant.
9. Add 5 ml of RPMI containing 1% BSA to the tube and mix well.
10. Centrifuge at 1,200 x rpm for five minutes to pellet the neutrophils.
11. Repeat steps 9 and 10 one more time.
12. Resuspend the cells in 20 ml RPMI containing 1% BSA. Mix well to ensure sufficient separation of the cells. The cells are now ready to be seeded and should be sufficient for two 96-well cell culture plates at a density of 1 x 10^5-5 x 10^5 cells/well.
**Treatment of Cells**

The following protocol is designed for a 96-well plate. For other sizes of plates the volume of medium/solution to apply to each well should be adjusted accordingly.

1. Seed a 96-well plate with the cells prepared above at 100 µl/well. Be sure to include two wells containing only culture medium for background controls.

2. Treat the cells with experimental compounds or vehicle for two to four hours, or for the period of time used in your typical experimental protocol, in a cell culture hood. To use the Cell-Based Assay PMA (Item No. 400145), dilute the PMA solution 1:10,000-1:50,000 into the culture medium. PMA at these concentrations causes a significant activation of neutrophil MPO release.

3. At the end of treatment, centrifuge the plate at 1,200 rpm for 10 minutes at 18-25°C.

4. The culture supernatant in each well is now ready for use in the MPO assay.

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**Plate Set Up**

There is no specific pattern for using the wells on the plate. We suggest that two wells contain culture medium without cells to be designated as background wells. Each sample should be assayed at least in duplicate. We suggest you record the contents of each well on the template sheet provided (see page 15).

**Pipetting Hints**

- It is recommended that a repeating pipettor be used to deliver reagents to the wells. This saves time and helps maintain more precise incubation times.
- Before pipetting each reagent, equilibrate the pipette tip in that reagent (i.e., slowly fill the tip and gently expel the contents, repeat several times).
- Do not expose the pipette tip to the reagent(s) already in the well.
Performing the Assay

Use the plates included in the kit to perform the assay described below.

1. **Positive Control Wells** - add 50 µl of positive control per well in the corresponding wells on the assay plate.

2. **Sample Wells** - Transfer 25 µl of culture supernatant from each well of the experimental plate to a corresponding well on the assay plate. Add 25 µl of the diluted Assay Buffer to each of the sample wells. The final volume for each sample well before addition of the substrate should be the same as that for the Positive Control Wells, which is 50 µl. Lesser amounts, for example 10 µl of culture supernatant, can be used when the concentration of myeloperoxidase released from stimulated cells is too high and has an absorbance >2 at 650 nm. If less sample is used, be certain to increase the amount of Assay Buffer accordingly.

3. **Sample Plus Inhibitor Wells** - Transfer 25 µl of culture supernatant from each well of the experimental plate to a corresponding well on the assay plate. Add 25 µl of the Cell-Based Assay Myeloperoxidase Inhibitor solution prepared above to each of the sample plus inhibitor wells.

4. **Addition of the MPO Substrate** - add 50 µl of TMB Substrate Solution to each well. Cover the plate with one of the plate covers included in the kit.

5. Incubate the plate at room temperature for 5-10 minutes.

6. Read the plate at absorbance 650 nm. **NOTE:** If calculating enzyme activity using the optional method on page 11, measure the absorbance at one minute and five minutes after addition of the MPO Substrate. The change in absorbance during that time can be determined using the following equation:

\[
\Delta A_{650} = \frac{A_{650} \text{(Time 5 min.)} - A_{650} \text{(Time 1 min.)}}{4 \text{ min.}}
\]

**Calculations (optional)**

*The following is an optional calculation that can be performed to convert \(A_{650}\) to units of enzyme activity (Units/ml; µmoles/min/ml). You may also choose to use the \(A_{650}\) value to compare enzyme activity between sample types (see Figure 1 on page 12).*

1. Determine the change in absorbance (\(\Delta A_{650}\)) per minute by reading the absorbance at one minute and five minutes after addition of the MPO Substrate. The change in absorbance during that time can be determined using the following equation:

\[
\Delta A_{650} = \frac{A_{650} \text{(Time 5 min.)} - A_{650} \text{(Time 1 min.)}}{4 \text{ min.}}
\]

2. Use the formula below to determine the myeloperoxidase activity. The reaction rate at 650 nm can be determined using an extinction coefficient of 3.9 x 10^4 M\(^{-1}\) cm\(^{-1}\).

\[
\text{Myeloperoxidase activity (µmoles/min./ml)} = \frac{\Delta A_{650}/\text{min.}}{(3.9 \times 10^4 \text{ M}\^{-1}\text{cm}\^{-1}) \times 0.4^{*} \text{ cm} \times 0.1 \text{ ml}} \times \frac{0.025^{**} \text{ ml}}{0.25 \text{ ml}} \times \text{Sample dilution}
\]

*Pathlength of sample in the well.

**This value corresponds to the amount of sample added to the well. Adjust this value as necessary if a sample volume other than 25 µl was used.
Figure 1. PMA stimulates neutrophil myeloperoxidase release from human neutrophils. Human neutrophils were isolated from freshly collected human blood according to the procedure described in the booklet. The cells were then plated in RPMI containing 1% BSA in a 96-well cell culture plate. Cells were treated with either vehicle or 25 nM PMA for 2.5 hours. The supernatant from each well (25 µl) was sampled and assayed for neutrophil myeloperoxidase according to the procedure described in the booklet.

### Performance Characteristics

<table>
<thead>
<tr>
<th>Control</th>
<th>Control + 4-ABH</th>
<th>PMA</th>
<th>PMA + 4-ABH</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.2</td>
<td>0.8</td>
<td>0.6</td>
</tr>
<tr>
<td>0.8</td>
<td>1.2</td>
<td>1.4</td>
<td>1.6</td>
</tr>
<tr>
<td>1.4</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>1.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.4</td>
</tr>
</tbody>
</table>

**Figure 1.**

**Performance Characteristics**

**Troubleshooting**

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Causes</th>
<th>Recommended Solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erratic values; dispersion of duplicates/triplicates</td>
<td>A. Poor pipetting/technique</td>
<td>A. Be careful not to splash the contents of the wells</td>
</tr>
<tr>
<td></td>
<td>B. Bubble in the well(s)</td>
<td>B. Carefully tap the side of the plate with your finger to remove bubbles</td>
</tr>
<tr>
<td>Erratic response curve of compound treatments</td>
<td>Unequal number of cells in each well</td>
<td>Make sure each well contains the same number of cells</td>
</tr>
<tr>
<td>High level of myeloperoxidase in control samples</td>
<td>Lysis of red blood cells is too long</td>
<td>Reduce the incubation time for red blood cell lysis</td>
</tr>
</tbody>
</table>

**Reference**

Related Products

- Catalase Assay Kit - Item No. 707002
- LDH Cytotoxicity Assay Kit - Item No. 10008882
- Myeloperoxidase Chlorination Assay Kit - Item No. 10006438
- Myeloperoxidase Inhibitor Screening Assay Kit - Item No. 700170
- Myeloperoxidase Peroxidation Assay Kit - Item No. 700160
- Neutrophil Elastase Activity Assay Kit - Item No. 600610
- Superoxide Dismutase Assay Kit - Item No. 706002
- WST-1 Cell Proliferation Assay Kit - Item No. 10008883
- WST-8 Cell Proliferation Assay Kit - Item No. 10010199
- Xanthine Oxidase Assay Kit - Item No. 10010895
- XTT Cell Proliferation Assay Kit - Item No. 10010200

Warranty and Limitation of Remedy

Cayman Chemical Company makes no warranty or guarantee of any kind, whether written or oral, expressed or implied, including without limitation, any warranty of fitness for a particular purpose, suitability and merchantability, which extends beyond the description of the chemicals hereof. Cayman warrants only to the original customer that the material will meet our specifications at the time of delivery. Cayman will carry out its delivery obligations with due care and skill. Thus, in no event will Cayman have any obligation or liability, whether in tort (including negligence) or in contract, for any direct, indirect, incidental or consequential damages, even if Cayman is informed about their possible existence. This limitation of liability does not apply in the case of intentional acts or negligence of Cayman, its directors or its employees.

Buyer’s exclusive remedy and Cayman’s sole liability hereunder shall be limited to a refund of the purchase price, or at Cayman’s option, the replacement, at no cost to Buyer, of all material that does not meet our specifications.

Said refund or replacement is conditioned on Buyer giving written notice to Cayman within thirty (30) days after arrival of the material at its destination. Failure of Buyer to give said notice within thirty (30) days shall constitute a waiver by Buyer of all claims hereunder with respect to said material.

For further details, please refer to our Warranty and Limitation of Remedy located on our website and in our catalog.