Myeloperoxidase (MPO) Activity Colorimetric Assay Kit

(introduction) Myeloperoxidase (MPO) is a peroxidase (EC 1.11.1.7) abundantly expressed in neutrophils. It is a lysosomal protein stored in the azurophilic granules of the neutrophil. MPO contains a heme which causes its green color in secretions rich in neutrophils, such as pus and some forms of mucus. MPO catalyzes the production of hypochlorous acid (HClO) from hydrogen peroxide (H2O2) and chloride anion, Cl- (or halide). MPO also oxidizes tyrosine to a tyrosyl radical using hydrogen peroxide as oxidizing agent. In BioVision’s MPO Assay Kit, HClO produced from H2O2 and Cl- reacts with taurine to generate taurine chloramines, which subsequently reacts with the TNB probe to eliminate color (λ = 412 nm). The kit provides a rapid, simple, sensitive, and reliable test suitable for high throughput activity assay of MPO. This kit can be used to detect MPO as low as 0.05 mU per well.

II. Kit Contents:

<table>
<thead>
<tr>
<th>Component</th>
<th>100 Assays</th>
<th>Cap Code</th>
<th>Part Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPO Assay Buffer</td>
<td>25 µl</td>
<td>WM</td>
<td>K744-100-1</td>
</tr>
<tr>
<td>DTNB Probe (100 mM)</td>
<td>50 µl</td>
<td>Red</td>
<td>K744-100-2</td>
</tr>
<tr>
<td>TCEP (50 mM)</td>
<td>50 µl</td>
<td>Clear</td>
<td>K744-100-3</td>
</tr>
<tr>
<td>MPO Substrate</td>
<td>50 µl</td>
<td>Blue</td>
<td>K744-100-4</td>
</tr>
<tr>
<td>Stop Mix</td>
<td>Lyophilized</td>
<td>Green</td>
<td>K744-100-5</td>
</tr>
<tr>
<td>MPO Positive Control (lyophilized)</td>
<td>1 vial</td>
<td>Purple</td>
<td>K744-100-6</td>
</tr>
</tbody>
</table>

III. Storage and Handling:

Store kit at -20°C protected from light. Allow Assay Buffer to warm to room temperature before use. Briefly centrifuge small vials before opening. Read the entire protocol prior to performing the assay.

IV. Reagent Preparation:

- TNB Reagent/Standard: TNB Reagent/Standard: TNB is easily oxidizable so it needs to be prepared from DTNB Probe as needed. Use the same day as prepared, discard any unused TNB reagent/standard. The amount of DTNB Probe for each well (standard, sample, and background control) is 0.5µl. The amount per well of TCEP is 0.5 µl and the Assay Buffer is 49 µl for a total of 50 µl per well. (Example: For 10 wells, take 5 µl DTNB Probe, 5 µl TCEP and 490 µl Assay Buffer, mix and set aside).
- MPO Substrate: Aliquot and store at -20°C. Stable for 2 months. Working solution: Add 5 µl MPO Substrate to 300 µl dH2O. Make fresh and discard unused portion.
- Stop Mix: Add 200 µl dH2O and dissolve. Aliquot and store at -20°C. Use within two months.
- MPO Positive Control: Reconstitute the positive control with 100 µl MPO Assay Buffer. Aliquot and store at -20°C. Use within two months.

V. MPO Assay Protocol:

1. Standard Curve Preparation: Add 150, 140, 130, 120, 110 and 100 µl of MPO Assay Buffer into a series of wells. The Standard will be added to the wells (0, 10, 20, 30, 40, 50 µl respectively) at the end of the sample incubation period (see 4 below).

2. Sample Preparation: Homogenize tissue or cells in 4 volumes of MPO Assay Buffer, centrifuge at 13,000g for 10 min to remove insoluble material. Serum samples can be directly diluted in the MPO Assay Buffer. Add 1-50 µl test samples in 96 well plate. For white blood cells, take 2 ml of blood and lyse RBC using RBC Lysis Buffer (Cat# 5831). Incubate for 10 min. at room temperature. Centrifuge at 400 x g for 5 min. and remove the supernatant carefully. Wash the pellet with 1 ml 1X PBS. Centrifuge at 400 x g for 5 min. and remove the supernatant carefully. Lyse the pellet using 200 µl MPO Assay Buffer. Keep on ice for 10 min. Centrifuge at 10,000 x g for 10 min. to remove insoluble material. Collect the supernatant. Add 1-10 µl of the WBC lysate into a 96-well plate. Prepare parallel sample well(s) as background control. Adjust the volume of background control and sample wells to 50 µl/well with Assay Buffer. We suggest testing several doses of a sample to ensure the readings are within the standard curve range.

VI. Related Products:

- NADPH/NADP Quantification Kit
- Fatty Acid Assay Kit
- Triglyceride Assay Kit
- Lipase Assay Kit
- Adipogenesis Assay Kit
- Lactate Assay Kit
- Glycogen Assay Kit

For Research Use Only! Not to be used on humans.
### GENERAL TROUBLESHOOTING GUIDE:

<table>
<thead>
<tr>
<th>Problems</th>
<th>Cause</th>
<th>Solution</th>
</tr>
</thead>
</table>
| Assay not working | • Use of ice-cold assay buffer  
• Omission of a step in the protocol  
• Plate read at incorrect wavelength  
• Use of a different 96-well plate | • Assay buffer must be at room temperature  
• Refer and follow the data sheet precisely  
• Check the wavelength in the data sheet and the filter settings of the instrument  
• Fluorescence: Black plates (clear bottoms) ; Luminescence: White plates ; Colorimeters: Clear plates |
| Samples with erratic readings | • Use of an incompatible sample type  
• Samples prepared in a different buffer  
• Cell/tissue samples were not completely homogenized  
• Samples used after multiple free-thaw cycles  
• Presence of interfering substance in the sample  
• Use of old or inappropriately stored samples | • Refer data sheet for details about incompatible samples  
• Use the assay buffer provided in the kit or refer data sheet for instructions  
• Use Dounce homogenizer (increase the number of strokes); observe for lysis under microscope  
• Aliquot and freeze samples if needed to use multiple times  
• Troubleshoot if needed  
• Use fresh samples or store at correct temperatures until use |
| Lower/Higher readings in Samples and Standards | • Improperly thawed components  
• Use of expired kit or improperly stored reagents  
• Allowing the reagents to sit for extended times on ice  
• Incorrect incubation times or temperatures  
• Incorrect volumes used | • Thaw all components completely and mix gently before use  
• Always check the expiry date and store the components appropriately  
• Always thaw and prepare fresh reaction mix before use  
• Refer datasheet & verify correct incubation times and temperatures  
• Use calibrated pipettes and aliquot correctly |
| Readings do not follow a linear pattern for Standard curve | • Use of partially thawed components  
• Pipetting errors in the standard  
• Pipetting errors in the reaction mix  
• Air bubbles formed in well  
• Standard stock is at an incorrect concentration  
• Calculation errors  
• Substituting reagents from older kits/lots | • Thaw and resuspend all components before preparing the reaction mix  
• Avoid pipetting small volumes  
• Prepare a master reaction mix whenever possible  
• Pipette gently against the wall of the tubes  
• Always refer the dilutions in the data sheet  
• Recheck calculations after referring the data sheet  
• Use fresh components from the same kit |
| Unanticipated results | • Measured at incorrect wavelength  
• Samples contain interfering substances  
• Use of incompatible sample type  
• Sample readings above/below the linear range | • Check the equipment and the filter setting  
• Troubleshoot if it interferes with the kit  
• Refer data sheet to check if sample is compatible with the kit or optimization is needed  
• Concentrate/Dilute sample so as to be in the linear range |

**Note:** The most probable list of causes is under each problem section. Causes/Solutions may overlap with other problems.