**DETECTION OF INTRACELLULAR ANTIGENS BY FLOW CYTOMETRY**

**FIX & PERM® CELL PERMEABILIZATION REAGENTS**

<table>
<thead>
<tr>
<th>Product Code</th>
<th>Fixation Medium (A)</th>
<th>Permeabilization Medium (B)</th>
<th>Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>GAS-003</td>
<td>1 x 5 ml</td>
<td>1 x 5 ml</td>
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<tr>
<td>GAS-004</td>
<td>4 x 5 ml</td>
<td>4 x 5 ml</td>
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<tr>
<td>GAS001S-5</td>
<td>1 x 5 ml</td>
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<tr>
<td>GAS001S-100</td>
<td>1 x 100 ml</td>
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<td>1000</td>
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<tr>
<td>GAS002S-5</td>
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<td>GAS002S-100</td>
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</tbody>
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Lot No.: See label  
Expiration: See label

**USE OF FIX & PERM REAGENTS:**

**FIX & PERM** reagents are intended for the fixation (Reagent A) and permeabilization (Reagent B) of cells in suspension. This procedure facilitates antibody access to intracellular structures and leaves the morphological scatter characteristics of the cells intact. Specific formulations reduce background staining and allow simultaneous addition of permeabilization medium and fluorochrome-labeled antibodies.

**PERMEABILIZATION AND STAINING PROCEDURE:**

1. For each sample to be analyzed add appropriate volume of the conjugated antibody directed to the cell surface marker(s) of interest and/or the appropriate isotype control(s) to a 5 ml, 12x75 mm tube.

2. Pipette appropriate volume of adjusted cells (equivalent to 1 x 10⁶ cells) into each tube containing the conjugated antibody or isotype control.

3. Vortex each tube gently to mix, and incubate for 15 minutes in the dark at room temperature.

4. Add 100 µl of Reagent A (Fixation Medium) and incubate for 15 minutes at room temperature.

5. Wash once in 3 ml PBS + 0.1% NaN₃ + 5% FBS.

6. Centrifuge for 5 minutes at 300-350 x g, aspirate the supernatant, and vortex to fully resuspend the cell pellet.

7. Add 100 µl of Reagent B (Permeabilization Medium) and the recommended volume of the FITC- and/or PE- conjugated intracellular antibody or the corresponding isotype control.

8. Vortex 1-2 seconds and incubate for 20 minutes.

9. Wash once in 3 ml PBS + 0.1% NaN₃ + 5% FBS.

10. Centrifuge for 5 minutes at 300-350 x g and aspirate the supernatant.

11. Resuspend cells in sheath fluid for immediate analysis or in 0.5 ml of 0.1% paraformaldehyde fixative solution for storage at 2-8°C in the dark. Fixed cells should be analyzed within 24 hours.

A modification of this protocol using precooled absolute methanol has been shown to give better results for certain cell cycle antigens when using FITC- conjugated antibodies. This modification is not recommended when using PE-conjugated antibodies. For full details, please refer to Appendix C, “Fix & Perm Applications Guide to Intracellular Flow Cytometry.”

**FLOW CYTOMETRIC ANALYSIS:**

**FIX & PERM** reagents are designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to the manufacturer's instructions. Typical staining and scatter patterns are shown on the next page.

**CLINICAL RESEARCH APPLICATIONS:**

Flow cytometric analysis with monoclonal antibodies has historically been restricted primarily to cell surface molecules. For this reason, intracellular antigens such as cytoplasmic or nuclear enzymes, oncoproteins, cytokines, immunoglobulins, etc., were largely excluded from such analysis. Also excluded have been cytometric studies designed to address the cytoplasmic localization of some well established membrane-associated molecules such as CD3 and CD22.

**FIX & PERM** reagents allow intracellular antigen analysis with the equivalent ease as cell surface antigens. The only prerequisite is the availability of suitable antibody conjugates. Most commercially available monoclonal antibody conjugates can be used with the **FIX & PERM** reagents. However, some determinants are sensitive to the fixation step. This and optimal fixation time may have to be empirically determined for each antibody conjugate.

The **COMBI-IC®** and **CYTO-IC®** antibodies offered as companion products to the **FIX & PERM** reagents have been optimized for use with this system.

**STORAGE AND STABILITY:**

**FIX & PERM** reagents should be stored and used at room temperature. They are stable for the period shown on the package label when stored as directed. Do not use reagents if a precipitate forms or discoloration occurs. All antibody combinations should be stored at 2-8°C in the dark.

**WARNING:** Reagent A of the **FIX & PERM** Kit contains formaldehyde which is toxic, allergenic and a suspected carcinogen. Avoid contact with eyes, skin and clothing. All antibodies contain sodium azide as a preservative.
Figure Legend: Peripheral blood mononuclear cells stained with FITC-conjugated mouse anti-human myeloperoxidase (MPO). Representative forward (FSC) and side (SSC) scatter patterns and reaction patterns are shown.

REFERENCES:


GENERAL PURPOSE REAGENTS. FOR IN VITRO DIAGNOSTIC USE.