

# FIX&PERM® CELL PERMEABILIZATION REAGENTS

For suspension stainings and flow cytometric analyses of intracellular antigens

## ANTIGENS BY FLOW CYTOMETRY

Product Code	Fixation Medium (A)	Permeabilization Medium (B)	Tests	
GAS-002	4 x 5 ml	4 x 5 ml	200	CE/IVD
GAS-002-1	1 x 100 ml	1 x 100 ml	1000	CE/IVD

### Each Kit Fix & Perm contains:

Reagent A (Fixation Medium)

Reagent B (Permeabilization Medium)

**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:

- 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.
- Pipette appropriate volume of adjusted cells (equivalent to  $1 \times 10^6$  cells) into each tube containing the conjugated antibody or isotype control.
- Vortex each tube gently to mix, and incubate for 15 minutes in the dark at room temperature.
- Add 100  $\mu$ l of Reagent A (Fixation Medium) and incubate for 15 minutes at room temperature.
- Wash once in 3 ml PBS + 0.1% NaN<sub>3</sub> + 5% FBS.
- Centrifuge for 5 minutes at 300-350 x g, aspirate the supernatant, and vortex to fully resuspend the cell pellet.
- Add 100  $\mu$ l of Reagent B (Permeabilization Medium) and the recommended volume of the FITC- and/or PE- conjugated intracellular antibody or the corresponding isotype control.
- Vortex 1-2 seconds and incubate for 20 minutes.
- Wash once in 3 ml PBS + 0.1% NaN<sub>3</sub> + 5% FBS.
- Centrifuge for 5 minutes at 300-350 x g and aspirate the supernatant.
- 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 such as BrdU, Ki-67, and PCNA when using FITC-conjugated antibodies.

This modification is not recommended when using PE-conjugated antibodies.

### Methanol Modification

- For each sample to be analyzed, add 100  $\mu$ l of adjusted cell volume (equivalent to  $1 \times 10^6$  cells) to an appropriate 5 ml, 12 x 75 mm tube.
- Add 100  $\mu$ l of Reagent A (Fixation Medium) and incubate for 2-3 minutes at room temperature.
- Add 4 ml of pre-cooled absolute methanol (0-4°C) and vortex.
- Incubate for an additional 10 minutes at 0-4°C.

- Centrifuge for 5 minutes at 300-350 x g and wash with wash medium PBS + 0.1% NaN<sub>3</sub> + 5% FBS.
- Add 100  $\mu$ l of Reagent B (Permeabilization Medium) and appropriate volume of intracellular antibody(ies) or corresponding isotype control(s).
- Vortex at low speed for 1-2 seconds and incubate for 30 minutes at room temperature.
- Wash once in 3 ml wash medium (PBS + 0.1% NaN<sub>3</sub> + 5% FBS).
- Centrifuge for 5 minutes at 300-350 x g and aspirate the supernatant.
- Resuspend cells in sheath fluid for immediate analysis, or fix in 0.5 ml of 0.1% paraformaldehyde and store at 2-8°C in the dark. Fixed cells should be analyzed within 18 hours.

### 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.

### 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.

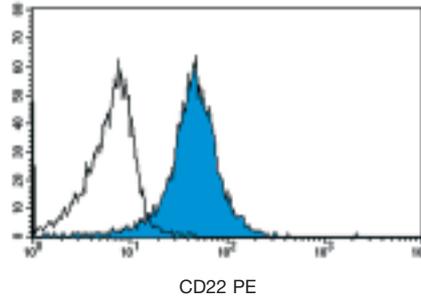
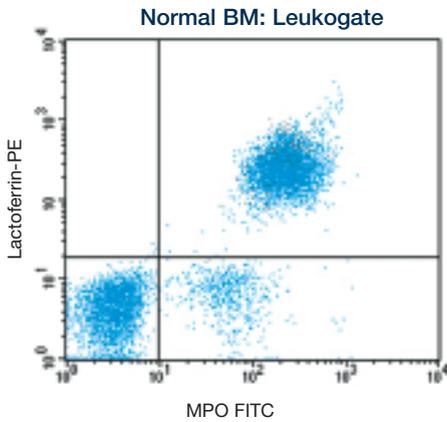
*FIX & PERM KIT COMPONENTS SHOULD NEVER BE FROZEN.*

**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.*

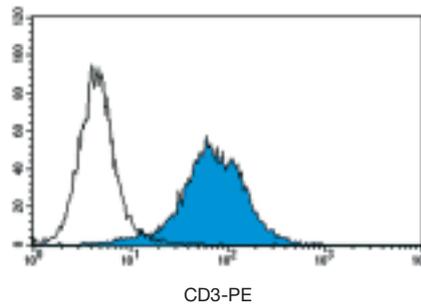
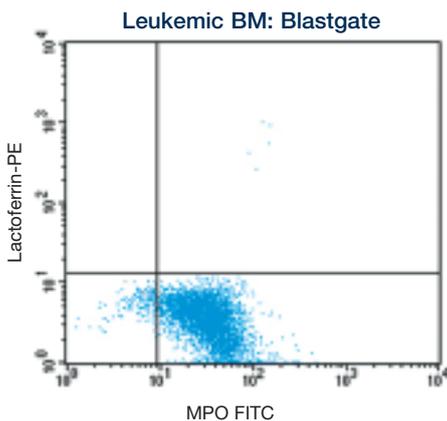
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# FIX&PERM<sup>®</sup> CELL PERMEABILIZATION REAGENTS

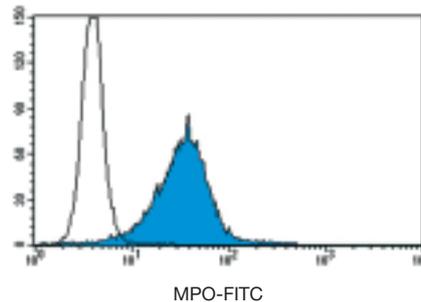
Please find below some staining examples with antibody conjugates:



Cytoplasmic staining with CD22-PE conjugate of undifferentiated leukemia cells of B-ALL type.



Cytoplasmic staining with CD3-PE conjugate of surface CD3 negative undifferentiated leukemia cells of T-ALL type.



Cytoplasmic staining with anti MPO-FITC conjugate of un-differentiated leukemia cells of AML type.

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