

DETECTION OF INTRACELLULAR ANTIGENS BY FLOW CYTOMETRY

FIX & PERM[®] CELL PERMEABILIZATION REAGENTS

Product Code	Fixation Medium (A)	Permeabilization Medium (B)	Tests
GAS003	1 x 5 ml	1 x 5 ml	50
GAS004	4 x 5 ml	4 x 5 ml	200
GAS001S5	1 x 5 ml		50
GAS001S100	1 x 100 ml		1000
GAS002S5		1 x 5 ml	50
GAS002S100		1 x 100 ml	1000

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 1×10^6 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 μ l of Reagent A (Fixation Medium) and incubate for 15 minutes at room temperature.
- Wash once in 3 ml PBS + 0.1% NaN₃ + 5% FBS.
- Centrifuge for 5 minutes at 300-350 x g, aspirate the supernatant, and vortex to fully resuspend the cell pellet.
- Add 100 μ 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% NaN₃ + 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 μ l of adjusted cell volume (equivalent to 1×10^6 cells) to an appropriate 5 ml, 12 x 75 mm tube.
- Add 100 μ 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% NaN₃ + 5% FBS.
- Add 100 μ 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% NaN₃ + 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.*

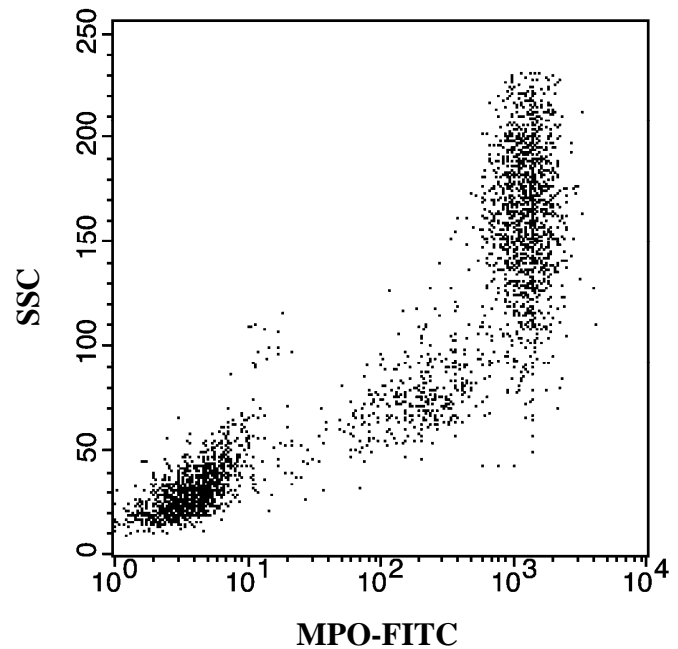
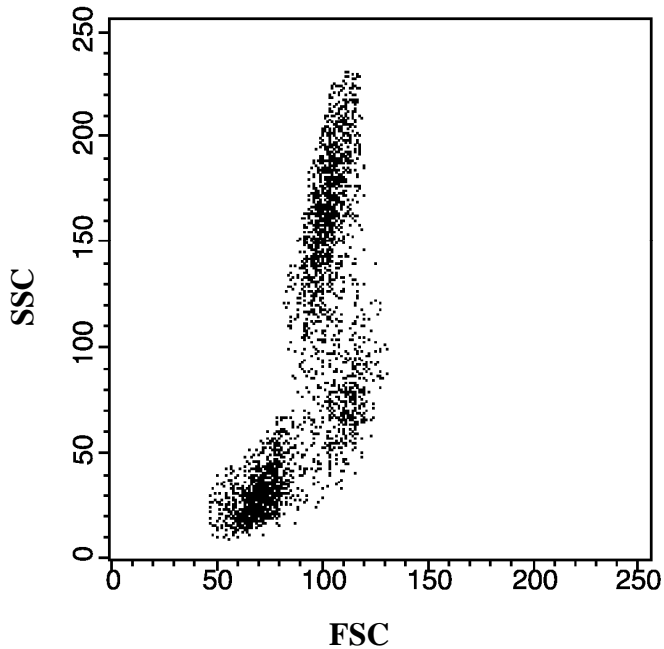















Figure Legend: Peripheral blood mononuclear cells stained with FITC-conjugated mouse anti-human myeloperoxidase (MPO). Representative forward (FSC) and side (SSC) scatter patterns and reaction patterns are shown.

REFERENCES:

1. Knapp, W., O. Majdic, and H. Strobl. 1993. Flow cytometric analysis of intracellular myeloperoxidase and lactoferrin in leukemia diagnosis. *Recent Results Cancer Res.* 131: 31-40.
2. Strobl, H., M. Takimoto, O. Majdic, G. Fritsch, C. Scheinecker, Höcker and W. Knapp. 1993. Myeloperoxidase expression in CD34+ normal human hemopoietic cells. *Blood* 82: 2069-2078.
3. Murao, S. I., F. J. Stevens, A. Ito, and E. Huberman. 1988. Myeloperoxidase: a myeloid cell nuclear antigen with DNA-binding properties. *Proc. Nat. Acad. Sci. USA* 85: 1232-1236.
4. Koefler, H. P., J. Ranyard, and M. Pertcheck. 1985. Myeloperoxidase: its structure and expression during myeloid differentiation. *Blood* 65: 484-491.
5. Campana, D., J. S. Thompson, P. Amiot, S. Brown, and G. Janossy. 1987. The cytoplasmic expression of CD3 antigens in normal and malignant cells of the T lymphoid lineage. *J. Immunol.* 138: 648-655.
6. Janossy, G, E. Coustan-Smith, and D. Campana. 1989. The reliability of cytoplasmic CD3 and CD22 antigen expression in the immunodiagnosis of acute leukemia: A study of 500 Cases. *Leukemia* 3: 170-181.

FIX & PERM reagent is produced for Invitrogen Laboratories by An Der Grub Bio Research GmbH, Austria.

Explanation of symbols

Symbol	Description	Symbol	Description
	Catalogue Number		Batch code
	Research Use Only		In vitro diagnostic medical device
	Use by		Temperature limitation
	Manufacturer		European Community authorised representative
	Without, does not contain		With, contains
	Protect from light		Consult accompanying documents
	Directs the user to consult instructions for use (IFU), accompanying the product.		

GENERAL PURPOSE REAGENT. FOR LABORATORY USE

www.invitrogen.com

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com