FIX & PERM™ Cell Permeabilization Reagents

Detection of intracellular antigens by flow cytometry

Catalog Numbers GAS003 and GAS004

Doc. Part No. L12001 **Pub. No.** MAN0026518 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

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.

Product description

FIX & PERM™ reagents allow analysis of intracellular antigen to proceed with the equivalent ease of 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.

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.

Contents and storage

Cat. No.	Fixation Medium (A)	Permeabilization Medium (B)	Storage
GAS003 (50 tests)	1 × 5 mL	1 × 5 mL	Room temperature. Do not freeze.
GAS004 (200 tests)	4 × 5 mL	4 × 5 mL	

Note: Fix & Perm reagents 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.

Permeabilize and stain cells

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, 12 × 75 mm tube.
2. Pipette appropriate volume of adjusted cells (equivalent to 1 x 10^6 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 of PBS with 0.1% NaN_3 and 5% FBS.
6. Centrifuge for 5 minutes at 300–350 × *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 of PBS with 0.1% NaN_3 and 5% FBS.
10. Centrifuge for 5 minutes at 300–350 × *g*, then 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. Analyze fixed cells within 24 hours.

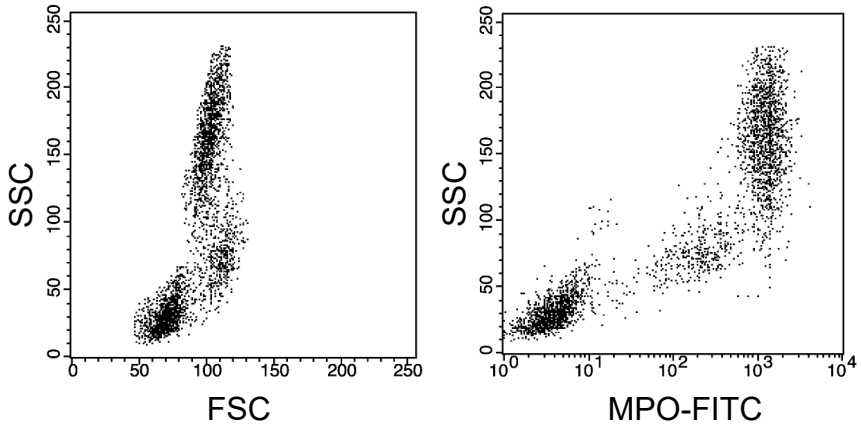
Permeabilize and stain cells (methanol supplemented protocol)

When analyzing certain cell cycle antigens such as BrdU, Ki-67, and PCNA with FITC-conjugated antibodies, a modification of the FIX & PERM™ protocol using precooled absolute methanol has been shown to give better results. The use of this modified protocol is not recommended when using PE-conjugated antibodies.

1. For each sample to be analyzed add 100 µL of adjusted cell volume (equivalent to 1 x 10^6 cells) to a 5 mL, 12 × 75 mm tube.
2. Add 100 µL of Reagent A (Fixation Medium) and incubate for 2–3 minutes at room temperature.
3. Add 4 mL of pre-cooled (0–4°C) absolute methanol and vortex.
4. Incubate for 10 minutes at 0–4°C.
5. Centrifuge for 5 minutes at 300–350 × *g*, then wash once in 3 mL of PBS with 0.1% NaN_3 and 5% FBS.
6. Add 100 µL of Reagent B (Permeabilization Medium) and the appropriate volume of intracellular antibody or corresponding isotype control.
7. Vortex 1–2 seconds at low speed and incubate for 30 minutes at room temperature.
8. Wash once in 3 mL of PBS with 0.1% NaN_3 and 5% FBS.
9. Centrifuge for 5 minutes at 300–350 × *g*, then aspirate the supernatant.
10. 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. Analyze fixed cells within 18 hours.

Analysis by flow cytometry

FIX & PERM™ reagents are designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to the manufacturer instructions. Typical staining and scatter patterns are shown in the following figure.



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

Shi W, Kasdan HL, Fridge A, Tai Y-C (2010) Four-part differential leukocyte count using μflow cytometer. Micro Electro Mechanical Systems (MEMS), 2010 IEEE 23rd International Conference 1019–1022.

O'Farrell A-M, Abrams TJ, Yuen HA, Ngai TJ, Louie SG, Yee KWH, Wong LM, Hong W, Lee LB, Town A, Smolich BD, Manning WC, Murray LJ, Heinrich MC, Cherrington JM (2003) SU11248 is a novel FLT3 tyrosine kinase inhibitor with potent activity in vitro and in vivo. *Blood* 101(9):3597–3605.

Little MA, Al-Ani B, Ren S, Al-Nuaimi H, Leite M Jr, Alpers CE, Savage CO, Duffield JS (2012) Anti-Proteinase 3 Anti- Neutrophil Cytoplasm Autoantibodies Recapitulate Systemic Vasculitis in Mice with a Humanized Immune System. *PLoS ONE* 7(1):e28626.

Young S-H, Wolfarth MG, Roberts JR, Kashon ML, Antonini JM (2013) Adjuvant effect of zymosan after pulmonary treatment in a mouse ovalbumin allergy model. *Exp Lung Res* 39(1):48–57.

Yassin LM, Londoño J, Montoya G, De Sanctis JB, Rojas M, Ramírez LA, García LF, Vásquez G (2011) Atherosclerosis development in SLE patients is not determined by monocytes ability to bind/endocytose Ox-LDL. *Autoimmunity* 44(3):201–210.

Antonini JM, Zeidler-Erdely PC, Young S-H, Roberts JR, Erdely A (2012) Systemic immune cell response in rats after pulmonary exposure to manganese-containing particles collected from welding aerosols. *J Immunotoxicol* 9(2):184–192.

Metzler B, Gfeller P, Bigaud M, Li J, Wieczorek G, Heusser C, Lake P, Katopodis A (2004) Combinations of Anti-LFA-1, Everolimus, Anti-CD40 Ligand, and Allogeneic Bone Marrow Induce Central Transplantation Tolerance through Hemopoietic Chimerism, Including Protection from Chronic Heart Allograft Rejection. *J Immunol* 173(11):7025–7036.

Baribaud F, Edwards TG, Sharron M, Brelot A, Heveker N, Price K, Mortari F, Alizon M, Tsang M, Doms RW (2001) Antigenically Distinct Conformations of CXCR4. *J Virol* 75(19):8957–8967.

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Revision history: Pub. No. MAN0026518

Revision	Date	Description
A.0	15 June 2022	New manual for FIX & PERM Cell Permeabilization Reagents skus GAS003 and GAS004.

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