### Overview

**Quantity:** 100 mL  
**Application:** Flow Cytometry (FACS)

### Product Details

**Brand:** FIX&PERM®  
**Specificity:** Biological fluids (blood, bone marrow, and others) must be collected under sterile conditions. Anticoagulation with EDTA or heparin is recommended. The samples should be stored at room temperature until used. For optimal results, samples should be processed and analyzed within 24 hours. Samples with high numbers of non-viable cells might cause false results, such cases require determination of cell viability with e.g. propidium iodide. All biological samples have to be handled with caution. Always consider them as potentially infective. Use appropriate precautions such as gloves, lab-coat, etc. The quality of each FIX&PERM® Lot is determined by fixation and permeabilization of well defined blood samples from representative donors and subsequent comparison of forward and side scatter characteristics of obtained leukocytes.

**Characteristics:** Intracellular staining of protein biomarkers using antibodies in flow cytometry has opened up a diversity of new options for phenotypic and functional characterisation of cells in both research and clinical diagnosis. Indeed, definitive phenotypic identification of certain cell types can require labelling of both cell surface and intracellular markers. Additionally, the cytoplasmic localisation of typical membrane molecules can prove to be a most reliable lineage marker, such as cytoplasmic CD3 and CD22 in undifferentiated leukaemia. In such cases, and for many other cell biological and functional investigations, intracellular immunostaining is a necessity. For this purpose we offer a class leading cell fixation and permeabilization kit - FIX&PERM® - which offers a number of advantages over other methods for intracellular staining of cells in flow cytometry.
Product Details

Mildly fixes cells, preserving their flow cytometric scatter characteristics
Allows simultaneous characterisation of both intracellular and cell surface markers
Rapid technique - whole procedure can be carried out in less than one hour, ready for immediate analysis or storage for 24 hours
Stringent QC procedures - the quality of each lot is determined using well-defined blood samples and subsequent comparison of scatter characteristics of obtained leukocyte populations, ensuring consistent and reliable results lot after lot
A range of intracellular antibodies with optimised protocols for use with FIX&PERM®

FIX&PERM® is a simple procedure making use of two reagents. Reagent A gently fixes cells, while Reagent B permeabilizes them. The specific formulations reduce background and allow simultaneous addition of permeabilization medium and fluorochrome labelled antibodies, allowing staining of intracellular structures such as cytoplasmic or nuclear enzymes, oncoproteins, cytokines, immunoglobulins, etc.

Components:
1 x 100 mL vial of Fixation Medium (Reagent A)

Application Details

Application Notes:
1. For each sample to be analyzed add 50 µL of whole blood, bone marrow or mononuclear cell suspension in a 5 mL tube
2. Add 100 µL of Reagent A (Fixation Medium, stored and used at room temperature)
3. Incubate for 15 minutes at room temperature
4. Add 5 mL phosphate buffered saline and centrifuge cells for 5 minutes at 300g
5. Remove supernatant and add to cell pellet 100 µL Reagent B (Permeabilization Medium) and 20 µL of the appropriate monoclonal antibody conjugate
6. Vortex at low speed for 1-2 seconds
7. Incubate for 15 minutes at room temperature
8. Wash cells with phosphate buffered saline as described above
9. Remove supernatant and resuspend cells in sheath fluid for immediate analysis or resuspend cells in 0.5 mL 1.0 % formaldehyde and store them at 2-8°C in the dark. Analyze fixed cells within 24 hours. Comments: Special cases (diluted bone marrow samples, other samples containing low soluble protein) might benefit from replenishment with plasma components before the FIX&PERM® treatment in order to create a milieu, which more closely resembles the situation in anti-coagulated blood. For that purpose addition of IgG preparations (e.g. Beriglobulin P, ZLB Behring, final concentration 10 mg/mL) and human serum albumin (e.g. human albumin \Behring\ 20 % - infusion solution, final concentration 40 mg/mL) is recommended.
Application Details

Comment: This FIX&PERM® Cell Permeabilization Kit contains 2 reagents: Fixation Medium (Reagent A) and Permeabilization Medium (Reagent B). It is intended for first fixing cells in suspension with Reagent A and then permeabilizing the cell membranes with Reagent B. This procedure gives antibodies access to intracellular structures and leaves the morphological scatter characteristics of cells intact. Specific formulations reduce background staining and allow simultaneous addition of permeabilization medium and fluorochrome labeled antibodies. FIX&PERM® is suitable for the analysis of normal and malignant leukocyte populations derived from various human biological samples (blood, bone marrow and others) using flow cytometry. Results must be put within the context of other diagnostic tests as well as the clinical history of the patient by a certified professional before final interpretation.

FIX&PERM® Reagents are designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to manufacturer’s instructions.

Assay Procedure:

Procedure: For each sample to be analysed use 50 µL of whole blood, bone marrow or mononuclear cell suspension in a 5 mL tube.
Add 100 µL of Reagent A (Fixation Medium, stored and used at room temperature)
Incubate for 15 minutes at room temperature.
Add 5 mL phosphate buffered saline and centrifuge cells for 5 minutes at 300 g.
Remove supernatant and add to cell pellet 100 µL Reagent B (Permeabilization Medium) and 20 µL of the appropriate monoclonal antibody conjugate.
Vortex at low speed for 1-2 seconds.
Incubate for 15 minutes at room temperature.
Wash cells with phosphate buffered saline as described above.
Remove supernatant and re-suspend cells in sheath fluid for immediate analysis or re-suspend cells in 0.5 mL 1.0 % formaldehyde and store at 2-8°C in the dark.
Analyse fixed cells within 24 hours.

Restrictions: For Research Use only.

Handling

Format: Liquid

Precaution of Use: Reagent A of FIX&PERM® Cell Permeabilization Kit contains formaldehyde and is labelled: Harmful. Formaldehyde is toxic, allergenic and a suspected carcinogen. Never pipette by mouth and avoid contact with eyes, skin and clothing. Proper handling procedures are recommended. As a main rule, persons under 18 years of age are not allowed to work with this product. Users
Handling

must be carefully instructed in the proper working procedure, the dangerous properties of the product and the necessary safety instructions. Please refer to the Material Safety Data Sheet (MSDS) for additional information. Dispose product remainders according to local regulations.

Storage: 4 °C

Storage Comment: FIX&PERM® Cell Permeabilization Kit reagents should be stored and used at room temperature. Do not freeze. Stability of the reagent: Please refer to the expiry date printed onto the vial. The use of the reagent after the expiration date is not recommended.

Publications

Product cited in:


There are more publications referencing this product on: Product page
Flow Cytometry

Image 1. Peripheral blood mononuclear cells stained with FITC-conjugated mouse anti–human myeloperoxidase (MPO). Representative forward scatter (FSC) and side scatter (SSC) patterns and reaction patterns are shown. FIX & PERM® cell fixation & permeabilization 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 fluorophore-labeled antibodies.

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Image 2. Two-day-old whole blood was lysed with ammonium chloride. Cells were first stained with CD13-APC conjugate. Cells were washed, stained with LIVE/DEAD® Fixable Near-IR dead cell stain, washed, and fixed with FIX & PERM® Reagent A. Cells were then washed, permeabilized with FIX & PERM® Reagent B, stained with MPO-FITC conjugate, and washed. Cells were analyzed on a BD™ LSRII flow cytometer. Gating on live cells (generally recommended, to eliminate dead cells) was performed. Further subgating is recommended in order to obtain the most accurate results.
**Flow Cytometry**

**Image 3.** Human peripheral blood from normal donors was stained using a combination of anti-human CD3 monoclonal antibody (clone: S4.1) for the cell surface antigens, and anti-human/mouse ZAP-70 monoclonal antibody (clone: 1E7.2), for intracellular detection. The cells were fixed and/or permeabilized using FIX & PERM® cell fixation & permeabilization reagents. The profiles represent lymphocyte gate.