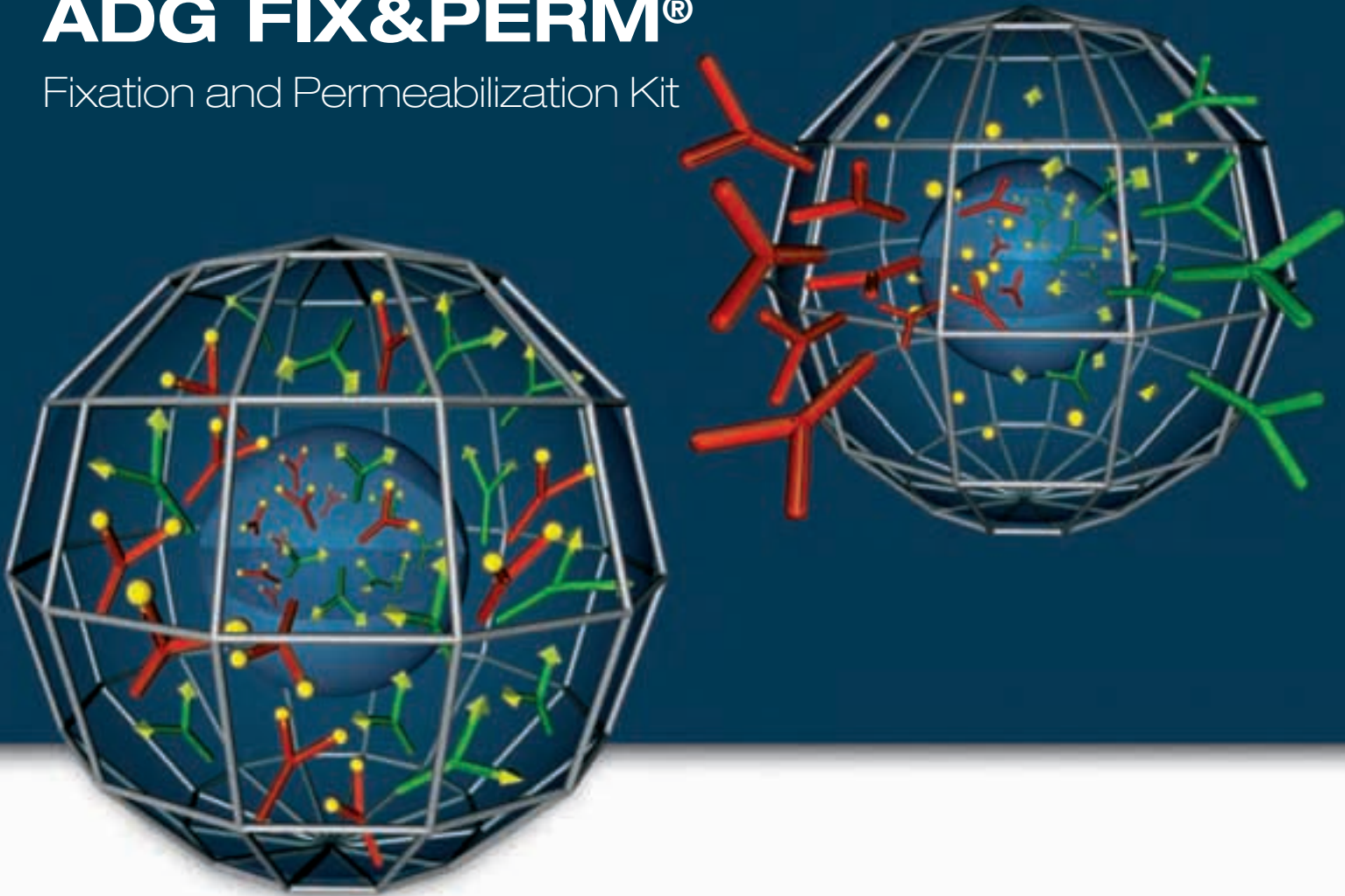


ADG FIX&PERM[®]

Fixation and Permeabilization Kit



- From AN DER GRUB, the pioneer in the field of intracellular flow cytometry.
- Worldwide recognized as superior reagent for intracellular flow cytometry.
- Well-suited for leukemia and lymphoma immunophenotyping.
- May be used with isolated cells or whole blood.
- For simultaneous detection of intracellular and surface antigens.
- Excellent scatter characteristics.
- Reliable and reproducible results.
- Rapid procedure.
- Easy to use and cost effective.

FIX&PERM® CELL PERMEABILIZATION KIT

For suspension stainings and flow cytometric analyses of intracellular antigens

Intended use

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.

Flow Cytometric Analysis

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

Permeabilization and Staining Procedure

- For each sample to be analyzed add 50 µl of whole blood, bone marrow or mononuclear cell suspension in a 5ml tube
- Add 100 µl of Reagent A (Fixation Medium, stored and used at room temperature)
- Incubate for 15 minutes at room temperature
- Add 5ml 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 ADG 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 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.

Samples

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.

Sensitivity

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.

Limitations of the technique

Flow cytometry should be performed by professional users only. Improper alignment of the flow cytometer, inaccurate compensation of fluorescence leaking into other channels as well as incorrect positioning of regions may lead to false results. Lysis of red cells might be impossible for various reasons. In such instances it is recommended to isolate mononuclear cells (MNC) via density gradient centrifugation prior to staining. Results will be

correct and reproducible as long as the procedures used respect the technical recommendations and obey good laboratory practice.

The FIX&PERM® solutions are provided in a concentration that will allow to fix and permeabilize human hematopoietic cells. It is therefore strongly recommended to stick to the working protocol in terms of concentration and volume regarding cells and antibody. The properties of FIX&PERM® have been determined using EDTA anticoagulated peripheral blood.

Precautions

For professional users only.

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 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.

Storage

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. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. Do not use reagents if a precipitate should form or discoloration occurs. If unexpected results are obtained which cannot be attributed to differences in laboratory procedures, please contact us.



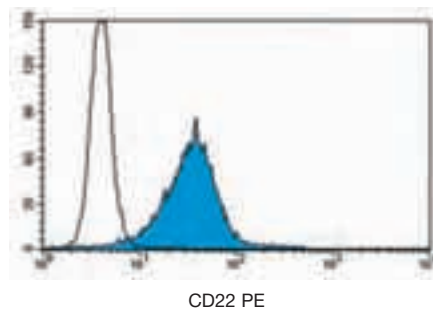
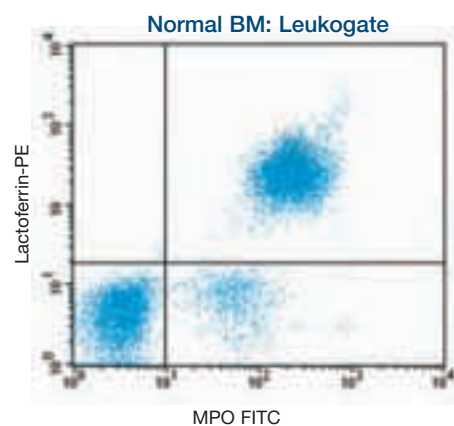
Clinical Research Applications

Flow cytometric analyses with monoclonal antibodies were so far mainly restricted to cell surface molecules. Intracellular structures such as cytoplasmic or nuclear enzymes, oncoproteins, cytokines, immunoglobulins etc. were largely excluded from such studies. Also excluded from flow cytometric studies were cytoplasmic localizations of

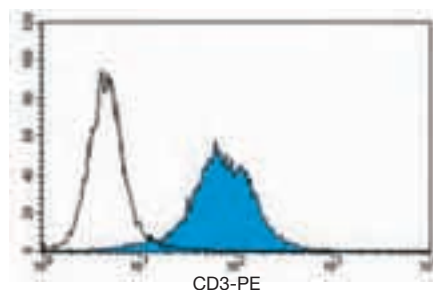
well established membrane molecules like CD3 and CD22, which, in their cytoplasmic form, are the most reliable lineage markers in undifferentiated leukemia. With the FIX&PERM® Kit flow cytometric analysis of intracellular antigens has become as easy as surface antigen studies. The only prerequisite is the availability of suitable antibody conju-

gates. Most of the available monoclonal antibody conjugates can be used with the FIX&PERM® Kit, some determinants are sensitive, however, to the fixation step involved. This and the optimal fixation time have to be tested for each reagent. Some staining examples with ADG antibody conjugates are shown below.

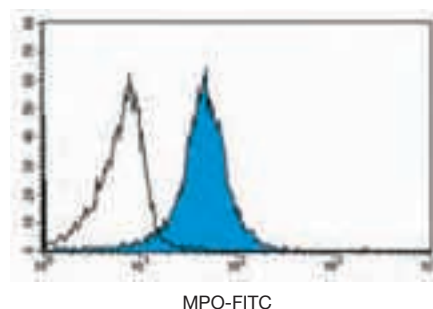
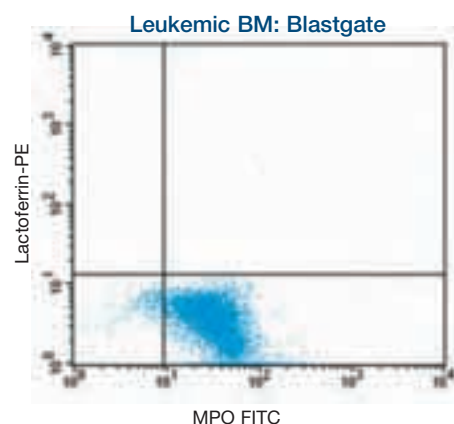
Please find below some staining examples with ADG antibody conjugates:



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



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



Cytoplasmic staining with ADG anti MPO-FITC conjugate of undifferentiated leukemia cells of AML type.

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FIX&PERM® Cell Permeabilization Reagents

| | Quantity | No. of Tests | Cat. No. |
|-----------------------------|------------|--------------|------------|
| FIX&PERM® Kit | 2 x 20 ml | 200 Tests | GAS-002 |
| FIX&PERM® Kit-1000 | 2 x 100 ml | 1.000 Tests | GAS-002-1 |
| FIX&PERM® Sample Kit | 2 x 5 ml | 50 Tests | GAS-002M |
| FIX&PERM® Solution A (Fix) | 100 ml | 1.000 Tests | GAS-002A-1 |
| FIX&PERM® Solution B (Perm) | 100 ml | 1.000 Tests | GAS-002B-1 |

AN DER GRUBs monoclonal Antibodies optimized for use with FIX&PERM® Cell Permeabilization Kit:

Single IC Antibodies/Conjugates

Single Antibodies/Conjugates for intracellular antigen detection

| Specificity | Host | Clone | Isotype | Format | Quantity | Cat. No. |
|--------------------------|-------|-------|---------|----------|-----------|----------|
| CD3 | Mouse | UCHT1 | IgG1 | purified | 0.2 mg | GM-4011 |
| | | | | FITC | 100 Tests | GM-4012 |
| | | | | PE | 100 Tests | GM-4013 |
| CD22 | Mouse | RFB4 | IgG1 | purified | 0.2 mg | GM-4051 |
| | | | | FITC | 100 Tests | GM-4052 |
| | | | | PE | 100 Tests | GM-4053 |
| Lactoferrin | Mouse | 4C5 | IgG1 | purified | 0.2 mg | GM-4111 |
| | | | | PE | 100 Tests | GM-4113 |
| Myeloperoxidase (MPO-C2) | Mouse | 8E6 | IgG1 | purified | 0.2 mg | GM-4191 |
| | | | | FITC | 100 Tests | GM-4192 |
| | | | | PE | 100 Tests | GM-4193 |
| Lysozyme | Mouse | LZ-2 | IgG1 | purified | 0.2 mg | GM-4131 |
| | | | | FITC | 100 Tests | GM-4132 |
| CD68 | Mouse | Ki-M7 | IgG1 | FITC | 100 Tests | GM-4152 |
| IL-1 beta | Mouse | FIB3 | IgG1 | FITC | 100 Tests | GM-4162 |
| IFN-gamma | Mouse | GZ4 | IgG1 | FITC | 100 Tests | GM-4172 |

Combi IC Antibodies/Conjugates

Combi Conjugated for intracellular antigen detection

| Specificity | Host | Clone | Isotype | Format | Quantity | Cat. No. |
|--------------------------|-------|-------|---------|--------|----------|----------|
| Negative Control | Mouse | | IgG1 | FITC | 50 Tests | GIC-201 |
| | Mouse | | IgG1 | PE | | |
| Lysozyme | Mouse | LZ-2 | IgG1 | FITC | 50 Tests | GIC-206 |
| Lactoferrin | Mouse | 4C5 | IgG1 | PE | | |
| Myeloperoxidase (MPO-C2) | Mouse | 8E6 | IgG1 | FITC | 50 Tests | GIC-212 |
| Lactoferrin | Mouse | 4C5 | IgG1 | PE | | |
| Myeloperoxidase (MPO-C2) | Mouse | 8E6 | IgG1 | FITC | 50 Tests | GIC-213 |
| CD3 | Mouse | UCHT1 | IgG1 | PE | | |
| Myeloperoxidase (MPO-C2) | Mouse | 8E6 | IgG1 | FITC | 50 Tests | GIC-214 |
| CD22 | Mouse | RFB4 | IgG1 | PE | | |

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