Instructions for Use
FIX&PERM®

For suspension stainings and flow cytometric analyses of surface membrane and intracellular antigens.

REF **GAS-002-1-CE/IVD** FIX&PERM® Kit 1000 Cell Fixation and Permeabilization kit

Solution A (Fixation Medium)	1 x 100 ml	1000 Tests
Solution B (Permeabilization Medium)	1 x 100 ml	1000 Tests

REF **GAS-002-CE/IVD** FIX&PERM® Cell Fixation and Permeabilization kit

Solution A (Fixation Medium)	4 x 5 ml	200 Tests
Solution B (Permeabilization Medium)	4 x 5 ml	200 Tests

REF **GAS-002M-CE/IVD** FIX&PERM® Sample Kit Cell Fixation and Permeabilization kit

Solution A (Fixation Medium)	1 x 5 ml	50 Tests
Solution B (Permeabilization Medium)	1 x 5 ml	50 Tests

REF **GAS-002A-1-CE/IVD** FIX&PERM® Solution A (Fix) Cell Fixation and Permeabilization kit

Solution A (Fixation Medium)	1 x 100 ml	1000 Tests

REF **GAS-002B-1-CE/IVD** FIX&PERM® Solution B (Perm) Cell Fixation and Permeabilization kit

Solution B (Permeabilization Medium)	1 x 100 ml	1000 Tests

IVD **In vitro diagnostic medical device**

Nordic Immunological Laboratories BV, Nordic-MUbio, Rangeerweg 5A, 6114 BC Susteren, The Netherlands

Intended Purpose

The device's intended purpose is to prepare cell suspension samples for flow cytometric analysis. Such analyses combined with (monoclonal) antibodies were long 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. By using FIX&PERM® flow cytometric analysis of intracellular (cytoplasmic and nuclear) antigens has become as easy as surface antigen studies. The FIX&PERM® solutions can be applied in both an automated as well as a non-automated setting to study peripheral blood cell samples, bone marrow aspirates, mononuclear cell suspensions or cell suspensions prepared from solid tissue and *in vitro* cultured cells.

This product is intended to be used for professional *in vitro* diagnostic use only.

Principle

FIX&PERM® contains 1 or 2 solutions: Fixation Medium (Solution A) and/or Permeabilization Medium (Solution B). It is intended for (first) fixing cells in suspension with Solution A and (then) permeabilizing the cell membranes with Solution B. This procedure does not only allow the immunostaining of cell surface antigens, but also gives antibodies access to intracellular structures and leaves the morphological scatter characteristics of cells intact. The specific formulation reduces background staining and allows simultaneous addition of the permeabilization medium and fluorochrome labelled antibodies. FIX&PERM® is suitable for the analysis of normal and malignant leukocyte populations derived from various human biological samples (peripheral blood cell samples, bone marrow aspirates, mononuclear cell suspensions or cell suspensions prepared from solid tissue and *in vitro* cultured cells) using flow cytometry. FIX&PERM® solutions are designed for use with all commercially available flow cytometers.

CONTENTS **Materials provided**

REF **GAS-002-1-CE/IVD** FIX&PERM® Kit 1000 Cell Fixation and Permeabilization kit

Solution A — Solution A (Fixation Medium) composition proprietary, contains 4-10% formaldehyde in phosphate buffered saline.
1 x 100 ml 1000 Tests

Solution B — Solution B (Permeabilization Medium) composition proprietary, contains 0.05% sodium azide.
1 x 100 ml 1000 Tests

REF **GAS-002-CE/IVD** FIX&PERM® Cell Fixation and Permeabilization kit

Solution A — Solution A (Fixation Medium) composition proprietary, contains 4-10% formaldehyde in phosphate buffered saline.

4 x 5 ml ml 200 Tests

Solution B — Solution B (Permeabilization Medium) composition proprietary, contains 0.05% sodium azide.

4 x 5 ml ml 200 Tests

REF **GAS-002M-CE/IVD** FIX&PERM® Sample Kit Cell Fixation and Permeabilization kit

Solution A — Solution A (Fixation Medium) composition proprietary, contains 4-10% formaldehyde in phosphate buffered saline.

1 x 5 ml 50 Tests

Solution B — Solution B (Permeabilization Medium) composition proprietary, contains 0.05% sodium azide.

1 x 5 ml 50 Tests

REF **GAS-002A-1-CE/IVD** FIX&PERM® Solution A (Fix) Cell Fixation and Permeabilization kit

Solution A — Solution A (Fixation Medium) composition proprietary, contains 4-10% formaldehyde in phosphate buffered saline.

1 x 100 ml 1000 Tests

REF **GAS-002B-1-CE/IVD** FIX&PERM® Solution B (Perm) Cell Fixation and Permeabilization kit

Solution B — Solution B (Permeabilization Medium) composition proprietary, contains 0.05% sodium azide.

1 x 100 ml 1000 Tests

Materials required but not provided

Use appropriate safety precautions such as wearing a lab-coat, gloves, safety goggles etc.
5 ml glass or plastic tubes
Pipettes, Vortex and Centrifuge
Flow cytometer and sheath fluid
Appropriately (fluorochrome) conjugated antibodies
Phosphate buffered saline (PBS)
1% Formaldehyde solution (optional)

Specimen Collection, Storage and Handling

Biological cell samples (peripheral blood cell samples, bone marrow aspirates, mononuclear cell suspensions or cell suspensions prepared from solid tissue and *in vitro* cultured cells) 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 in a separate sample with e.g. propidium iodide without fixation and permeabilization with FIX&PERM®. All biological samples have to be handled with caution. Always consider them as potentially infective. Use appropriate precautions such as gloves, lab coat, etc.

Fixation, Permeabilization and Staining Procedure

The FIX&PERM® solutions are ready to use.

- For each sample to be analysed add 50 µl of whole blood, bone marrow aspirate, mononuclear cell suspension, or cell suspensions prepared from solid tissue and *in vitro* cultured cells in a 5ml tube
- Add 100 µl of Solution 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 300xg
- Remove supernatant and add to cell pellet 100 µl Solution B (Permeabilization Medium) and 20 µl of the appropriate 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. Analyse 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 10mg/ml) and human serum albumin (e.g. human albumin “Behring” 20% - infusion solution, final concentration 40mg/ml) is recommended.

Perfomance Characteristics

FIX&PERM® has been shown in a vast number of publications (see selected references below) to allow successful immunostaining of cell surface markers and intracellular antigens in different types of cells derived from peripheral blood or bone marrow and at the same time leave the scatter characteristics of these cell types intact. As a result the different cell types and

their maturation stages can be quantified by flow cytometric techniques in normal and (pre)malignant blood and bone marrow aspirates. For illustrations of representative performance characteristics see examples below. The performance 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, as well as immunostaining efficiency for several membranous and cytoplasmic antigens. Deviations for the 7 parameters determined between subsequent lots are all less than 10%.

Limitations of the technique

With the FIX&PERM® flow cytometric analysis of intracellular antigens has become as easy as surface antigen studies. The only prerequisite is the availability of suitable antibody conjugates. Most of the available (monoclonal) antibody conjugates can be used with FIX&PERM®, some determinants are sensitive, however, to the fixation step involved. This and the optimal fixation time have to be tested for each solution. Some staining examples with antibody conjugates are shown below.

FIX&PERM® solutions are designed for use with all commercially available flow cytometers. Alignment and compensation should be performed according to manufacturer´s instructions. 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 anti-coagulated peripheral blood and bone marrow aspirates.

Warnings and Precautions

For professional users only.

Solution A of FIX&PERM® 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 Safety Data Sheet (SDS) for additional information. Dispose product remainders according to local regulations.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the EU Member State in which the user and/or the patient is established.

Only for Solution A: hazardous substance content 4-10% Formaldehyde

Danger

H341: Suspected of causing genetic defects (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)

H350: May cause cancer (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)

H317: May cause an allergic skin reaction

P201: Obtain special instructions before use.

P280: Wear protective gloves/protective clothing/eye protection/face protection.

P308+P313: IF exposed or concerned: Get medical advice/attention.

P333+P313: If skin irritation or rash occurs: Get medical advice/attention.

P362+P364: Take off contaminated clothing and wash it before reuse.

Storage

FIX&PERM® solutions should be stored and used at room temperature (18-24°C). Do not freeze. Stability of the solution: Please refer to the expiry date printed onto the vial. The use of the solution after the expiration date is not recommended. If solutions are stored under any conditions other than those specified, the conditions must be verified by the user. Do not use the solutions if a precipitate should form or discoloration occurs.

Storage conditions after opening of the vials are the same as for unopened vials.

If unexpected results are obtained which cannot be attributed to differences in laboratory procedures, please contact us.

Warranty

The products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, that extend beyond the description on the label of the product. Nordic-MUbio's sole liability is limited to either replacement of the products or refund of the purchase price. Nordic-MUbio is not liable for property damage, personal injury, or economic loss caused by the product.

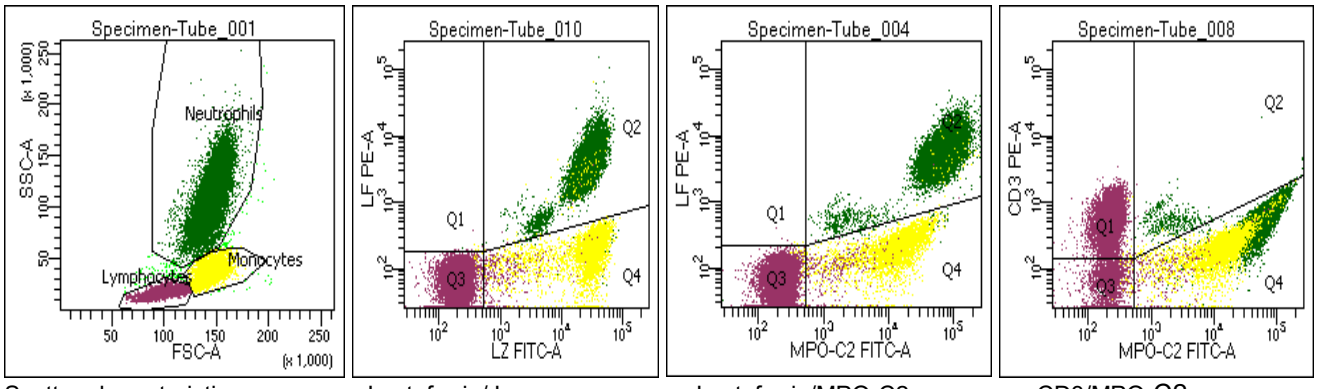
Selected References

- Groeneveld, K, te Marvelde, JG, van den Beemd, MW, Hooijkaas, H, van Dongen, JJ (1996) *Leukemia* **10,** 1383-9.
- Haranaga, S., Yamaguchi, H., Friedman, H., Izumi, S., & Yamamoto, Y. (2001) *Infect Immun* **69**, 7753-9.
- Hegazy, A. N. & Klein, C. (2008) *Leukemia* **22**, 2070-9.

- Kappelmayer, J., Gratama, J. W., Karaszi, E., Menendez, P., Ciudad, J., Rivas, R. & Orfao, A. (2000) *J Immunol Methods* **242,** 53-65.
- Kline, MP, Rajkumar, SV, Timm, MM, Kimlinger, TK, Haug, JL, Lust, JA, Greipp, PR, Kumar,S (2007) *Leukemia* **21**, 1549-60
- Knapp, W., Majdic, O. & Strobl, H. (1993) *Recent Results Cancer Res* **131,** 31-40.
- Knapp, W., Strobl, H. & Majdic, O. (1994) *Cytometry* **18,** 187-98.
- Knapp, W., Strobl, H., Scheinecker, C., Bello-Fernandez, C. & Majdic, O. (1995) *Ann Hematol* **70,** 281-96.
- Konikova, E., Glasova, M., Kusenda, J. & Babusikova, O. (1998) *Neoplasma* **45,** 282-91.
- Lanza, F., Latorraca, A., Moretti, S., Castagnari, B., Ferrari, L. & Castoldi, G. (1997) *Cytometry* **30,** 134-44.
- Millard, I., Degrave, E., Philippe, M. & Gala, J. L. (1998) *Clin Chem* **44,** 2320-30.
- Mestrum S.G.C., R.B.Y. Vanblarcum, R.J.M. Drent, B.T. Boonen, W.L.W. van Hemert, F.C.S. Ramaekers, A.H.N. Hopman, M.P.G. Leers. Cytometry A (2022) in press.
- Mestrum SGC, de Wit NCJ, Drent RJM, Hopman AHN, Ramaekers FCS, Leers MPG. Cytometry B Clin Cytom. 2021;100(3):322-330.
- Mestrum SGC, Cremers EMP, de Wit NCJ, Drent RJM, Ramaekers FCS, Hopman AHN, Leers MPG. Leuk Res. 2022;113:106789.
- Mestrum SGC, Cremers EMP, de Wit NCJ, Drent RJM, Ramaekers FCS, Hopman AHN, Leers MPG. Data Brief. 2022 Feb 22;41:107976
- Nakase, K., Sartor, M. & Bradstock (1998) *Cytometry* **34,** 198-202.
- Nies KPH, Kraaijvanger R, Lindelauf KHK, Drent RJMR, Rutten RMJ, Ramaekers FCS, Leers MPG. Cytometry A. (2018) 93, 1097-1105.
- Pascale, F., Contreras, V., Bonneau, M., Courbet, A., Chilmonczyk, S., Bevilacqua, C., Epardaud, M., Niborski, V., Riffault, S., Balazuc, A. M., Foulon, E., Guzylack-Piriou, L., Riteau, B., Hope, J., Bertho, N., Charley, B. & Schwartz-Cornil, I. (2008) *J Immunol* **180**, 5963-72
- Pickl, W. F., Majdic, O., Kohl, P., Stockl, J., Riedl, E., Scheinecker, C., Bello-Fernandez, C. & Knapp, W. (1996) *J Immunol* **157,** 3850-9.
- Riera-Sans, L., & Behrens, A. (2007) *J Immunol* **178**, 5690-700
- Roberts, J. L., Lengi, A., Brown, S. M., Chen, M., Zhou, Y. J., O'Shea, J. J. & Buckley, R. H. (2004) *Blood* **103**, 2009-18
- Sargent, R. L., Craig, F. E. & Swerdlow, S. H. (2009) *Int J Clin Exp Pathol* **2**, 574-82
- Scheinecker, C., Strobl, H., Fritsch, G., Csmarits, B., Krieger, O., Majdic, O. & Knapp, W. (1995) *Blood* **86,** 4115-23.
- Sedlmayr, P., Grosshaupt, B. & Muntean, W. (1996) *Cytometry* **23,** 284-9.
- Strobl, H. & Knapp, W. (2004) *J Biol Regul Homeost Agents* **18,** 335-9.
- Strobl, H., Scheinecker, C., Csmarits, B., Majdic, O. & Knapp, W. (1995) *Br J Haematol* **90,** 774-82.
- Strobl, H., Scheinecker, C., Riedl, E., Csmarits, B., Bello-Fernandez, C., Pickl, W. F., Majdic, O. & Knapp, W. (1998) *J Immunol* **161,** 740-8.
- Strobl, H., Takimoto, M., Majdic, O., Fritsch, G., Scheinecker, C., Hocker, P. & Knapp, W. (1993) *Blood* **82,** 2069-78.
- Wang, X., Chang, X., Facchinetti, V., Zhuang, Y. & Su, B. (2009) *J Immunol* **182**, 3597-608

Representative Examples

Tested sample: whole blood (EDTA), treated with the FIX&PERM® Cell Fixation and Permeabilization Kit and immunostained for surface marker CD3, and cytoplasmic antigens Lactoferrin (LF), Lysozyme (LZ) and Myeloperoxidase (MPO-C2).

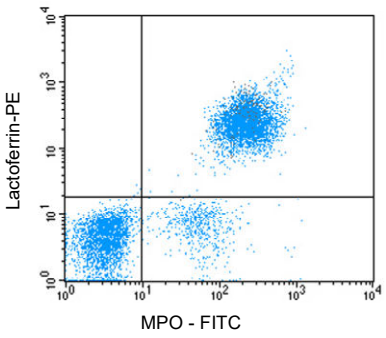


Scatter characteristics | Lactoferrin/ Lysozyme | Lactoferrin/MPO-C2 | CD3/MPO-C2

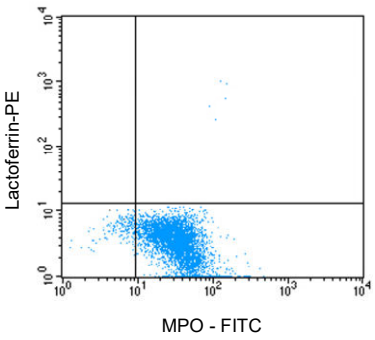
Result: Good quality intracellular and membranous staining patterns with both PE- and FITC-conjugated monoclonal antibodies. Scatter characteristics allow a good separation of leukocyte subpopulations.

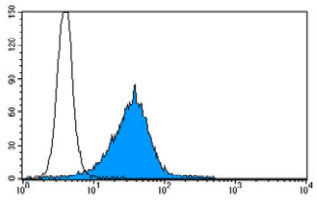
Tested samples: bone marrow aspirates from a non-malignant case (normal BM), and from several leukemia patients (leukemic BM, B-ALL, T-ALL and AML) treated with the FIX&PERM® Cell Fixation and Permeabilization Kit and immunostained for surface markers CD3 and CD22, and cytoplasmic antigens Lactoferrin and Myeloperoxidase (MPO).

Normal bone marrow: Leukogate

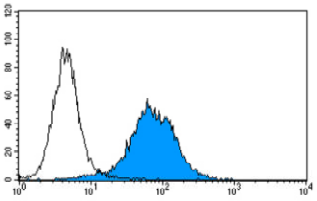


Leukemic bone marrow: Blastgate

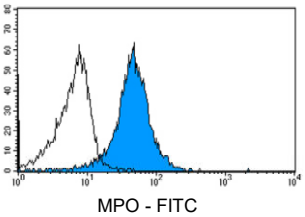




Cytoplasmic staining with CD22-PE conjugate of undifferentiated leukemia cells of B-ALL type.



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



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

Result: Good quality intracellular and membranous staining patterns with both PE- and FITC-conjugated monoclonal antibodies allowing the differential diagnosis of the different types and stages of leukemia.

Date of Issue
Version 2: May 22, 2022
Introduced modifications: This version has been amended to be IVD-R compliant