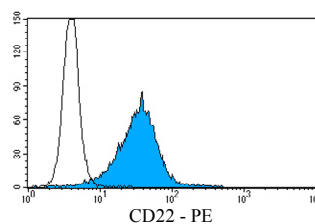
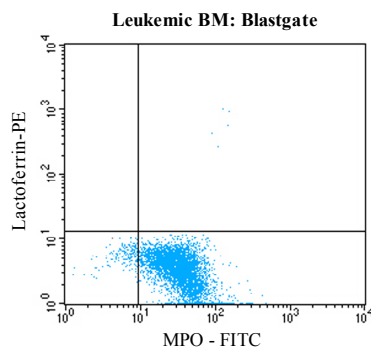
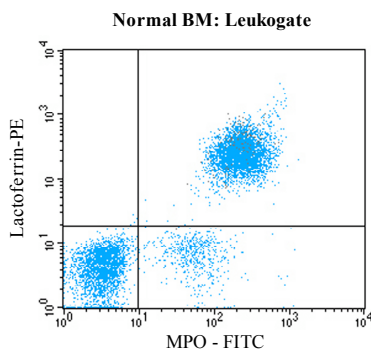


CELL PERMEABILIZATION KIT

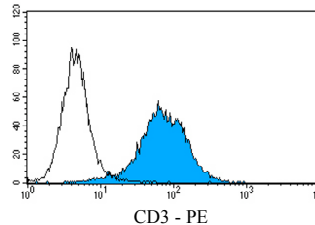
For suspension stainings and flow cytometric analyses of intracellular antigens.

Reagents provided:

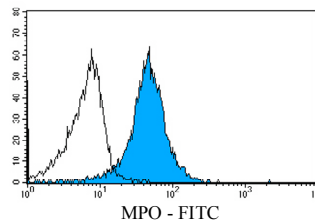
Reagent A (Fixation Medium)	Cat. No.: GAS-002	4 x 5 ml	200 Tests
Reagent B (Permeabilization Medium)	Cat. No.: GAS-002	4 x 5 ml	200 Tests



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 un-differentiated leukemia cells of AML type

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.

Introduction (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 conjugates. 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 above.

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.

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.

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.

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.

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 anti-coagulated peripheral blood.

Precautions

For professional users only.

Reagent A of FIX&PERM® Cell Permeabilization Kit contains formaldehyde. Formaldehyde is toxic, allergenic and a suspected carcinogen. Avoid contact with eyes, skin and clothing. Proper handling procedures are recommended.

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 condition 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.

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. ADG's sole liability is limited to either replacement of the products or refund of the purchase price. ADG is not liable for property damage, personal injury, or economic loss caused by the product.

Selected References

- Gerna, G., Percivalle, E., Lilleri, D., Lozza, L., Fornara, C., Hahn, G., Baldanti, F. & Revello, M. G. (2005) *J Gen Virol* **86**, 275-84.
- Groeneveld, K., te Marvelde, J. G., van den Beemd, M. W., Hooijkaas, H. & van Dongen, J. J. (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, M. P., Rajkumar, S. V., Timm, M. M., Kimlinger, T. K., Haug, J. L., Lust, J. A., Greipp, P. R. & 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., Degraeve, E., Philippe, M. & Gala, J. L. (1998) *Clin Chem* **44**, 2320-30.
- Nakase, K., Sartor, M. & Bradstock (1998) *Cytometry* **34**, 198-202.
- Pascale, F., Contreras, V., Bonneau, M., Courbet, A., Chilmonecnyk, 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

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