IOTest®

MPO-FITC

REF IM1874
100 tests; 2 mL
20 µL / test

BECKMAN COULTER

IOTest
Conjugated Antibody

IVD CE

ENGLISH	Specifications
Specificity	MPO
Clone	CLB-MPO-1
Hybridoma	SP 2/0 x CAF
Immunogen	Purified myeloperoxidase
Immunoglobulin	IgG1
Species	Mouse
Source	Ascites
Purification	Protein A affinity chromatography
Fluorochrome	Fluorescein isothiocyanate (FITC)
λ excitation	488 nm
Emission peak	525 nm
Buffer	PBS pH 7.2 plus 2 mg / mL BSA and 0.1% NaN_3

USE

This fluorochrome-conjugated antibody permits the identification and numeration of cell populations expressing the myeloperoxidase (MPO) antigen present in human biological samples using flow cytometry.

PRINCIPLE

This test is based on the ability of specific monoclonal antibodies to bind to the antigenic determinants expressed by leucocytes on the internal surface of the plasma membrane, in the cytoplasm or in the nucleus.
Following a membrane fixation/permeabilization step, internal staining of the leucocytes is performed by incubating the sample with the IOTest reagent. The leucocytes are then analyzed by flow cytometry.
The flow cytometer measures light diffusion and the fluorescence of cells. It makes possible the delimitation of the population of interest within the electronic window defined on a histogram, which correlates the orthogonal diffusion of light (Side Scatter or SS) and the diffusion of narrow-angle light (Forward Scatter or FS). Other histograms combining two of the different parameters available on the cytometer can be used as supports in the gating stage depending on the application chosen by the user.
The fluorescence of the delimited cells is analyzed in order to distinguish the positively-stained events from the unstained ones. The results are expressed as a percentage of positive events in relation to all the events acquired by the gating.

EXAMPLES OF CLINICAL APPLICATIONS

Analysis of the intracellular expression of MPO by flow cytometry is useful for the identification of hematopoietic neoplasia.
The anti-myeloperoxidase is one of the most specific marker for the characterization of the myeloid origin of acute leukemias (1 – 4).

STORAGE AND STABILITY

The conjugated liquid forms must be kept at between 2 and 8°C and protected from light, before and after the vial has been opened.
Stability of closed vial: see expiry date on vial.
Stability of open vial: the reagent is stable for 90 days.

PRECAUTIONS

1. Do not use the reagent beyond the expiry date.
2. Do not freeze.
3. Let it come to room temperature (18 – 25°C) before use.
4. Minimize exposure to light.
5. Avoid microbial contamination of the reagents, or false results may occur.
6. Antibody solutions containing sodium azide (NaN_3) should be handled with care. Do not ingest and avoid contact with the skin, mucosa and eyes.
   Moreover, in an acid medium, sodium azide can form the potentially dangerous hydrazoic acid. If it needs to be disposed of, it is recommended that the reagent be diluted in a large volume of water before pouring it into the drainage system so as to avoid the accumulation of sodium azide in metal pipes and to prevent the risk of explosion.
7. All blood samples must be considered as potentially infectious and must be handled with care (in particular: the wearing of protective gloves, gowns and goggles).
8. Never pipette by mouth and avoid all contact of the samples with the skin, mucosa and eyes.
9. Blood tubes and disposable material used for handling should be disposed of in ad hoc containers intended for incineration.

SAMPLES

Venous blood or bone marrow samples must be taken using sterile tubes containing an EDTA salt as the anticoagulant. The use of other anticoagulants is not recommended.
The samples should be kept at room temperature (18 – 25°C) and not shaken. The samples should be homogenized by gentle agitation prior to taking the test sample.
The samples must be analyzed within 24 hours of venipuncture.

METHODOLOGY

NECESSARY MATERIAL NOT SUPPLIED

- Sampling tubes and material necessary for sampling.
- Automatic pipettes with disposable tips for 20, 100 and 500 µL.
- Plastic haemolysis tubes.
- Calibration beads: Flow-Set™ Fluorospheres (Ref. 6607007).
- To obtain optimal results, the following reagents are recommended:
  - IntraPrep™ Fixation/Permeabilization reagent (Ref. A07802 or A07803).
  - Leucocyte fixation reagent. For example: IOTest 3 Fixative Solution (Ref. A07800).
- Isotypic control: IOTest reagent. IgG1-FITC (Ref. A07795).
- Buffer (PBS: 0.01 M sodium phosphate; 0.145 M sodium chloride; pH 7.2).
- Centrifuge.
- Automatic agitator (Vortex type).
- Flow cytometer.

PROCEDURE

For the following procedure, the use of the IntraPrep Fixation/Permeabilization reagent (Ref. A07802 or A07803) is recommended.
For each sample analyzed, in addition to the test tube, one control tube is required in which the cells are mixed with the isotypic control IgG1-FITC (Ref. A07795).
For a blood sample, optimal staining is obtained using a number of leucocytes between 3 and 10 x 10^3 cells / µL. If the leucocyte concentration is greater than 10 x 10^3 cells / µL, dilute. In the case of a cell suspension, optimal staining is obtained with 5 x 10^3 cells / µL; dilute if necessary.

1. Add 50 µL of blood sampled into EDTA or 5 x 10^5 cells to 2 tubes (one test tube, one control tube).
2. Add to each tube 100 µL of IntraPrep reagent 1 (Fixation).
3. Vortex the tubes vigorously for 3 to 5 seconds.
4. Incubate for 15 minutes at room temperature (18 – 25°C) protected from light.
5. Add 4 mL of PBS to each tube.
6. Centrifuge for 5 minutes at 300 x g at room temperature. Remove the supernatant by aspiration.
7. Add to each tube 100 µL of IntraPrep reagent 2 (Permeabilization). Let mix by diffusion. DO NOT VORTEX.
8. Incubate for 5 minutes at room temperature WITHOUT SHAKING.
9. Shake the tubes carefully and manually for 2 to 3 seconds.
10. Add 20 µL of the specific IOTest conjugated antibody to each test tube, and 20 µL of the isotypic control to the control tube. Vortex the tubes gently
11. Incubate for 15 to 20 minutes at room temperature protected from light.
12. Add 4 mL of PBS and centrifuge at 300 x g for 5 minutes at room temperature.
13. Remove the supernatant by aspiration and resuspend the cell pellet in 0.5 to 1 mL of IOTest 3 Fixative Solution (Ref. A07800) at its working concentration (1X).
14. The preparations are ready for cytometric analysis.

NOTE: If the preparations are to be stored for more than 2 hours prior to cytometric analysis, it is advisable to store them at 2-8°C and protected from light. The preparations thus stored do not keep, however, for more than 24 hours.

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PERFORMANCE

SPECIFICITY

Myeloperoxydase (MPO) is an intracellular enzymatic protein expressed at an early stage in the differentiation of the myeloid cell line.
The two subunits (α and β) have a molecular weight of 55 and 15 kDa, respectively (6, 7).
MPO is synthesized primarily during the formation of promyelocytes, the stage during which azurophile granules, or primary granules, are formed (7 – 9). MPO is a major constituent of the azurophile granules of polynuclear neutrophils and macrophages, but is expressed neither by lymphocytes nor by erythrocytes (7, 9, 10).
The monoclonal antibody CLB-MPO-1 recognizes the MPO as well as the MPO precursor (proMPO; the inactive form of the enzyme).

LINEARITY

To test the linearity of staining of this reagent, a sample containing positive cells (permeabilized granulocytes from normal whole blood) and a negative cell line (FRN17.4.14.33) were mixed in different proportions with a constant final number of cells, so that the positive cells / negative cell line ratio of the mixture ranged from 0 to 100%.
Aliquots were stained using the procedure described above and linear regression between the expected values and the observed values was calculated.

Specificity	Linear regression	Linearity (R^2)
MPO	Y = 0.9845 X + 1.052	0.9992

EXPECTED VALUES

Each laboratory must compile a list of reference values based upon a group of healthy donors from the local population. This must be done by taking age, sex and ethnic group into account, as well as any other potential regional differences.
In our laboratories, the whole blood samples of 10 healthy adults were used. The results obtained for the count of the positive events of interest are given in the table below:

Monocytes	Number	Mean (%)	SD	CV (%)
MPO^+	10	90.45	6.97	7.70

Granulocytes	Number	Mean (%)	SD	CV (%)
MPO^+	10	99.82	0.24	0.24

INTRA-LABORATORY REPRODUCIBILITY

On the same day and using the same cytometer, 12 measurements of the positivity of a sample containing positive cells (permeabilized peripheral blood from the same donor) were carried out. The results obtained are summarized in the following table:

Positive cells	Number	Mean (%)	SD	CV (%)
MPO^+ Monocytes	12	96.89	0.41	0.42
MPO^+ Granulocytes	12	99.96	0.03	0.03

INTER-LABORATORY REPRODUCIBILITY

On the same day and on the same sample containing positive cells (permeabilized peripheral blood from the same donor), 12 measurements of the positivity were carried out by two technicians and the preparations analyzed using two different cytometers. The results obtained are summarized in the following tables:

Cytometer n° 1:

Positive cells	Number	Mean (%)	SD	CV (%)
MPO^+ Monocytes	12	96.89	0.41	0.42
MPO^+ Granulocytes	12	99.96	0.03	0.03

Cytometer n° 2:

Positive cells	Number	Mean (%)	SD	CV (%)
MPO^+ Monocytes	12	96.96	0.75	0.78
MPO^+ Granulocytes	12	99.95	0.03	0.03

LIMITATIONS OF THE TECHNIQUE

1. Flow cytometry may produce false results if the cytometer has not been aligned perfectly, if fluorescence spillover have not been correctly compensated for and if the regions have not been carefully positioned.
2. Accurate and reproducible results will be obtained as long as the procedures used are in accordance with the technical insert leaflet and compatible with good laboratory practices.
3. The conjugated antibody of this reagent is calibrated so as to offer the best specific signal/non-specific signal ratio. Therefore, it is important to adhere to the reagent volume/sample volume ratio in every test.
4. In the case of a hyperleucocytosis, dilute the specimen in PBS so as to obtain a value of approximately 5 x 10^9 leucocytes/L.
5. In certain disease states, such as severe renal failure or haemoglobinopathies, lysis of red cells may be slow, incomplete or even impossible. In this case, it is recommended to isolate mononucleated cells using a density gradient (Ficoll, for example) prior to staining.

MISCELLANEOUS

See the Appendix for examples and references.

TRADEMARKS

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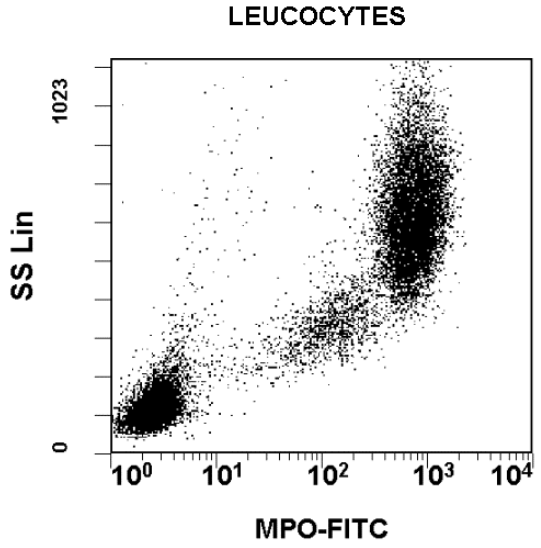
APPENDIX TO REF IM1874

EXAMPLE

The graph below is a biparametric representation (Side scatter versus Fluorescence Intensity) of whole blood permeabilized with IntraPrep. Staining is with IOTest MPO-FITC Conjugated Antibody (Ref. IM1874). All leucocytes are represented.

Acquisitin and analysis were performed with CYTOMICS FC 500 equipped with CXP Software.



REFERENCES

1. Rothe, G., Schmitz, G. Adorf, D., Barlage, S., Gramatzki, M., Hanenberg,H., Höffkes H.G., Janossy, G., Knüchel, R., Ludwig, W.D., Nebe, T., Nerl, C., Orfao, A., Serke, S., Sonnen, R., Tichelli, A., Wörmann, B., "Consensus protocol for the flow cytometric immunophenotyping of hematopoietic malignancies", 1996, Leukemia, 10, 877-895.
2. Stewart, C.C., Behm, F.G., Carey, J.L., Cornbleet, J., Duque, R.E., Hudnall, S.D., Hurtubise, P.E., Loken, M., Tubbs, R.R., Wormsley, S., "U.S. Canadian consensus recommendations on the immunophenotypic analysis of hematologic neoplasia by flow cytometry: selection of antibody combinations", 1997, Cytometry, 30, 231-235.
3. Bain, B.J., Barnett, D;, Linch, D., Matutes, E., Reilly, J.T., “Revised guideline on immunophenotyping in acute leukemias and chronic lymphoproliferative disorders”, 2002, Clin. Lab; Haem., 24, 1-13.
4. Orfao, A., Ruiz-Arguelles, A., Lacombe, F., Ault, K., Basso, G., Danova, M., "Flow Cytometry: its applications in hematology", 1995, Haematologica, 80, 69-81.
5. Nauseef, W.M., Olsson, I., Arnljots, K., "Biosynthesis and processing of myeloperoxidase: a marker for myeloid cell differentiation", 1988, Eur. J. Haematol., 40, 97-110.
6. Koeffler, H.P., Ranyard, J., Pertcheck, M., "Myeloperoxidase: its structure and expression during myeloid differentiation", 1985, Blood, 2, 65, 484-491.
7. Cramer, E., Pryzwansky, K.B., Villeval, J.L., Testa, U., Breton-Gorius, J., "Ultrastructural localization of lactoferrin and myeloperoxidase in human neutrophils by immunogold", 1985, Blood, 2, 65, 423-432.
8. Borregaard, N., Cowland, J.B., "Granules of the human neutrophilic polymorphonuclear leukocyte", 1997, Blood, 10, 89, 3503-3521.
9. Strobl, H., Takimoto, M., Majdic, O., Fritsch, G., Scheinecker, C., Höcker, P., Knapp, W., "Myeloperoxidase expression in CD34+ normal human hematopoietic cells", 1993, Blood, 7, 82, 2069-2078.
10. Murao, S.I., Stevens, F.J., Ito, A., Huberman, E., "Myeloperoxidase: a myeloid cell nuclear antigen with DNA-binding properties", 1988, Proc. Natl. Acad. Sci. USA, 85, 1232-1236.