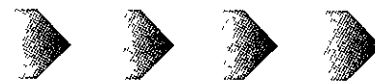


PN IM2743**50 tests****20 µL / test****Myeloperoxidase-FITC****Lactoferrin-PE****IOTest[®]**
Conjugated Antibodies**For Research Use Only. Not for use in diagnostic procedures.****SPECIFICITY**

Myeloperoxidase (MPO) is an heterodimeric glycoprotein of 150 kDa with an $\alpha 2\beta 2$ structure. The two subunits (α and β) have molecular weights of 55 and 15 kDa, respectively (1, 2)

MPO synthesis occurs in bone marrow at an early stage of myeloid lineage differentiation. MPO is specifically expressed during promyelocytic formation, the stage at which azurophilic granules (or primary granules) are formed (1, 3, 4). MPO is still found in mature myeloid cells, then constituting the major constituent of azurophilic granules of neutrophils (4, 5, 2)

MPO acts as a potent microbicidal agent catalyzing the formation of hypochlorous acid (HOCl) in the presence of active oxygen (H_2O_2) (1, 4)

MPO is stored in polymorphonuclear neutrophilic granules and on macrophages, but is not expressed in lymphocytes, platelets and erythrocytes (2)

Lactoferrin is a 76 kDa single chain polypeptide with iron-binding properties. This protein shows structural similarities to transferrin, the plasma iron-transport protein (6, 7, 8)

Lactoferrin synthesis occurs at the myelocytic stage during the polymorphonuclear neutrophil (PMN) ontogeny and constitutes a component of secondary (or specific) granules located in the cytoplasm (4). Unlike myeloperoxidase, lactoferrin is absent from azurophilic granules and appears at a later step of maturation in the granulocytic differentiation (7, 3). Consequently, among peripheral whole blood leucocytes, lactoferrin expression is restricted to the neutrophil subset (7). The intracellular location of lactoferrin within azurophilic granules is unique. However, lactoferrin is found in some physiological fluids such as saliva, tears, semen, pancreatic secretion, colostrum and serum (7)

In normal bone marrow samples, the majority of cells are of myeloid ontogeny and, then express myeloperoxidase. Among these myeloperoxidase-expressing cells, lactoferrin expression is restricted to the more mature subset of cells

Lactoferrin is involved in various immunoregulatory functions associated to anti-infective and inflammatory responses such as *in vitro* antibody synthesis, cytokines production, NK-cell cytotoxicity, complement activation and lymphocytes proliferation (6, 7)

CLB-MPO1 monoclonal antibody recognizes the human intracellular myeloperoxidase and the precursor of MPO (proMPO) which is enzymatically inactive. CLB-13.17 monoclonal antibody recognizes intracellular lactoferrin. A cell permeabilization procedure is therefore required to address the cytoplasmic compartment in flow cytometric studies

REAGENTS

MPO-FITC	Lactoferrin-PE
CLB-MPO1	CLB13.17
IgG2a (mouse)	IgG1 (mouse)
SP2/0 x CAF spleen cells	SP2/0 x CAF spleen cells

Conjugations • Fluorescein isothiocyanate (FITC) is conjugated at 3-5 moles of FITC per mole of Ig
Excitation wavelength 488 nm
Maximum emission wavelength 525 nm
Main emission color Green

• R-phycoerythrin (PE) is conjugated at 0.7-1 mole of PE per mole of Ig
Excitation wavelength 488 nm
Maximum emission wavelength 575 nm
Main emission color Orange-red

Buffer 2 mg/mL bovine serum albumin in phosphate-buffered saline containing 0.1% sodium azide

APPLICATIONS

- Flow cytometric analysis of intracellular myeloperoxidase and lactoferrin
- Identification and characterization of specific stages of maturation of myeloid lineage
- Research studies on polymorphonuclear granules characterization

The IOTest dual color combination MPO-FITC / Lactoferrin-PE is optimized to detect intracellular myeloperoxidase and lactoferrin after formaldehyde / saponin-based permeabilizing procedures on whole blood samples, bone marrow samples and density purified cell-suspensions. IntraPrep™ Permeabilization Reagent is recommended (PN IM2388, PN IM2389).

STATEMENT OF WARNINGS

- 1 This reagent contains 0.1% sodium azide. Sodium azide under acid conditions yields hydrazoic acid, an extremely toxic compound. Azide compounds should be flushed with running water while being discarded. These precautions are recommended to avoid deposits in metal piping in which explosive conditions can develop. If skin or eye contact occurs, wash excessively with water.
- 2 Specimens, samples and all material coming in contact with them should be handled as if capable of transmitting infection and disposed of with proper precautions.
- 3 Never pipet by mouth and avoid contact of samples with skin and mucous membranes.
- 4 Do not use antibody beyond the expiration date on the label.
- 5 Do not expose reagents to strong light during storage or incubation.
- 6 Avoid microbial contamination of reagents or incorrect results might occur.

STORAGE CONDITIONS AND STABILITY

Each reagent is stable up to the expiration date when stored at 2-8°C. Do not freeze. Minimize exposure to light.

REAGENT PREPARATION

No reconstitution is necessary. This monoclonal antibody may be used directly from the vial. Bring reagent to 20-25°C prior to use.

PROCEDURE

This reagent is designed for flow cytometric studies after permeabilization of the cells.

The following is the recommended permeabilization procedure using IntraPrep™ Permeabilization Reagent (PN IM2388, PN IM2389), for combined membrane and intracytoplasmic staining with conjugated antibodies.

- 1 Dispense 50 µL of whole blood (or 5 x 10⁵ white blood cells) into two tubes for each sample.
 - One membrane and cytoplasmic staining test tube = assay tube
 - One membrane and cytoplasmic control tube (isotypic control or specific antibody used for control) = control tube
- 2 Add 20 µL (or 10 µL, depending on the manufacturer recommendations) of membrane-specific conjugated monoclonal antibody to the assay tubes, and 20 µL (or 10 µL, depending on the manufacturer recommendations) of appropriate membrane isotypic control (or conjugated specific antibody used for the control tube) to the control tubes.
- 3 Vigorously vortex tube by tube.
- 4 Incubate for 15 minutes at room temperature (18-25°C) in the dark.
- 5 Add 100 µL of IntraPrep Reagent 1 to each tube.
- 6 Vigorously vortex tube by tube.
- 7 Incubate for 15 minutes at room temperature in the dark.
- 8 Add 4 mL of PBS.
- 9 Centrifuge for 5 minutes at 300 x g at room temperature.
- 10 Discard supernatant (by aspiration).
- 11 Add 100 µL of IntraPrep Reagent 2 to each tube. Let mixing occurs WITHOUT VORTEXING.
- 12 Incubate for 5 minutes at room temperature WITHOUT VORTEXING.
- 13 Gently agitate (manually), for 1 to 2 seconds.
- 14 Add 20 µL (or 10 µL, depending on the manufacturer recommendations) of intracellular conjugated specific antibody to the assay tubes, and 20 µL (or 10 µL, depending on the manufacturer recommendations) of the appropriate intracellular control reagent (e.g. conjugated isotypic control or conjugated specific antibody used for control) to the control tubes.
- 15 Gently vortex tube by tube.
- 16 Incubate for 15 minutes at room temperature in the dark.
- 17 Add 4 mL of PBS.

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**COULTER**

PARTNERS IN CELL ANALYSIS

**IMMUNOTECH**
A COULTER COMPANY

PN IM2743

50 tests

20 µL / test

Myeloperoxidase-FITC

Lactoferrin-PE

For Research Use Only. Not for use in diagnostic procedures.

- 18 Centrifuge for 5 minutes at 300 x g at room temperature
19. Discard supernatant (by aspiration)
- 20 Resuspend cells in 500 µL of PBS, containing 0.5% formaldehyde and proceed to flow cytometry analysis

The specimen should be analyzed within two hours of IntraPrep treatment when stored at 18–25°C. Otherwise, fixed preparations should be stored at 2–8°C in the dark and analyzed within 24 hours.

If the membrane staining is not required, start the permeabilization procedure at step 5 by adding 100 µL of IntraPrep Reagent 1 to a volume of 50 µL of whole blood (or 5×10^5 cells / test) and continue as further described.

EXAMPLE DATA

The graphs below are biparametric representations (Fluorescence Intensity versus Fluorescence Intensity) of a permeabilized normal whole blood sample. Staining is with MPO-FITC / Lactoferrin-PE dual color reagent (PN IM2743). Gate is on leucocytes. Permeabilization is done using IntraPrep Permeabilizing Reagent (PN IM2388 and IM2389).

- Upper right quadrant (2) contains MPO- and Lactoferrin-double positive leucocytes corresponding to granulocytes
- Lower left quadrant (3) contains MPO- and Lactoferrin-double negative leucocytes constituted of lymphocytes and debris
- Lower right quadrant (4) contains MPO-positive, Lactoferrin-negative leucocytes clustering monocytes

Figure 1
Acquisition is with a COULTER® EPICS® XL flow cytometer. Analysis is with the XL System II™ software.

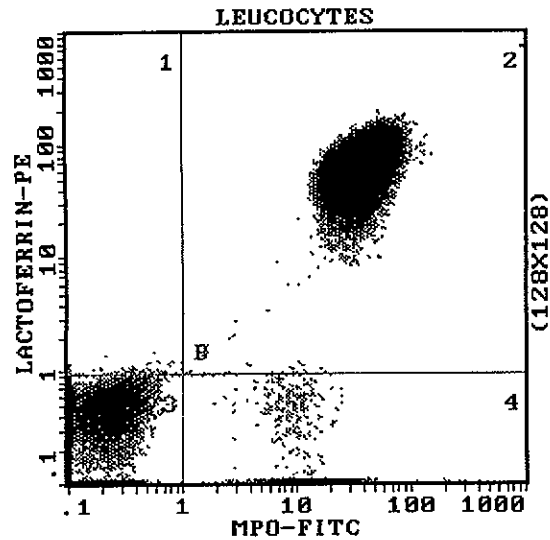
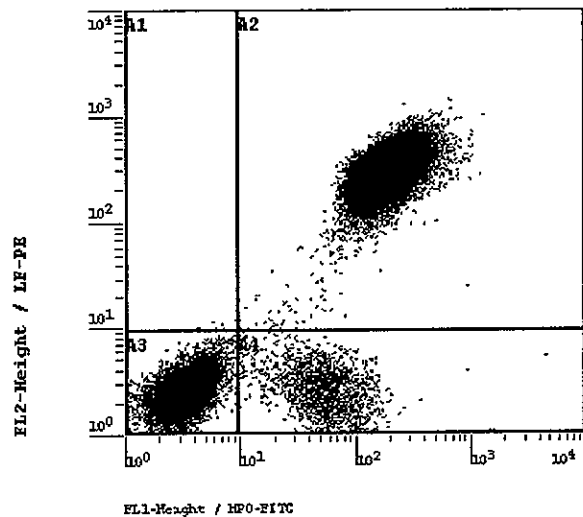


Figure 2
Acquisition is with a Becton Dickinson FACScan™ flow cytometer equipped with LYSYS II™ software. Analysis is with EXPO2™ Cytometer software (Coulter PN 6605434).

(1) 010998 003 FL1-H/EL2-H UnGated



SELECTED RESEARCH REFERENCES

- 1 [3587] 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
- 2 [368] Koeffler, H P, Ranyard, J, Pertcheck, M, "Myeloperoxidase: its structure and expression during myeloid differentiation", 1985, *Blood*, 2, 65, 484-491
- 3 [2745] 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
- 4 [3191] Borregaard, N, Cowland, J B, "Granules of the human neutrophilic polymorphonuclear leukocyte", 1997, *Blood*, 10, 89, 3503-3521
- 5 [3177] Strobl, H, Takimoto, M, Majdic, O, Fritsch, G, Scheinecker, C, Hocker, P, Knapp, W, "Myeloperoxidase expression in CD34+ normal human hematopoietic cells", 1993, *Blood*, 7, 82, 2069-2078
- 6 [3192] Brock, J, "Lactoferrin: a multifunctional immunoregulatory protein?", 1995, *Immunol Today*, 9, 16, 417-419.
- 7 [2847] Rado, T A, Bollekens, J, St Laurent, G, Parker, L, Benz, E J Jr, "Lactoferrin biosynthesis during granulocytopoiesis", 1984, *Blood*, 5, 64, 1103-1109
- 8 [3193] Srivastava, C H, Rado, T A, Bauerle, D, Broxmeyer, H E, "Regulation of human bone marrow lactoferrin and myeloperoxidase gene expression by tumor necrosis factor-α", 1991, *J Immunol*, 3, 146, 1014-1019

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