

COMBI-IC REAGENT

Myeloperoxidase-C2/CD22(MPO-C2/CD22)



Available Form:

Fluorescein/PE Conjugated mAb mixture

Cat. No.: GIC-214

'50 Tests



Specification

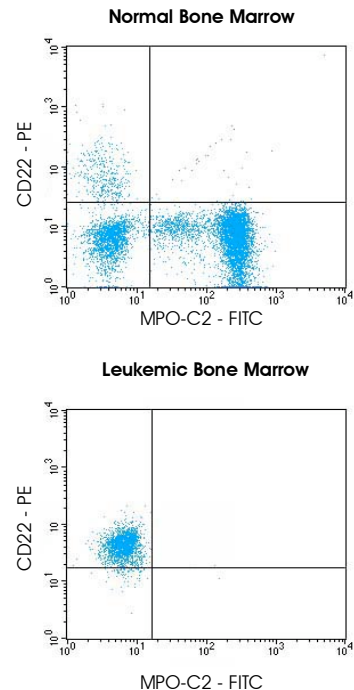
MPO-Fluorescein Antibody

Specificity (Synonyms): Human Myeloperoxidase (MPO-C2)
Clone: 8E6
Immunogl. Class: IgG1
Species: Mouse
Purification: Chromatography
Fluorochrome: FITC conjugated

LF-Phycoerythrin Antibody

Specificity (Synonyms): Human CD22
Clone: RFB4
Immunogl. Class: IgG1
Species: Mouse
Purification: Chromatography
Fluorochrome: FITC conjugated

Storage Buffer: PBS pH 7.2, 1 mg/ml BSA, 0.1% NaN₃



Introduction

Myeloperoxidase (MPO) is a glycoprotein present in the azurophil (primary) granules of myeloid cells, which appears in the myeloblast stage of myeloid cell differentiation. MPO is the most common functional protein of myeloid cells and is involved in the inflammatory response. It helps to kill microbes by breaking down peroxide in the presence of halide ions, contributing to the bactericidal function of granulocytes. The primary translation product of MPO undergoes glycosylation with production of the 89 kDa heme-free apopro-MPO form followed by incorporation of heme and conversion into the enzymatically active pro-MPO form. Subsequently, pro-MPO becomes targeted to azurophil granules where final processing occurs to produce mature dimeric MPO consisting of the 59-64 kDa MPO α -chain and the 14 kDa MPO β -chain. Precursor B-cells are surface-CD22 negative, but cytoplasmic CD22 positive. Mature B-lymphocytes express CD22 also on their surface. The combined staining for MPO and CD22 allows the distinction of mature/immature myeloid cells and B-lymphocytes.

Intended use

The MPO-C2/CD22 COMBI-IC mAb permits the identification and enumeration of normal and malignant myelomonocytic cells and B lymphoid committed cells in human blood and bone marrow 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. Analyses performed with this antibody should be paralleled by positive and negative controls. If unexpected results are obtained which cannot be attributed to differences in laboratory procedures, please contact us.

Specificity

Antibody MPO-C2 (clone 8E2) reacts with human myeloperoxidase (MPO) expressed by normal and malignant myelomonocytic cells. The CD22 mAb (clone RFB4) recognizes cytoplasmatic CD22 in precursor B-cells and surface as well cytoplasmatic CD22 on mature B-cells.

Storage

AN DER GRUB monoclonal antibody reagents contain optimal concentrations of affinity-purified antibody. For stability reasons monoclonal antibody solution contains sodium azide. These reagents should be stored at 2-8°C (DO NOT FREEZE!) and protected from prolonged exposure to light before and after the vial has been opened. 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. Analyses performed with this antibody should be paralleled by positive and negative controls.

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

- In combination with our Permeabilization Kit FIX&PERM® (Cat. No. GAS-002) intracellular MPO-C2 and CD22 can be easily stained in cell suspensions.
- For each sample to be analyzed add 50 μ l of whole blood, bone marrow or mononuclear cell suspension in a 5ml tube
- Add 100 μ l 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 μ l Reagent B (Permeabilization Medium) and 20 μ l of the MPO-C2/CD22 COMBI-IC 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.

Sensitivity

The sensitivity of MPO-C2/CD22 mAb is determined by staining well-defined blood samples from representative donors with serial-fold mAb dilutions to obtain a titration curve that allows

relating the mAb concentration to the percentage of stained cells and geometric MFI (mean fluorescence intensity). For this purpose, a mAb-concentration range is selected to include both the saturation point (i.e. the mAb dilution expected to bind all epitopes on the target cell) and the detection threshold (i.e. the mAb dilution expected to represent the least amount of mAb needed to detect an identical percentage of cells). In practice, 50 µl of leukocytes containing 10⁷ cells/ml are stained with 20 µl mAb of various dilutions to obtain a titration curve and to identify the saturation point and detection threshold. The final concentration of the product is then adjusted to be at least 3-fold above the detection threshold. In addition and to control lot-to-lot variation, the given lot is compared and adjusted to fluorescence standards with defined intensity.

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 antibody is provided in a concentration that will allow to unequivocally detect specific cells. It is therefore strongly recommended to stick to the staining protocol in terms of concentration and volume regarding cells and antibody.

The therapeutic use of antibodies might influence the recognition of target-antigens by this antibody. The reaction pattern of MPO-C2/CD22 mAb alone is not sufficient to diagnose "leukemia". Combination with other antibodies in multi-color stainings is strongly recommended. The properties of this mAb have been determined using EDTA anti-coagulated peripheral blood.

Precautions

For professional users only.

This reagent contains sodium azide. To avoid the development of hazardous conditions, reagents containing azide should be diluted in running water prior to be discarded. Similar to the work with other biological products, proper handling procedures are recommended.

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.

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For In Vitro Diagnostic Use

For professional use only.

Explanation of symbols

REF	Catalog number
IVD	In vitro diagnostic medical device
①	Consult instructions for use
2°-8°	Temperature limitation
☀	Keep away from sunlight
LOT	Batch code
🕒	Use by
⚠	Contains sufficient for (N) Tests
🏭	Manufacturer