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Mouse anti CD22, conjugated to FITC

 nordicmubio.com/products/Mouse-anti-CD22-conjugated-to-FITC/GM-4052

Catalog number: **GM-4052**

Clone	RFB4
Isotype	IgG1
Product Type	Primary Antibodies
Units	2ml (100 Tests)
Host	Mouse
Species Reactivity	Human
Application	Flow Cytometry Immunofluorescence

Background

The epitope recognized by antibody RFB4 is a 135 kDa type I integral membrane protein expressed by human B-cells. Precursor B-cells are surface-CD22 negative, but cytoplasmic CD22 positive. Mature B-lymphocytes express CD22 also on their surface. The RFB4 antibody permits the identification and enumeration of human B-cells using flow cytometry. Results must be interpreted 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.

Product

2 ml of FITC-conjugated anti CD22 (clone RFB4) in PBS pH 7.2, 1% BSA, and 0.05% NaN₃, approximately 100 tests.

Product Form: FITC

Formulation: PBS pH 7.2, 1% BSA, 0.05% NaN₃

Purification Method: Purified by Chromatography

Specificity

The CD22 mAb (clone RFB4) recognizes surface CD22 expressed by mature peripheral B-cells and cytoplasmatic CD22 expressed by precursor B-cells. The sensitivity of RFB4 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 μ l of leukocytes containing 10^7 cells/ml are stained with 20 μ l 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.

Applications

Staining Procedure for Surface CD22: Direct Immunofluorescence (Staining Procedure)
Nordic-MUBio fluorochrome labeled antibodies are designed for use with either whole blood or isolated mononuclear cell (MNC) preparations Proposed staining procedure for whole blood in short: - For each sample add 50 μ l of EDTA anti-coagulated blood to a 3-5 ml tube - Add 20 μ l of the appropriate Nordic-MUBio monoclonal antibody conjugate - Incubate the tube for 15 minutes at 4°C or at room temperature in the dark - Add 100 μ l Nordic-MUBio-LYSE (Cat.No. GAS-003) to each tube and incubate for 10 minutes at room temperature - Add 3-4 ml of distilled water and vortex, incubate for 5-10 minutes at room temperature - Centrifuge tube for 5 minutes at 300 g - Aspirate supernatant and resuspend pellet in 0.3 ml of sheath fluid - Analyze immediately or store samples at 2-8°C in the dark and analyze within 24 hours For "No-Wash" protocol please refer to www.nordicmubio.com Proposed staining procedure for MNC in short: - Carefully add 20 μ l antibody conjugate and 50-100 μ l MNC to the bottom of a tube - Vortex at low speed for 1-2 seconds - Incubate for 15-30 minutes at 2-8°C or at room temperature - Centrifuge tubes for 5 minutes at 300 g - Remove supernatant, resuspend cells in 2-5 ml of phosphate buffered saline (PBS) and centrifuge cells again for 5 minutes at 300 g - Remove supernatant and resuspend cells in sheath fluid for immediate analysis or resuspend cells in 0.5 ml 1 % formaldehyde and store them at 2-8°C in the dark. - Analyze fixed cells within 24 hours Indirect Immunofluorescence (Staining Procedure) - Mix 20 μ l Nordic-MUBio purified antibody with 50 μ l whole blood or MNC suspension - Incubate for 15 minutes at 2-8°C - Wash cells with phosphate buffered saline (PBS) - Add to cell pellet 20 μ l of affinity purified, fluorochrome labeled F(ab')₂ anti mouse Ig antibodies - Incubate for 15 minutes at 2-8°C - Wash cells with phosphate buffered saline (PBS) or proceed as described for direct staining Staining Procedure for Cytoplasmatic CD22: Permeabilization and Staining Procedure - In combination with our Permeabilization Kit FIX&PERM® (Cat. No. GAS-002) intracellular 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 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 CD22 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.

Storage

Nordic-MUBio monoclonal antibody reagents contain optimal concentrations of affinity-purified antibody. For stability reasons this 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. If a slight precipitation occurs upon storage, this should be removed by centrifugation. It will not affect the performance or the concentration of the product. 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.

Caution

This product is intended FOR RESEARCH USE ONLY, and FOR TESTS IN VITRO, not for use in diagnostic or therapeutic procedures involving humans or animals. It may contain hazardous ingredients. Please refer to the Safety Data Sheets (SDS) for additional information and proper handling procedures. Dispose product remainders according to local regulations. This datasheet is as accurate as reasonably achievable, but Exalpha Biologicals accepts no liability for any inaccuracies or omissions in this information.

References

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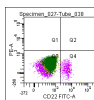
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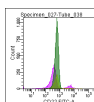
NM_Sodium Azide



Flow cytometric analysis of a blood sample after immunostaining with GM-4052 (CD22-FITC).



Flow cytometric analysis of a normal blood sample after immunostaining with GM-4052 (CD22-FITC).



Flow cytometric analysis of a normal blood sample after immunostaining with GM-4052 (CD22-FITC).