3050 Spruce Street
Saint Louis, Missouri 63103 USA
Telephone 800-325-5832 • (314) 771-5765
Fax (314) 286-7828
email: techserv@sial.com
sigma-aldrich.com

ProductInformation

MONOCLONAL ANTI-HUMAN CD15
CLONE DU-HL60-3
BIOTIN CONJUGATE
Purified Mouse Immunoglobulin

Product No. **B0906**

Product Description

Monoclonal Anti-Human CD15 (mouse IgM isotype) is derived from the DU-HL60-3 hybridoma produced by the fusion of mouse myeloma cell line and splenocytes from BALB/c mice immunized with the human promyelocyte cell line HL60.[1] The isotype is determined using the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of ε-amino caproyl biotin to purified CD15 monoclonal antibody.

Monoclonal Anti-Human CD15 antibody recognizes the CD15 antigen (3FA-L, Le^x, X-hapten, SSEA)[1]. The CD15 antigen is expressed on approximately 90% human circulating granulocytes (membranes and granules), 30-60% of circulating monocytes and is absent from normal lymphocytes. CD15 antigen is also expressed on Reed-Sternberg cells of Hodgkin's disease, on T cell lymphomas including mycosis fungoides and on some leukemias. Outside the hematopoietic system, CD15 expression is described in certain normal and neoplastic epithelial cells and in astrocytes. CD15 antibodies recognize the tri-saccharide 3-fucosyllactosamine (3-Fl) which is present in lacto-N-fucopentaose III and in the blood group antigen X-hapten. At least 5 major CD15 antigens (105, 135, 165, 185, 220 kDa) are present on the surface membranes of polymorphonuclear cells. The hapten occurs also in glycolipids. The epitope recognized by monoclonal DU-HL60-3 is resistant to formalin fixation and paraffin embedding.

Product Profile

When assayed by flow cytometric analysis, using 10 µl of the antibody per 1 X 10^6 cells and ExtrAvidin®-FITC Conjugate (Product No. E 2761), a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels.

Uses

Biotin Monoclonal Anti-Human CD15 antibody may be used for:

1. Enumeration of human granulocytes and monocytes in bone marrow, blood and other body fluids.
2. Identification and localization of normal and malignant cells of the myeloid lineage.
3. Characterization of leukemias.
4. Myeloid cell differentiation studies.

In order to obtain best results in different techniques and preparations, it is recommended that each individual user determine their optimum working dilutions by titration assay.

Storage/Stability

Store at 2-8 °C.
If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Reagents

The conjugate is provided as purified immunoglobulin (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Precautions and Disclaimer

Due to the sodium azide content a material safety sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous and safe handling practices.

Procedure for Indirect Immunofluorescent Staining using Biotinylated Primary Antibodies

Reagents and Materials Needed but Not Supplied

1. a. Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A or heparin anticoagulant **or**
   b. Human cell suspension (e.g., peripheral blood mononuclear cells isolated on Histopaque® Stock No. 1077-1).
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated avidin derivative diluted to recommended working dilution in diluent. Appropriate products for use are ExtrAvidin-FITC (Product No. E 2762), Streptavidin-FITC (Product No. S 3402), Streptavidin-PE (Product No. S 3762), or Streptavidin-Quantum Red™ (Product No. S2899).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. 0.2% Trypan blue (Product No. T 0776) in 0.01M phosphate buffered saline, pH 7.4.
9. 2% paraformaldehyde in PBS.
10. Whole blood lysing solution.
11. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **or**
   b. Adjust cell suspension to 1 x 10^7 cells/ml in diluent. Cells should be >90% viable as determined by dye exclusion (e.g., trypan blue). For each sample, add 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µl of biotinylated monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 – 22 ° C) for 30 minutes.
3. After 30 minutes, add 2 ml of diluent to all tubes.
4. Pellet cells by centrifugation at 500 x g for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Repeat washing procedure (steps 4-6) twice.
8. After the last wash, resuspend the cells in 100 µl of the fluorochrome conjugated avidin derivative at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 – 22 °C) for 30 minutes. Protect from light at this and all subsequent steps.
   Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then pellet and wash cells as in steps 4-6 twice, and proceed to step 10.
9. Centrifuge and wash as in steps 4-6 twice.
10. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored before analyzing) and analyze in a flow cytometer according to manufacturer's instructions.

Reference

1. McKolanis, J. R., et al., In: Leucocyte Typing II, Reinherz, E. L., et al. (Eds.), pp. 387-394, (Springer Verlag, New York 1986).

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