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
Purified Mouse Immunoglobulin

Product No. **C 7798**

Product Description

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

Monoclonal Anti-Human CD15 recognizes the CD15 antigen (3FAL, Le^x, X-hapten, SSEA). The CD15 antigen is expressed on approximately 90% of human circulating granulocytes (membranes and granules), 30-60% of circulating monocytes, and is absent from normal lymphocytes and platelets. It 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 has been described in certain normal and neoplastic epithelial cells and astrocytes. Antibodies to CD15 recognize the trisaccharide 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 this clone is resistant to formalin fixation, paraffin embedding and pronase treatment. Neuraminidase treatment increases reactivity in monocytes.

Monoclonal Anti-Human CD15 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.
5. Enrichment or depletion of myeloid cells.

Reagent

The product is provided as purified antibody (50-200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

Precautions

Due to the sodium azide content, a material safety data 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.

Performance

When assayed by flow cytometric analysis, using 5 µl of the antibody to stain 1 x 10^6 cells, a fluorescence intensity is observed similar to that obtained with saturating monoclonal antibody levels. The percent population positive is also at the maximum percentage positive using saturating monoclonal antibody levels.

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

Storage

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

Procedure for Indirect Immunofluorescent Staining

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® (Product Code 1077-1)).

2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1%NaN_3.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Product No. F2883 FITC-Sheep Anti-Mouse IgG (whole molecule), F(ab´)$_2$ fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. M5909).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Product No. T0776) in 0.01 M phosphate buffered saline, pH 7.4.
10. 2% paraformaldehyde in PBS.
11. Whole blood lysing solution.
12. 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 5 µl of monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently. Incubate the cells at room temperature (18 – 22 °C) for 30 minutes.
   Proper controls to be included for each sample are:
   a. Autofluorescence control: 5 µl diluent in place of monoclonal antibody.
   b. Negative staining control 1: 5 µl isotype-matched non-specific mouse immunoglobulin (Product No. M5909) at the same concentration as test antibody.
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).
8. After the second wash, resuspend the cells in 100 µl of the fluorochrome conjugated secondary - antibody 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.
9. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10.
   b. If a mononuclear cell suspension is used, proceed to Step 10.
10. Add 2 ml diluent to all tubes.
11. Wash as in steps 4-6 twice.
12. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer's instructions.

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein. It should be isotype-matched to the primary antibody, not specific for human cells and of the same concentration as the primary antibody. The degree of autofluorescence or negative control reagent fluorescence will vary with the type of cells under study and the sensitivity of the instrument used.

For fluorescence analysis of cells with Fc receptors, the use of isotype matched negative control is mandatory. In some systems it may be necessary to incubate the cells in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

References

1. McKolanis, J., et al., in Leukocyte Typing II, Reinherz, E., et al (Eds.), Springer Verlag, New York, 387 (1986).
2. Leucocyte Typing IV, Knapp, W., et al., (eds.), Oxford Press, New York, 1079 (1989).
3. Civin, C., et al., Blood, **57**, 842 (1981).
4. Majdic, O., et al., Blood, **58**, 1127 (1981).

5. Huang, L., et al., Blood, **61**, 1020 (1983).
6. Howie., A., et al., J. Clin. Pathol., **37**, 555 (1984).
7. Gooi, H., et al., Clin. Exp. Immunol., **60**, 126 (1985).
8. Skubitz, K. and Snook II, R., J. Immunol., **139**, 1631 (1987).
9. Skubitz, K., et al., J. Immunol., **141**, 4318 (1988).
10. Skubitz, K., et al., in Leukocyte Typing IV, Knapp, W., et al., (eds.), Oxford Press, New York (1989).