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ProductInformation

MONOCLONAL ANTI-HUMAN CD16
CLONE BL-LGL/1
Purified Mouse Immunoglobulin

Product Number **C4071**

Product Description

Monoclonal Anti-Human CD16 (mouse IgG1 isotype) is derived from the BL-LGL/1 hybridoma produced by the fusion of mouse myeloma cell line NS-1 and splenocytes from BALB/c mice immunized with human LGL-cells. The isotype is determined using the Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

Monoclonal Anti-Human CD16 recognizes the human CD16 antigen (also known as the low affinity receptor for complexed IgG, or Fcγ-RIII) expressed on Natural Killer (NK) cells, a macrophage subpopulation and weakly on granulocytes. The human CD16 molecule exhibits two truncated Ig-like domains. In NK cells and macrophages, a transmembrane form (50-80 kDa) with a 25 amino acid cytoplasmic tail is present. This form is non-covalently associated with other receptors (FcεRIγ or the TcR? chain). The human CD16 molecule in granulocytes is a glycosyl-phosphatidyl-inositol (GPI) - linked form.[2] CD16 is a low affinity receptor for aggregated IgG. The transmembrane form binds complexed IgG and mediates phagocytosis and antibody-dependent cellular cytotoxicity.[3] The GPI-linked form is unable to induce any signal or functional effect. In frozen sections of liver and thymocytes, most macrophages are recognized by this antibody. Staining of some subepithelial tonsillar macrophages and of splenic red pulp macrophages is reported.[4] The antibody does not block immune complex binding. It can be used in flow cytometry, immunocytology and immunohistology on frozen sections.

Reagents

Monoclonal Anti-Human CD16 is provided as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide as a preservative.

Precautions and Disclaimer

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 hazards and safe handling practices.

Storage/Stability

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

Product Profile

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.

Uses

Monoclonal Anti-Human CD16 may be used for:

1. Detection and enumeration of NK cells in blood and tissue sections in health and disease.
2. Studies of FCγRIII function.
3. Typing of Chronic Myeloid Leukemia.

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.

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®(Sigma Stock No. 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. F 2883 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. M5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Product No. T 0776) 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. M 5284) 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. Hogg, N., Immunol. Today, **9**, 185 (1988).
2. Lanier, L. L., et al., J. Immunol., **141**, 3478 (1988).
3. Ravetch, J. V., and Kinet, J. P., Annu. Rev. Immunol., **9**, 457 (1991).
4. Leucocyte Typing IV, Oxford University Press, 578 (No. 401), (1989).

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