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Product Information

Monoclonal Anti CD16-FITC
produced in mouse
clone 3G8, purified immunoglobulin

Product Number **F3668**

Product Description

Monoclonal Anti-CD16 (mouse IgG1 isotype) is derived from the 3G8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized CD2F1 mouse. Viable human polymorphonuclear cells were used as the immunogen.[1] The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Product Number ISO2. The product is prepared by conjugation of fluorescein isothiocyanate isomer I to Protein A purified Monoclonal Anti-Human CD16. The product is purified by gel filtration and contains no detectable free FITC.

Monoclonal Anti-CD16-FITC recognizes the human CD16 antigen expressed on Natural Killer (NK) cells, granulocytes and a macrophage subpopulation.

Monoclonal Anti-CD16-FITC is a homogenous population of antibody molecules which may be used for:

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

The human CD16 antigen (also known as the low affinity receptor for complexed IgG or Fc(-RIII) is expressed on Natural Killer (NK) cells, granulocytes, a macrophage subpopulation, cultured monocytes and on a small subset of T cells.[2] The human CD16 molecule exhibits two truncated Ig-like domains. In NK cells, macrophages and cultured monocytes a transmembrane form $Fc(RIII_a$, (50-80 kDa) with a 25 amino acid cytoplasmic tail is present. This form is non-covalently associated with other receptors (FcεRIγ chain or the TcRζchain). The human CD16 molecule in granulocytes is a glycosyl-phosphatidyl-inositol (GPI) - linked form, $FC(RIII_b$.[3] CD16 is a low affinity receptor for aggregated IgG. The transmembrane form binds complexed IgG and mediates phagocytosis and antibody-dependent cellular cytotoxicity (ADCC).[4] Monoclonal Anti-CD16 (Clone 3G8)[1,5,6,7] is a pan anti-CD16 antibody which reacts with both NA1 and NA2 neutrophils and lymphocytes. It strongly labels neutrophils in tissue frozen sections. Liver Kupffer cells are weakly stained. The epitope recognized by the antibody seems to reside near the IgG binding site of FC(RIII. The antibody functionally blocks binding of soluble immune complexes to granulocytes and inhibits E-IgG rosettes. It induces Ca^{2+} increase in neutrophils.

Reagents

Supplied 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

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Product Profile

F/P Molar Ratio: 3 – 8

Antibody Performance

When assayed by flow cytometric analysis (with a FACScan flow cytometer) using 10 µL of the antibody to stain $1X10^6$ cells or 100 µL whole blood, 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.

Storage

Store at 2–8 °C. Do not freeze. Protect from prolonged exposure to light. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure for Direct 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 Coe 1077-1)).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Product No. F 6397).
4. 12 x 75 mm test tubes.
5. Adjustable micropipette.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Product No. T 0776), 0.2% in 0.01 M 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 (trypan blue). For each sample, add 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µL of conjugate to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 to 22 °C) for 30 minutes.
   Proper controls to be included for each sample are:
   a. An autofluorescence control: 10 µL of Diluent in place of monoclonal antibody followed by steps 3 - 8.
   b. A negative staining control: 10 µL of FITC conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3 - 8.
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 of Diluent.
7. Repeat washing procedure (steps 3-6) twice. **Note**: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then repeat steps 3-6 twice, and proceed to step 8
8. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored beforeanalyzing) 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. Fleit, H., et al., *Proc. Nat. Acad. Sci. USA*, **79**, 3275 (1982).
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3. Lanier, L., et al., *J. Immunol.*, **141**, 3478 (1988).
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6. Fleit, H., et al., in: Leucocyte Typing IV, Knapp, W., et al., (eds.), Oxford University Press, Oxford (No. N409), p 579 (1989).
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