Product No. F-5773
Lot 016H4811

Monoclonal Anti-Human CD11c FITC Conjugate

Purified Mouse Immunoglobulin
Clone 3.9

Monoclonal Anti-Human CD11c (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes of BALB/c mice immunized with rheumatoid synovial fluid cells followed by fibronectin purified human monocytes.[1,2] The isotype is determined using Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I to purified CD11c monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The product is provided as purified antibody (200 µg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Description

Monoclonal Anti-Human CD11c antibody recognizes the CD11c, 150 kD, α-chain of the CD11c/CD18 complex, an α/β heterodimeric glycoprotein which belongs to the β_2 integrins family.[1-3] It is also known as p150,95. Human CD11c is constitutively present on monocytes, macrophages, dendritic reticular cells, NK cells and certain cytotoxic T-cells.[2-7] Low levels of CD11c are expressed on polymorphonuclear cells. It is also strongly expressed on hairy cell leukemia cells. B cell prolymphocytic leukemia cells also carry this antigen. Expression on a B cell subset is weak. A large proportion of CD11c/CD18 is stored in intracellular compartments and translocated to the cell surfaces upon stimulation by inflammatory mediators, resulting in enhanced adhesiveness of the cells to vascular endothelial cells. CD11c/CD18 is a lipopolysaccharide receptor. It also mediates the attachment of unopsonized bacteria and fungi to leukocytes. Monoclonal Anti-CD11c stains macrophages in acetone-fixed frozen sections of human tissues (lung, brain, colon, skin, spleen, thymus, tonsil). The epitope recognized by the antibody is formalin sensitive. The antibody reduces adhesion of TNF-stimulated polymorphonuclears to surfaces coated with fibrinogen.[8]

Uses

FITC Monoclonal Anti-Human CD11c may be used for:

1. Characterization of leukemias and lymphomas.
2. Detection and monitoring of leukocyte adhesion deficiencies.
3. Studies of cell adhesion and effector-target interactions.

F/P Molar Ratio: 6.2

Performance

When assayed by flow cytometric analysis, using 10 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 techniques and preparations it is recommended that each individual user determine their optimum working dilutions by titration assay.

Storage

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.

* 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 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® (Sigma Product No. 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, Sigma Product No. F-6397).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma Product No. T-0776), 0.2% in 0.01 M PBS, 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 conjugate 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. An autofluorescence control: 10 µl diluent in place of monoclonal antibody, followed by steps 3 - 7.
   b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (Sigma Product No. F-6397) at the same concentration as test antibody followed by steps 3 - 7.
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of diluent to all tubes.
5. Pellet cells by centrifugation at 500 x G for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. 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 binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the 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.

References

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