SIGMA CHEMICAL COMPANY

Product No. F-2787
Lot 104H4851

Monoclonal Anti-Human CD11a
FITC Conjugate

Purified Mouse Immunoglobulin
Clone DF1524

Monoclonal Anti-Human CD11a (mouse IgG2b isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with human KG1 cultured cells. The isotype is determined using the Sigma ImmunoType™ Kit (Sigma Stock No. ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Sigma Stock No. ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I with purified CD11a monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The antibody is provided (300 µg/ml) as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA with 0.1% sodium azide (see MSDS)* as a preservative.

Description

FITC Monoclonal Anti-Human CD11a recognizes the human CD11a antigen expressed on lymphocytes, granulocytes, monocytes and macrophages. The human CD11a[2,3,4] antigen is the 180kD integrin α^L subunit of the non-covalently associated 180/95 kD heterodimeric glycoprotein also known as CD11a/CD18 or Leu-CAMa complex, or the lymphocyte function associated antigen-1 (LFA-1). The LFA-1 complex is an adhesion molecule involved in promotion of adhesion of cells to the vascular endothelium in association with its ligand CD54 (I-CAM-1). LFA-1 is important in cell-cell adhesion, T and B cell proliferation and T cell mediated cytolysis, and NK killing and ADCC of granulocytes and monocytes. Cells of patients with some leukemias, lymphomas and Leukocytic Adhesion Deficiency may lack LFA-1 or LFA-1α^L. Differential expression of LFA-1 on leukemic cells and lymphomas may be correlated to the aggressiveness of pathology. Besides I-CAM-1, LFA-1 interacts also with I-CAM-2 and I-CAM-3 (CD50). CD11a is expressed by virtually all peripheral blood leukocytes: lymphocytes, granulocytes, monocytes and macrophages. CD11a levels are increased on memory T cells and on monocytes following interferon treatment. The antibody inhibits aggregation response of monocytes but not that of FMLP treated granulocytes. It blocks LFA-1 mediated aggregation of SKW3 T Lymphoma cells. The antibody detects CD11a in cell suspensions and frozen lymphoid tissue sections but not in routine formalin-fixed, paraffin-embedded tissues.

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.

F/P Molar Ratio: 3.6

Uses

Monoclonal Anti-Human CD11a may be used for:
1. Detection and enumeration of leuknocytes in blood and tissues.
2. Studies of cell-cell interactions in health and disease.
3. Studies of transplantation rejection.
4. Studies of leukemias and lymphomas.

Storage

Store at 0-5°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 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.

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 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: 0.01M 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-6647).
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-6647) 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

1. Leucocyte Typing IV, Oxford University Press, pp. 548, 553, 691, (No. N222) (1989).
2. Kishimoto, T., et al., Adv. Immunol., **46,** 149 (1989).
3. Arnaout, M.A., Immunol. Rev., **114,** 145 (1990).
4. Clayberger, C., et al., Blood, **72,** 1431 (1980).

 Issued 12/94.