Product No. C-0551
Monoclonal Anti-Human CD11b
Purified Mouse Immunoglobulin
Clone 44

Lot 072H4838

Monoclonal anti-Human CD11b antibody (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with human monocytes.[1,2] The isotype is determined using the 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 provided as purified antibody (200 μg/ml) in 0.01M phosphate buffered saline pH 7.4 containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Description

Monoclonal anti-Human CD11b[1-4] antibody recognizes the 165-170 kD α-chain of the CD11b/CD18 complex, an α/β heterodimeric glycoprotein which belongs to the β2 integrin family. It is also known as Mac-1, CR3, MO-1 and C3bi receptor. CD11b[5,6] is expressed on the surface of circulating monocytes, granulocytes and certain NK cells. It is also present on subsets of macrophages. In granulocytes, it is present in subcellular granules and is translocated to the surface after activation.[7] Surface expression of CD11b/CD18 is capable of both functional and quantitative upregulation. CD11b/CD18 functions as a receptor for C3bi, clotting factor X, fibrinogen,and ICAM-1.[8-10] CD11b/CD18 is involved in a variety of cell-cell and cell-substrate interactions such as attachment and phagocytosis of particles coated with C3bi by granulocytes and macrophages and phagocytosis of opsonized pathogens. It also plays a role in the initiation of a coagulation protease cascade and in cell migration mechanisms. The endothelial cell counter-receptor for CD11b/CD18 is ICAM-1.
Monoclonal anti-Human CD11b can be used to stain acetone-fixed cryostat sections or cell preparations. The epitope recognized by the antibody is formalin sensitive.

Performance

When assayed by flow cytometric analysis, using 1 μg 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 CD11b may be used for:
1. Studies of cell adhesion and migration.
2. Detection and monitoring of leukocyte adhesion deficiencies.
3. Blood coagulation studies.

Storage

Store at 0-5°C. 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 hazardous 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 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 Product No. 1077-1).
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3, prechilled to 4°C.
3. Fluorochrome conjugated anti-mouse secondary antibody in diluent to recommended working dilution (Sigma Product No. F-2883 FITC Sheep anti-Mouse IgG (whole molecule), $F(ab')_2$ Fragment of Affinity Isolated Antibody is recommended). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype matched non-specific mouse immunoglobulin (negative control, Sigma Product No. M-5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Refrigerated centrifuge.
8. Counting chamber.
9. Trypan blue (Sigma Product No. T-0776), 0.2% in 0.01M phosphate buffered saline, pH 7.4.
10. Whole blood lysing solution.
11. Flow cytometer or fluorescent microscope.

Procedure

Prechill medium, perform all staining steps on ice and in a refrigerated centrifuge (2-6°C).

1. a. Use 100µl of whole blood **or**
   b. Adjust cell suspension to 2 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 50µl or 1 x 10^6 cells per tube.
2. Prepare dilutions of Monoclonal anti-Human CD11b in diluent to give a concentration of 20 µg/ml, i.e. 1:10 dilution of antibody. The CD11b antibody is provided at 200µg/ml.
3. Add 50µl of diluted monoclonal antibody to tube(s) containing cells to be stained, i.e. 1µg of monoclonal antibody per 1 x 10^6 cells in a final volume of 100µl. Tap tube gently to mix. Incubate the cells on ice for 30 minutes.
   Proper controls to be included for each sample are:
   a. An autofluorescence control: 50µl diluent in place of secondary antibody and monoclonal antibody.
   b. A negative staining control: 50µl isotype-matched non-specific mouse immunoglobulin at a concentration of 20µg/ml.
4. Pellet cells by centrifugation at 500 x g, 2-6°C, for 10 minutes.
5. Remove supernatant by careful aspiration.
6. Resuspend cells in 2 ml diluent.
7. Pellet cells as in step 4 and repeat washing procedure (steps 5-6) twice.
8. After the last wash, resuspend the cells in 50µl of the fluorochrome conjugated second antibody (diluted in diluent containing BSA) at the recommended concentration, except for autofluorescence controls. Resuspend cells for autofluorescence controls in diluent. Incubate on ice for 30 minutes. Protect from light at this and all subsequent steps.
   Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then pellet and wash cells as in steps 4-6 twice, and procede to step 10.
9. Centrifuge and wash as in steps 4-6 twice. Keep cells cold until analyzed.
10. After last wash, resuspend cells in 0.5 ml of diluent 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 maybe necessary to incubate the monoclonal antibody and cells (at step 4) in 10-20% normal serum from the second antibody host species in order to decrease non-specific staining with the conjugated second antibody.

References

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