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ProductInformation

Monoclonal Anti-Human CD11b
FITC Conjugate
Clone ICRF44
Purified Mouse Immunoglobulin

Product Number **F2648**

Product Description

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 the human monocytes. The isotype is determined using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion assay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2). The product is prepared by conjugation of fluorescein isothiocyanate (FITC) to purified CD11b monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC. No free FITC is detectable.

FITC Conjugated 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.

Monoclonal Anti-Human CD11b[1-4] antibody recognizes the 165-170 kDa 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.

Reagents

The product is provided as purified antibody 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.

Procedure

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 (peripheral blood mononuclear cells isolated on HISTOPAQUE® (Product Code 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 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 (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 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 - 7.
   b. A negative staining control: 10 µl of FITC conjugated, isotype-matched non-specific mouse immunoglobulin (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. Then proceed to Step 4.
   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.

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 that 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.

Product Profile

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

References

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