ProductInformation

Product No. B-0531
Monoclonal Anti-Human CD11b
Biotin Conjugate
Purified Mouse Immunoglobulin
Clone 44

Lot 023H4847

Monoclonal Anti-Human CD11b (mouse IgG1 isotype) is derived from the hybridoma produced by the fusion of mouse myeloma cell line and splenocytes from BALB/c mice immunized with human monocytes. 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 prepared by conjugation of ε-amino caproyl biotin to purified CD11b monoclonal antibody. The conjugate is provided as purified immunoglobulin (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 CD11b 165-170 kD α-chain of the CD11b/CD18 complex, an α/β heterodimeric glycoprotein which belongs to the β2 integrins 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 in 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-CD11b antibody 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 10 µl of the antibody per 1 X 10^6 cells and ExtrAvidin®-FITC Conjugate (Sigma Product No. E-2761), 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

Biotin Monoclonal Anti-Human CD11b antibody may be used for:

1. Studies of cell adhesion and migration.
2. Detection and monitoring of leukocyte adhesion
3. Blood coagulation studies.

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 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 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 Indirect Immunofluorescent Staining using Biotinylated Primary Antibodies

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 Stock No. 1077-1).
2. Diluent: 0.01M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated avidin derivative diluted to recommended working dilution in diluent. Appropriate products for use are ExtrAvidin®-FITC (Sigma Product No. E-2762), Streptavidin-FITC (Sigma Product No. S-3402), Streptavidin-PE (Sigma Product No. S-3762), or Streptavidin-Quantum Red™ (Sigma Product No. S-2899).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. 0.2% Trypan blue (Sigma Product No. T-0776) in 0.01M 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 (e.g., trypan blue). For each sample, add 100 µl or 1 x 10^6 cells per tube.
2. Add 10 µl of biotinylated monoclonal antibody to tube(s) containing cells to be stained. Vortex tube gently to mix. Incubate the cells at room temperature (18 - 22°C) for 30 minutes.
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 diluent.
7. Repeat washing procedure (steps 4-6) twice.
8. After the last wash, resuspend the cells in 100 µl of the
e recommended concentration. For the autofluorescence control, add 100 µl
2°C) for 30 minutes. Protect from light at this and all subsequent steps.

Note: If whole blood is used, use lysing solution after
ns, then pellet and wash cells as in steps 4-6 twice, and proceed to step 10.

9. Centrifuge and wash as in steps 4-6 twice.
10. After last wash, resuspend cells in 0.5 ml of diluent or
re analyzing) and analyze in a flow cytometer according to manufacturer's

References

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