Product No. F-5898
Lot 054H4861

Monoclonal Anti-Human CD36
FITC Conjugate

Purified Mouse Immunoglobulin
Clone SMØ

Monoclonal Anti-Human CD36 (mouse IgM isotype) is derived from the SMØ hybridoma produced by the fusion of mouse myeloma cells and splenocytes from mice immunized with human tonsil followed by peripheral blood monocytes. 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 CD36 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound FITC, no free FITC is detectable. The antibody is provided (100 µg/ml) as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Description

FITC Monoclonal Anti-Human CD36 recognizes the CD36 (88-90 kD) human cell surface glycoprotein.[1,2] CD36 (Platelet GPIV/GPIIIB) is a single chain membrane glycoprotein found on platelets and their precursors, some endothelial cells, adipocytes and several human tumor cell lines. It is expressed by neoplastic vascular endothelial cells and well differentiated tumors of adipose tissues. It is not found on lymphocytes or on adult erythrocytes. CD36 is a putative platelet adhesion receptor for thrombospondin[3] and an endothelial receptor for *Plasmodium falciparum*-infected red blood cells[4,5] and possibly for collagen.[6] It is involved in platelet-platelet, platelet-monocyte and platelet-tumor cell interactions. FITC Monoclonal Anti-Human CD36 recognizes CD36-transfected cells, but does not react with SDS-denatured CD36 under reducing or non-reducing conditions. It inhibits collagen induced platelet aggregation and is lytic to platelets in plasma. The antibody inhibits ingestion of apoptotic polymorphonuclear cells by macrophages. The antibody partially blocks adhesion of *P. falciparum*-infected red blood cells to immobilized CD36. It may be used in ELISA and on acetone- fixed frozen sections. The conformational epitope recognized by this antibody is sensitive to routine formalin-fixation and paraffin-embedding.

F/P Molar Ratio: 6.4

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.

Uses

FITC Monoclonal Anti-Human CD36 may be used for:

1. Studies of CD36 function in monocytes, platelets and endothelial cells.
2. Investigations of surface alterations in human erythrocytes infected with *Plasmodium falciparium* and their interaction with endothelial cells.

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-7022).
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-7022) 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% para-formaldehyde. 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. Hogg, N., et al., Immunology, **53**, 753 (1984).

2. Leukocyte Typing IV, Knapp, W., et al. (eds.), Oxford University Press, Oxford, pp. 960 (1989).
3. Oquendo, P., et al., Cell, **58**, 95 (1989).
4. Lawler, J., Blood, **67**, 1197 (1986).
5. Hovard, R.J. and Gilladoga, A.D., Blood, **74**, 2603 (1989).
6. Tandon, N., et al., J. Biol. Chem., **264**, 7576 (1989).