MONOCLONAL ANTI-HUMAN CD44
CLONE A3D8
BIOTIN CONJUGATE
Purified Mouse Immunoglobulin

Product No. **B1156**

Monoclonal Anti-Human CD44 (mouse IgG1 isotype) is derived from the A3D8 hybridoma produced by the fusion of mouse myeloma cell line and splenocytes from BALB/c mice immunized with circulating human Sezary T cells.[1] 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 CD44 monoclonal antibody. The conjugate is provided as purified immunoglobulin in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 0.1% sodium azide (see MSDS)* as a preservative.

Description
Monoclonal Anti-Human CD44 antibody[1,2,3,4] recognizes the CD44 80-95 kD human cell surface glycoprotein. CD44 antigen (Pgp-1, ECM-III, HUTCH-1, Hermes antigens) is a transmembraneous molecule with extensive O-linked glycosylation. CD44 antigen is expressed on a variety of cell types including peripheral blood leukocytes (B and T lymphocytes, monocytes, granulocytes) and red cells and as a soluble form in plasma. It is a backbone molecule for the frequent In^a and the rare In^b group antigens which are expressed on a variety of peripheral blood and hematopoietic cells. CD44 is also detectable in bone marrow nucleated cells, medullary thymocytes, liver Kupffer cells, fibroblasts, corneal cells, a subset of pancreatic acinar cells and brain cells. It is also weakly expressed in platelets. CD44 molecules mediate a variety of functions: leukocytes-endothelial cell binding, lymphocyte homing, extracellular matrix binding, enhancement of T cell activation and adhesion to monocytes.[3] The epitope recognized by monoclonal A3D8 is sensitive to formalin fixation and paraffin embedding.

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 CD44 antibody may be used for:
1. Studies of cell-cell and cell substrate interactions in a variety of normal, inflamed and neoplastic tissues.
2. Studies of lymphocyte homing.
3. Studies of functional activation of T cells.

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 2-8°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: 0.01 M 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. E2762), Streptavidin-FITC (Sigma Product No. S3402), Streptavidin-PE (Sigma Product No. S3762), or Streptavidin-Quantum

Red™ (Sigma Product No. S2899).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. 0.2% Trypan blue (Sigma Product No. T0776) in 0.01 M 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 fluorochrome conjugated avidin derivative at the recommended concentration. For the autofluorescence control, add 100 µl of diluent. Incubate at room temperature (18 - 22°C) for 30 minutes. Protect from light at this and all subsequent steps.
9. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10.
   b. If a mononuclear cell suspension is used, proceed to Step 10.
10. Centrifuge and wash as in steps 4 - 6 twice.
11. After last wash, resuspend cells in 0.5 ml of diluent or 2% paraformaldehyde (if cells are stored before analyzing) and analyze in a flow cytometer according to manufacturer's instructions.

References

1. Telen, M., et al., J. Clin. Invest., **71**, 1878 (1983).
2. Haynes, B., et al., Immunology Today, **10**, 423 (1989).
3. Hale, L., et al., J. Immunol., **143**, 3944 (1989).
4. Denning, S., et al., J. Immunol., **144**, 7 (1990).

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