MONOCLONAL ANTI-HUMAN CD44 CLONE A3D8
R-Phycoerythrin Conjugate
Purified Mouse Immunoglobulin

Product No. **P7312**

Monoclonal Anti-CD44 (mouse IgG1 isotype) is derived from the A3D8 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with circulating malignant human Sezary T cells. The isotype is determined using 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 R-Phycoerythrin (R-PE) to purified CD44 monoclonal antibody. The conjugate is then purified by gel filtration to remove unbound R-PE, The conjugate is provided in 0.01M phosphate buffered saline, pH 7.4, containing 1% BSA with 15 mM sodium azide (see MSDS)* as a preservative.

Description
Monoclonal Anti-Human CD44 recognizes the CD44 human cell surface glycoprotein. CD44 (PgP-1, ECM-III, HUTCH-1, Hermes antigens) is a transmembraneous 80-95 kD glycoprotein with extensive O-linked glycosylation. The extracellular domain has 6 potential glycosylation sites. It is widely distributed on many tissues and in 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 molecules mediate a variety of functions: leukocyte-endothelial cell binding, lymphocyte homing, extracellular matrix binding, enhancement of T cell activation and adhesion to monocytes. Monoclonal antibodies to CD44 are powerful tools in the analysis of these functions. The CD44 antigen is expressed on a variety of cell types including peripheral blood leukocytes (B and T lymphocytes, monocytes, granulocytes) and red cells. It is also weakly expressed on platelets. The antibody is also reactive with bone marrow nucleated cells, medullary thymocytes, liver Kupffer cells, fibroblasts, corneal cells, epidermal keratinocytes, synovial cells, a subset of pancreatic acinar cells and brain cells. The epitope recognized by this clone is sensitive to formalin fixation and paraffin embedding.

Performance
When assayed by flow cytometric analysis, using 10 µl of the antibody to stain 1 x 10^6 cells, a fluorescence intensity and percent positive are observed similar to that obtained with saturating monoclonal antibody levels.

Antibody Concentration will be stated on certificate of analysis.

F/P Molar Ratio: 1 to 4

Uses
Monoclonal Anti-Human CD44 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.

Storage
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.

* 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 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: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. R-PE conjugated, isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. P4685).
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue (Sigma Product No. T0776), 0.2% 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 conjugate 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.
   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 - 8.
   b. A negative staining control: 10 µl of R-PE conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3 - 8.
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 3-6) twice.
   Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then repeat steps 3-6 twice, and proceed to step 8.
8. 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.

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 may be necessary to incubate the cells (at step 2 before adding monoclonal antibody) 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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