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Product Information

Monoclonal Anti-CD44, clone A3D8
produced in mouse, purified immunoglobulin

Catalog Number **C7923**

Product Description

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 by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2.

Monoclonal Anti-CD44 recognizes the CD44 human cell surface glycoprotein. CD44 (PgP-1, ECM-III, HUTCH-1, Hermes antigens) is a transmembraneous 80-95 kDa 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: eukocyte-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, granuloctes) 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.

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.

Reagent

The product is provided as purified antibody (200 μg/ml) in 0.01 M phosphate buffered saline, pH 7.4, containing 1% BSA and 15 mM sodium azide as a preservative.

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Performance

When assayed by flow cytometric analysis using 5 μL of the antibody to stain 1×10^6 cells, a fluorescence intensity is observed similar to that obtained with 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.

Storage/Stability

Store at 2-8 °C for up to one month.

For extended storage, the solution may be frozen in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Procedure

Reagents and Equipment Needed but Not Provided

- Whole human blood collected by standard clinical blood evacuation tubes with EDTA, ACD-A, or heparin anticoagulant **or**
  Human cell suspension (e.g. peripheral blood mononuclear cells isolated on HISTOPAQUE®, Catalog Number 10771.

- Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1%NaN_3.
- Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent, e.g., Anti-Mouse IgG (whole molecule) $F(ab')_2$ fragment-FITC, Catalog Number F2883. Aggregates in conjugates should be removed by centrifugation immediately prior to use.
- Isotype-matched, non-specific mouse immunoglobulin (negative control, Catalog Number M5284.
- 12 x 75 mm test tubes
- Adjustable micropipette
- Centrifuge
- Counting chamber
- 0.2% Trypan blue, Catalog Number 302643, in 0.01 M phosphate buffered saline, pH 7.4.
- 2% paraformaldehyde in PBS
- Whole blood lysing solution
- Flow cytometer.

Procedure

1. Use 100 µL of whole blood **or**
   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 1×10^6 cells per tube.
2. Add 5 µL of monoclonal antibody 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. Autofluorescence control: 5 µL of diluent in place of monoclonal antibody.
   b. Negative staining control: 5 µl of isotype-matched non-specific mouse immunoglobulin, Catalog Number M5284, at the same concentration as test antibody.
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 of diluent.
7. Repeat washing procedure (steps 4-6).
8. After the second wash, resuspend the cells in 100 µL of the fluorochrome conjugated secondary antibody 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. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10. **or**
   If a mononuclear cell suspension is used, proceed to Step 10.
10. Add 2 ml of diluent to all tubes.
11. Wash as in steps 4-6 twice.
12. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde 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 in 10-20% normal serum from the second antibody host species (at step 2 before adding monoclonal antibody) in order to decrease non-specific staining with the conjugated second antibody.

References

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