ProductInformation

Monoclonal Anti-Human CD9
Purified Mouse Immunoglobulin
Clone DU-ALL-1

Product No. **C7548**
Lot 073H4808

Monoclonal Anti-Human CD9 (mouse IgG1 isotype) is derived from the DU-ALL-1 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from BALB/c mice immunized with fresh common acute lymphoblastic leukemia cells. The isotype is determined using Sigma ImmunoType[tm] 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 provided as purified antibody (200 μg/ml) 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 CD9 recognizes the CD9 human cell surface glycoprotein. CD9 is a 24 kD single-chain membrane protein lacking a transmembrane moiety and N-linked sugars. It is reported to be associated with protein kinase activity and with mitogenicity for a pancreatic adenocarcinoma cell line. This antigen is expressed mainly on pre-B cells, monocytes and platelets. Many CD9 monoclonal antibodies induce platelet activation. The purified non-heated CD9 monoclonal is capable of aggregating platelets. The antibody reacts with pre-B cells, monocytes, platelets, non-T, non-B ALL and some AML. It does not react with mature B or T lymphocytes or erythrocytes. It is reactive with many non-hematopoietic cells (e.g. fibroblasts, epithelium, tubular epithelium, arteriolar smooth muscle). The epitope recognized by this clone is sensitive to formalin fixation and paraffin embedding.

Performance
When assayed by flow cytometric analysis, 5 μl of the monoclonal antibody will stain 1 x 10^6 cells with FITC-Sheep Anti-Mouse IgG (whole molecule), $F(ab')_2$ fragment of Affinity Isolated Antibody). positive that is similar to that observed with saturating amounts of monoclonal antibody.

Uses
Monoclonal Anti-CD9 may be used for:
1. Identification and characterization of leukemias.
2. *In vitro* elimination of leukemic cells.
3. Platelet activation studies.
4. Purification of CD9 antigen.

Storage
Store at 2-8EC. 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 Indirect Immunofluorescent Staining

Reagents and Materials Needed but Not Supplied
1. a. Whole human blood collected by standard
   clinical technique (e.g. collected with EDTA, ACD-A or heparin anticoagulant **or**
   b. Human cell suspension (e.g. peripheral blood mononuclear cells isolated on HISTOPAQUE[TM] 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[TM]) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Sigma Product No. F2883 FITC-Sheep Anti-Mouse IgG (whole molecule), $F(ab')_2$ fragment of Affinity Isolated Antibody). Aggregates in conjugates should be removed by centrifugation immediately prior to use.
4. Isotype-matched, non-specific mouse immunoglobulin (negative control, Sigma Product No. M5284).

5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Sigma Product No. T0776) in

10. 2% paraformaldehyde in PBS.
11. Whole blood lysing solution.
12. Flow cytometer.

Procedure

1. a. Use 100 µl of whole blood **or**

b. Adjust cell suspension to 1 x 10^7 cells/ml in

e exclusion (e.g., trypan blue). For each sample, add 100 µl

2. Add 5 µl of monoclonal antibody to tube(s) con-

ıcubate the cells at room temperature (18 - 22EC) for 30 min-

Proper controls to be included for each sample are:

a. Autofluorescence control: 5 µl diluent in

b. Negative staining control 1: 5 :l isotype-

duct No. 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

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

recommended concentration. For the autofluorescence

re (18 - 22EC) for 30 minutes. Protect from light at this and

Note: If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, 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 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 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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