SIGMA CHEMICAL COMPANY

Product No. C-9062
Lot 084H8966

Monoclonal Anti-Human CD29
Purified Mouse Immunoglobulin
Clone P4C10

Monoclonal Anti-Human CD29, clone P4C10 (mouse isotype IgG1) is derived from the hybridoma produced by the fusion of mouse myeloma cell line NS-1/FOX-NY and splenocytes from RBF/Dn mice immunized with non-trypsinized HT1080 cells. The product is provided as purified immunoglobulin (300 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

CD29 is expressed on resting and activated leukocytes and on non-lymphoid cells. CD29 is the β_1 subunit of integrin. Integrins share the common structural feature of two non-covalently associated subunits, α and β. There are at least eight β-subunits which show strong homology to each other and 12 α-subunits which exhibit less sequence conservation. The amino acid sequence homology of the β-subunits is between 40-48%. All β-subunits contain 56 conserved cysteines arranged in four repeating units. The N-terminus is in the extracellular domain. The cytolasmic domain is generally short, 40-50 amino acids long, except for β_4. Phosphorylated serine and tyrosine have been reported in the cytoplasmic domains of the integrin β subunits. CD29 polypeptide β_1 has potential tyrosine phosphorylation sites and an apparent MW of 110 kD in non-reduced SDS-PAGE. In contrast to earlier classification schemes, individual α-subunits have been shown to associate with more than one type of β-subunit. CD29 is known to associate with $\alpha_{1\text{-}6}$CD49a-f. The non-covalent complexes of CD49a-f/CD29 are also known as very late antigens (VLA). Interaction of the cytoplasmic domain of CD29 and other β subnits with the cytoskeleton has been proposed as a mechanism for linking the cytoskeleton with the extracellular matrix. Integrin molecules bind a wide variety of extracellular matrix proteins such as collagen, laminin, fibronectin, thrombospondin, vitronectin and von Willebrand factor. Integrins also function in cell-cell adhesion. The cell surface ligands for integrins are members of the immunoglobulin superfamily, Cell Adhesion Molecules (CAM).

Performance

When assayed by flow cytometric analysis, using 5 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

Monoclonal Anti-Human CD29 antibody may be used for:

1. Inhibition of cell-cell adhesion.
2. Flow cytometry/immunocytochemistry identification and localization of CD29 expression.

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 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.

Procedure for Indirect Immunofluorescent Staining

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.

Reagents and Materials Needed but Not Supplied (cont.)

3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. Sigma Product No. F-2883 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. M-5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipet.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (Sigma Product No. T-0776) in 0.01 M phosphate buffered saline, pH 7.4.
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 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 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 diluent in place of monoclonal antibody.
   b. Negative staining control: 5 µl isotype-matched non-specific mouse immunoglobulin (Sigma Product No. M-5284) 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 the cells in 100 µl of the fluorochrome conjugated secondary antibody at the recommended concentration. For the a
7. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 8.
8. Add 2 ml diluent to all tubes.
9. Wash as in steps 4-5.
10. After last wash, resuspend cells in 0.5 ml of 2% paraformaldehyde and analyze in a flow cytometer according to manufacturer's

Quality Control

It is advisable to run the appropriate negative controls. Negative controls establish background fluorescence and non-specific staining 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 m

References

Hemler, H., Ann. Rev. Immunol., **8**, 365 (1990).
Carter, W., et al., J. Cell Biology, **110**, 1387 (1990).