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Product Information

Monoclonal Anti-CD31 (PECAM-1)-FITC
Clone WM-59
produced in mouse, purified immunoglobulin

Catalog Number **F8402**

Product Description

Monoclonal Anti-CD31 (PECAM-1) (mouse IgG1 isotype) is derived from the WM-59 hybridoma produced by the fusion of mouse myeloma cells and splenocytes from an immunized mouse. The human cell line RC-2A originally derived from myeloid leukemia cells was used as the immunogen. The isotype is determined by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents, Catalog Number ISO2. The product is prepared by conjugation of fluorescein isothiocyanate (FITC) Isomer I with Protein A purified CD31 monoclonal antibody.

Monoclonal Anti-CD31 (PECAM-1)- FITC recognizes the human CD31 antigen expressed on platelets, endothelial cells, myeloid cells, B lymphocytes and certain T lymphocyte subsets.

The human CD31 antigen (Platelet Endothelial Cell Adhesion Molecule, PECAM-1, endoCAM. gpIIa, hec7) is a 130-140 kDa single chain integral membrane glycoprotein member of the immunoglobulin gene superfamily of cell adhesion molecules. It consists of six Ig-like loops in the extracellular domain, a trans-membrane domain and a relatively long cytoplasmic domain.[1,2] Human CD31 is expressed on platelets, myeloid cells, B lymphocytes, certain T-lymphocyte cell subsets, bone marrow precursor cells and NK cells. CD31 is abundantly expressed in endothelial cells. It becomes localized to the intracellular junctions in monolayers of cultured endothelial cells.[3] CD31 (PECAM-1) functions in homophilic and heterophilic cell-cell adhesion and cell signalling activities. It plays a major role in the transmigration of monocytes, neutrophils and NK cells between the endothelial cell junctions into the subendothelial matrix. The $\alpha_v\beta_3$ (CD51/61) integrin has been suggested to interact with CD31.[4] CD31 also binds to glycosaminoglycans (GAG).[5] Endothelial CD31 is phosphorylated on tyrosine and serine residues. Tyrosine dephosphorylation, following integrin engagement, probably plays a role during endothelial cell engagement. Phosphorylation of serine residues in the cytoplasmic domain of platelet CD31 occurs following activation. CD31 (PECAM-1) is possibly involved in some of the interactive events taking place during cardiovascular development inflammation, thrombosis, wound healing and angiogenesis. Monoclonal Anti-CD31 (PECAM-1) was shown to increase the rate of homotypic aggregation induced in U937 cells by TGFβ1.[6] Its binding to platelets is reported to be enhanced following their washing and concomitant activation.[6]

Reagents

Supplied 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.

Storage/Stability

Store at 2-8 °C. Do not freeze. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use.

Note: Store product protected from light.

Product Profile

Monoclonal Anti-CD31 (PECAM-1)- FITC may be used for the detection and enumeration of CD31 cells in blood and tissues, and for studies of CD31 function in cell-cell interactions.

When assayed by flow cytometric analysis (with a FACScan flow cytometer) using 10 µL of the antibody to stain 1 x 10^6 cells or 100 µL whole blood, 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.

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®, Catalog Number 10771).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1% NaN_3.
3. FITC conjugated, isotype-matched, non-specific mouse immunoglobulin, negative control, Catalog Number F6397.
4. 12 x 75 mm test tubes.
5. Adjustable micropipet.
6. Centrifuge.
7. Counting chamber.
8. Trypan blue, Catalog Number T0776, 0.2% in 0.01 M PBS, 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 (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. 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 – 7.
   b. A negative staining control: 10 μL of FITC conjugated, isotype-matched non-specific mouse immunoglobulin at the same concentration as test antibody followed by steps 3 - 7
3. a. If whole blood is used, use lysing solution after incubation and wash cells according to manufacturer's instructions.
   b. If a mononuclear cell suspension is used, proceed to Step. 4.
4. Add 2 ml of diluent to all tubes.
5. Pellet cells by centrifugation at 500 x *g* for 10 minutes.
6. Remove supernatant by careful aspiration.
7. Resuspend cells in 0.5 ml of 2% paraformaldehyde. 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 binding of the primary and secondary antibodies. The ideal negative control reagent is a mouse monoclonal or myeloma protein which has no reactivity with human cells. It should be isotype-matched to the antibody and of the same concentration and F/P molar ratio as the 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.

References

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6. Leucocyte Typing V, Oxford University Press, pp 1259-1265, (PO25) (1995).

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