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# ProductInformation

**MONOCLONAL ANTI-HUMAN CD31 (PECAM-1)**
**Clone WM-59**
Purified Mouse Immunoglobulin

Product Number **C 7714**

## Product Description

Monoclonal Anti-Human CD31 (PECAM-1) (mouse IgG1 isotype) is produced 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 using Sigma ImmunoType™ Kit (Product Code ISO-1) and by a double diffusion immunoassay using Mouse Monoclonal Antibody Isotyping Reagents (Product Code ISO-2).

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

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

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 6 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 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 is possibly involved in some of the interactive events taking place during cardiovascular development, inflammation, thrombosis, wound healing and angiogenesis. Monoclonal Anti-Human CD31 increased the rate of homotypic aggregation induced in U937 cells by TGFβ1.[6] Its binding to platelets was enhanced following their washing and concomitant activation.[6]

## Reagents

The product is Protein A purified and provided in 0.01 M phosphate buffered saline pH 7.4 containing 1% BSA and 15 mM sodium azide as a preservative.

## Precautions and Disclaimer

Due to the sodium azide content a material safety 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.

## Storage/Stability

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

## Procedure

Indirect 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® (Product Code1077-1)).
2. Diluent: 0.01 M phosphate buffered saline (PBS), pH 7.4, containing 1% BSA and 0.1%$NaN_3$.
3. Fluorochrome (FITC, PE, or Quantum Red™) conjugated anti-mouse secondary antibody diluted to recommended working dilution in diluent (e.g. 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, Product No. M 5284).
5. 12 x 75 mm test tubes.
6. Adjustable micropipette.
7. Centrifuge.
8. Counting chamber.
9. 0.2% Trypan blue (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 (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 to 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 1: 5 µl isotype-matched non-specific mouse immunoglobulin (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 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. a. If whole blood is used, use lysing solution after incubation according to manufacturer's instructions, then proceed to Step 10.
   b. 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.

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.

**Product Profile**

When assayed by flow cytometric analysis (with a FACScan flow cytometer) using 5 µl of the antibody to stain 1 x $10^6$ or 100 µl whole blood 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.

**References**


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