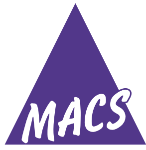

CD146 antibodies
human

CD146-FITC	130-092-851
CD146-PE	130-092-853
CD146-APC	130-092-849
CD146-Biotin	130-092-852
CD146 pure	130-092-850

Contents

1. Description
   - 1.1 Background information
   - 1.2 Applications
   - 1.3 Recommended antibody dilution
   - 1.4 Reagent requirements
2. General protocol for immunofluorescent staining
3. Examples of immunofluorescent staining with CD146 antibodies
4. References

1. Description

Components 1 mL CD146 antibodies, human: monoclonal CD146 antibodies conjugated to fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.

Clone 541-10B2 (isotype: mouse IgG1).

Capacity 100 tests or up to 10^9 total cells.

Product format Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background information

CD146 (also known as MUC18, MCAM, Mel-CAM, and S-Endo-1) is a transmembrane glycoprotein belonging to the immunoglobulin superfamily and contains 5 extracellular immunoglobulin (Ig)-like domains.[1,2] CD146 possesses a limited tissue distribution, including endothelial cells, smooth muscle cells, follicular dendritic cells, melanoma cells, and a sub-population of activated T lymphocytes.[1,3] CD146 is also expressed on circulating endothelial cells (cECs) and a subpopulation of endothelial progenitor cells (cEPCs)[4] as well as on marrow stromal cells (MSCs)[5].
CD146 functions as a Ca^{2+}-independent cell adhesion molecule that is involved in heterophilic cell-cell interactions[6], and may be involved in the extravasion and/or homing of activated T cells[3]. CD146 expression in melanoma is also directly associated with tumor growth and metastasis.[7,8]

1.2 Applications

- Identification and enumeration of CD146$^+$ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Human endothelial cells can be isolated by using the CD31 MicroBead Kit, human (# 130-091-935).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD146 conjugate	FITC	PE	APC	Biotin
Flow cytometry[a]				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells[b]	1:11	1:11	1:11	1:11

a) Given antibody dilutions are for a cell concentration of up to 10^7 cells/100 µL of buffer.
b) For optimal results, cells must be stained prior to fixation.

1.4 Reagent requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS) pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS™ Rinsing Solution (# 130-091-222). Keep buffer cold (2−8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) Anti-Biotin-FITC (# 130-090-857), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856) as secondary antibody reagent in combination with CD146-Biotin.
- (Optional) CD31-PE (# 130-092-653) or CD31-APC (# 130-092-652).
- (Optional) Anti-FITC MicroBeads (# 130-048-701), Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

2. General protocol for immunofluorescent staining

▲ Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD146 antibody.

140-001-904.02

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5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.

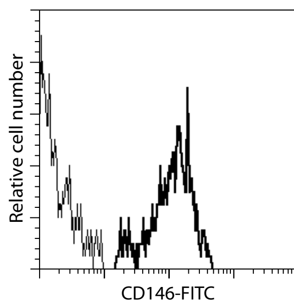
7. (Optional) If CD146-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody (Anti-Biotin-FITC, Anti-Biotin-PE, or Anti-Biotin-APC), and continue as described in steps 5 and 6.

8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

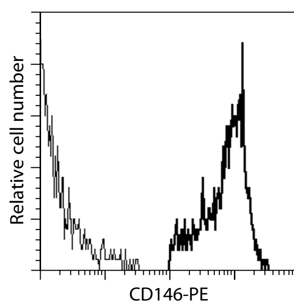
3. Examples of immunofluorescent staining with CD146 antibodies

Human umbilical vein endothelial cells (HUVECs) were passaged three times and then stained with CD146 antibodies conjugated to FITC (a), PE (b), or APC (c), and analyzed by flow cytometry (black line). Cells stained with CD146-Biotin (d) were also stained with Anti-Biotin-PE (black line). Fc receptor–mediated binding was prevented by using FcR Blocking Reagent. Unstained HUVECs were used as a negative control (grey line). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

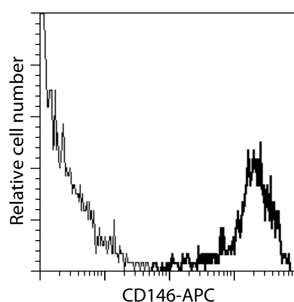
(a) HUVECs stained with CD146-FITC.



(b) HUVECs stained with CD146-PE.



(c) HUVECs stained with CD146-APC.



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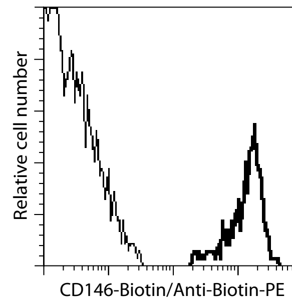
(d) HUVECs stained with CD146-Biotin as well as Anti-Biotin-PE.



4. References

1. Bardin, N. *et al.* (2001) Identification of CD146 as a component of the endothelial junction involved in the control of cell-cell cohesion. Blood 98: 3677–3684.
2. Yan, X. *et al.* (2003) A novel anti-CD146 monoclonal antibody, AA98, inhibits angiogenesis and tumor growth. Blood 102: 184–191.
3. Pickl, W.F. *et al.* (1997) MUC18/MCAM (CD146), an activation antigen of human T lymphocytes. J. Immunol. 158: 2107–2115.
4. Delorme, B. *et al.* (2005) Presence of endothelial progenitor cells, distinct from mature endothelial cells, within human CD146$^+$ blood cells. Thromb. Haemost. 94: 1270–1279:
5. Filshie, R. J. A. *et al.* (1998) MUC18, a member of the immunoglobulin superfamily, is expressed on bone marrow fibroblasts and a subset of hematological malignancies. Leukemia 12: 414–421.
6. Shih, J.M. (1999) The role of CD146 (Mel-CAM) in biology and pathology. J. Pathol. 189: 4–11.
7. Xie, S. *et al.* (1997) Expression of MCAM/MUC18 by human melanoma cell leads to increased tumor growth and metastasis. Cancer Res. 57: 2295–2303.
8. Satyamoorthy, K. *et al.* (2001) Mel-CAM-specific genetic suppressor elements inhibit melanoma growth and invasion through loss of gap junctional communication. Oncogene 20: 4676–4684.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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