

# CD146 (LSEC) antibodies, mouse

**For research use only**

9 µg equal 60 tests, 30 µg equal 200 tests. One test corresponds to labeling of 10<sup>6</sup> cells.

| Product                        | Content        | Order no.   |
|--------------------------------|----------------|-------------|
| CD146 (LSEC)-FITC <sup>1</sup> | 9 µg in 300 µL | 130-102-790 |
| CD146 (LSEC)-FITC <sup>1</sup> | 30 µg in 1 mL  | 130-102-230 |
| CD146 (LSEC)-PE                | 9 µg in 300 µL | 130-102-844 |
| CD146 (LSEC)-PE                | 30 µg in 1 mL  | 130-102-319 |
| CD146 (LSEC)-APC               | 9 µg in 300 µL | 130-102-846 |
| CD146 (LSEC)-APC               | 30 µg in 1 mL  | 130-102-277 |
| CD146 (LSEC)-VioBlue           | 9 µg in 300 µL | 130-103-378 |
| CD146 (LSEC)-VioBlue           | 30 µg in 1 mL  | 130-102-739 |
| CD146 (LSEC)-PerCP-Vio700      | 9 µg in 300 µL | 130-103-865 |
| CD146 (LSEC)-PerCP-Vio700      | 30 µg in 1 mL  | 130-103-795 |
| CD146 (LSEC)-Biotin            | 9 µg in 300 µL | 130-101-991 |
| CD146 (LSEC)-Biotin            | 30 µg in 1 mL  | 130-101-866 |

<sup>1</sup>Not recommended for cells that are labeled with MACS MicroBeads using the same antigen.

## 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.

## Technical data and background information

|                                        |                                                                                                                                                  |
|----------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Antigen</b>                         | CD146 (LSEC)                                                                                                                                     |
| <b>Clone</b>                           | ME-9F1                                                                                                                                           |
| <b>Isotype</b>                         | rat IgG2ak                                                                                                                                       |
| <b>Isotype control</b>                 | Rat IgG2a – isotype control antibodies                                                                                                           |
| <b>Alternative names of antigen</b>    | MCAM, 1-gicerin, CD149, MUC18, S-endo, s-gicerin, Endo-CAM, Mel-CAM                                                                              |
| <b>Molecular mass of antigen [kDa]</b> | 69                                                                                                                                               |
| <b>Distribution of antigen</b>         | bone marrow, dendritic cells, endothelial cells, epithelial cells, fibroblasts, mesenchymal stem cells, ES and iPS cells, smooth muscle, T cells |
| <b>Product format</b>                  | Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.                                                                  |
| <b>Fixation</b>                        | The antibody is suited for staining of formaldehyde-fixed cells.                                                                                 |
| <b>Storage</b>                         | Store protected from light at 2–8 °C. Do not freeze.                                                                                             |

The CD146 (LSEC) antibody (clone ME-9F1) binds to the CD146 antigen, which is expressed on mouse endothelial cells, including liver sinusoidal endothelial cells (LSECs), smooth muscle cells, and the basal membrane.<sup>1</sup>

LSECs are microvascular endothelial cells lining the hepatic sinusoidal wall. Their strategic positioning favors a tight interaction with lymphocytes migrating through the liver. They possess a high capacity for antigen uptake and processing. However, in contrast to professional antigen-presenting cells (e.g. dendritic cells), they express only low levels of costimulatory molecules.<sup>2</sup>

## Reagent requirements

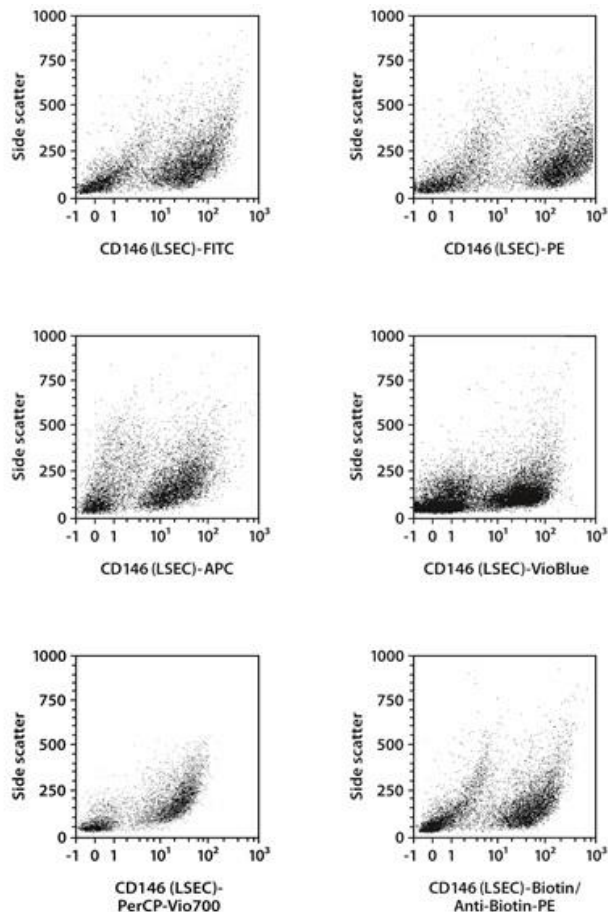
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).  
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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.

## Protocol for cell surface staining

- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10<sup>6</sup> cells/50 µL of buffer.
  - Volumes given below are for up to 10<sup>6</sup> nucleated cells. When working with fewer than 10<sup>6</sup> 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<sup>6</sup> 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<sup>6</sup> nucleated cells per 45 µL of buffer.
  4. Add 5 µL of the antibody.
  5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).  
Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice requires increased incubation times.
  6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
  7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of fluorochrome-conjugated anti-biotin antibody, 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.

## Examples of immunofluorescent staining

Mouse liver cells were stained with CD146 (LSEC) antibodies. Cells stained with CD146 (LSEC)-Biotin were also stained with Anti-Biotin-APC and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



## References

1. **Harder et al.** (1991) Dissection of murine lymphocyte-endothelial cell interaction mechanisms by SV-40-transformed mouse endothelial cell lines: novel mechanisms mediating basal binding, and alpha 4-integrin-dependent cytokine-induced adhesion. *Exp. Cell Res.* 197: 259–267.
2. **Diehl, L. et al.** (2008) Tolerogenic maturation of liver sinusoidal endothelial cells promotes B7-homolog 1-dependent CD8<sup>+</sup> T cell tolerance. *Hepatology* 47: 296–305.

## Warranty

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