

## 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 (LSEC) antibodies
4. References

## 1. Description

<b>Components</b>	1 mL CD146 (LSEC) antibodies, mouse: monoclonal CD146 antibodies conjugated to fluorescein isothiocyanate (FITC) or biotin.
<b>Clone</b>	ME-9F1 (isotype: rat IgG2a).
<b>Capacity</b>	100 tests or up to 10 <sup>9</sup> total cells.
<b>Product format</b>	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.
<b>Storage</b>	Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

### 1.1 Background information

The CD146 (LSEC) antibody recognizes 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> LSECs are supposed to mainly contribute to the control of immune responses against circulating soluble antigens in the liver.

### 1.2 Applications

- Identification and enumeration of CD146<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Mouse CD146<sup>+</sup> cells can be isolated by using, for example, CD146 (LSEC) MicroBeads, mouse (# 130-092-007).

### 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

CD146 (LSEC) conjugate	FITC	Biotin
<b>Flow cytometry<sup>a</sup></b>		
- In general	1:11	1:11
- Formaldehyde-fixed cells	n. r.	1:11
- CD146 (LSEC) MicroBead-labeled cells	1:11	1:11
<b>Immunohistochemistry<sup>b</sup></b>		
a) Given antibody dilutions are for a cell concentration of up to 10 <sup>7</sup> cells/100 µL of buffer.		
b) The optimal antibody dilution should be determined.		
n. r.: not recommended		

### 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 mouse serum albumin, mouse serum, or fetal bovine serum. 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) 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 (LSEC)-Biotin.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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

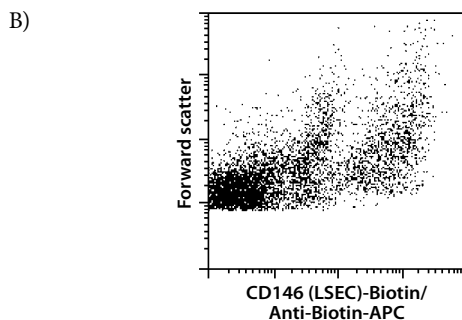
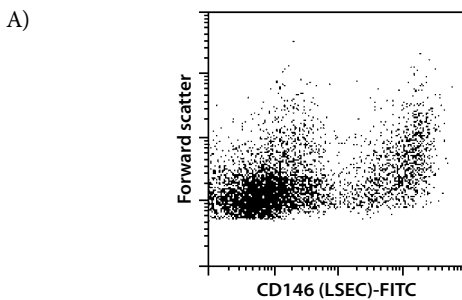
▲ Volumes given below are for up to 10<sup>7</sup> nucleated cells. When working with fewer than 10<sup>7</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes, accordingly (e.g. for 2×10<sup>7</sup> nucleated cells, use 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  $10^7$  nucleated cells per 100  $\mu\text{L}$  of buffer.
4. Add 10  $\mu\text{L}$  of the CD146 (LSEC) antibody.  
▲ **Note:** See table for exceptions.
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  $300\times g$  for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD146 (LSEC)-Biotin was used, resuspend the cell pellet in 100  $\mu\text{L}$  of buffer, add 10  $\mu\text{L}$  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 (LSEC) antibodies

Mouse liver cells were stained with CD146 (LSEC) antibodies conjugated to FITC (A) or biotin and Anti-Biotin-APC (B) and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



### 4. References

1. Harder, R. *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.
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.

All protocols and data sheets are available at [www.miltenyibiotec.com](http://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.

#### Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

autoMACS and MACS are registered trademarks of Miltenyi Biotec GmbH.

Copyright © 2009 Miltenyi Biotec GmbH. All rights reserved.