



# CD105 antibodies mouse

CD105-PE	130-092-929
CD105-APC	130-092-930
CD105-Biotin	130-092-927
CD105 pure	130-092-926

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## 1. Description

<b>Components</b>	1 mL CD105 antibodies, mouse: monoclonal CD105 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL.
<b>Clone</b>	MJ7/18 (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 CD105 antibody (clone MJ7/18) recognizes the CD105 antigen, also known as endoglin. CD105 is a proliferation-associated and hypoxia-inducible protein, abundantly expressed in angiogenic endothelial cells. In mouse bone marrow, CD105 is also expressed on a population of Sca-1<sup>+</sup> hematopoietic stem cells (HSCs). This population has a long-term repopulating (LTR) capacity and is therefore termed LTR-HSCs<sup>1,2</sup>. Isolation of CD105<sup>+</sup>Sca-1<sup>+</sup> LTR-HSCs from mouse bone marrow cells can be obtained by combination of CD105 MultiSort Kit (PE), mouse (# 130-092-924) and Anti-Sca-1 MicroBead Kit (FITC), mouse (# 130-092-529). A special protocol is available at [www.miltenyibiotec.com/protocols](http://www.miltenyibiotec.com/protocols).

### 1.2 Applications

- Identification and enumeration of CD105<sup>+</sup> cells by flow cytometry or fluorescence microscopy.
- Identification and enumeration of CD105<sup>+</sup>Sca-1<sup>+</sup> double-positive LTR-HSCs.
- Identification and enumeration of CD105<sup>+</sup>CD146 (LSEC)<sup>+</sup> double-positive liver sinusoidal endothelial cells (LSEC).

### 1.3 Recommended antibody dilution

For antibody labeling of mouse cells.

CD105 conjugate	PE	APC	Biotin
<b>Flow cytometry<sup>a</sup></b>			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells <sup>b</sup>	1:11	1:11	1:11

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

▲ **Note:** The use of CD105-PE is not recommended to stain endothelial cells due to cellular autofluorescence being detectable in the PE channel in flow cytometry.

### 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 CD105-Biotin.  
▲ **Note:** The use of Anti-Biotin-PE as secondary antibody reagent is not recommended for endothelial cells due to autofluorescence.
- (Optional) Anti-PE MicroBeads (# 130-048-801), Anti-APC MicroBeads (# 130-090-855), or Anti-Biotin MicroBeads (# 130-090-485).
- (Optional) Anti-Sca-1-FITC (# 130-093-222), Anti-Sca-1-PE (# 130-093-224), Anti-Sca-1-APC (# 130-093-223), CD146 (LSEC)-FITC (# 130-092-026), or CD146 (LSEC)-Biotin (# 130-092-025).
- (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<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).

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1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10<sup>7</sup> nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD105 antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

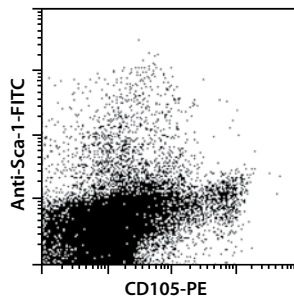
▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

6. Wash cells by adding 1–2 mL of buffer per 10<sup>7</sup> cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD105-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.

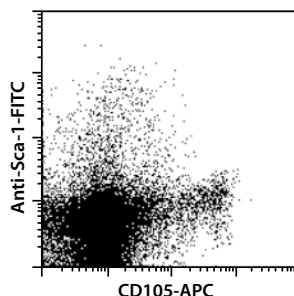
### 3. Examples of immunofluorescent staining with CD105 antibodies

Mouse bone marrow cells were stained with CD105 antibodies conjugated to PE (a) or APC (b) as well as with Anti-Sca-1-FITC (# 130-093-222) and analyzed by flow cytometry. Cells stained with CD105-Biotin (c) were stained with Anti-Biotin-PE (# 130-090-756) and Anti-Sca-1-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

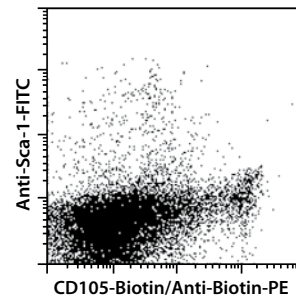
(a) Mouse bone marrow cells stained with CD105-PE.



(b) Mouse bone marrow cells stained with CD105-APC.



(c) Mouse bone marrow cells stained with CD105-Biotin and Anti-Biotin-PE.



### 4. References

1. Chen, C. Z. *et al.* (2002) Identification of endoglin as a functional marker that defines long-term repopulating hematopoietic stem cells. *Proc. Natl. Acad. Sci. U S A* 99: 15468–15473. [939]
2. Chen, C. Z. *et al.* (2003) The endoglin(positive) sca-1(positive) rhodamine(low) phenotype defines a near-homogeneous population of long-term repopulating hematopoietic stem cells. *Immunity* 19: 525–533.

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.

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