

CD105 antibodies

human

CD105-PE	130-094-941
CD105-APC	130-094-926
CD105-Biotin	130-094-916

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1. Description

Components	1 mL CD105 antibodies, human: monoclonal CD105 antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin.
Clone	43A4E1 (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

The CD105 antigen, also known as endoglin, serves as a receptor for the growth and differentiation factors TGF- β ₁ and TGF- β ₃. An epitope of CD105 is recognized by the SH-2 antibody¹, which was raised against human mesenchymal stromal cells (MSC) that show mesodermal differentiation capacity.² Further, CD105 is also expressed on mature endothelial cells and on some leukemic cells of B lymphoid and myeloid origin.

1.2 Applications

- Identification and enumeration of CD105⁺ cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS[®] Separations by flow cytometry or fluorescence microscopy, e.g., isolated human endothelial cells using CD105 MicroBeads.
- Studies on mesengensis.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD105 conjugate	PE	APC	Biotin
Flow cytometry ^a			
- In general	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11
- CD105 MicroBead-labeled cells	1:11	1:11	1:11
Immunohistochemistry ^c			

- a) The indicated antibody dilutions are for up to 10⁷ cells/100 μ L of buffer.
 b) For optimal results, cells must be stained prior to fixation.
 c) The optimal antibody dilution should be determined by the user.

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 (FBS). Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) Anti-Biotin-VioBlue[®] (# 130-094-669), 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.
- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (Optional) CD45-FITC (# 130-080-202) or CD45-PE (# 130-080-201). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Mouse IgG1-FITC (# 130-092-213), Mouse IgG1-PE (# 130-092-212), or Mouse IgG1-APC (# 130-092-214) for isotype control.
- (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.

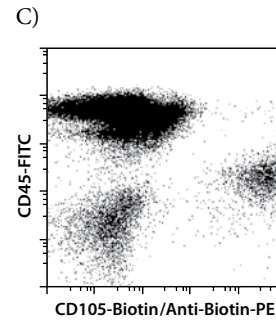
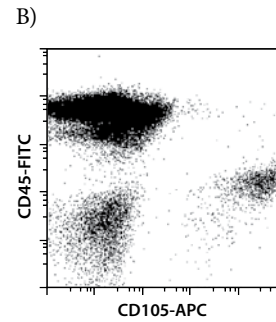
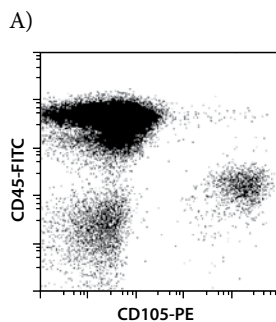
2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10^7 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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 μ L of buffer.
4. Add 10 μ L of the CD105 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 $300 \times g$ for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD105-Biotin was used, resuspend the cell pellet in 100 μ L of buffer, add 10 μ L of anti-biotin antibody (Anti-Biotin-VioBlue, 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

Human peripheral blood mononuclear cells (PBMCs) were spiked with human umbilical vein endothelial cells (HUVECs) and stained with CD105 antibodies conjugated to PE (A) or APC (B) as well as with CD45-FITC (# 130-080-202) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cells labeled with CD105-Biotin (C) were stained with Anti-Biotin-PE (# 130-090-756) as well as CD45-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

1. Barry, F. P. *et al.* (1999) The monoclonal antibody SH-2, raised against human mesenchymal stem cells, recognizes an epitope on endoglin (CD105). *Biochem Biophys Res. Commun.* 265: 134–139.
2. Majumdar, M. K. *et al.* (2000) Isolation, characterization, and chondrogenic potential of human bone marrow - derived multipotential stromal cells. *J. Cell Physiol* 185: 98–106.

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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