

CD133/1 (AC133) antibodies

human

CD133/1 (AC133)-PE	130-080-801
CD133/1 (AC133)-APC	130-090-826
CD133/1 (AC133)-Biotin	130-090-664
CD133/1 (AC133) pure	130-090-422

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1. Description

Components	1 mL CD133/1 (AC133) antibodies, human: monoclonal CD133/1 (AC133) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 50 µg/mL.
Clone	AC133 (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 molecule is a 5-transmembrane cell surface antigen with a molecular weight of 117 kD.¹ The CD133/1 (clone AC133) antibody recognizes an epitope of the CD133 antigen^{2,3}. This epitope is called epitope 1 to distinguish it from another epitope (epitope 2) recognized by the clone 293C3. In the hematopoietic system, CD133 expression is restricted to a subset of CD34^{bright} stem and progenitor cells in human fetal liver, bone marrow, cord blood, and peripheral blood⁴. Additionally, CD133 is expressed by a small portion of CD34⁻ cells in these tissues⁵. The CD34⁺CD133⁺ cell population, which includes CD34⁺CD38⁻ cells, was shown to be capable of repopulating NOD/SCID mice.⁶ CD133 has also been found to be expressed on circulating endothelial progenitor cells^{7,8} and fetal neural stem cells^{9,10} as well as on other tissue-specific stem cells, such as renal¹¹, prostate¹², and corneal¹³ stem cells. The putative murine homologue prominin is expressed on neuroepithelial and epithelial mouse cells.¹⁴

1.2 Applications

- Identification and enumeration of CD133/1 (AC133)⁺ cells by flow cytometry or fluorescence microscopy.
- Studies of hematopoiesis, phenotyping of hematopoietic stem cells.

- Studies on phenotyping of hematologic malignancies.
- Phenotyping of endothelial progenitor cells (EPCs).
- Studies of nonhematopoietic stem cells.
- Studies of tissue-specific stem cells.

A special protocol for the immunohistochemical analysis of paraffin-embedded tissue sections is available from our website.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD133/1 (AC133) 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
Immunohistochemistry ^c			

- a) The indicated antibody dilutions are for up to 10⁷ cells/100 µL 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) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor-mediated antibody labeling.
- (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 CD133/1 (AC133)-Biotin.
- (Optional) CD34-FITC (# 130-081-001), CD34-PE (# 130-081-002), CD34-APC (# 130-090-954), CD117 (A3C6E2)-PE (# 130-091-734), CD117 (A3C6E2)-APC (# 130-091-733), CD117 (AC1126)-PE (# 130-091-735), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), or CD45-APC (# 130-091-230). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Mouse IgG1-PE (# 130-092-212), Mouse IgG1-APC (# 130-092-214), or Mouse IgG1-Biotin (# 130-093-018) 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

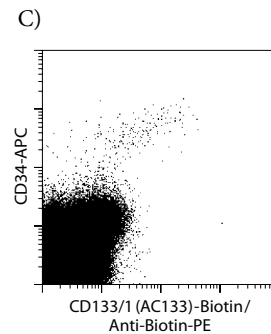
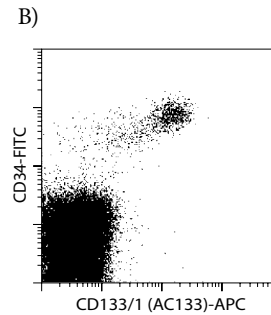
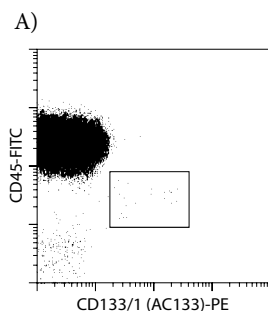
▲ Volumes given below are for up to 10^7 nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated 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 80 μ L of buffer.
4. Add 20 μ L of FcR Blocking Reagent.
5. Add 10 μ L of the CD133/1 (AC133) antibody.
6. 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.
7. Wash cells by adding 1–2 mL of buffer and centrifuge at $300 \times g$ for 10 minutes. Aspirate supernatant completely.
8. (Optional) If CD133/1 (AC133)-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 6 and 7.
9. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD133/1 (AC133) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD133/1 (AC133) antibodies conjugated to PE (a), APC (b), or Biotin (c). Cells stained with CD133/1 (AC133)-PE were counterstained with CD45-FITC (#130-080-202) and cells labeled with CD133/1 (AC133)-APC were counterstained with CD34-FITC (#130-081-001). For cells stained with CD133/1 (AC133)-Biotin, Anti-Biotin-PE was used as secondary antibody and cells were counterstained with CD34-APC (#130-090-954). Cells were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



4. References

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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.

Warranty

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