



CD133/2 (AC141) antibodies human

CD133/2 (AC141)-PE
CD133/2 (AC141) pure

130-080-901
130-090-423

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- Studies of hematopoiesis.
- Phenotyping of hematopoietic stem cells.
- Studies on phenotyping of hematologic malignancies.
- Studies of nonhematopoietic stem cells.

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD133/2 (AC141) conjugate	PE
Flow cytometry^a	
- In general	1:11
- Formaldehyde-fixed cells ^b	1:11
- CD133 MicroBead-labeled cells	1:11

a) Given antibody dilutions are for a cell concentration of up to 10⁷ cells/100 μL of buffer.
b) For optimal results, cells must be stained prior to fixation.

1. Description

Components 1 mL CD133/2 (AC141) antibodies, human: monoclonal CD133/2 (AC141) antibodies conjugated to R-phycoerythrin (PE). The unconjugated (pure) antibody is supplied at a concentration of 50 μg/mL.

Clone AC141 (isotype: mouse IgG1).

Capacity 100 tests or up to 10⁹ 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 CD133/2 (clone AC141) antibody recognizes an epitope of the CD133 antigen^{1,2}. This epitope is called epitope 2 to distinguish it from another epitope (epitope 1) recognized by the clones AC133 and W6B3C1. 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 blood³. Additionally, CD133 is expressed by a small portion of CD34⁻ cells in these tissues⁴. The CD34⁺CD133⁺ cell population, which includes CD34⁺CD38⁻ cells, was shown to be capable of repopulating NOD/SCID mice⁵. Recently, CD133 has also been found to be expressed on endothelial precursor cells^{6,7} and fetal neural stem cells⁸ as well as on developing epithelium⁹. In contrast to the other CD133 clones, the antibody clone AC141 shows cross-reactivity with the intracellular protein cytokeratin 18.¹⁰

The molecule is a novel 5-transmembrane cell surface antigen with a molecular weight of 117 kDa¹¹. The putative murine homologue, prominin, which is expressed on neuroepithelial and epithelial mouse cells, was identified¹².

1.2 Applications

▲ For evaluation of cells isolated with the CD133 MicroBead Kit (# 130-050-801), the use of clone 293C3 is 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 human serum albumin, human serum, or fetal bovine serum. 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) CD34-FITC (# 130-081-001), CD34-PE (# 130-081-002), CD34-APC (# 130-090-954), CD45-FITC (# 130-080-202), CD45-PE (# 130-080-201), CD45-APC (# 130-091-230), or CD45-VioBlue (# 130-092-880).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without fixation.

2. General protocol for immunofluorescent staining

▲ Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ 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 2×10⁷ 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. Add 20 μL of FcR Blocking Reagent and resuspend up to 10⁷ nucleated cells in 80 μL of buffer.

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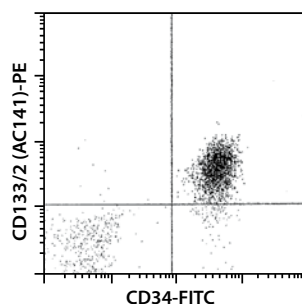
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4. Add 10 μ L of the CD133/2 (AC141) antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °C).

▲ **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 10^7 cells and centrifuge at $300\times g$ for 10 minutes. Aspirate supernatant completely.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD133/2 (AC141) antibodies

Human CD133⁺ cells were isolated from non-mobilized peripheral blood cells using the CD133 MicroBead Kit and an MS Column. Isolated cells were stained with CD133/2 (AC141)-PE and CD34-FITC (clone AC136), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



4. References

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12. Weigmann, A. *et al.* (1998) AC133 hematopoietic stem cell antigen: human homologue of mouse kidney prominin or distinct member of a novel protein family? *Proc. Natl. Acad. Sci. USA* 94: 12425–12430.

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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