

CD133/2(293C3)-PE	130-090-853
CD133/2(293C3)-APC	130-090-854
CD133/2(293C3)-Biotin	130-090-852
CD133/2(293C3) pure	130-090-851

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1. Description

Clone 293C3 (isotype: mouse IgG2b).

Product format 1 mL CD133/2 (293C3) antibodies, human: monoclonal CD133/2 (293C3) antibodies conjugated to R-phycoerythrin (PE), allophycocyanin (APC), or biotin. The unconjugated (pure) antibody is supplied at a concentration of 50 µg/mL.

Antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.

Product size 100 tests or up to 10⁹ total cells.

Storage Store protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

CD133 is a novel 5-transmembrane cell surface antigen with a molecular weight of 117 kD.¹ The CD133/2 (clone 293C3) antibody recognizes an epitope of the CD133 antigen^{2,3}. This epitope is called epitope 2 to distinguish it from another epitope (epitope 1) recognized by the clone AC133 and clone 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 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 Recently, 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, which is expressed on neuroepithelial and epithelial mouse cells, was recently identified.¹⁴

Product applications

- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Cells can be isolated by using CD133 MicroBead Kit (# 130-050-801), CD34 MicroBead Kit (# 130-046-702, 130-046-703), CliniMACS AC133 MicroBeads (# 195-01), or CliniMACS AC133 Complete Kit (# 196-01).

- 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.

1.2 Recommended antibody dilution

For antibody labeling of human cells.

CD133/2 (293C3)	PE	APC	Biotin
Flow cytometry^a			
- in general	1:11	1:11	1:11
- formaldehyde-fixed cells ^b	1:11	1:11	1:11
- CD133 MicroBead-labeled cells	1:11	1:11	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.

1.3 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 (4–8 °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 calf 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), 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).
- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling 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).

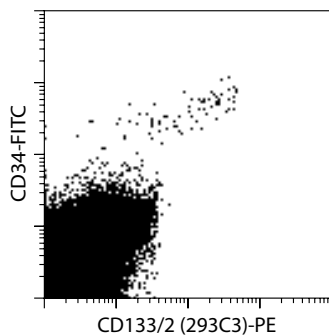
1. Resuspend up to 10⁷ nucleated cells per 80 µL of buffer.
2. Add 20 µL of FcR Blocking Reagent.

3. Add 10 μL of the CD133/2 (293C3) antibody.
4. Mix well and refrigerate for 10 minutes in the dark (4–8 $^{\circ}\text{C}$).
 ▲ **Note:** Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
5. 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.
6. (Optional) If CD133/2 (293C3)-Biotin was used, resuspend the cell pellet in 100 μL of buffer, add 10 μL of anti-biotin antibody (Anti-Biotin-FITC # 130-090-857, Anti-Biotin-PE # 130-090-756, or Anti-Biotin-APC # 130-090-856), and continue as described in steps 4 and 5.
7. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

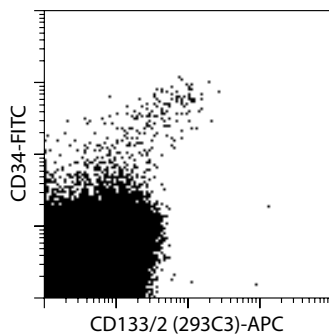
3. Examples of immunofluorescent staining with CD133/2 (293C3) antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD133/2 (293C3) antibodies conjugated to PE (a) or APC (b) as well as CD34-FITC (# 130-081-001), and analyzed by flow cytometry. Cells stained with CD133/2 (293C3)-Biotin (c) were stained with Anti-Biotin-PE and CD34-FITC. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

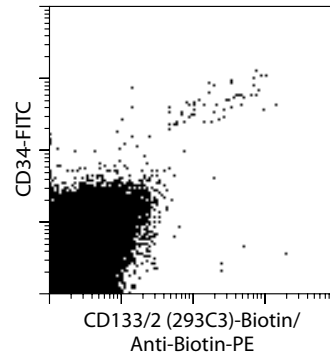
- (a) Human PBMCs stained with CD133/2 (293C3)-PE and CD34-FITC.



- (b) Human PBMCs stained with CD133/2 (293C3)-APC and CD34-FITC.

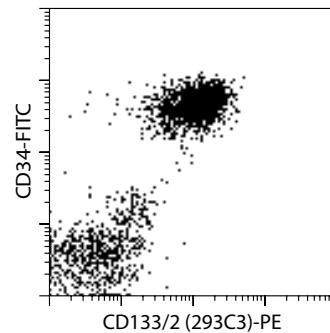


- (c) Human PBMCs stained with CD133/2 (293C3)-Biotin/Anti-Biotin-PE and CD34-FITC.

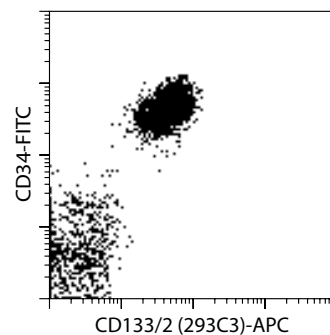


CD133⁺ cells were isolated from human non-mobilized PBMCs with the CD133 MicroBead Kit (# 130-050-801), stained with CD133/2 (293C3)-PE and CD34-FITC (a), or CD133/2 (293C3)-APC and CD34-FITC (b), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

- (a) CD133⁺ cells stained with CD133/2 (293C3)-PE and CD34-FITC.



- (b) CD133⁺ cells stained with CD133/2 (293C3)-APC and CD34-FITC.



4. References

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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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