Miltenyi Biotec

CD133/2 (293C3) antibodies
human

Contents

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.

1. Description

This product is for research use only.

Components Monoclonal CD133/2 (293C3) antibodies, human conjugated to:

Conjugate	Order no. 1 mL (100 tests)	Order no. 300 µL (30 tests)
VioBright™ FITC	130-104-273	130-104-322
PE	130-090-853	130-098-046
APC	130-090-854	130-098-129
PE-Vio® 615	130-107-453	130-107-507
PE-Vio® 770	130-104-117	130-104-155
Biotin	130-090-852	130-099-093
pure	130-090-851	–

Clone 293C3 (isotype: mouse IgG2b).

Capacity 1 mL: 100 tests or up to 10^9 total cells

300 µL: 30 tests or up to 3×10^8 total cells.

The unconjugated (pure) antibody is supplied at a concentration of 50 µg/mL.

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

- Antigen: CD133/2 (293C3)
- Synonym: AC133; Prominin-1
- Expression patterns: CD133 is a novel 5-transmembrane cell surface antigen with a molecular weight of 117 kDa.[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 blood[4]. Additionally, CD133 is expressed by a small portion of $CD34^-$ cells in these tissues[5]. 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 renal[11], prostate[12], and corneal[13] stem cells. The putative murine homologue, prominin, which is expressed on neuroepithelial and epithelial mouse cells, was recently identified.[14]

1.2 Applications

- Identification and enumeration of $CD133/2\ (293C3)^+$ cells by flow cytometry.
- Evaluation of MACS® Separations by flow cytometry or fluorescence microscopy. Cells can be isolated by using CD133 MicroBead Kit, human – lyophlized (# 130-097-049), CD34 MicroBead Kit (# 130-046-702, 130-046-703), CliniMACS® CD133 (# 172-01), or CliniMACS CD133 Complete Kit (# 130-090-734).

1.3 Recommended antibody dilution

The recommended antibody dilution for all CD133/2 (293C3) conjugates is **1:11 for up to 10^7 cells/100 µL** of buffer for labeling of cells and subsequent analysis by flow cytometry.

The antibody is suited for staining of formaldehyde-fixed cells.

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). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) FcR Blocking Reagent, human (# 130-059-901) to avoid Fc receptor–mediated antibody labeling.

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140-001-916.04

- (Optional) Tandem Signal Enhancer, human (# 130-099-888) to reduce non-specific binding of tandem dye–conjugated antibodies to human cells, especially to monocytes.
- (Optional) Conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with CD133/2 (293C3)-Biotin.
- (Optional) For antibodies for additional staining or for isotype control, refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) 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

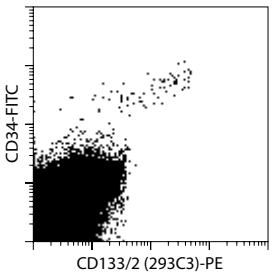
Volumes given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^7 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD133/2 (293C3) 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×g for 10 minutes. Aspirate supernatant completely.
7. (Optional) If CD133/2 (293C3)-Biotin was used, resuspend the cell pellet in 100 µL of buffer, add 10 µL of anti-biotin antibody, 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. Example of immunofluorescent staining with CD133/2 (293C3) antibodies

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



For more examples please refer to the respective product page at www.miltenyibiotec.com/antibodies.

4. References

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Refer to www.miltenyibiotec.com for all data sheets and protocols.

Warranty

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