CD133/2 (293C3) antibodies, human

For research use only

6
One test corresponds to labeling of up to 10 cells in a total volume of 100 µL

Product	Content	Order no.
CD133/2 (293C3)-APC	for 30 tests	130-113-746
CD133/2 (293C3)-VioBright FITC	for 30 tests	130-113-750
CD133/2 (293C3)-VioBright FITC	for 100 tests	130-113-188
CD133/2 (293C3)-PE	for 30 tests	130-113-748
CD133/2 (293C3)-PE	for 100 tests	130-113-186
CD133/2 (293C3)-APC	for 100 tests	130-113-184
CD133/2 (293C3)-PE-Vio770	for 30 tests	130-113-749
CD133/2 (293C3)-PE-Vio770	for 100 tests	130-113-187
CD133/2 (293C3)-Biotin	for 30 tests	130-113-747
CD133/2 (293C3)-Biotin	for 100 tests	130-113-185

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.

Technical data and background information

Antigen	CD133/2 (293C3)
Clone	293C3
Isotype	mouse IgG2bκ
Isotype control	Mouse IgG2b – isotype control antibodies
Alternative names of antigen	PROM1, AC133, CORD12, MCDR2, MSTP061, PROML1, RP41, STGD4
Entrez Gene ID	8842
Molecular mass of antigen [kDa]	117
Distribution of antigen	brain, endothelial cells, epithelial cells, heart, hematopoietic stem cells, kidney, liver, lung, pancreas, placenta, ES and iPS cells, red blood cells
Product format	Reagents are supplied in buffer containing stabilizer and 0.05% sodium azide.
Fixation	The antibody is suited for staining of formaldehyde-fixed cells.
Storage	Store protected from light at 2–8 °C. Do not freeze.

CD133 is a novel 5-transmembrane cell surface antigen with a molecular weight of 117 kDa. CD133/2 (293C3) antibodies recognize epitope 2 of the human CD133 antigen (CD133/2). 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. Additionally, CD133 is expressed by a small portion of $CD34^-$ cells in these tissues. The $CD34^+CD133^+$ cell population, which includes $CD34^+CD38^-$ cells, was shown to be capable of repopulating NOD/SCID mice. Recently, CD133 has also been found to be expressed on circulating endothelial progenitor cells, tissue-specific stem cells, cancer stem cells from tumor tissues, fetal neural stem cells, and ES and iPS cell–derived cells as well as on other tissue-specific stem cells, such as renal, prostate, and corneal stem cells. The putative murine homologue, prominin, which is expressed on neuroepithelial and epithelial mouse cells, was dentified.

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.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated 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.

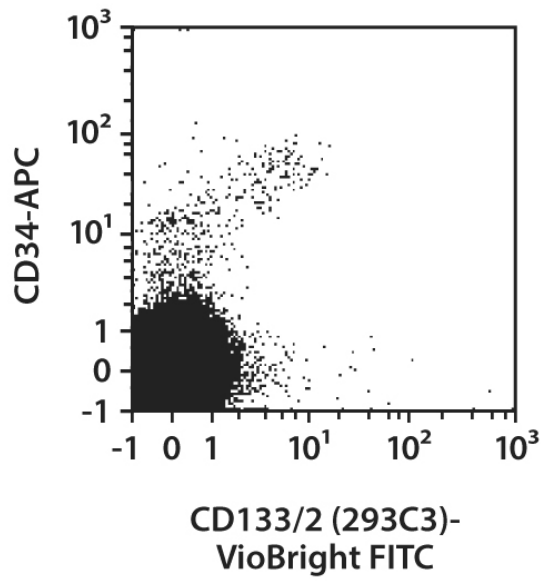
Protocol for cell surface staining

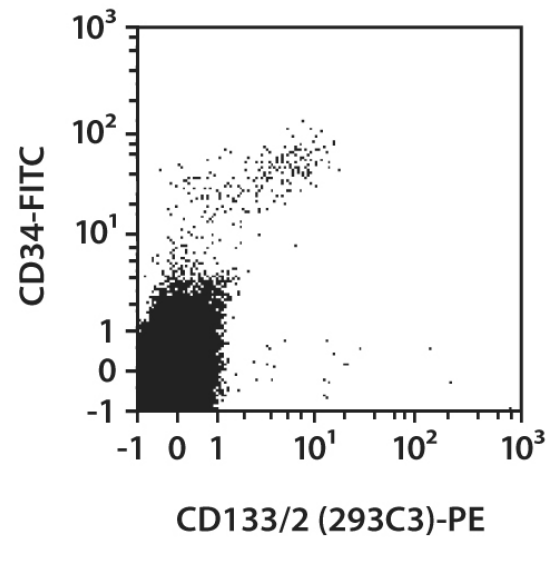
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:50 for up to 10^6 cells/100 µL.
- Volumes given below are for up to 10^6 nucleated cells. When working with fewer than 10^6 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

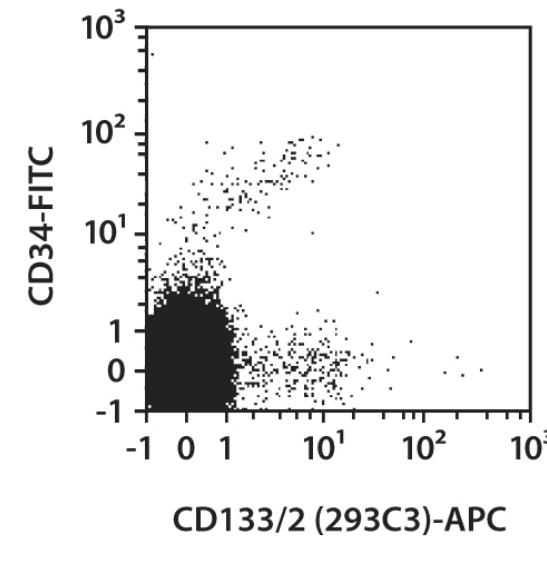
1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10^6 nucleated cells per 98 µL of buffer.
4. Add 2 µL of the antibody.
5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2−8 °C).
   Note: Higher 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 biotinylated antibody was used, resuspend the cell pellet in buffer and stain with fluorochrome-conjugated anti-biotin antibody according to the manufacturer's recommendations.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

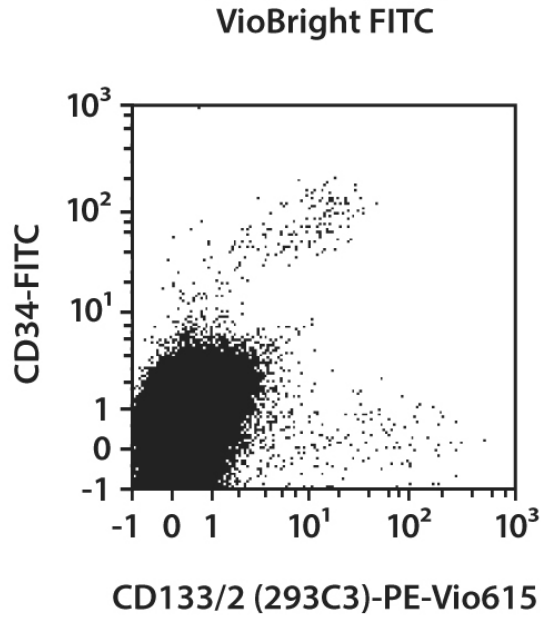
Examples of immunofluorescent staining

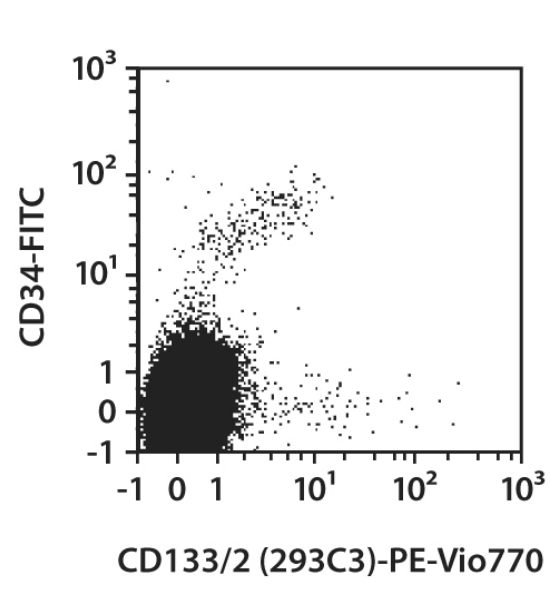
Human peripheral blood mononuclear cells (PBMCs) were stained with 133/2 (293C3) antibodies as well as with CD34 and CD45 antibodies and analyzed by flow cytometry using the MACSQuant® Analyzer. The Tandem Signal Enhancer has been used to increase binding specificity of tandem-dye–conjugated antibodies. For all other conjugates the FcR Blocking Reagent has been used to avoid Fc receptor–mediated antibody labeling. A pre-gate of $CD45_+$ cells was used. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence or 4',6-diamidino-2-phenylindole (DAPI) fluorescence, as in the case of tandem conjugates.

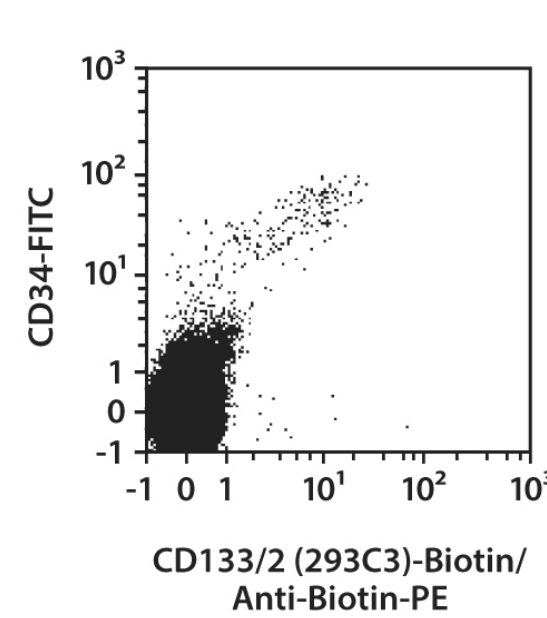












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Miltenyi Biotec GmbH | Friedrich-Ebert-Straße 68 | 51429 Bergisch Gladbach | Germany | Phone +49 2204 8306-0 | Fax +49 2204 85197 | macs@miltenyibiotec.de | www.miltenyibiotec.com Miltenyi Biotec provides products and services worldwide. Visit **www.miltenyibiotec.com/local** to find your nearest Miltenyi Biotec contact.