

CD133/1 (AC133) antibodies, human

For research use only

One test corresponds to labeling of up to 10^7 cells in a total volume of 100 μ L.

Product	Content	Order no.
CD133/1 (AC133)-VioBright FITC ¹	for 30 tests	130-105-226
CD133/1 (AC133)-VioBright FITC ¹	for 100 tests	130-105-225
CD133/1 (AC133)-PE ¹	for 30 tests	130-098-826
CD133/1 (AC133)-PE ¹	for 100 tests	130-080-801
CD133/1 (AC133)-APC ¹	for 30 tests	130-098-829
CD133/1 (AC133)-APC ¹	for 100 tests	130-090-826
CD133/1-PE-Vio615 ¹	for 30 tests	130-107-509
CD133/1-PE-Vio615 ¹	for 100 tests	130-107-455
CD133/1-PE-Vio770 ¹	for 30 tests	130-101-652
CD133/1-PE-Vio770 ¹	for 100 tests	130-101-676
CD133/1 (AC133)-Biotin ¹	for 30 tests	130-098-897
CD133/1 (AC133)-Biotin ¹	for 100 tests	130-090-664
CD133/1 (AC133) pure	50 μ g in 1 mL	130-090-422

¹Not recommended for cells that are labeled with MACS MicroBeads using the same antigen.

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	CD133/1 (AC133)
Clone	AC133
Isotype	mouse IgG1 κ
Isotype control	Mouse IgG1 – isotype control antibodies
Alternative names of antigen	PROM1, AC133, CORD12, MCDR2, MSTP061, PROML1, RP41, STGD4
Molecular mass of antigen [kDa]	95
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	Antibodies are supplied in buffer containing stabilizer and 0.05% sodium azide.

Fixation	Cells should be stained prior to fixation, if formaldehyde is used as a fixative.
Storage	Store protected from light at 2–8 °C. Do not freeze.

CD133, formerly known as AC133, recognizes epitope 1 of the CD133 antigen. It is a marker that is frequently found on multipotent progenitor cells, including immature hematopoietic stem and progenitor cells. In the hematopoietic system, CD133 is expressed on a small portion of CD34⁻ cells as well as on a subset of CD34^{bright} stem and progenitor cells in human fetal liver, bone marrow, cord blood, and peripheral blood. CD133 has also been found to be expressed on circulating endothelial progenitor cells, fetal neural stem cells, other tissue-specific stem cells, such as renal, prostate, and corneal stem cells, cancer stem cells from tumor tissues, as well as ES and iPS cell-derived cells.

Reagent requirements

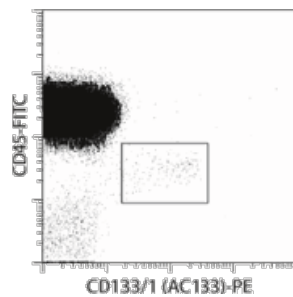
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS[®] BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[®] Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca²⁺ or Mg²⁺ 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

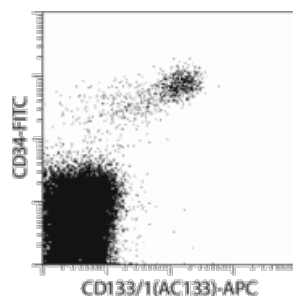
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:11 for up to 10⁷ cells/100 µL of buffer.
 - Volumes given below are for up to 10⁷ nucleated cells. When working with fewer than 10⁷ cells, use the same volumes as indicated. When working with higher 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. Resuspend up to 10⁷ nucleated cells per 100 µL of buffer.
 4. Add 10 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 100 µL of buffer, add 10 µL of fluorochrome-conjugated 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.

Examples of immunofluorescent staining

Staining of non-mobilized PBMCs with CD133/1-PE and CD45-FITC.



Human peripheral blood mononuclear cells (PBMCs) were stained with CD133/1 (AC133) antibodies conjugated to APC, as well as with CD34-FITC. The FcR Blocking Reagent has been used to avoid Fc receptor-mediated antibody labeling. Cells were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



References

1. **Galic et al.** (2006) T lineage differentiation from human embryonic stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 103: 11742–11747.
2. **Yin, A. H. et al.** (1997) AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* 90: 5002–5012.
3. **Piechaczek, C.** (2001) CD133. *J. Biol. Regul. Homeost. Agents* 15: 101–102.
4. **Gallacher, L. et al.** (2000) Isolation and characterization of human CD34(-)Lin(-) and CD34(+)Lin(-) hematopoietic stem cells using cell surface markers AC133 and CD7. *Blood* 95: 2813–2820.
5. **Bühring, H. J. et al.** (1999) Expression of novel surface antigens on early hematopoietic cells. *Ann. N. Y. Acad. Sci.* 872: 25–39.
6. **Gehling, U. M. et al.** (2000) In vitro differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* 95: 3106–3112.
7. **Peichev, M. et al.** (2000) Expression of VEGFR-2 and AC133 by circulating human CD34(+) cells identifies a population of functional endothelial precursors. *Blood* 95: 952–958.
8. **Uchida, N. et al.** (2000) Direct isolation of human central nervous system stem cells. *Proc. Natl. Acad. Sci. U.S.A.* 97: 14720–14725.
9. **Cummings, B. J. et al.** (2005) Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. *Proc. Natl. Acad. Sci. U.S.A.* 102: 14069–14074.
10. **Bussolati, B. et al.** (2005) Isolation of renal progenitor cells from adult human kidney. *Am. J. Pathol.* 166: 545–555.
11. **Richardson, G. et al.** (2004) CD133, a novel marker for human prostatic epithelial stem cells. *J. Cell. Sci.* 117: 3539–3545.
12. **Thill, M. et al.** (2004) Identification of a population of CD133⁺ precursor cells in the stroma of human cornea. *Invest. Ophthalmol. Vis. Sci.* 45: 3519.
13. **Giebel B. et al.** (2004) Segregation of lipid raft markers including CD133 in polarized human hematopoietic stem and progenitor cells. *Blood* 104(8): 2332–2338.
14. **Ingram, D.A. et al.** (2004) Identification of a novel hierarchy of endothelial progenitor cells using human peripheral and umbilical cord blood. *Blood* 104(9): 2752–2760.
15. **Alvarez, D.F. et al.** (2008) Lung microvascular endothelium is enriched with progenitor cells that exhibit vasculogenic capacity. *Am. J. Physiol. Lung Cell Mol. Physiol.* 294(3): L419–L430.
16. **Wagner, W. et al.** (2004) Molecular evidence for stem cell function of the slow-dividing fraction among human hematopoietic progenitor cells by genome-wide analysis. *Blood* 104(3): 675–686.
17. **Miraglia, S. et al.** (1997) A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood* 90(12): 5013–5021.
18. **de Wynter, E. A. et al.** (1998) CD34⁺AC133⁺ cells isolated from cord blood are highly enriched in long-term culture-initiating cells, NOD/SCID-repopulating cells and dendritic cell progenitors. *Stem Cells* 16: 387–396.
19. **Weigmann, A. et al.** (1997) Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. *Proc. Natl. Acad. Sci. U.S.A.* 94: 12425–12430.
20. **Immervoll, H. et al.** (2008) Expression of the "stem cell marker" CD133 in pancreas and pancreatic ductal adenocarcinomas. *BMC Cancer* 8: 48.
21. **Duda, D.G. et al.** (2007) A protocol for phenotypic detection and enumeration of circulating endothelial cells and circulating progenitor cells in human blood. *Nat. Protoc.* 2(4): 805–810.
22. **Margolis, D. J. et al.** (2009) Phase I study of H5.020CMV.PDGF-beta to treat venous leg ulcer disease. *Mol. Ther.* 17(10): 1822–1829.
23. **Metsuyanin, S. et al.** (2009) Expression of stem cell markers in the human fetal kidney. *PLoS One* 4(68): e6709.
24. **Maxwell, S. A. et al.** (2009) 14-3-3zeta. *J. Biol. Chem.* 284(33): 22379–22389.
25. **Lin, L. et al.** (2009) The STAT3 inhibitor NSC 74859 is effective in hepatocellular cancers with *Oncogene* 28(7): 961–972.
26. **Fonseca, A. V. et al.** (2010) Polarization and migration of hematopoietic stem and progenitor cells rely on the J. *Biol. Chem.* 285(41): 31661–31671.

27. **Estes, M. L. et al.** (2010) Application of polychromatic flow cytometry to identify novel subsets of circulating cells with angiogenic potential. *Cytometry A* 77(9): 831–839.
28. **Mak, A. B. et al.** (2011) CD133 protein N-glycosylation processing contributes to cell surface recognition of the primitive cell marker AC133 epitope. *J. Biol. Chem.* 286(47): 41046–41056.
29. **Fusi, A. et al.** (2011) Expression of the stem cell markers nestin and CD133 on circulating melanoma cells. *J. Invest. Dermatol.* 131(2): 487–494.
30. **Fredebohm, J. et al.** (2012) Establishment and characterization of a highly tumorigenic and cancer stem cell enriched pancreatic cancer cell line as a well defined model system. *PLoS One* 7(11): e48503.
31. **Ferro, F. et al.** (2012) Dental pulp stem cells differentiation reveals new insights in Oct4A dynamics. *PLoS One* 7(7): e41774.
32. **Clark, P. A. et al.** (2012) Activation of multiple ERBB family receptors mediates glioblastoma cancer stem-like cell resistance to EGFR-targeted inhibition. *Neoplasia* 14(5): 420–428.
33. **Wong, C. K. E. et al.** (2012) Levels of a subpopulation of platelets, but not circulating endothelial cells, predict early treatment failure in prostate cancer patients after prostatectomy. *Br. J. Cancer* 107(9): 1564–1573.
34. **Qi, Y. et al.** (2012) Inhaled NO contributes to lung repair in piglets with acute respiratory distress syndrome via increasing circulating endothelial progenitor cells. *PLoS One* 7(3): e33859.
35. **Yuanjie Hu et al.** (2013) Tumor-specific chromosome mis-segregation controls cancer plasticity by maintaining tumor heterogeneity. *PLoS One* 8(11): e80898.
36. **Balasubramanian, P. et al.** (2013) AQP9 expression in glioblastoma multiforme tumors is limited to a small population of astrocytic cells and CD15(+)/CalB(+) leukocytes. *PLoS One* 8(9): e75764.
37. **Rheinbay, E. et al.** (2013) An aberrant transcription factor network essential for Wnt signaling and stem cell maintenance in glioblastoma. *Cell Rep* 3(5): 1567–1579.
38. **Walton, R. M. et al.** (2013) Postnatal neural precursor cell regions in the rostral subventricular zone, hippocampal subgranular zone and cerebellum of the dog (*Canis lupus familiaris*). *Histochem. Cell Biol.* 139(3): 415–429.
39. **Xiaofeng Ye et al.** (2013) The effect of Heparin-VEGF multilayer on the biocompatibility of decellularized aortic valve with platelet and endothelial progenitor cells. *PLoS One* 8(1): e54622.
40. **Tea Soon Prk et al.** (2013) Efficient and simultaneous generation of hematopoietic and vascular progenitors from human induced pluripotent stem cells. *Cytometry A* 83(1): 114–126.
41. **Behbod, F. et al.** (2009) An intraductal human-in-mouse transplantation model mimics the subtypes of ductal carcinoma in situ. *Breast Cancer Res.* 11(5): R66.
42. **Steinmetz, N. F. et al.** (2011) Two domains of vimentin are expressed on the surface of lymph node, bone and brain metastatic prostate cancer lines along with the putative stem cell marker proteins CD44 and CD133. *Cancers (Basel)* 3(3): 2870–2885.
43. **Liu, H.** (2013) Single-cell clones of liver cancer stem cells have the potential of differentiating into different types of tumor cells. *Cell Death Dis* 4: e857.
44. **Czyz, M. et al.** (2013) Parthenolide reduces the frequency of ABCB5-positive cells and clonogenic capacity of melanoma cells from anchorage independent melanospheres. *Cancer Biol. Ther.* 14(2): 135–145.
45. **Tsang, H. et al.** (2013) Role of asymmetric methylarginine and connexin 43 in the regulation of pulmonary endothelial function. *Pulm Circ* 3(3): 675–691.
46. **Yu Sun et al.** (2014) Slug overexpression induces stemness and promotes hepatocellular carcinoma cell invasion and metastasis. *Oncol Lett* 7(6): 1936–1940.
47. **Schiffer, D.** (2014) Stem cell niches in glioblastoma: a neuropathological view. *Biomed Res Int* 2014: 725921.

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