



Miltenyi Biotec

CD34 antibodies

human

CD34-VioBlue®	130-095-393
CD34-FITC	130-081-001
CD34-PE	130-081-002
CD34-APC	130-090-954

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1. Description

Components	1 mL CD34 antibodies, human: monoclonal CD34 antibodies conjugated to VioBlue®, fluorescein isothiocyanate (FITC), R-phycoerythrin (PE), or allophycocyanin (APC).
Clone	AC136 (isotype: mouse IgG2a).
Capacity	100 tests or up to 10 ⁹ 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 CD34 antigen is a single chain transmembrane glycoprotein, expressed on human hematopoietic stem and progenitor cells, endothelial progenitor cells, vascular endothelial cells, embryonic fibroblasts and some cells in fetal and adult nervous tissue. The antigen is absent on fully differentiated hematopoietic cells such as normal peripheral blood lymphocytes, monocytes, granulocytes, erythrocytes and platelets. Clone AC136 recognizes a class III epitope of the CD34 antigen and has a similar specificity as the CD34 monoclonal antibody clone 8G12 (HPCA-2™).

1.2 Applications

- Identification and enumeration of CD34⁺ cells by flow cytometry or fluorescence microscopy.
- Flow cytometric or fluorescence microscopic analysis of CD34⁺ cells separated by using, for example, CD34 MicroBead Kit (# 130-046-702, # 130-046-703), Indirect CD34 MicroBead Kit (# 130-046-701), CD34 MultiSort Kit (# 130-056-701), CD133 MicroBead Kit (# 130-050-801), CliniMACS® CD34 Reagent (# 171-01), CliniMACS CD133 Reagent (# 172-01), or CliniMACS CD133 Complete Kit (# 196-01).
- Studies of hematopoiesis.
- Phenotyping of hematopoietic stem cells.

- Studies on phenotyping of hematologic malignancies.
- Studies of nonhematopoietic stem cells.
- Studies on endothelial cells and endothelial progenitor cells (EPCs).

1.3 Recommended antibody dilution

For antibody labeling of human cells.

CD34 conjugate	VioBlue	FITC	PE	APC
Flow cytometry ^a				
- In general	1:11	1:11	1:11	1:11
- Formaldehyde-fixed cells ^b	1:11	1:11	1:11	1:11
- CD34 MicroBead-labeled cells	1:11	1:11	1:11	1:11

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

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) CD45-FITC (# 130-080-202) or CD45-PE (# 130-080-201). For more information about antibodies refer to www.miltenyibiotec.com.
- (Optional) Mouse IgG2a-VioBlue (# 130-094-671), Mouse IgG2a-FITC (# 130-091-837), Mouse IgG2a-PE (# 130-091-835), or Mouse IgG2a-APC (# 130-091-836) for isotype control.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD 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 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).

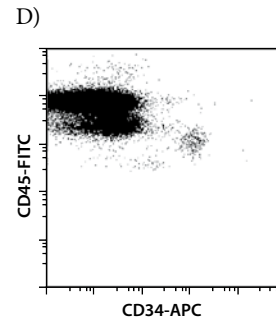
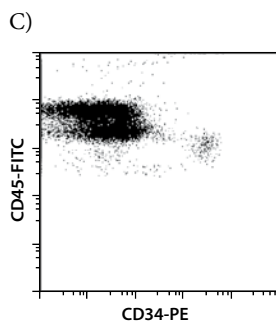
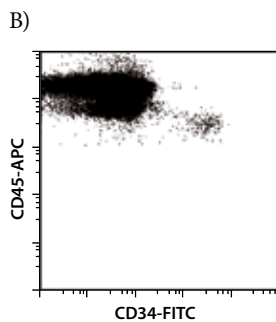
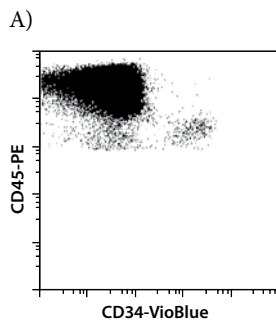
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1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend up to 10⁷ nucleated cells per 100 μL of buffer.
4. Add 10 μL of the CD34 antibody.
5. Mix well and incubate for 10 minutes 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. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with CD34 antibodies

Human peripheral blood mononuclear cells (PBMCs) were stained with CD34 antibodies conjugated to VioBlue (A), FITC (B), PE (C), or APC (D) as well as with CD45-FITC (# 130-080-202) or CD45-PE (# 130-080-201) and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.



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.

Warranty

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