Miltenyi Biotec

CD34 Stem Cell Analysis Cocktail, anti-human

Order no. 130-093-427

Contents

1. **Description**
   - 1.1 **Background information**
   - 1.2 **Applications**
   - 1.3 **Reagent and instrument requirements**
2. **Protocol**
   - 2.1 **Immunofluorescent staining of nucelated cells**
   - 2.2 **Flow cytometric data aquisition**
   - 2.3 **Data analysis**
   - 2.4 **Determination of CD34$^+$ cell frequencies**

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	**0.5 mL CD34 Stem Cell Analysis Cocktail, anti-human** containing:
	CD34 Antibody, anti-human, PE (clone: AC136, isotype: mouse IgG2a)
	CD45 Antibody, anti-human, FITC (clone: MB4-6D6, isotype: mouse IgG1κ)
	CD45 Antibody, anti-human, VioBlue® (clone: 5B1, isotype: mouse IgG2a)
Capacity	50 tests or up to 5×10^8 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 Stem Cell Analysis Cocktail, anti-human allows the optimal identification of human hematopoietic stem and progenitor cells (CD34$^+$CD45$^{+/dim}$) which have been isolated using MACS Technology. CD45-VioBlue is included in the cocktail as a trigger to restrict analysis to leukocytes only. This enables the straightforward and automated identification of leukocytes using the MACSQuant Analyzer. The CD45-VioBlue antibody recognizes a different epitope from the CD45-FITC antibody.

1.2 Applications

- Evaluation and quality control of MACS Separations of human hematopoietic stem and progenitor cells using either the CD34 MicroBead Kit, human (# 130-046-703) or the Indirect CD34 MicroBead Kit, human (# 130-046-701).

1.3 Reagent and instrument 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 (FBS). Buffers or media containing Ca2+ or Mg2+ are not recommended for use.

- Flow cytometer equipped with a blue (488 nm) and a violet (405 nm) laser, e.g., MACSQuant Analyzer 10 (# 130-096-343) or MACSQuant Analyzer 16 (# 130-109-803).
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells without fixation.
- (Optional) MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

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2. Protocol

2.1 Immunofluorescent staining of nucleated cells

▲ 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 (e.g. for 2×10^6 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 10^6 nucleated cells per 100 µL of buffer.
4. Add 10 µL of the CD34 Stem Cell Analysis Cocktail, anti-human.
6. 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.
7. Wash cells by adding 1–2 mL of buffer per 10^6 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry. To exclude dead cells and debris from the analysis, add propidium iodide to an end concentration of 1 µg/mL to each tube directly before data acquisition.

2.2 Flow cytometric data aquisition

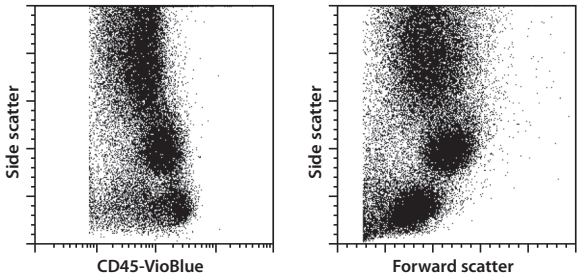
▲ For automated flow cytometric analysis using the MACSQuant Analyzer flow cytometers, the Express Mode **MC_CD34_h** can be used. Express Modes are unique add-on features for the MACSQuantify™ Software. They are standardized data analysis tools that are optimized to automate flow cytometric measurements and analyses via predefined experiment settings, acquisition, and automated gating. Derived from mathematical algorithms, they reduce human error and therefore increase experimental reproducibility.
For details refer to the MACSQuant user manual, the MACSQuantify Software guide, or visit www.macsquant.com. For more information on the usage of Express Modes refer to the application note "How to use a MACSQuant® Instrument Express Mode in Custom Login" in the Resources section at www.miltenyibiotec.com.

▲ The gating strategy outlined below is applicable for the analysis of cells isolated using either the CD34 MicroBead Kit, human (# 130-046-702), or the Indirect CD34 MicroBead Kit, human (# 130-046-701). Always analyze both the starting cell fraction (before separation) and the target cell fraction (after separation) in order to be able to calculate the recovery and purity of target cells after separation. Analysis of the non-target cell fraction (negative fraction; after separation) is optional.

▲ **Note:** If CD45-FITC or FSC/SSC has been used for leukocyte exclusion, the gating strategy must be adjusted accordingly.

1. Set the instrument to a standard 3-color data acquisition protocol. Make sure the calibration and compensation settings have been optimized. Set the instrument to collect at least 100,000 cells in the original cell fraction in order to receive >100 $CD34^+$ target cells. For the MACSQuant Analyzer, choose an appropriate analysis volume. The number of events per second should not exceed 2,000.
2. Define an appropriate threshold based on CD45-VioBlue vs. SSC signals for the exclusion of debris and erythrocytes from the data acquisition. Ensure that the CD45-VioBlue trigger is set to only exclude $CD45^-$ cells but not $CD45^{dim}$ cells. Note that $CD34^+$ hematopoietic cells express CD45 at a lower fluorescence intensity than lymphocytes (A). Due to the detection of autofluorescence of small particles and debris in the violet laser channel, events with very low signal in the FSC channel should be excluded from the analysis (B).

A) B)



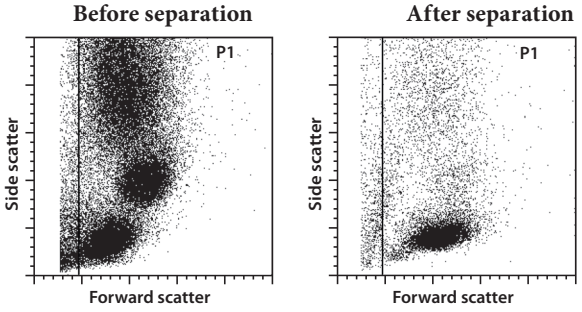
3. For manual gating create a population list as follows:

Population	Parameter/label	Definition
Total cells (excluding debris)	FSC/SSC	P1
Viable leukocytes	CD34-PE/Propidium iodide	P1/P2
$CD34^+$ target cells	CD34-PE/SSC	P1/P2/P3
(Optional) Verification of target cells based on ISHAGE* guidelines.	CD45-FITC/SSC	P1/P2/P3/P4
	FSC/SSC	P1/P2/P3/P4/P5

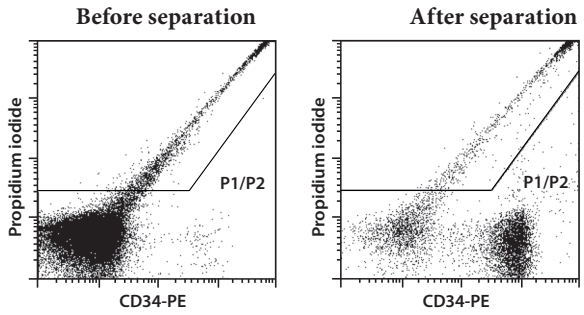
* ISHAGE = International Society of Hematotherapy and Graft Engineering

2.3 Data analysis

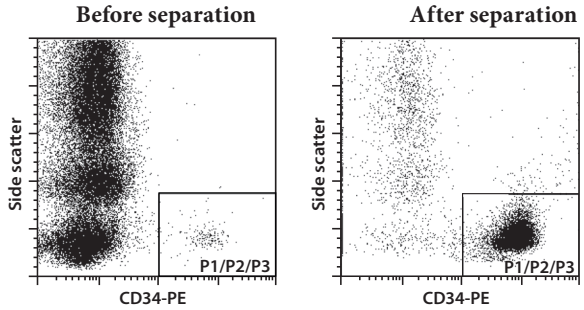
1. Create a FSC vs. SSC dot plot and draw region P1 to exclude debris.



2. Create a CD34-PE vs. propidium iodide (PI) dot plot on the cells within P1 to exclude dead cells. If using 7-AAD for dead cell exclusion, create a 7-AAD versus SSC dot plot. The cells within this region should all be viable $CD45^+$ cells and belong to the P1/P2 population. The gate statistic of this dot plot is used for subsequent statistical analysis.

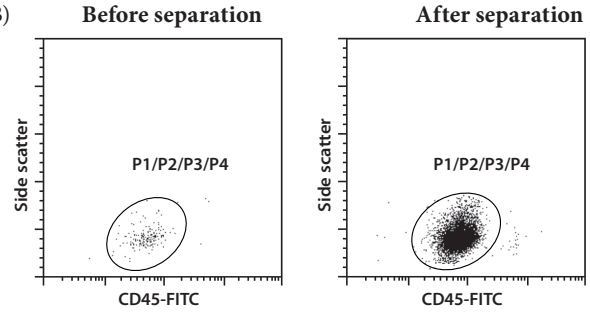


3. Create a CD34-PE vs. SSC dot plot on the cells within P1/P2 to select CD34-PE$^+$ cells.

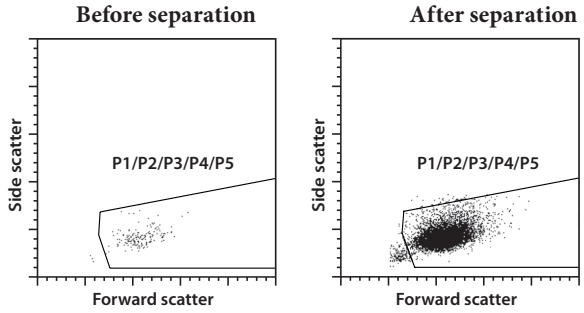


4. (Optional) Create a CD45-FITC vs. SSC dot plot on the cells within P1/P2/P3. Draw a region to exclude non-specifically stained cells. Target cells belonging to the P1/P2/P3/P4 population form a cluster with characteristic low scatter and dim CD45 fluorescence.

B)



5. (Optional) Create a FSC vs. SSC dot plot on cells within P1/P2/P3/P4. Draw a region to identify all CD34$^+$ cells, taking into consideration that CD34$^+$ cells show a slightly higher FSC than small lymphocytes.



2.4 Determination of CD34$^+$ cell frequencies

Using the population statistics table calculate the following:

1. Percentage of viable leukocytes (PI$^-$CD45$^+$ cells) amongst total cells (debris excluded).

$$= \frac{\text{No. of viable leukocytes (P2)}}{\text{Total no. of cells (P1)}} \times 100$$

2. Purity of CD34$^+$ cells amongst leukocytes (CD45$^+$ cells).

Percentage of CD34$^+$ cells (viable CD45$^+$CD34$^+$ cells) amongst leukocytes (viable CD45$^+$ cells)

$$= \frac{\text{No. of CD34}^+\text{ cells (P3)}}{\text{No. of viable leukocytes (P2)}} \times 100$$

3. Total number of CD34$^+$ cells

= Percentage of viable CD34$^+$ cells × total number of leukocytes

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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