Miltenyi Biotec

CD34/CD133 Stem Cell Analysis Cocktail Kit, anti-human

Order no. 130-092-882

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

0.5 mL CD34/CD133 Stem Cell Analysis Cocktail, anti-human containing:

CD133/2 Antibody, anti-human, PE (clone: 293C3, isotype: mouse IgG2b)

CD34 Antibody, anti-human, APC (clone: AC136, isotype: mouse IgG2a)

CD45 Antibody, anti-human, FITC (clone: MB4-6D6, isotype: mouse IgG1)

CD45 Antibody, anti-human, VioBlue® (clone: 5B1, isotype: mouse IgG2a)

0.5 mL CD34/CD133 Stem Cell Control Cocktail, anti-human containing:

CD133/2 Antibody, anti-human, PE (clone: 293C3, isotype: mouse IgG2b)

CD34 Antibody, anti-human, APC (clone: AC136, isotype: mouse IgG2a)

CD45 Antibody, anti-human, FITC (clone: MB4-6D6, isotype: mouse IgG1)

CD45 Antibody, anti-human, VioBlue® (clone: 5B1, isotype: mouse IgG2a)

CD133/2 Antibody, anti-human, pure (clone: 293C3, isotype: mouse IgG2b)

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/CD133 Stem Cell Analysis Cocktail Kit, anti-human is designed to enable the easy and rapid evaluation of stem and progenitor cells enriched with the CD133 MicroBead Kit.

The CD34/CD133 Stem Cell Analysis Cocktail Kit, anti-human consists of the CD34/CD133 Stem Cell Analysis Cocktail, anti-human and the CD34/CD133 Stem Cell Control Cocktail, anti-human. The analysis cocktail includes CD133, CD34, and CD45 antibodies for optimal identification of hematopoietic stem and progenitor cells. The control cocktail in addition contains excess unconjugated CD133 antibody to block staining of CD133$^+$ cells. This enables a reliable identification of false-positive events and their exclusion from the analysis.

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. Alternatively, the threshold for leukocyte restriction can also be set on forward scatter signals.

1.2 Applications

- Evaluation and quality control of MACS Separations of human hematopoetic stem and progenitor cells using the CD133 MicroBead Kit, human – lyophilized (# 130-097-049).

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 red (640 nm), 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) when using the MACSQuant Express Mode, or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.
- (Optional) MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900) for optimal compensation of the fluorescence spillover from fluorochrome-conjugated antibodies.

140-001-931.05

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

2. Protocol

2.1 Immunofluorescent staining of nucleated cells

▲ Prepare two samples of the original fraction. One sample is stained with the CD34/CD133 Stem Cell Analysis Cocktail, anti-human and the other one with the CD34/CD133 Stem Cell Control Cocktail, anti-human.

▲ 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/CD133 Stem Cell Analysis Cocktail, anti-human or 10 µL of the CD34/CD133 Stem Cell Control Cocktail, anti-human, respectively.
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.

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 at a final 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_CD133_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.

▲ If analyses are performed using the Express Mode, these have to be done as a group analysis.

Grouping of samples required?	Yes
Samples IDs pre-defined by the Express Mode	Minimum: 2 Maximum: 4
Samples IDs pre-defined by the Express Mode	Control* Original fraction/Before separation Negative fraction Positive fraction/After separation
Exclusion trigger/threshold	CD45-VioBlue

* This is a mandatory sample in the Express Mode; use the CD34/CD133 Stem Cell Control Cocktail, anti-human for staining.

▲ For manual data analysis using flow cytometers other than the MACSQuant Analyzer, follow the protocol provided below.

▲ The gating strategy outlined below is applicable for the analysis of cells enriched with the CD133 MicroBead Kit, human – lyophilized (# 130-097-049). Always analyze both the original cell fraction (before separation) and the target cell fraction (positive 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 FSC/SSC or CD45-FITC have been used for leukocyte exclusion, the gating strategy must be adjusted accordingly.

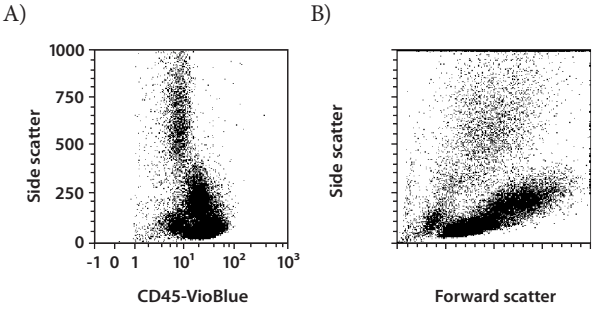
1. Set the instrument to a standard 3-color data acquisition protocol. Make sure the calibration and compensation settings have been optimized. Set the instrument to collect at least 100,000 cells in the original cell fraction in order to receive >100 $CD133^+$ target cells. For the MACSQuant Analyzer, choose an appropriate analysis volume. The number of events per second should not exceed 2,000. Analyze samples in the following order:

   1. Cells before separation, stained with the CD34/CD133 Stem Cell Control Cocktail, anti-human.

   2. Cells before separation, stained with the CD34/CD133 Stem Cell Analysis Cocktail, anti-human.

   3. Cells after separation, stained with the CD34/CD133 Stem Cell Analysis Cocktail, anti-human.

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 $CD133^+$ and $CD34^+$ hematopoietic stem and progenitor 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).



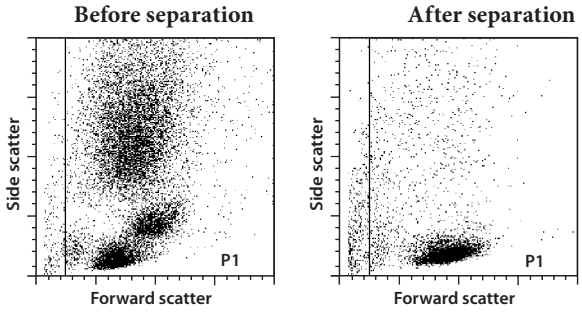
3. For manual gating create a population list as follows:

Population	Parameter/label	Definition
Total cells (excluding debris)	FSC/SSC	P1
Viable leukocytes	CD133/2-PE/ Propidium iodide	P1/P2
CD34$^+$ target cells	CD34-APC/SSC	P1/P2/P3
CD133$^+$ target cells	CD133/2-PE/CD34-APC	P1/P2/P3/P4
(Optional) Verification of target cells based on ISHAGE* guidelines.	CD45-FITC/SSC	P1/P2/P3/P4/P5
	FSC/SSC	P1/P2/P3/P4/P5/P6

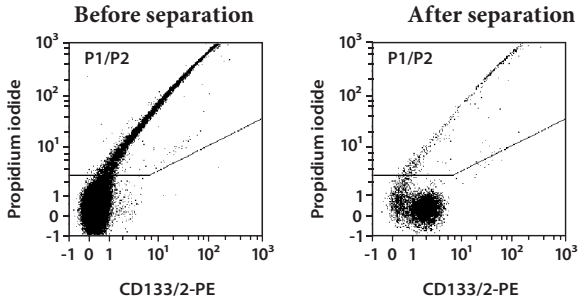
* ISHAGE = International Society of Hematotherapy and Graft Engineering

2.3 Data analysis

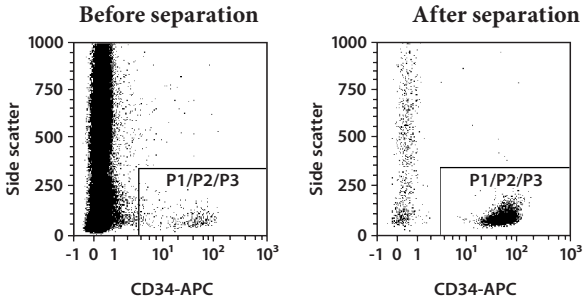
1. Create a FSC vs. SSC dot plot and draw region P1 to exclude debris.



2. Create a CD133/2-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.

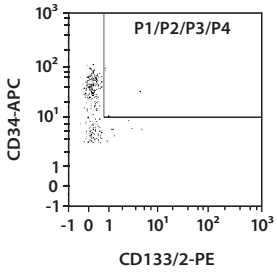


3. Create a CD34-APC vs. SSC dot plot on the cells within P1/P2 to select CD34-APC$^+$ cells.

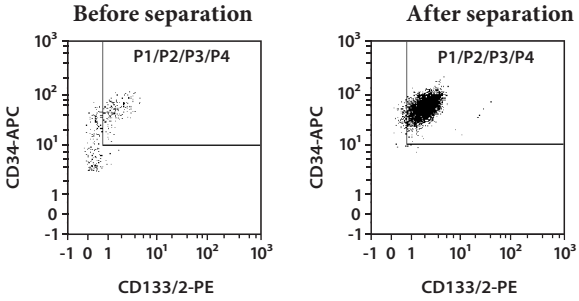


4. Create a CD34-APC vs. CD133/2-PE dot plot. In the control sample stained with the CD34/CD133 Stem Cell Control Cocktail, anti-human, define region P1/P2/P3/P4 to exclude CD133$^-$ cells (A). Apply region P1/P2/P3/P4 to the samples stained with the CD34/CD133 Stem Cell Analysis Cocktail, anti-human to identify CD133$^+$ target cells (B).

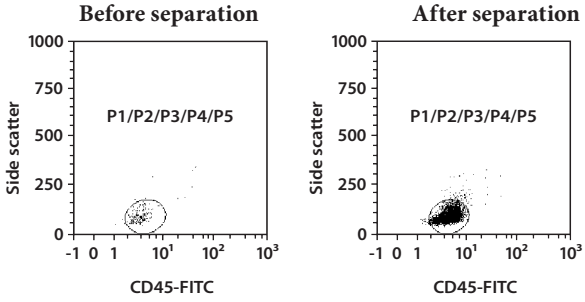
A)



B)

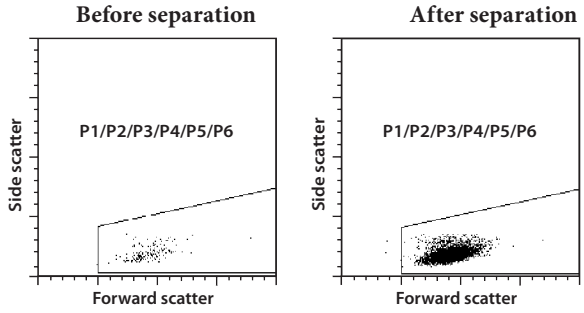


5. (Optional) Create a CD45-FITC vs. SSC dot plot on the cells within P1/P2/P3/P4. Draw a region to exclude non-specifically stained cells. Target cells belonging to the P1/P2/P3/P4/P5 population form a cluster with characteristic low scatter and dim CD45 fluorescence.



140-001-931.05

6. (Optional) Create a FSC vs. SSC dot plot on cells within P1/P2/P3/P4/P5. Draw a region to identify all CD133$^+$ cells, taking into consideration that CD133$^+$ cells show a slightly higher FSC than small lymphocytes.



2.4 Determination of CD133$^+$ 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 CD133$^+$ cells amongst leukocytes (CD45$^+$ cells).

   Percentage of CD133$^+$ cells (viable CD45$^+$CD34$^+$CD133$^+$ cells) amongst leukocytes (viable CD45$^+$ cells)

$$= \frac{\text{No. of CD133}^+\text{ cells (P6)}}{\text{No. of viable leukocytes (P2)}} \times 100$$

3. Total number of CD133$^+$ cells

   = Percentage of viable CD133$^+$ 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.

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

autoMACS, MACS, MACSQuant, MACSQuantify, the Miltenyi Biotec logo, and VioBlue are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.