Miltenyi Biotec

Proposal for CD34$^+$ cell, T cell, and B cell determination by flow cytometry after CliniMACS® CD34$^+$ enrichment

Contents

1. **Description**
   - 1.1 **Purpose**
   - 1.2 **Reagent and instrument requirements**
   - 1.3 **Preparation of solutions and staining cocktails**
2. **Staining protocol**
   - 2.1 **Staining with staining cocktail "SC panel" to determine cell count, viability, and frequency of CD34$^+$ cells**
   - 2.2 **Staining with staining cocktail "ImCC" to determine the frequency of T, B, NK, and NKT cells, monocytes, and granulocytes**
3. **Example of flow cytometric data acquisition and analysis**
   - 3.1 **Instrument settings for cell count and frequency**
   - 3.2 **Data acquisition for "SC panel" and "ImCC"**
   - 3.3 **Creation of dot plots**
4. **Analysis of results**

Important note

The present protocols are research protocols, based on laboratory experience. Miltenyi Biotec cannot and will not accept any liability as to the outcome of procedures. The procedures are for research use only, not for clinical use.

1. Description

1.1 Purpose

This protocol describes the quantification of CD34$^+$ cells, T cells, and B cells before and after an automated separation process using the CliniMACS Plus or the CliniMACS Prodigy® Instrument.
The described staining and analysis strategy was optimized according to the special requirements of flow cytometric detection suitable for a very low percentage of remaining T cells in the final product and a low percentage of CD34$^+$ cells in the starting material. In addition, it enables the characterization of the composition (immunophenotyping) of the starting cell product and all fractions obtained during a CliniMACS Separation.

1.2 Reagent and instrument requirements

- Flow cytometer equipped with three lasers (405 nm, 488 nm, and 635 nm to allow simultaneous analysis of VioBlue®, VioGreen™, FITC, PE, PerCP, PE-Vio® 770, APC, and APC-Vio 770) and appropriate software, e.g., MACSQuant® Analyzer 10 installed with a compatible MACSQuantify™ Software version.
- (Optional) MACS Comp Bead Kit, anti-mouse Igκ (# 130-097-900) and MACS Comp Bead Kit, anti-REA (# 130-104-693)
- Automated hematology analyzer
- Tabletop centrifuge
- CliniMACS PBS/EDTA Buffer CR/GMP (# 220-001-827)
- Human serum albumin (HSA)
- Red Blood Cell Lysis Solution (10×) (# 130-094-183)
- Double-distilled water (ddH_2O)
- CD45 Antibody, anti-human, FITC, REAfinity™ (# 130-110-631)
- CD34 Antibody, anti-human, APC, REAfinity (# 130-120-514)
- 7-AAD Staining Solution (# 130-111-568)
- StainExpress™ Immune Cell Composition Cocktail, human (# 130-127-637) ("ImCC")

  Alternatively, use single fluorochrome-conjugated antibodies for "ImCC":

Product	Clone	Order no.
CD45 Antibody, anti-human, VioBlue, REAfinity	REA747	130-110-637
CD4 Antibody, anti-human, VioGreen, REAfinity	REA623	130-113-230
CD3 Antibody, anti-human, FITC, REAfinity	REA613	130-113-138
CD16 Antibody, anti-human, PE, REAfinity	REA423	130-113-393
CD56 Antibody, anti-human, PE, REAfinity	REA196	130-113-312
CD19 Antibody, anti-human, PE-Vio 770, REAfinity	REA675	130-113-647
CD14 Antibody, anti-human, APC, REAfinity	REA599	130-110-520
CD8 Antibody, anti-human, APC-Vio 770, REAfinity	REA734	130-110-681

Table 1: List of fluorochrome-conjugated antibodies for "ImCC".

1.3 Preparation of solutions and staining cocktails

▲ Solutions and staining cocktails should be prepared freshly. Discard unused solutions and cocktails at the end of the day.

- 1× Red Blood Cell Lysis Solution:
  Prepare 1× Red Blood Cell Lysis Solution by diluting Red Blood Cell Lysis Solution (10×) 1:10 with ddH_2O, e.g., dilute 2.5 mL Red Blood Cell Lysis Solution (10×) with 22.5 mL of ddH_2O and mix well. Adjust for multiple samples accordingly. Store and use at room temperature (+19 to +25 °C).
- Buffer: CliniMACS PBS/EDTA Buffer CR/GMP supplemented with HSA to a final concentration of 0.5% (w/v). Store at +2 to +8 °C.

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

CL_SP78.01

- Staining cocktail for stem cell panel ("SC panel")

  Prepare following volumes per sample type to a final volume of 40 µL:

  - 2 µL CD45 Antibody, anti-human, FITC, REAfinity
  - 2 µL CD34 Antibody, anti-human, APC, REAfinity
  - 10 µL 7-AAD Staining Solution
  - 26 µL buffer

2. Staining protocol

An analysis of $CD34^+$ cells, T cells, and B cells is performed by fluorochrome-conjugated monoclonal antibody labeling of the target cells followed by determination using a flow cytometer.
For analysis purpose take aliquots (approximately 0.5 mL of starting material and optional original fraction, at least 1.5 mL of the Non-Target Cell Bag fraction, and at least 3×10^6 white blood cells (WBCs) of the waste and Target Cell Bag fraction) from the samples listed below (table 2).

▲ **Note:** The bag labels of the CliniMACS Plus and the CliniMACS Prodigy Tubing Sets are different. These are listed in table 3.

Sample type	Referred to as
Leukapheresis product	Starting material (Start)*
CD34-enriched cells after CliniMACS Plus / CliniMACS Prodigy enrichment	Target Cell Bag (TCB) fraction*: $CD34^+$ cells
$CD34^-$ cells after CD34 enrichment	Non-Target Cell Bag (NTCB) fraction: $CD34^-$ cells
(Optional) CD34-labeled leukapheresis product prior to enrichment	Original (Ori) fraction
(Optional) Waste Bag fraction	Waste Bag (waste) fraction

* It is recommended to always analyze triplicates.

Table 2: List of required samples for staining.

Bag content	CliniMACS Prodigy	CliniMACS Plus
Enriched $CD34^+$ cells	Target Cell Bag	Cell Collection Bag
$CD34^-$ cells	Non-Target Cell Bag	Negative Fraction Bag
Buffer waste	Waste Bag	Waste Bag

Table 3: Bag label naming overview of individual bags for CliniMACS Plus and CliniMACS Prodigy Tubing Sets.

2.1 Staining with staining cocktail "SC panel" to determine cell count, viability, and frequency of $CD34^+$ cells

▲ All samples must be processed and analyzed within 24 hours.

1. Label all tubes (5 mL tubes) with "SC panel" and additionally as follows:
   1) 3× Start
   2) 3× TCB fraction: $CD34^+$ cells
   3) NTCB fraction: $CD34^-$ cells
   4) (Optional) Ori fraction
   5) (Optional) Waste fraction
2. Transfer 100 µL of each fraction into the appropriate tube.

   ▲ **Note:** If the cell count of a sample exceeds 1×10^8/mL dilute the sample 1:10 with buffer and take 100 µL of the diluted sample for staining.
3. Add 40 µL of the prepared staining cocktail "SC panel" into each tube. The final volume should be 140 µL.
4. Vortex and incubate for 10 minutes in the dark at +2 to +8 °C.
5. Add 1860 µL of 1× Red Blood Cell Lysis Solution to the tubes containing the Start, NTCB fraction, and Ori fraction (if applicable). The final volume is now 2000 µL per tube (dilution factor 1:20).
6. Add 860 µL 1× Red Blood Cell Lysis Solution to the TCB fraction and waste fraction. The final volume is now 1000 µL per tube (dilution factor 1:10).
6. Mix well and incubate for 10 minutes in the dark at room temperature.

   ▲ **Note:** If a sample is not light red and transparent after 10 minutes, incubate for additional 2 minutes.
7. Analyze samples by flow cytometry within one hour after staining procedure.

2.2 Staining with staining cocktail "ImCC" to determine the frequency of T, B, NK, and NKT cells, monocytes, and granulocytes

1. Label all tubes (5 mL tubes) with "ImCC" and additionally as follows:
   1) Start
   2) 3× TCB: $CD34^+$ cells
   3) NTCB: $CD34^-$ cells
   4) (Optional) Ori fraction
   5) (Optional) Waste fraction
2. Transfer required volume for 2×10^6 leukocytes of each fraction into the appropriate tube and wash cells by adding 1 mL of prepared CliniMACS PBS/EDTA Buffer CR/GMP containing 0.5% HSA (w/v).
3. Centrifuge cells at 300×g for 10 minutes at room temperature. Aspirate supernatant completely.
4. Resuspend cells in 100 µL of prepared CliniMACS PBS/EDTA Buffer CR/GMP containing 0.5% HSA (w/v).
5. Add 100 µL of the cell suspension to one tube of StainExpress Immune Cell Composition Cocktail.
6. Mix well and incubate for 10 minutes in the dark at +2 to +8 °C.
7. Lyse the red blood cells by adding 2 mL of 1× Red Blood Cell Lysis Solution.
8. Mix well and incubate for 10 minutes in the dark at room temperature.

   ▲ **Note:** If a sample is not light red and transparent after 10 minutes, incubate for additional 2 minutes.
9. Centrifuge cells at 300×g for 5 minutes at room temperature. Aspirate supernatant completely.
10. Resuspend cells in 0.5 mL of CliniMACS PBS/EDTA Buffer CR/GMP containing 0.5% HSA (w/v).
11. Analyze samples by flow cytometry.

    ▲ **Note:** Store sample in the refrigerator until analysis.

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

3. Example of flow cytometric data acquisition and analysis

The following protocol has been designed for data acquisition and analysis of all samples with commercially available analysis software, e.g., MACSQuantify Software.

3.1 Instrument settings for cell count and frequency

Choose an appropriate setting for analysis of human leukocytes with an 8-color panel. An appropriate instrument setting can be achieved by automatic compensation with VioBlue-, VioGreen-, FITC-, PE-, PerCP-, PE-Vio 770–, APC-, and APC-Vio 770–conjugated antibodies in combination with the MACS Comp Bead Kits and MACSQuant Flow Cytometers. For detailed information about compensation using the MACS Comp Bead Kits refer to respective data sheets.

- Use the starting material stained with the "SC panel" and the starting material stained with the "ImCC" to adjust the instrument stettings for each staining.
- Set threshold (trigger) on forward scatter (FSC) and adjust scatter settings to exclude debris from data acquisition.
- Ensure that no leukocyte population is excluded from analysis.
- To obtain accurate results, check the compensation for all eight fluorescence channels.

3.2 Data acquisition for "SC panel" and "ImCC"

The samples are analyzed using a MACSQuant Analyzer after adjustment of the regions according to table 4 (refer to sections 3.3.1 and 3.3.2):

Sample	Panel "ImCC"		Panel "SC panel"	
	Uptake volume (µL)	Measure rate (cells/s)	Uptake volume (µL)	Measure rate (cells/s)
Starting material	100	2000	50–450**	Medium
ddH_2O*	200	High*	–	–
Target Cell Bag fraction	450	2000	50–450**	Medium
Non-Target Cell Bag fraction	100	2000	450	Medium
(Optional) Ori	100	2000	50–450**	Medium
Buffer Waste Bag fraction	200	2000	300	Medium

* To avoid carryover, rinsing of MACSQuant Analyzer or the measuring of ddH_2O prior to measurement of the target cell fraction is recommended. Rinse MACSQuant Analyzer by, e.g., choosing **Extended mode** instead of **Standard mode** for washing between samples.

** Adjust uptake volume in accordance to measure at least 3000 viable $CD34^+$ cells.

Table 4: Overview of flow cytometric analysis.

3.3 Creation of dot plots

3.3.1 Creation of dot plots for panel "SC panel"

For data acquisition create following dot plots, regions (R) are set according to dot plots shown below. The x-axis is mentioned first.

A Plot: Forward scatter (FSC) versus side scatter (SSC) (R1)
B Plot: CD45-FITC versus SSC (R2)
C Plot: 7-AAD Staining Solution versus SSC (R3)
D Plot: CD34-APC versus SSC (R4)
E Plot: CD45-FITC versus SSC (R5)
F Plot: CD34 cell determination: FSC versus SSC (R6)
G Plot: 7-AAD Staining Solution versus CD34-APC (R7)

Definition of logical gates

Define and label the gates as described in table 5:

Gate	Label	Definition
G1	Debris exclusion	R1
G2	$CD45^+$ cells	R1*R2
G3	Viable $CD45^+$ cells	R1*R2*R3
G4	$CD34^+$ cells SSC^{low}	R1*R2*R4
G5	$CD34^+CD45^{+dim}$ cluster control	R1*R2*R4*R5
G6	$CD34^+$ scatter control	R1*R2*R4*R5*R6
G7	Viable $CD34^+$ cells	R1*R2*R4*R5*R6*R7

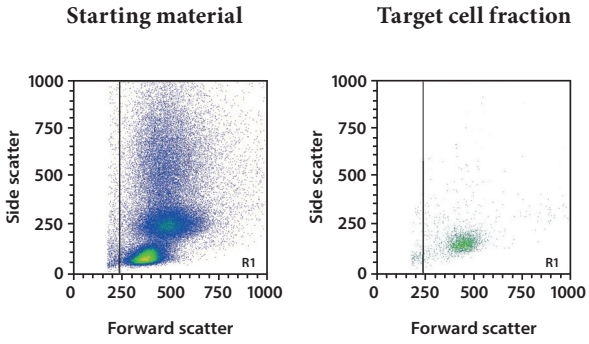
Table 5: Overview of the definitions and labels of "SC panel" gates.

Description of detailed gating strategy

A Plot: Forward scatter (FSC) versus side scatter (SSC) – Debris exclusion

Activated gate: no gate

R1 is defined to identify a cluster of events meeting the scatter profile of leukocytes, while excluding the debris. For more details refer to the MACSQuant Analyzer user manual.

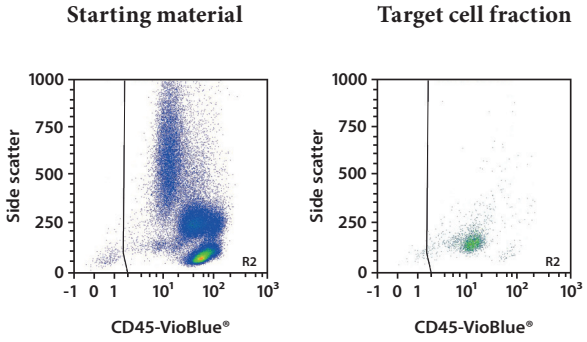


Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

B Plot: CD45-FITC versus SSC – Identification of CD45$^+$ cells

Activated gate: R1 = leukocytes

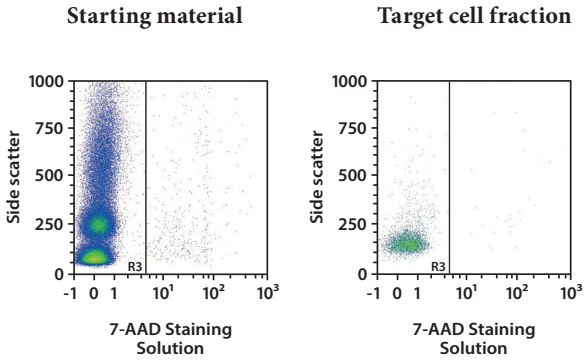
Set R2 thereby excluding all CD45$^-$ events from analysis while all CD45$^+$ cells are included. CD34$^+$ progenitor cells express CD45 with lower staining intensity than lymphocytes. Carefully set region R2 without excluding relevant events from analysis.



C Plot: 7-AAD Staining Solution versus SSC – Identification of viable CD45$^+$ cells

Activated gate: R1*R2 = CD45$^+$ cells

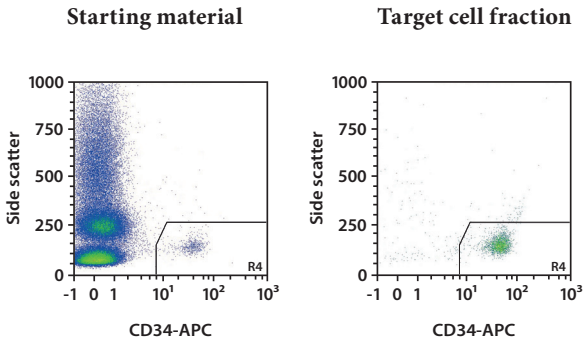
Define R3 thereby including all 7-AAD$^-$ viable leukocytes.



D Plot: CD34-APC versus SSC – Identification of CD34$^+$ cells

Activated gate: R1*R2 = CD45$^+$ cells

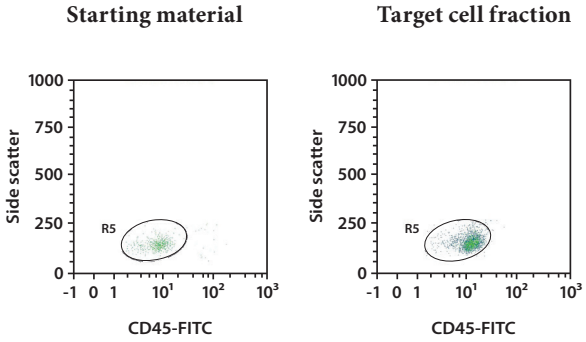
Define R4 thereby including all CD34$^+$ cells with a low SSC.



E Plot: CD34 cluster control: CD45-FITC versus SSC – Identification of CD34$^+$CD45^{dim} cells

Activated gate: R1*R2*R4 = CD34$^+$SSC^{low} cells

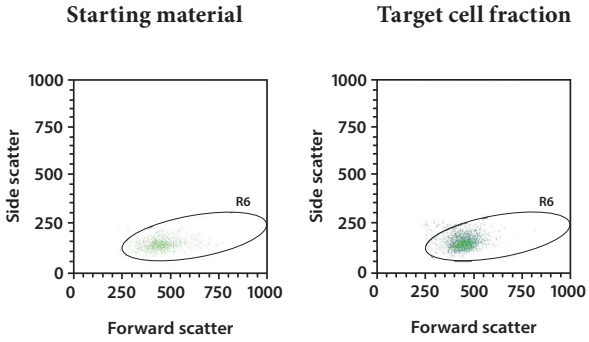
CD34$^+$ cells form a cluster with low SSC and dim CD45 fluorescence and are included in R5. Non-specifically stained events are excluded from this region.



F Plot: Final cell determination plot: FSC versus SSC – Identification of CD34$^+$ cells

Activated gate: R1*R2*R4*R5 = CD34$^+$CD45^{dim} cluster control

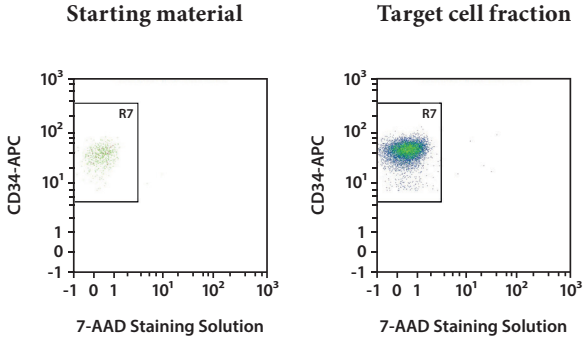
Define R6 that identifies a cluster of events meeting the scatter profile of leukocytes of CD34$^+$ progenitor cells. Cells clustered in R6 exhibit slightly higher forward scatter compared to small lymphocytes and uniformly low side scatter. Any events outside R6 are not included in the % viable CD34$^+$ cells determination.



G Plot: 7-AAD Staining Solution versus CD34-APC – Identification of viable CD34$^+$ cells

Activated gate: R1*R2*R4*R5*R6 = CD34$^+$ scatter control

Define R7 thereby including all viable CD34$^+$ 7-AAD$^-$ cells.



3.3.2 Creation of dot plots for panel "ImCC"

For data acquisition create following dot plots (the regions are set according to dot plots shown below). The x-axis is mentioned first.

A Plot: FSC versus SSC (R1)
B Plot: CD45-VioBlue versus SSC (R2)
C Plot: 7-AAD Staining Solution versus SSC (R3)
D Plot: CD14-APC versus SSC (R4, R5, and R6)
E Plot: CD19-PE-Vio 770 versus CD3-FITC (R7 and R8)
F Plot: CD8-APC-Vio 770 versus CD4-VioGreen (R9 and R10)
G Plot: CD16/CD56-PE versus SSC (R11 and R12)
H Plot: CD16/CD56-PE versus CD3-FITC (R13 and R14)

Definition of logical gates

Define and label the gates as described in table 6:

Gate	Label	Definition
G1	Debris exclusion	R1
G2	$CD45^+$ cells	R1*R2
G3	Viable leukocytes	R1*R2*R3
G4	Lymphocytes	R1*R2*R3*R4
G5	$CD14^-$ cells	R1*R2*R3*R5
G6	$CD14^+$ cells	R1*R2*R3*R6
G7	$CD3^+$ cells	R1*R2*R3*R4*R7
G8	$CD19^+$ cells	R1*R2*R3*R4*R8
G9	$CD4^+$ cells	R1*R2*R3*R4*R7*R9
G10	$CD8^+$ cells	R1*R2*R3*R4*R7*R10
G11	$CD16/CD56^-$ SSC^{high} cells	R1*R2*R3*R5*R11
G12	$CD16/CD56^+$ SSC^{high} cells	R1*R2*R3*R5*R12
G13	$CD3^-CD16/CD56^+$ cells	R1*R2*R3*R4*R13
G14	$CD3^+CD16/CD56^+$ cells	R1*R2*R3*R4*R14

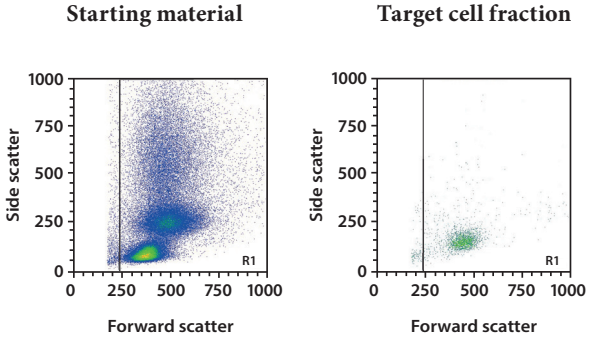
Table 6: Overview of the definitions and labels of "ImCC" gates.

Description of detailed gating strategy

A Plot: Forward scatter (FSC) versus side scatter (SSC) – Debris exclusion

Activated gate: no gate

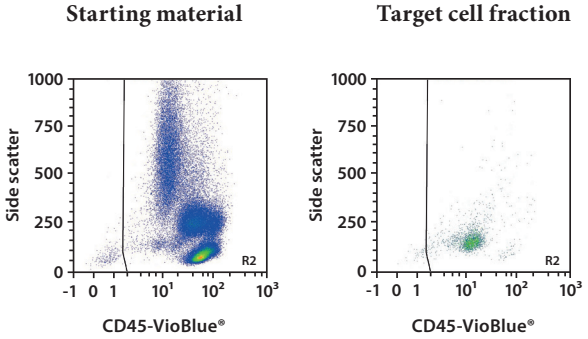
R1 is defined to identify a cluster of events meeting the scatter profile of leukocytes, while excluding the debris. For more details refer to the MACSQuant Analyzer user manual.



B Plot: CD45-VioBlue versus SSC – Identification of $CD45^+$ cells

Activated gate: R1 = leukocytes

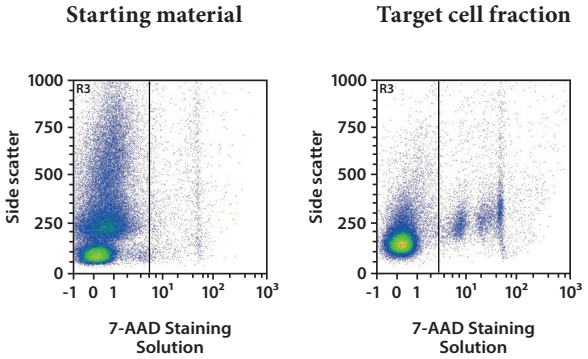
Set R2 thereby excluding all $CD45^-$ events from analysis while all $CD45^+$ cells are included. $CD34^+$ progenitor cells express CD45 with lower staining intensity than lymphocytes. Carefully set region R2 without excluding relevant events from analysis.



C Plot: 7-AAD Staining Solution versus SSC – Identification of viable leukocytes

Activated gate: R1*R2 = leukocytes

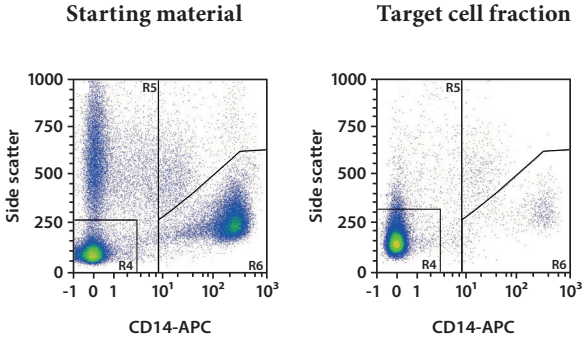
Define R3 thereby including all $7\text{-}AAD^-$ viable leukocytes.



D Plot: CD14-APC versus SSC – Identification of $CD14^+$ and $CD14^-$ cells and lymphocytes

Activated gate: R1*R2*R3 = viable leukocytes

Define R4 thereby including all lymphocytes (SSC^{low} and $CD14^-$), R5 including all $CD14^-$ cells (non-monocytes), and R6 including all $CD14^+$ cells with medium SSC (monocytes).

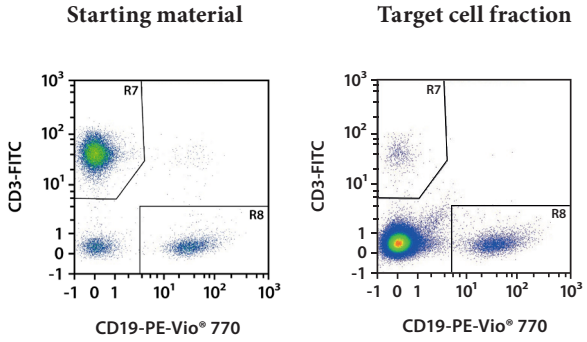


Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

E Plot: CD19-PE-Vio 770 versus CD3-FITC – Identification of $CD3^+$ and $CD19^+$ cells

Activated gate: R1*R2*R3*R4 = lymphocytes

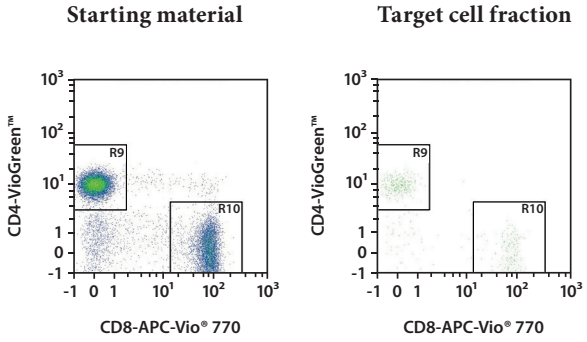
Define R7 thereby including all $CD3^+$ cells and R8 including all $CD19^+$ cells.



F Plot: CD8-APC-Vio 770 versus CD4-VioGreen – Identification of $CD4^+$ and $CD8^+$ T cells

Activated gate: R1*R2*R3*R4*R7 = $CD3^+$ cells

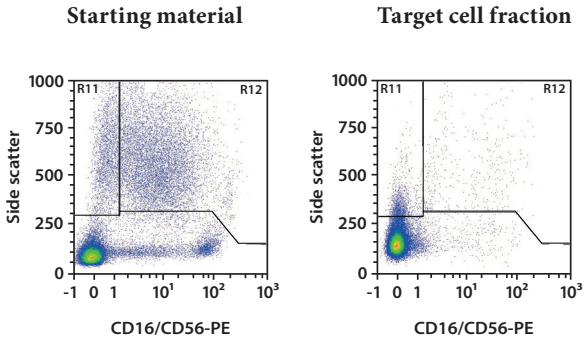
Define R9 thereby including $CD4^+$ T cells and R10 including $CD8^+$ T cells.



G Plot: CD16/CD56-PE versus SSC – Identification of $CD16^-$ and $CD16^+$ cells

Activated gate: R1*R2*R3*R4*R5 = $CD14^-$ cells (non-monocytes)

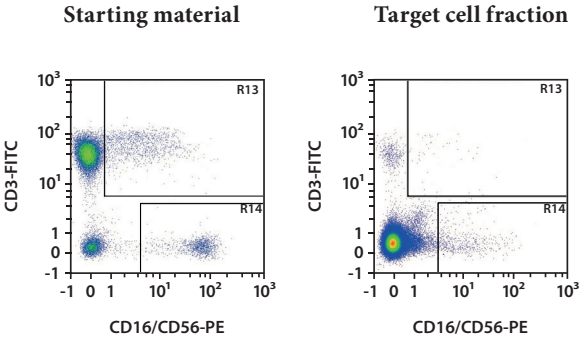
Define R11 thereby including all $CD16/CD56^-SSC^{high}$ cells (eosinophils) and R12 including all $CD16/CD56^+SSC^{high}$ cells (neutrophils).



H Plot: CD16/CD56-PE versus CD3-FITC – Identification of $CD3^-/CD56^+$ and $CD3^+/CD56^+$ cells

Activated gate: R1*R2*R3*R4 = lymphocytes

Define R13 thereby including all $CD3^+CD16^+/CD56^+$ cells (NKT cells) and R14 including all $CD3^-CD16^+/CD56^+$ cells (NK cells).



4. Analysis of results

Calculations of cell counts for $CD34^+$, $CD3^+$ T cells, and $CD19^+$ B cells and log depletion for $CD3^+$ T cells and $CD19^+$ B cells

For analysis of results, display the statistic of the panel "SC panel" with following parameters:
Count, count/mL, and percentage of superior gate (%-#)

The superior gate (%-#) is a parameter of the MACSQuantify Software and describes the percentage of cells that were loaded from the respective upstream gate.

For analysis of results, display the statistic of the panel "ImCC" with following parameters:
Count, percentage of superior gate (%-#), and percentage of superior gate-2 (%-2)

The percentage of superior gate-2 (%-2) is a parameter of the MACSQuantify Software and refers to the viable $CD45^+$ cells from the respective upstream gate R3 for all gates except $CD4^+$ versus $CD8^+$ cells.

(Optional) The percentage of superior gate-3 (%-3) refers to the viable $CD45^+$ cells and can be displayed in the statistics of the MACSQuantify Software if required.

Starting material is used as an example in the following statistics (tables 7 and 8). The other fractions can be analysed in the same way.

Name	%-#	Count	Count/mL
R1	98.46	452281	1.01×10^6
R2	99.74	451118	10^6
R3	99.37 ❶	448285 ❸	9.96×10^5 ❷
R4	0.66	2997	6.66×10^3
R5	96.60	2895	6.43×10^3
R6	99.21	2872	6.38×10^3
R7	99.76 ❺	2865 ❹	6.37×10^3

Table 7: Example of statistics for panel "SC panel" based on the starting material ample.

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

Name	%-#	%-2	Count
R1	98.335	–	288835
R2	99.34	94.73	286934
R3	97.53	96.89	279846
R4	43.31	42.24	121200
R5	62.95	61.39	176150
R6	34.24	33.39	95820
R7	77.10	33.39 ❻	93449
R8	12.18	5.27 ❼	14757
R9	58.86	45.38	55004
R10	33.23	25.62	31056
R11	6.39	4.02	11262
R12	22.94	14.44	40413
R13	5.96	2.58	7223
R14	5.63	2.44	6828

Table 8: Example of statistics for panel "ImCC" based on the starting material sample.

The following calculations in grey boxes show general formula for calculation. The purple boxes show examplary calculation for the starting material.

4.1 Calculation of leukocyte cell count

The dilution factor is calculated by multiplying an (optional) pre-dilution factor of the sample (no pre-dilution: factor = 1) by the sample volume applied to flow cytometer, e.g., 2000 µL, divided by the sample volume used for the staining, e.g., 100 µL. The dilution factor in the standard application without a pre-dilution is 20.

Dilution factor = Pre-dilution factor × 2000 µL/sample volume (µL)

Dilution factor = 1 × 2000 µL/100µL = 20

The viability of leukocytes can be adopted from the R3 %-# (❶) statistics in table 7.

Viability of leukocytes = R3 %-#

Viability of leukocytes = 99.37 % (❶)

The viable leukocyte concentration (cells/mL) can be derived by multiplying R3 count/mL (❷) from table 7 with the dilution factor (20).

Viable leukocytes (cells/mL) = R3 count/mL × dilution factor

Viable leukocytes (cells/mL) = 9.96×10^5/mL × 20 = 1.99×10^7/mL

The total leukocyte count can be calculated by multiplying the number of viable leukocytes (cells/mL) by sample volume (mL).

Total leukocyte count = Viable leukocytes/mL × sample volume (mL)

Total leukocyte count = 1.99×10^7/mL × 249 mL = 4.96×10^9

4.2 Calculation of CD34$^+$ cell count

The percentage of viable CD34$^+$ cells can be calculated by multiplying R7 count (❹) from table 7 by 100% and dividing this by R3 count (❸) from table 7.

$$\textbf{Viable CD34}^+\textbf{ cells (\%)} = \frac{\text{Viable CD34}^+\text{ cells (R7 count)}}{\text{Viable leukocytes (R3 count)}} \times 100\%$$

$$\textbf{Viable CD34}^+\textbf{ cells (\%)} = \frac{2865}{448285} \times 100\% = 0.64\%$$

The total count of CD34$^+$ cells can be calculated by multiplying percentage of viable CD34$^+$ cells with total leukocyte count divided by 100%.

$$\textbf{Total CD34}^+\textbf{ cell count} = \frac{\text{Viable CD34}^+\text{ cells (\%)} \times \text{total leukocyte count}}{100\%}$$

$$\textbf{Total CD34}^+\textbf{ cell count} = \frac{0.64\% \times 4.96\times10^9}{100\%} = 3.17\times10^7$$

The viability of CD34$^+$ cells (%) can be taken from the statistic R7 %-# (❺) from table 7.

Viability of CD34$^+$ cells (%) = R7 %-#

Viability of CD34$^+$ cells (%) = 99.76%

The yield of CD34$^+$ cells (%) can be calculated by dividing total CD34$^+$ cell count of Target Cell Bag (TCB) fraction (mean of all taken aliquots) by total CD34$^+$ cell count of starting material (mean of all taken aliquots), followed by multiplication with 100%.

Example for calculation:
Mean of total CD34$^+$ cell count of all TCB fractions = 2.01×10^7
Mean of total CD34$^+$ cell count of all starting material = 3.17×10^7

$$\textbf{Yield of CD34}^+\textbf{ cell (\%)} = \frac{\text{Total CD34}^+\text{ cell count of TCB}}{\text{Total CD34}^+\text{ cell count of starting material}} \times 100\%$$

$$\textbf{Yield of CD34}^+\textbf{ cell (\%)} = \frac{2.01\times10^8}{3.43\times10^8} \times 100\% = 63.4\%$$

4.3 Calculation of CD3$^+$ T cell count

The total CD3$^+$ T cell count can be calculated by multiplying frequency of CD3$^+$ cells (%) R7 %-2 (❻) from table 8 by total viable leukoyte count divided by 100%.

$$\textbf{Total CD3}^+\textbf{ cell count} = \frac{\text{Frequency of CD3}^+\text{ cells (\%)} \times \text{total leukocyte count}}{100\%}$$

$$\textbf{Total CD3}^+\textbf{ cell count} = \frac{33.39\% \times 4.96\times10^9}{100\%} = 1.66\times10^9$$

Unless otherwise specifically indicated, all Miltenyi Biotec products and services are for research use only and not for diagnostic or therapeutic use.

4.4 Calculation of CD3$^+$ T cell –logP value

The CD3$^+$ T cell –log P value describes the depletion efficiency of CD3$^+$ T cells. For determination, the total CD3$^+$ T cell count of Target Cell Bag fraction is divided by total CD3$^+$ T cell count of starting material fraction and then the –log of the quotient is calculated.

Example for calculation:
Total CD3$^+$ cell count of TCB fraction = 4.55×10^4
Total CD3$^+$ cell count of starting material = 1.66×10^9

$$\text{–logP (CD3}^+\text{ T cells)} = -\log\left(\frac{\text{Total CD3}^+\text{ T cell count of TCB}}{\text{Total CD3}^+\text{ T cell count of starting material}}\right)$$

$$\text{–logP (CD3}^+\text{ T cells)} = -\log\left(\frac{4.55\times10^4}{1.66\times10^9}\right) = 4.6$$

4.5 Calculation of CD19$^+$ B cell count

The total CD19$^+$ B cell count in the sample can be calculated by multiplying the frequency of CD19$^+$ cells (%) R8 %-2 (❼) from table 8 by total viable leukocyte count divided by 100%.

$$\text{Total CD19}^+\text{ cell count} = \frac{\text{Frequency of CD19}^+\text{ cells (\%)} \times \text{total leukocyte count}}{100\%}$$

$$\text{Total CD19}^+\text{ cell count} = \frac{5.27\% \times 4.96\times10^9}{100\%} = 2.61\times10^8$$

4.6 Calculation of CD19$^+$ B cell –logP value

The CD19$^+$ B cell –log P value describes the depletion efficiency of CD19$^+$ B cells. For determination the total CD19$^+$ B cell count of TCB fraction is divided by total CD19$^+$ B cell count of starting material fraction and then the –log of the quotient is calculated.

Example for calculation:
Total CD19$^+$ cell count of TCB fraction = 3.85×10^5
Total CD19$^+$ cell count of starting material = 2.61×10^8

$$\text{–logP (CD19}^+\text{ B cells)} = -\log\left(\frac{\text{Total CD19}^+\text{ B cell count of TCB}}{\text{Total CD19}^+\text{ B cell count of starting material}}\right)$$

$$\text{–logP (CD3}^+\text{ T cells)} = -\log\left(\frac{3.85\times10^5}{2.61\times10^8}\right) = 2.8$$

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

The CliniMACS System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485.

In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. The CliniMACS Reagents and Biotin Conjugates are intended for in vitro use only and are not designated for therapeutic use or direct infusion into patients. The CliniMACS Reagents in combination with the CliniMACS System are intended to separate human cells. Miltenyi Biotec as the manufacturer of the CliniMACS System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit. For the manufacturing and use of target cells in humans, the national legislation and regulations – e.g. for the EU the Directive 2004/23/EC ("human tissues and cells"), or the Directive 2002/98/EC ("human blood and blood components") – must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS System.

In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application, Investigational Device Exemption (IDE) or FDA approval.

CR/GMP Products are designed, manufactured and tested under an ISO 13485 quality management system and are in compliance with relevant GMP guidelines. They are designed following the recommendations of USP <1043> on ancillary materials. The manufacturing and testing of this product is compliant with the requirements laid down in the Ph.Eur. Chapter 5.2.12.

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.

Trademarks

CliniMACS, CliniMACS Prodigy, MACS, MACSQuant, MACSQuantify, the Miltenyi Biotec logo, REAfinity, Vio, and VioGreen are registered trademarks or trademarks of Miltenyi Biotec B.V. & Co. KG and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Copyright © 2024 Miltenyi Biotec and/or its affiliates. All rights reserved.