
CliniMACS[®]

User Manual US Edition

- Software 2.40 -



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Analysis	- 4

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Disconnect bags and record process code	- 3
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Separation sequence	- 1
Disconnect bags and record process code	- 3
Unload tubing set and shutdown	- 4
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1 General

1.1 Introduction

The CliniMACS® System offers a set of tools making high quality standard cell separations available for therapeutic applications. The CliniMACS® System is based on the magnetic cell separation technology (MACS Technology) developed by Miltenyi Biotec GmbH. Miltenyi has made this technology available for clinical applications meeting the requirements of European Regulatory Standards.

The CliniMACS® components - CliniMACS®^{plus} Instrument, CliniMACS® Reagents, CliniMACS® Tubing Sets, and CliniMACS® PBS/EDTA Buffer - enable the user to enrich or deplete human cells from heterogeneous hematologic cell populations under GMP conditions.

Before using the CliniMACS® System or any CliniMACS® component outside the European Economic Community, the regulatory approval of the CliniMACS® System or any CliniMACS® component in your country must be confirmed.

Note

In Chapter 4, “Four STEPS to your target cells” is a detailed description on how to use the CliniMacs User Manual.

Limited Warranty

Should the CliniMACS® System be used in a manner not explicitly described in this manual, all warranties will be null and void.

1.2 Service information

CliniMACS® Technical Support

For any information about the CliniMACS® Systems and their components, please contact our CliniMACS® Technical Support:

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Fax +1-530-888-8925

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www.miltenyibiotec.com

CliniMACS®^{plus} Instrument information

Please record below the model and serial number located on the back of the CliniMACS®^{plus} Instrument. Refer to these numbers, whenever you call regarding information or service on the instrument.

Model no : _____

Serial no : _____

Software version: _____

The software version is shown during the start-up phase of the CliniMACS®^{plus} Instrument (see screen no 1.1, chapter 1.8).

1.3 Warnings and precautions

- CliniMACS® plus Instrument

1. Read and observe all operating instructions carefully (see Fig. 1.1).
2. Equipment safety will be compromised if it is not used according to the manufacturer's instructions
3. Risk of serious personal injury! The CliniMACS® plus Instrument is equipped with an extremely powerful magnet. Keep any magnetic information carriers (such as credit cards, magnetic tapes and floppy disks), any electronic equipment (such as hearing aids, pacemakers, cerebral/brain shunts, measuring and control instruments, computers, and watches) and magnetizable tools and objects at a distance of at least 30 cm from the device. These items may be affected or damaged by the magnetic field (see Fig. 1.2).
4. Medical electrical equipment needs special precautions regarding electromagnetic compatibility (EMC) and needs to be installed and put into service according to the EMC information provided in the accompanying documents. Portable and mobile RF communications equipment can affect medical electrical equipment.
5. The CliniMACS® plus Instrument is a protection class I device and may only be plugged into an outlet with a grounded connection.
6. Before cleaning or maintenance of the CliniMACS® plus Instrument, the power cord cable should be disconnected
7. To disconnect the CliniMACS® plus Instrument from the power supply unplug the power cord. Only use the originally supplied power cord
8. To prevent the risk of an electric shock, do not remove the back cover of the CliniMACS® plus Instrument. The instrument may be opened and any spare parts may be exchanged by authorized personnel only
9. Movement or vibration may affect the CliniMACS® plus Instrument. Do not place the instrument next to any equipment that vibrates or can cause the instrument to move
10. There are no interior components which can be serviced or calibrated by the operator
11. Never leave the CliniMACS® plus Instrument unattended during a run. If an error occurs, the cell separation will be interrupted at the current step and the operator will have 600 seconds to correct certain errors. If the instrument has not been restarted after this time period, the run will be aborted



Fig 1.1: Attention, see instructions for use.



Fig 1.2: Contains a strong permanent magnet.

12. The pump door should not be left open at any time during a run. If left open for more than 600 seconds, the run in process will be aborted.
13. Do not open the door of the peristaltic pump when it is moving. Keep away from all moving parts.
14. Fluid containers must be handled with caution when near the CliniMACS® plus Instrument. Avoid spills. Do not operate the instrument if it has been exposed to moisture. Avoid ingress of any liquid into the valves.
15. After running a patient sample and prior to decontamination, the CliniMACS® plus Instrument should be treated as a biohazard.
16. The CliniMACS® plus Instrument may be used repeatedly. It is not intended for disposal after single use. It must be returned to Miltenyi Biotec for final disposal.
17. Only supplies (e.g. CliniMACS® Tubing Sets) recommended by the manufacturer may be used.

Caution

Changes or modifications not expressly approved by the manufacturer of the CliniMACS® System could invalidate the user's authority to operate this system.

1.4 Technical specifications

- **Model:**

CliniMACS® plus Instrument, Model CS2-CE/UL (REF 151-01).

-

Description:

The CliniMACS® plus Instrument is an electromechanical device incorporating a permanent magnet, a peristaltic pump, pinch valves and electronics.

- **Dimensions:**

70 cm (W), 90-140 cm (H), 60 cm (D)

- **Weight:**

35 kg

- **Input voltage:**

100-240 VAC (Single phase alternating current)

- **Power consumption:**

180 VA

- **Power source:**

Uninterruptable power source. Reliable, noise free utility is recommended. Recommended UPS: APC Smart-UPS 1500VA USB & Serial 230 V, manufactured by APC (American Power Conversion).

- **Frequency:**
50/60 Hz
- **Fuses:**
2 × T4A/250V, 5 × 20 mm
Use only fuses with UL and European approvals, acc. IEC 127-2/ III, EN 60127-2/III, DIN 41662.
- **Standards:**
The CliniMACS® plus Instrument, Model CS2-CE/UL, has been tested to and satisfies the requirements for EN 61010-1 for electrical safety and the requirements for EN 60601-1-2 and CISPR 22 for electromagnetic compatibility. Additionally, it has been tested to and satisfies the requirements for UL 3101-1 (File No. E188423) and for CAN/CSA-C22.2 No. 1010.1 (File No. 98SC02331). Therefore, it is listed as laboratory equipment.
- **Conditions for operation** (CliniMACS® plus Instrument only):
+10 °C to +30 °C with 0% to 85% humidity
- **Conditions for storage** (CliniMACS® plus Instrument only):
-10 °C to +60 °C with 0% to 85% humidity, when contained and sealed in the outer packaging provided by the manufacturer
- **Instrument power inlet IEC-320-C13, power cords:**
A country specific power cord is supplied with the CliniMACS plus Instrument.
- **Power connection** (see Fig. 1.3):
The power connection module is located at the rear of the CliniMACS® plus Instrument. Viewed from behind, the connection consists of three sections. The left section is the recessed male 3-pin connector to which the power cord is attached. The center section is the main power ON/OFF switch. When positioned to the left, the switch is 'OFF' (0). When positioned to the right, the switch is 'ON' (I).

The right section is the fuse box. The CliniMACS® plus Instrument must be unplugged and switched off before opening the fuse box. To open it, a thin-bladed screwdriver is inserted into the slot and twisted to release the catch. To replace the fuses, remove the fuses from the rear, insert new fuses and slide the module back in until the latch clicks to the closed position. The module will only slide in one direction. Only fuses with UL and European approvals are to be used.
- **Protection class:**
The CliniMACS® plus Instrument is a protection class I device (acc. to DIN 61140) and may only be plugged into an outlet with a grounded conductor. The protection category according to DIN EN 60529 is IPX 0.

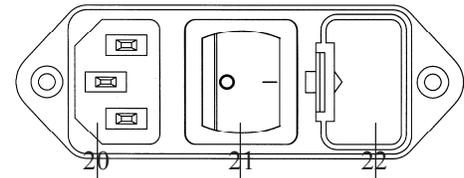


Fig 1 3: Rear view of the CliniMACS® plus Instrument.
20 Power connection, recessed male 3-pin connector
21 Main power ON/OFF switch
22 Fuse block

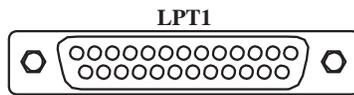


Fig 1.4: Parallel printer port, SUBD 25, female connector.

- **Printer port:**

There is a printer port (see Fig. 1.4) situated at the rear of the CliniMACS® plus Instrument next to the power connection. However, a printer is currently not supported. Please **do not connect** any printer.

- **Interferences:**

This equipment has been tested and found to comply with the limits for a class B digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference in a residential installation. This equipment generates, uses and can radiate radio frequency energy and, if not installed and used in accordance with the instructions, may cause harmful interference to radio communications. However, there is no guarantee that interference will not occur in a particular installation. If this equipment does cause harmful interference to radio or television reception, which can be determined by turning the equipment off and on, the user is encouraged to try to correct the interference by one or more of the following measures:

- Redirect or relocate the receiving antenna.
- Increase the space between the equipment and receiver.
- Connect the equipment to an outlet which is not on the same circuit as the receiver.
- Consult the dealer or an experienced radio/TV technician for help.

1.5 Unpacking the CliniMACS® plus Instrument

Unpacking the CliniMACS® plus Instrument should be performed by two people, according to the following instructions

1. Cut the plastic straps using a pair of scissors (see Fig. 1.5).

Caution

- Wear safety glasses. The straps are wrapped under tension.

Note

- Visually inspect and note any significant damage to the package before unpacking. Damage may require inspection by a representative of the shipping company.

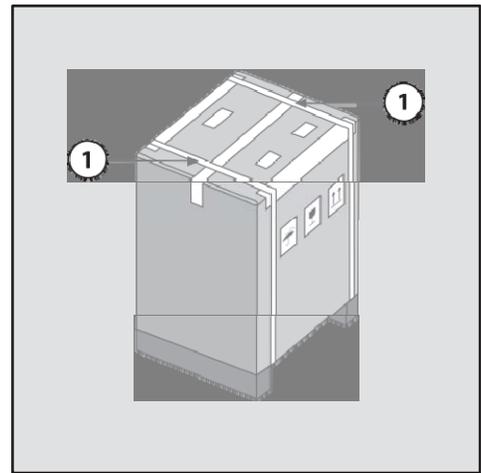


Fig 1.5: Cut plastic straps.

2. Open the top carton by cutting the adhesive tape (see Fig 1.6).

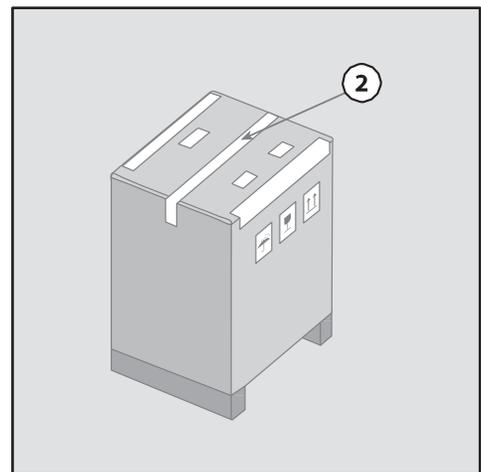


Fig 1.6: Open top carton.

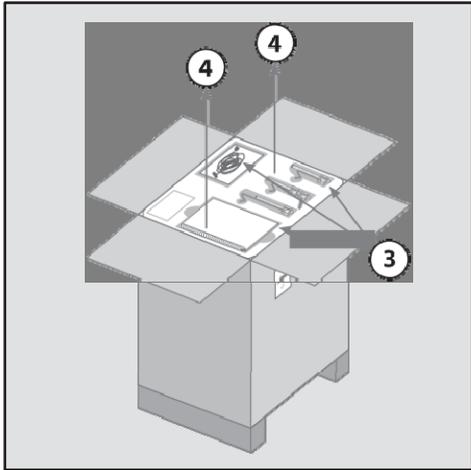


Fig 1.7: Remove the parts and the protective foam.

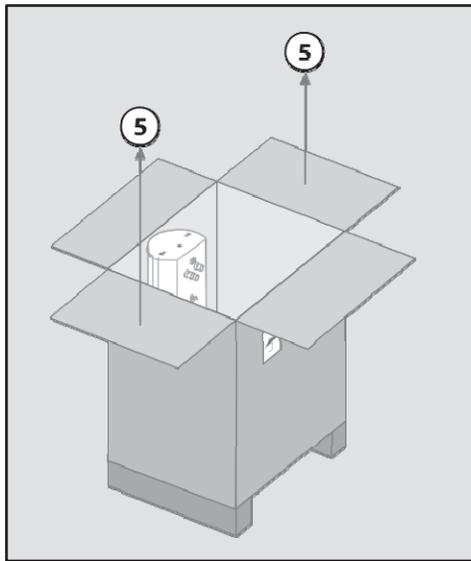


Fig 1.8: Lift the top carton.

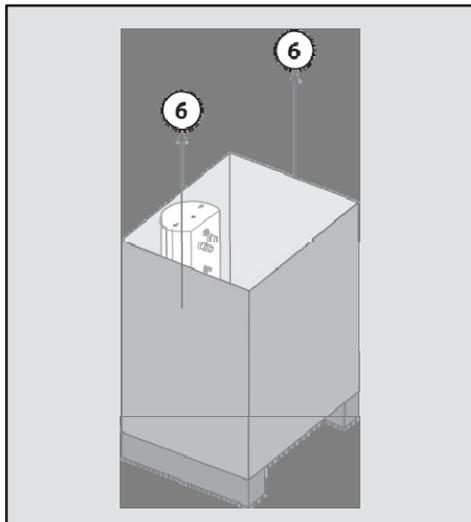


Fig 1.9: Remove the inner carton.

3. Open the carton and remove the parts (power cord, bag hangers) from the protective foam (see Fig. 1.7).

4. Remove the protective foam.

5. Lift the top carton vertically off the pallet (see Fig. 1.8).

6. Remove the inner carton (see Fig. 1.9).

7. Unwrap the large shipping bag (see Fig. 1.10). Two people should carefully lift the CliniMACS® plus Instrument onto a flat, stable surface which is capable of supporting 100 kg. The instrument should be lifted under each of the four corners at the base of the instrument.
8. To maintain ventilation, place the instrument at least 10 cm away from the wall.

Note

- Do not locate the CliniMACS® plus Instrument next to any vibrating equipment which might cause movement during operation.

9. Attach bag hangers. Tighten rods with clockwise twists until hand tight (see Fig 1.11). The height of the bag hangers can be adjusted by pressing the bag hanger clamps (1, Fig. 1.11).

10. A stabilization foot (1, Fig. 1.12) is included in your delivery. Please keep this foot for later installation by our Technical Service or an authorized representative. The foot will be installed at the back of the instrument.

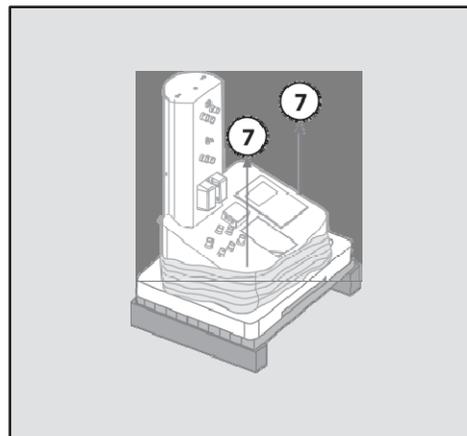


Fig 1.10: Lift the CliniMACS® plus Instrument.

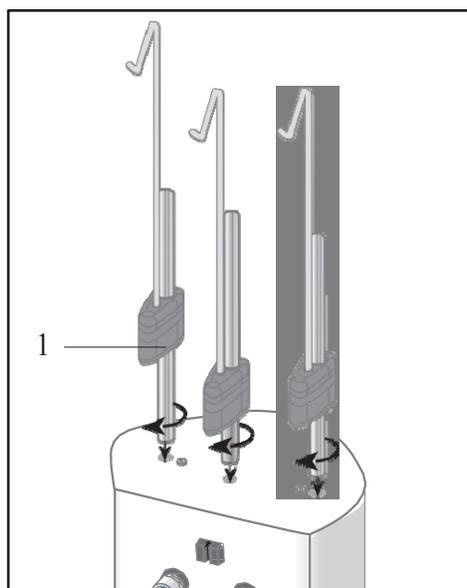


Fig 1.11: Attach bag hangers.
1 Bag hanger clamp

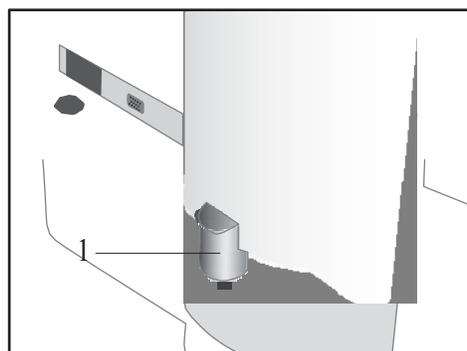


Fig 1.12: Rear of CliniMACS® plus Instrument with installed stabilization foot.
1 Stabilization foot

1.6 Cleaning and maintenance of the CliniMACS®^{plus} Instrument

Caution

- Clean the CliniMACS®^{plus} Instrument only when it is switched off and the power cord is unplugged
- Avoid ingress of any liquid into the valves

Cleaning

The surface of the CliniMACS®^{plus} Instrument should be cleaned at regular intervals and after each application with an antiseptic solution, e.g. ¹Bacillol® plus or ²Meliseptol®, according to standard procedures for device decontamination.

Do not use other cleaning agents or an excessive amount of water. After cleaning, dry all excess liquid from the valves, pumphead etc.

Maintenance

The CliniMACS®^{plus} Instrument does not contain operator service-able parts. Routine and preventative maintenance procedures should be conducted by the manufacturer's authorized service personnel at least once a year. Calibration is not required.

¹Bacillol® is a registered trademark of Bode Chemie Hamburg, Hamburg.

²Meliseptol® is a registered trademark of B. Braun Melsungen AG, Melsungen.

1.7 Description of the CliniMACS® plus Instrument (Model CS2-CE/UL)

The CliniMACS® plus Instrument is an electromechanical device intended to separate human cells from heterogeneous hematologic cell populations in combination with the CliniMACS® Reagents, CliniMACS® Tubing Sets, and CliniMACS® PBS/EDTA Buffer

The key components of the CliniMACS® plus Instrument are an integrated computer, a magnetic separation unit, a peristaltic pump, a liquid sensor, and pinch valves

The integrated microcomputer controls all electromechanical components of the instrument and directs the system to perform procedures in a standard sequence. The keypad and display guide the operator through the set-up procedure and allow monitoring of automatic system operations (see Fig 1.13).

The magnetic separation unit includes the movable permanent magnet and the selection column holder for the selection column.

During the separation, the peristaltic pump controls the flow rate through the CliniMACS® Tubing Sets. The liquid sensor monitors the flow of labeled cell suspension into the tubing set. Disruption of continuous fluid flow through the sensor automatically advances the separation program to the next phase of the separation process.

Eleven pinch valves ensure controlled flow of buffer and cell suspension throughout the procedure.

The CliniMACS® plus Instrument and CliniMACS® Tubing Sets allow the operator to perform cell separations in a closed and sterile system.

The CliniMACS® plus Instrument software offers the operator the choice between various separation programs. For further details see chapter 4.

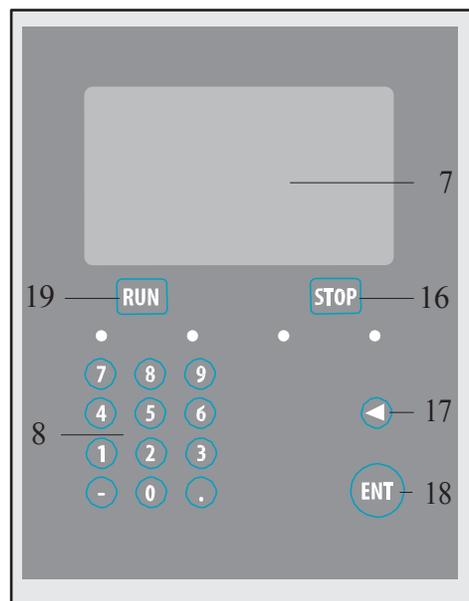
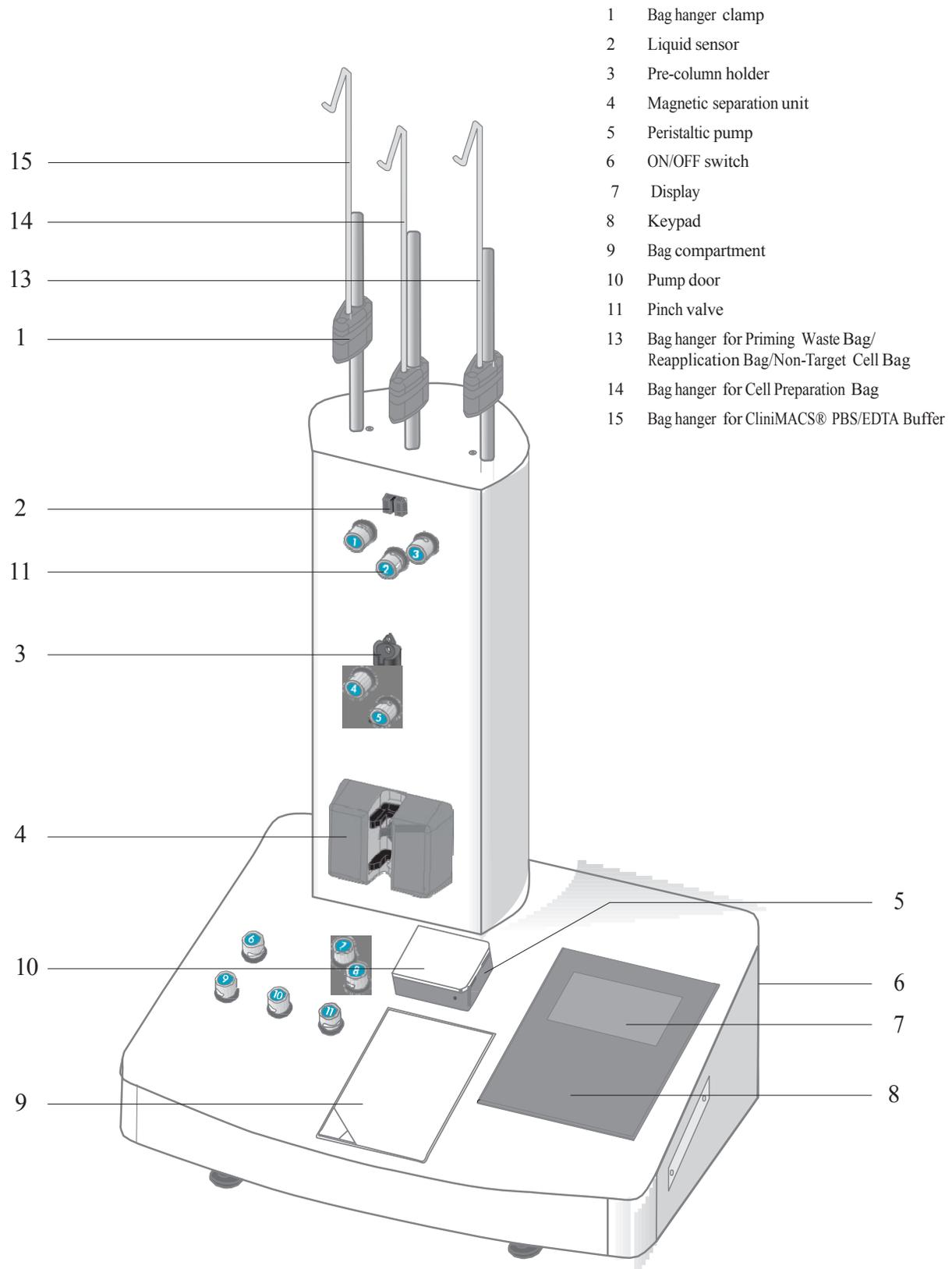


Fig 1 13: Keypad of the CliniMACS® plus Instrument.
 7 Display
 8 Keypad
 16 Stop Key
 17 Undo Key
 18 Enter Key
 19 Run Key

Important

- This CliniMACS® User Manual only contains the instructions for **clinical applications** using the **CliniMACS® Reagents**

For **non-clinical applications** using the **CliniMACS® MicroBeads for Research** Use refer to the *General Instructions*, the respective special protocols and the data sheets, provided by the technical service of Miltenyi Biotec



- 1 Bag hanger clamp
- 2 Liquid sensor
- 3 Pre-column holder
- 4 Magnetic separation unit
- 5 Peristaltic pump
- 6 ON/OFF switch
- 7 Display
- 8 Keypad
- 9 Bag compartment
- 10 Pump door
- 11 Pinch valve
- 13 Bag hanger for Priming Waste Bag/
Reapplication Bag/Non-Target Cell Bag
- 14 Bag hanger for Cell Preparation Bag
- 15 Bag hanger for CliniMACS® PBS/EDTA Buffer

Fig 1.14: The CliniMACS®plus Instrument, Model CS2-CE/UL

1.8 Installation of the CliniMACS® plus Instrument

Connect and switch on the CliniMACS® plus Instrument

Connect the CliniMACS® plus Instrument to an uninterruptable power supply using the supplied power cord Switch on the instrument by using the ON/OFF switch (Fig. 1.15) located on the right hand back panel of the instrument.

For safety, the CliniMACS® plus Instrument should be turned off after each run and the power cord cable should be disconnected during the instrument clean-up procedures

The CliniMACS® plus Instrument will perform self-checking procedures and the window will display screen no 1.1. The program will automatically be loaded and screen no 1.2 will appear in the display window

If the CliniMACS® plus Instrument does not start up, switch the instrument off and disconnect it from the power supply Check the power cord connection and the fuses, which are located on the right hand back panel (see chapter 1.4). Then switch on the instrument again.

If the CliniMACS® plus Instrument does not start correctly or the window displays an error message, note the error message number, switch the instrument off and contact our technical service representative

Proper training is required to operate the CliniMACS® plus Instrument. Read the instructions carefully Further training by our technical service or an authorized representative may be required This will include details on instrument operation and sample handling

Language selection and service menu

The CliniMACS® plus Instrument provides a menu to set-up the language and a service menu (see chapter 1.9 “General menus to set-up the CliniMACS® plus Instrument”).

To enter these menus wait until screen no 1.2 appears in the display window and DO NOT press ‘ENT’ as shown on the window display (Screen no 1.2).

To start the general menus, please refer to chapter 1.9 on the next page

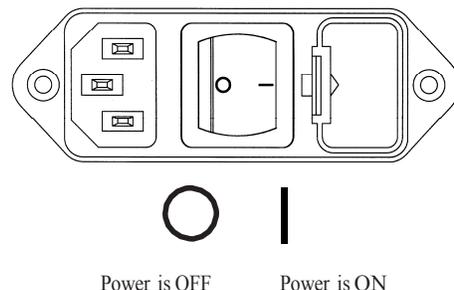
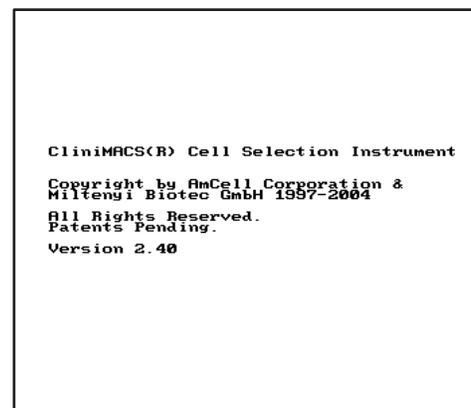


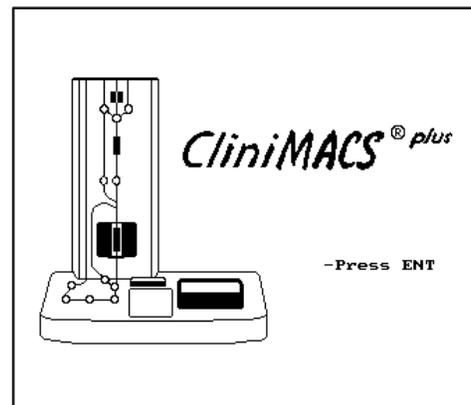
Fig 1.15: Switch on the CliniMACS® plus Instrument.



Screen no. 1.1

Important

- The actual CliniMACS® software version number is indicated in screen no 1.1.



Screen no. 1.2

1.9 CliniMACS®^{plus} Instrument - Guidance and Manufacturer’s Declaration

Warning Labeling, Guidance and Manufacturer’s Declaration in Accompanying Documents of Medical Electrical Equipment According to IEC 60601-1-2:2007

Instructions for use

Medical electrical equipment needs special precautions regarding electromagnetic compatibility (EMC) and needs to be installed and put into service according to the EMC information provided in the accompanying documents. Portable and mobile RF communications equipment can affect medical electrical equipment.

Technical Description

EMC compliance with IEC 60601-1-2:2007 has been attested for the provided power cable. The use of other power cables may result in increased electromagnetic emissions or decreased immunity of the CliniMACS®^{plus} Instrument. If the provided power cable is missing, please contact Miltenyi Biotec for information on a replacement part.

Guidance and Manufacturer’s Declaration – Electromagnetic Emissions		
The CliniMACS® ^{plus} Instrument is intended for use in the electromagnetic environment specified below. The customer or the user of the CliniMACS® ^{plus} Instrument should assure that it is used in such an environment.		
Emissions Test	Compliance	Electromagnetic Environment – Guidance
RF Emissions CISPR 11	Group 1	The CliniMACS® ^{plus} Instrument uses RF energy only for its internal function. Therefore, its RF emissions are very low and are not likely to cause any interference in nearby electronic equipment.
RF Emissions CISPR 11	Class B	The CliniMACS® ^{plus} Instrument is suitable for use in all establishments, including domestic establishments and those directly connected to the public low voltage power supply network that supplies buildings used for domestic purposes.
Harmonic Emissions IEC 61000-3-2	Class A	
Voltage Fluctuations/ Flicker Emissions IEC 61000-3-3	Complies	

Table 1 1: Guidance and Manufacturer’s Declaration – Electromagnetic Emissions

The CliniMACS®^{plus} Instrument should not be used adjacent to or stacked with other equipment and that if adjacent or stacked use is necessary, the CliniMACS®^{plus} Instrument should be observed to verify normal operation in the configuration in which it will be used.

Based on technical limitations of the internal power supply voltage, interruptions on power supply input lines for longer than 10 ms may lead to cessation of the separation process (power failure). The separation process cannot be resumed after a power failure. It is recommended that the CliniMACS®^{plus} Instrument is powered from an uninterruptible power supply or a battery that starts up within 10 ms.

Guidance and Manufacturer's Declaration – Electromagnetic Immunity			
The CliniMACS® plus Instrument is intended for use in the electromagnetic environment specified below. The customer or the user of the CliniMACS® plus Instrument should assure that it is used in such an environment.			
Immunity Test	IEC 60601 Test Level	Compliance Level	Electromagnetic Environment Guidance
Electrostatic Discharge (ESD) IEC 61000-4-2	± 6 kV contact ± 8 kV air	± 6 kV contact ± 8 kV air	Floors should be wood, concrete or ceramic tile. If floors are covered with synthetic material, the relative humidity should be at least 30 %.
Electrical Fast Transient/Burst IEC 61000-4-4	± 2 kV for power supply lines ± 1 kV for input/output lines	± 2 kV for power supply lines ± 1 kV for input/output lines	Mains power quality should be that of a typical commercial or hospital environment.
Surge IEC 61000-4-5	± 1 kV line(s) to line(s) ± 2 kV line(s) to earth	± 1 kV line(s) to line(s) ± 2 kV line(s) to earth	Mains power quality should be that of a typical commercial or hospital environment.
Voltage Dips, Short Interruptions and Voltage Variations on Power Supply Input Lines IEC 61000-4-11	<5 % U_T (>95 % dip in U_T) for 0.5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles <5 % U_T (>95 % dip in U_T) for 5 s	<5 % U_T (>95 % dip in U_T) for 0.5 cycle 40 % U_T (60 % dip in U_T) for 5 cycles 70 % U_T (30 % dip in U_T) for 25 cycles	Mains power quality should be that of a typical commercial or hospital environment but if the user of the CliniMACS® plus Instrument requires continued operation during power mains interruptions longer than 10 ms, it is recommended that the CliniMACS® plus Instrument be powered from an uninterruptible power supply or a battery that starts up within 10 ms.
Power Frequency (50/60 Hz) Magnetic Field IEC 61000-4-8	3 A/m	3 A/m	Power frequency magnetic fields should be at levels characteristic of a typical location in a typical commercial or hospital environment.
NOTE U_T is the a.c. mains voltage prior to application of the test level.			

Table 1 2: Guidance and Manufacturer's Declaration – Electromagnetic Immunity

Successful cell separation treatment is the essential performance criteria. Repetitive failure of critical components may impair successful cell separation. Regardless of electromagnetic interference, isolated cells intended for human applications, have to be examined regarding quality and quantity in view of the intended use.

Critical Component Failures

- Solenoid valves: Changes in state (On/Off) due to EMI
- Peristaltic pump: Direction change or interruption without resume
- Magnet motor drive: Does not reach intended position (On/Off)
- PC:
 - Changes in programmable parameters
 - Change of operating mode (exiting program)
 - Irregular error messages
 - Stop or interruption of any intended operation
 - Initiation of any unintended operation
- Display: Long time freeze or black out (> 5 s)
- Power supply: No stop or interruption of any intended operation (if Uninterruptible Power Supply is used)

Guidance and Manufacturer's Declaration – Electromagnetic Immunity			
The CliniMACS® plus Instrument is intended for use in the electromagnetic environment specified below. The customer or the user of the CliniMACS® plus Instrument should assure that it is used in such an environment.			
Immunity Test	IEC 60601 Test Level	Compliance Level	Electromagnetic Environment Guidance
Conducted RF IEC 61000-4-6	3 Vrms 150 kHz to 80 MHz	3 V	Portable and mobile RF communications equipment should be used no closer to any part of the CliniMACS® plus Instrument, including cables, than the recommended separation distance calculated from the equation applicable to the frequency of the transmitter. Recommended Separation Distance $d = 1.2 \sqrt{P}$ $d = 1.2 \sqrt{P}$ 80 MHz to 800 MHz $d = 2.3 \sqrt{P}$ 800 MHz to 2.5 GHz where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer and d is the recommended separation distance in metres (m). Field strengths from fixed RF transmitters, as determined by an electromagnetic site survey, ^a should be less than the compliance level in each frequency range. ^b Interference may occur in the vicinity of equipment marked with the following symbol: 
Radiated RF IEC 61000-4-3	10 V/m 80 MHz to 2.5 GHz	3 V/m	
NOTE 1: At 80 MHz and 800 MHz, the higher frequency range applies.			
NOTE 2: These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.			
^a Field strengths from fixed transmitters, such as base stations for radio (cellular/cordless) telephones and land mobile radios, amateur radio, AM and FM radio broadcast and TV broadcast cannot be predicted theoretically with accuracy. To assess the electromagnetic environment due to fixed RF transmitters, an electromagnetic site survey should be considered. If the measured field strength in the location in which the CliniMACS® plus Instrument is used exceeds the applicable RF compliance level above, the CliniMACS® plus Instrument should be observed to verify normal operation. If abnormal performance is observed, additional measures may be necessary, such as re-orienting or relocating the CliniMACS® plus Instrument.			
^b Over the frequency range 150 kHz to 80 MHz, field strengths should be less than 3 V/m.			

Table 1 3: Guidance and Manufacturer's Declaration – Electromagnetic Immunity

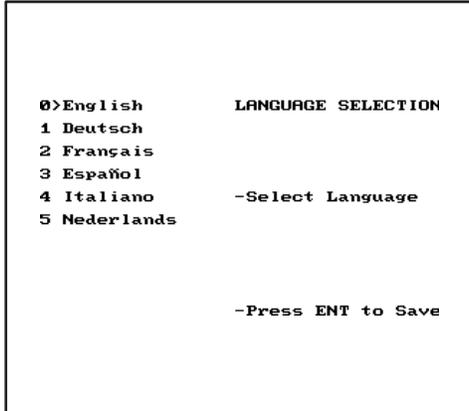
Recommended Separation Distances Between Portable and Mobile RF Communications Equipment and the CliniMACS® plus Instrument			
The CliniMACS® plus Instrument is intended for use in an electromagnetic environment in which radiated RF disturbances are controlled. The customer or the user of the CliniMACS® plus Instrument can help prevent electromagnetic interference by maintaining a minimum distance between portable and mobile RF communications equipment (transmitters) and the CliniMACS® plus Instrument – as recommended below, according to the maximum output power of the communications equipment.			
Rated Maximum Output Power of Transmitter (W)	Separation Distance According to Frequency of Transmitter (m)		
	150 kHz to 80 MHz $d = 1,2 \sqrt{P}$	80 MHz to 800 MHz $d = 1,2 \sqrt{P}$	800 MHz to 2,5 GHz $d = 2,3 \sqrt{P}$
0.01	0.12	0.12	0.23
0.1	0.38	0.38	0.73
1	1.2	1.2	2.3
10	3.8	3.8	7.2
100	12	12	23
For transmitters rated at a maximum output power not listed above, the recommended separation distance d in metres (m) can be estimated using the equation applicable to the frequency of the transmitter, where P is the maximum output power rating of the transmitter in watts (W) according to the transmitter manufacturer.			
NOTE 1: At 80 MHz and 800 MHz, the separation distance for the higher frequency range applies.			
NOTE 2: These guidelines may not apply in all situations. Electromagnetic propagation is affected by absorption and reflection from structures, objects and people.			

Table 1.4: Recommended Separation Distances Between Portable and Mobile RF Communications Equipment and the CliniMACS® plus Instrument

1.10 General menus to set-up the CliniMACS®plus Instrument

Language selection

The language selection menu allows the operator to change the language used in the display. It is possible to choose between English, German, French, Spanish, Italian and Dutch. To change the language, wait until the window displays screen no. 1.2 and DO NOT press 'ENT' then.



Screen no. 1.3

To start language selection, press

2

The window will display screen no. 1.3 "Language selection". To select a language, press the corresponding number.

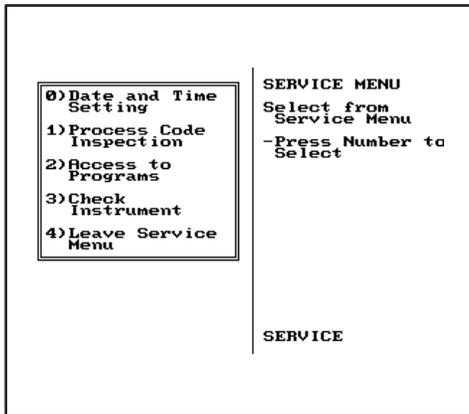
To save the language, press

ENT

The window will automatically display screen no. 1.1 again, now in the chosen language.

Service Menu

The service menu contains some programs that might be useful for the operator.



Screen no. 1.4

To open the service menu folder, wait until the window displays screen no. 1.2 and DO NOT press 'ENT' then.

Then press

5

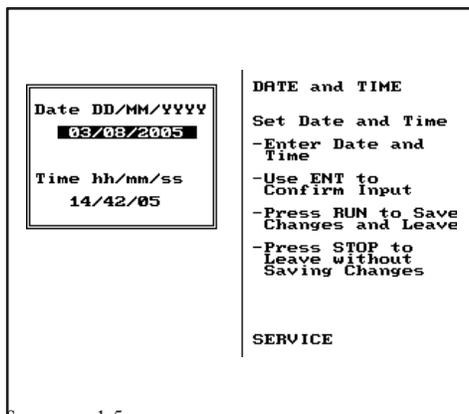
The window will display screen no. 1.4 "Service Menu".

DATE AND TIME SETTING

To set date and time, press

0

Follow the instructions shown on screen no. 1.5. Date or time can be changed, when the respective field is highlighted by the black bar.



Screen no. 1.5

To move the black bar between date and time input, press 'ENT'.

Enter the current date (order: day/month/year) and time (order: hours/minutes/seconds). A wrong input can be amended by pressing "Undo" (17, Fig. 1.13).

To save the data and leave, press

RUN

If you want to leave the program without saving the changes that have been done, press 'STOP'. After pressing 'RUN', the program will automatically return to the service menu.

PROCESS CODE INSPECTION

The operator is able to call up the process codes of the last 15 CliniMACS® operations. A process code is saved when the operator has started the installation of the CliniMACS® Tubing Set or when the EMERGENCY PROGRAM (see trouble shooting) has been used. Saving a process code is independent from whether the separation sequence has been completed or interrupted.

To call up a process code, press 1

The process codes of the last 15 CliniMACS® operations are listed chronologically as shown on screen no. 1.6. The list starts with the most recent one.

To return to the service menu, press ENT

ACCESS TO PROGRAMS

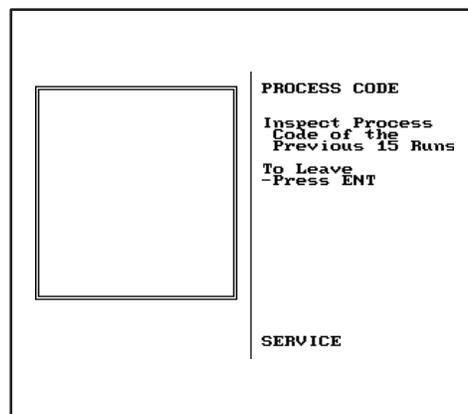
Activation of additional separation programs. Contact our Technical Service for further information and instructions to continue. If you have entered this program by mistake, leave the program by pressing 'STOP'.

CHECK INSTRUMENT

In case of a suspected malfunction of the CliniMACS® plus Instrument contact our Technical Service representative. If an instrument check is indicated our product specialist will assist you in performing the instrument check sequence. If you have entered this program by mistake, leave the program by pressing 'STOP'.

To leave the service menu, press 4

The program will return to the initial screens no. 1.1 and 1.2.



Screen no. 1.6

Note

- Important data collected by the instrument software during a CliniMACS® Separation are saved within the process code.
- It is strongly recommended to record the process code.

2 Glossary

2.1 Glossary of symbols

European conformity approval with ID number 0123 (ID number of Notified Body: “TÜV SÜD Product Service GmbH, Munich”) Medical device according to MDD 93/42/EEC.



UL listing mark, listed as laboratory equipment.



Attention, see instructions for use



Contains a strong permanent magnet. Do



not reuse



Do not use if package is damaged



Sterile



Sterile Manufactured aseptically, sterile filtrated, filled aseptically



Sterile Method of sterilization using steam or dry heat.



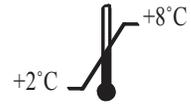
Sterile Method of sterilization using ethylene oxide



Use-by date



Temperature limitation +2 °C to +8 °C.



Contains phthalates: di(2-ethylhexyl)phthalate (DEHP)



Date of manufacture



Manufacturer



Instrument power is OFF



Instrument power is ON.



Reference number (REF).



Serial number



Part number



Batch code



2.2 Glossary of terms

Apheresis	The method of collecting blood in which whole blood is withdrawn, a desired component selected and retained, and the remainder of the blood returned to the donor
Bag compartment	Compartment of the CliniMACS® plus Instrument in which the Negative Fraction Bag and Buffer Waste Bag are placed
Bag hanger	Support on the CliniMACS® plus Instrument to mount the Cell Preparation Bag, Non-Target Cell Bag, Priming Waste Bag, Reapplication Bag and buffer bag
Buffer Waste Bag	Waste bag to collect buffer during cell separation using the CliniMACS® plus Instrument.
CD3 antigen	The CD3 antigen is present on mature human T cells, thymocytes and a subset of NK cells. It is associated with the T cell receptor (TCR) and is responsible for the signal transduction of the TCR. The CD3 antigen is a complex of five invariable chains: γ , δ , ϵ , ζ and η . The CD3 antibody recognizes all T cells, i.e. it reacts with 70-80% of human peripheral blood lymphocytes and with 65-85% of thymocytes. The epitope recognized by the antibody is located on the ϵ -chain of the CD3 complex.
CD4 antigen	The CD4 antigen is expressed on most thymocytes and approximately two-thirds of peripheral blood T cells; it is also expressed on monocytes and macrophages. CD4 is an accessory molecule in the recognition of foreign antigens in association with MHC class II. Moreover, CD4 is a receptor for HIV-1.
CD8 antigen	The CD8 antigen is a disulfide-linked dimer, which exists either as a CD8 α homodimer or as a CD8 α/β heterodimer. CD8 is strongly expressed on CD8 positive T cells and thymocytes and on a subset of NK cells and CD8 positive γ/δ T cells. CD8 acts as a co-receptor with MHC class I-restricted T cell receptors in antigen recognition.
CD14 antigen	The CD14 antigen is a 53-55 kD cell membrane glycoprotein expressed on monocytes. The CD14 antigen is known as a receptor for complex of LPS and LPS binding protein (LBP).
CD19 antigen	The CD19 antigen is a critical signal transduction molecule that regulates B lymphocyte development, activation, and differentiation. As a B cell lineage marker, CD19 is expressed from the early pro-B cell stage to the B cell lymphoblast stage but the expression is downregulated upon B cell maturation to plasma cells. The CD19 antigen is further expressed on most malignant B cells and a subset of follicular dendritic cells.
CD25 antigen	The CD25 antigen, the low affinity interleukin-2 receptor alpha chain (IL-2R α), is expressed on activated T and B cells, NK cells and monocytes, as well as on regulatory CD4 positive T cells. The CD25 antigen shows three epitope regions A, B and C. The antibody of the CliniMACS® CD25 Reagent recognizes epitope A of the CD25 molecule.

CD34 antigen	The CD34 antigen is a highly glycosylated 115 kD type 1 integral membrane protein of unknown function which is expressed on 1% to 4% of normal bone marrow cells and less than 0.2% of normal peripheral blood leukocytes, on subsets of bone marrow stromal cells, and on small vessel endothelium of various tissues
CD45RA antigen	The CD45RA antigen is expressed on naive CD4 and CD8 positive T cells, as well as on subsets of B cells, NK cells, and monocytes. It is an isoform of the common leukocyte antigen CD45, a transmembrane protein tyrosine phosphatase
CD56 antigen	The CD56 antigen is a member of the NCAM-family (Neural Cell Adhesion Molecule-family) which is expressed within the hematopoietic system on NK, NKT cells
CD133 antigen	The CD133 antigen is a 5-transmembrane cell surface antigen with a molecular weight of 117 kD and is expressed on 30-60% of all CD34 positive cells including CD34 bright cells
Cell Collection Bag	Bag in which the purified target cells are accumulated after separation.
Cell Preparation Bag	Bag into which leukapheresis material is transferred and in which magnetic labeling and washing of cells are performed
CliniMACS®	CliniMACS® is a registered trademark of Miltenyi Biotec GmbH.
CliniMACS® Anti-Biotin Reagent	Reagent for magnetic labeling of cells primarily labeled with biotinylated antibodies or ligands
CliniMACS® CD3 Reagent	Reagent for magnetic labeling of cells expressing the CD3 antigen.
CliniMACS® CD4 Reagent	Reagent for magnetic labeling of cells expressing the CD4 antigen.
CliniMACS® CD8 Reagent	Reagent for magnetic labeling of cells expressing the CD8 antigen.
CliniMACS® CD14 Reagent	Reagent for magnetic labeling of cells expressing the CD14 antigen.
CliniMACS® CD19 Reagent	Reagent for magnetic labeling of cells expressing the CD19 antigen.
CliniMACS® CD25 Reagent	Reagent for magnetic labeling of cells expressing the CD25 antigen.
CliniMACS® CD34 Reagent	Reagent for magnetic labeling of cells expressing the CD34 antigen.
CliniMACS® CD45RA Reagent	Reagent for magnetic labeling of cells expressing the CD45RA antigen.
CliniMACS® CD56 Reagent	Reagent for magnetic labeling of cells expressing the CD56 antigen.
CliniMACS® CD133 Reagent (formerly AC133 Reagent)	Reagent for magnetic labeling of cells expressing the CD133 antigen.
CliniMACS® Cytokine Capture System (IFN-gamma)	Combination of reagents, consisting of the ClinMACS® IFN-gamma Catchmatrix Reagent and the ClinMACS® IFN-gamma Enrichment Reagent. With the ClinMACS® Cytokine Capture System (IFN-gamma), IFN-gamma secreting cells can be enriched after <i>in vitro</i> restimulation.

CliniMACS® Depletion Tubing Set	Set of tubes, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place, especially designed for the specific depletion needs
CliniMACS® IFN-gamma Catchmatrix Reagent	Component of the CliniMACS® Cytokine Capture System (IFN-gamma) for surface labeling of leucocytes with IFN-gamma specific antibodies
CliniMACS® IFN-gamma Enrichment Reagent	Component of the CliniMACS® Cytokine Capture System (IFN-gamma) for magnetic labeling of IFN-gamma bound to the CliniMACS® IFN-gamma Catchmatrix Reagent on the IFN-gamma secreting cells
CliniMACS® ^{plus} Instrument	Magnetic cell separation instrument based on the MACS® Technology
CliniMACS® PBS/EDTA Buffer	Buffer used for cell preparation and cell separation with the CliniMACS® System: PBS (phosphate buffered saline), supplemented with 1 mM EDTA, pH 7.2. Before use, CliniMACS® PBS/EDTA Buffer must be supplemented with pharmaceutical grade HSA to a final concentration of 0.5% (weight/volume, i.e. 5 g HSA per liter buffer).
CliniMACS® Tubing Set, CliniMACS® Tubing Set LS	Set of tubes, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place
Cryopreservation	Preservation of cells by freezing at very low temperatures EDTA Ethylene-diamine-tetra-acetic acid
×g	Multiples of the earth's gravitational acceleration. Heat
sealer	Heating device used to sterile seal PVC tubing
Hematopoietic progenitor cells	Progenitor cells of lymphoid, myeloid, and erythroid lineages
HSA	Human serum albumin. FDA-licensed HSA is necessary as a buffer supplement when used with the CliniMACS® System.
Labeling	Reaction of cells with magnetic labeling reagent, e.g. CliniMACS® CD34 Reagent to CD34 positive cells
Leukapheresis	Apheresis collecting leukocytes
Liquid sensor connector	Component of the CliniMACS® ^{plus} Instrument that detects liquid in the tubing Luer screw coupling, part of the tubing set.
Magnetic antibody	A super-paramagnetically labeled antibody
Monoclonal antibodies	A single type of antibody that is directed against a specific epitope (antigen, antigenic determinant) and is produced by a single clone of B cells or a single hybridoma cell line, which is formed by the fusion of a lymphocyte cell with a myeloma cell. Some myeloma cells synthesize single antibodies naturally
Negative Fraction Bag	Bag of the CliniMACS® Tubing Set and CliniMACS® Tubing Set LS containing the non-target cell fraction.

Non-Target Cell Bag	Bag of the CliniMACS® Depletion Tubing Set containing the non-target cell fraction.
Orbital rotator	Device used to mix leukapheresis product during the reaction with CliniMACS® Reagents
Peristaltic pump	Tubing pump Pump used in the CliniMACS® plus Instrument to control the flow rate of fluid in the tubing set.
Plasma extractor	Device used to extract liquid from the Cell Preparation Bag after cell washing Plasma
Waste Bag	Waste bag to collect excess plasma prior to the labeling procedure
Pre-column	First column in the CliniMACS® Tubing Set and the CliniMACS® Tubing Set LS, serves as filter to trap cells having non-specific interactions with the column matrix.
Pre-column holder	Support mounted on the CliniMACS® plus Instrument that holds the pre-column in place
Pre-system filter	40 µm filter device between Cell Preparation Bag and pre-column used to trap clumps and cell debris
Priming	Step prior to cell separation in which buffer is flushed through the tubing set.
Priming Waste Bag	Bag in which buffer from priming step is collected
Pump safety switch	Sensor that prevents pump operation when the pump door is open.
Reapplication Bag	Bag of the CliniMACS® Depletion Tubing Set in which the unlabeled cells are collected temporarily during the separation. The unlabeled cells from the Reapplication Bag are applied onto the separation column twice, to ensure high purity of the target cells
Retaining ring	Part of a tubing set that enables the pump tubing to remain in its proper location.
rpm	Revolutions per minute
Sampling Site Coupler	Injection port, e.g. for removal of samples or addition of CliniMACS® Reagents to the Cell Preparation Bag.
Selection buffer	See CliniMACS® PBS/EDTA Buffer
Separation column	Column in which magnetically labeled cells are separated when exposed to the magnetic field
Separation column holder	Moulded guides in the magnet housing that holds the separation column in place
Selection column	See separation column. Selection
column holder	See separation column holder

Separation program	Software program designed for the enrichment or depletion of magnetically labeled cell subsets from a mixed cell population. The operator can choose from a menu of separation programs depending on the intended separation.
Separation reagent	Reagent for magnetic labeling of cells, e.g. CliniMACS® CD34 Reagent. T-
fitting	T-shaped fitting on a tubing set where three tubes meet.
Transfer Bag	Bag with a tubing and a spike at the end
Wash Waste Bag	Collection bag in which the wash supernatant is collected by separation from the sedimented cell suspension after centrifugation steps during sample preparation.
WBC	White blood cells

3 Customer information

3.1 MACS® Magnetic Cell Separation

MACS® Magnetic Cell Sorting has been well proven to be a powerful tool for the separation of many cell types, in research laboratories as well as in clinical applications. Cell mixtures can be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, e.g. human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations.

3.2 The CliniMACS® System

The CliniMACS® System consists of a computer controlled instrument (CliniMACS® plus Instrument), sterile, magnetic labeling reagents (CliniMACS® Reagents), sterile tubing sets (CliniMACS® Tubing Sets), and sterile buffer (CliniMACS® PBS/EDTA Buffer).

3.3 CliniMACS® Reagents and Biotin Conjugates

The CliniMACS® Reagents are dark amber colored, non-viscous, colloidal solutions, containing the cell specific antibody conjugates in buffer. The conjugates consist of the antibody chemically coupled to superparamagnetic particles.

The CliniMACS® Biotin Conjugates are clear and colorless solutions containing antibody covalently linked to biotin in buffer.

The antibodies are highly specific, making labeling of rare target cells possible.

No materials of animal or human origin are used in the whole manufacturing process of the CliniMACS® Reagents and Biotin Conjugates.

Note

- For warnings and precautions concerning CliniMACS® Reagents and Biotin, please refer to the respective section in chapter „Four STEPS to your target cells“, STEP 1.

3.4 CliniMACS® Tubing Sets

Important

- Humanitarian Device: For use with the CliniMACS® CD34 Reagent System. The effectiveness of the device has not been demonstrated for its indication.

- Investigational Device: For use with CliniMACS® Reagent Systems other than the CliniMACS® CD34 System, Limited by Federal Law to Investigational use

Intended use

The CliniMACS® Tubing Sets intended for *in vitro* enrichment or depletion of human cells from heterogeneous haematologic cell populations in combination with a CliniMACS® ^{plus} Instrument, CliniMACS® Reagents, and CliniMACS® PBS/EDTA Buffer

Precautions

- Aseptic working procedures must be applied for the unpacking, assembly and use of the tubing set.

- Before human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity

- For the manufacturing and use of target cells in humans, the national legislations and regulations must be followed

- Any clinical application of the separated cells is exclusively within the responsibility of the user

- The separation of cells using the tubing sets must be performed by trained operators only

- All materials which have come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed

- Do not connect directly to a patient.

- Do not store blood, blood fractions or cell fractions in the tubing set.

- For additional safety, the integrity test must be performed as described in STEP 3. When the CliniMACS® ^{plus} Instrument is equipped with software version 2.2x, please contact the technical service for further information.

Warnings

- Prior to opening the tray holding a tubing set, inspect the pack- age for damage, punctures or tears. Damaged packaging could indicate that the tubing set is no longer sterile and therefore must not be used. Do not use if any leaks are observed during priming or separations.
- CliniMACS® Tubing Sets are for single use only (see Fig 3.1). The reuse of tubings sets or parts of them leads to the endanger- ment of the patient due to biological contamination and to inefficient cell separation.

Further information

- Do not use the CliniMACS® Tubing Sets after the use-by date printed on the tray label (see Fig. 3.2).
- Do not use the CliniMACS® Tubing Sets if package is damaged (see Fig. 3.3). Use undamaged and sealed packages only.
- Some components of the device contain the phthalate di(2-ethylhexyl)phthalate (DEHP) as softener (see Fig. 3.4). The tubing sets do not release critical concentrations of the plas- ticizer DEHP. No risk caused by DEHP was identified including children (pre- and postnatal).
- CliniMACS® Tubing Sets have been sterilized with ethylene oxide (EO) (see Fig. 3.5).
- Store at room temperature.



Fig 3.1: Do not reuse.



Fig 3.2: Use-by date.



Fig 3.3: Do not use if package is damaged.



Fig 3.4: Contains phthalates: di(2-ethylhexyl) phthalate (DEHP).



Fig 3.5: Sterile. Method of sterilization using ethylene oxide.

Important

- Humanitarian Device: For use with the CliniMACS® CD34 Reagent System. The effectiveness of the device has not been demonstrated for its indication.
- Investigational Device: For use with CliniMACS® Reagent Systems other than the CliniMACS® CD34 System, Limited by Federal Law to Investigational use
- Before use, CliniMACS® PBS/EDTA Buffer must be supplemented with HSA to a final concentration of 0.5% (w/v). Note that **HSA is not a component of the CliniMACS® System**. Only FDA-licensed HSA should be used. Carefully read the package insert of the HSA used; in particular the section regarding hypersensitivity reactions and the risk of infection that HSA as a blood-derived product brings to all patients. All risks arising from these materials must be evaluated by the user.
- Prior to target cell infusion the CliniMACS® PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS® System is responsible for the selection of an appropriate medium for infusion.



Fig 3.6: Sterile. Method of sterilization using steam (autoclaving) or dry heat.

3.5 CliniMACS® PBS/EDTA Buffer

Intended purpose

The CliniMACS® PBS/EDTA Buffer is intended for the *in vitro* separation of human cells in combination with the CliniMACS® System.

Precautions

- For the manufacturing and use of target cells in humans, the national legislations and regulations must be followed
- Any clinical application of the separated cells is exclusively within the responsibility of the user
- The separation of human cells using the CliniMACS® System components must be performed by trained operators only
- All materials which have come into contact with blood and blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed

Warnings

- For *in vitro* use only. Not for use in any other cell processing, therapeutic or diagnostic application. Not for parenteral application. Do not infuse into patients
- **Drugs may be incompatible with the buffer. Do not add any drugs to the buffer during CliniMACS® Separation, storage, or transfusion of the target cell fraction.**

Further information

- The CliniMACS® PBS/EDTA Buffer is tested for endotoxins
- Do not use the CliniMACS® PBS/EDTA Buffer after the use-by date printed on the bag label (see Fig. 3.2).
- Do not use the CliniMACS® PBS/EDTA Buffer if package is damaged (see Fig. 3.3). Use undamaged and sealed bags only
- CliniMACS® PBS/EDTA Buffer has been sterilized by autoclaving (see Fig. 3.6).
- Store at room temperature. Do not freeze the CliniMACS® PBS/EDTA Buffer

3.6 Additional materials required

In addition to the CliniMACS® Products, additional materials may be required for a CliniMACS® Separation.

- Transfer bags, suitable for centrifugation
Transfer Bag 150 mL, Miltenyi Biotec, REF 183-01, or equivalent,
Transfer Bag 600 mL, Miltenyi Biotec, REF 190-01, or equivalent,
Transfer Bag 1000 mL, Miltenyi Biotec, REF 180-01, or equivalent,
Transfer Bag 400 mL, Baxter, Ref No R4R2074, or equivalent
- Cell culture bags, gas-permeable
Cell Differentiation Bag 100 mL, Miltenyi Biotec, REF 200-074-101, or equivalent.
- Sampling site coupler
Sampling Site Coupler, Miltenyi Biotec, REF 189-01, or equivalent.
- Plasma transfer set
Transfer Set Coupler/Coupler, Miltenyi Biotec, REF 186-01, or equivalent.
- Luer/Spike Interconnector
Luer/Spike Interconnector, Miltenyi Biotec, REF 187-01.
- Pre-system filter
Pre-system Filter, Miltenyi Biotec, REF 181-01, or
Blood Transfusion Filter, Pall, Ref. No SQ40S.
- 200 µm in-line blood filter
Blutset, Baxter, Ref. No RMC5849, or equivalent.
- Human serum albumin (HSA)
Only FDA-licensed HSA should be used
- Clinical grade immunoglobulin G
Use IgG of pharmaceutical grade quality only, which is available as approved drug in your country, e. g. ¹ Gamunex® 10%, or equivalent.
- Locking forceps or slide clamps
Locking forceps, Qosina: Part No 16093, or equivalent.
- Syringes and needles
Appropriate syringes (1 mL, 10 mL, 20 mL, 50 mL) and hypodermic 20 gauge needles
- Sample tubes
- Application specific materials:
Individual biotinylated cell specific antibody or ligand; AB serum or, alternatively, autologous serum; cell culture medium.

Note

- For information on the amount of materials required for an application or further specific materials, please refer to the instructions of the respective application given in chapter “Four STEPS to your target cells” (STEP 1).

3.7 Equipment required

- Uninterruptable power supply, rated at a minimum of 180 VA (APC Smart-UPS 1500VA USB & Serial 230 V or equivalent).
- Laminar flow hood
- Sterile tubing connector (Terumo Sterile Connection Device, ¹TSCD® SC-201, or equivalent).
- Orbital rotator (Lab-Line, Model 4635, or equivalent)
- Centrifuge (Sorvall, Model RC3, or equivalent) and buckets for centrifugation with aerosol containment caps
- Plasma extractor (Terumo Equipment, Plasma Separation Stand, Ref. No 1ME*ACS201, or equivalent).
- Table top balance (Mettler Toledo, Ref No 11274-998, or equivalent) with 1 kg capacity; resolution to 0.1 g
- Tubing heat sealer (Baxter, Hematron III, Ref. No FDR4360, or equivalent).
- Tubing stripper (Baxter, Ref. No RAR4415, or equivalent).
- Biohazard waste containers
- Hemostats

Additional equipment required for an application using the CliniMACS® Cytokine Capture System (IFN-gamma):

- 24 cell culture dish (²Corning® Costar® 24 cell culture plate, Sigma Aldrich, Ref. N° CLS3526, or equivalent).
- MiniMACS Separation Unit, Miltenyi Biotec, REF 130-042-102.
- MS Columns, Miltenyi Biotec, REF 130-042-201).
- Pre-Separation Filter, Miltenyi Biotec, REF 130-041-407.
- MACSmix device (MACSmix Tube Rotator, Miltenyi Biotec, REF 130-090-753).

¹ TSCD® is a registered trademark of Terumo Corporation, Tokyo, Japan

² Corning® and Costar® are registered trademarks of Corning Incorporated, Corning, NY, U.S.A.

3.8 Warnings and precautions

1. All separation procedures must be performed by trained operators only. The operator training will be provided by our Technical Service representative.
2. Before human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity.
3. **For the manufacturing and use of target cells in humans, the national legislations and regulations must be followed.**
4. Any clinical application of the separated cells is exclusively within the responsibility of the user.
5. All blood products and materials which have come into contact with blood and blood products, must be treated as infectious material. Regulations for the handling of infectious material must be observed.
6. All cell preparation and labeling procedures must be performed at room temperature (+19°C to +25°C). Higher ambient temperature results in less purity and yield of the target cells.
7. All tubing, fittings, valves, the pre-column, and the separation column should be checked thoroughly for leaks during the priming step.
8. All bags, including those used in sample preparation, should be preserved until final analysis of the collected cells has been completed and successful separation of the target cells has been confirmed.

Limited warranty

Should the CliniMACS® System be used in a manner not explicitly described in this manual, all warranties will be null and void.

Warnings and precautions

- Handling of biohazardous material

- To avoid contamination of the leukapheresis product, cellular starting product or the bone marrow aspirate, all preparation steps should be performed using aseptic techniques
- The operator performing the cell separation must be trained in the proper use of the equipment and in the handling of blood products and bone marrow aspirate
- The operator performing the cell separation should wear appropriate clothing (e.g. lab coat, gloves and eye glasses or goggles) when working with a patient sample and handling potentially biohazardous material
- All blood products must be treated as a potential biohazard. Leukapheresis product, blood product, bone marrow aspirate, collected cells, used buffer, used tubing set and other materials that have been in contact with these fluids must be treated as biohazardous materials according to standard hospital or institutional requirements
- Disposable materials should be treated according to standard hospital or institutional requirements for biohazardous materials
- The CliniMACS® plus Instrument should be considered a potential biohazard after each separation run and cleaned with an aqueous biocidal detergent (e.g. Bacillol® plus or Meliseptol®, see also chapter “1.6 Cleaning and maintenance of the CliniMACS® plus Instrument”) according to standard hospital or institutional requirements

Warnings and precautions

- Leukapheresis product

- The leukapheresis product should be collected according to standard hospital or institutional leukapheresis procedures in standard leukapheresis collection bags Labeling and separation of cells should begin as soon as possible after the leukapheresis product has been collected Prior to the cell labeling procedure, no additional anticoagulants or blood additives (heparin, etc) should be included beyond those normally used during leukapheresis
- The leukapheresis product container should be labeled with patient identification, time, date and place of collection according to procedures specified for use with the clinical protocol
- For transportation, the leukapheresis product should be packed in insulated containers and should be kept at controlled room temperature (+19 °C to +25 °C) according to standard hospital or institutional blood collection procedures approved for use with the clinical protocol Do not refrigerate The cell concentration should not exceed 0.2×10^9 cells per mL during transportation.
- Avoid intensive mixing of the leukapheresis product.
- If the leukapheresis product has to be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C) During storage, the concentration of leukocytes should never exceed 0.2×10^9 cells per mL.
- Cells should be stored in autologous plasma If the cell concentration is higher than 0.2×10^9 cells per mL, dilute the product with autologous plasma.

Important

- Labeling and separation of cells should begin as soon as possible after the leukapheresis product has been collected The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure

Important

- Labeling and separation of cells should begin as soon as possible after the cellular starting product has been collected. The cellular starting product should not be older than 24 hours when starting the labeling and separation procedure.

Warnings and precautions**- Cellular starting product**

- The cellular starting product (e.g. leukapheresis harvest, PBMC etc.) should be collected according to standard hospital or institutional procedures in standard collection bags. Labeling and separation of cells should begin as soon as possible after the product has been collected. Prior to the cell labeling procedure, no additional anticoagulants or blood additives should be included.
- The container for the cellular starting product should be labeled with patient identification, time, date and place of collection according to procedures specified for use with the clinical protocol.
- For transportation, the cellular starting product should be packed in insulated containers and should be kept at controlled room temperature (+19 °C to +25 °C) according to standard hospital or institutional blood collection procedures approved for use with the clinical protocol. Do not refrigerate. The cell concentration should not exceed 0.2×10^9 cells per mL during transportation.
- Avoid intensive mixing of the cellular starting material.
- If the cellular starting product has to be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C). During storage, the concentration of leukocytes should never exceed 0.2×10^9 cells per mL.
- Cells should be stored in autologous plasma. If the cell concentration is higher than 0.2×10^9 cells per mL, dilute the cellular starting product with autologous plasma.

Warnings and precautions

- Bone marrow aspirate

- Bone marrow aspirate should be collected according to standard hospital or institutional procedures in heparin-coated containers (e.g. 5 mL syringes). Labeling and separation of cells should begin as soon as possible after the bone marrow cells have been collected. Prior to the cell labeling procedure, no additional anticoagulants or blood additives (heparin, etc.) should be included beyond those normally used during bone marrow aspiration.
- The container should be labeled with patient identification, time, date and place of collection according to procedures specified for use with the clinical protocol.
- For transportation, the bone marrow aspirate should be packed in insulated containers and should be kept at controlled temperature (+4 °C).
- Avoid intensive mixing of the bone marrow aspirate.
- If the bone marrow aspirate has to be stored overnight, it should be kept at controlled temperature (+4 °C). During storage, the concentration of bone marrow cells should not exceed 0.2×10^9 cells per mL.
- If the CD133 positive cells have to be stored overnight, they should be kept in 0.9% NaCl supplemented with 10% autologous or AB serum at controlled temperature (+4 °C). During storage, the concentration of the cells should not exceed 2×10^5 cells per mL to 1×10^6 cells per mL.

Important

- Labeling and separation of cells should begin as soon as possible after the bone marrow cells have been collected. The bone marrow cells should not be older than 24 hours when starting the labeling and separation procedure.

3.9 Labeling of cells with CliniMACS® Reagents

Using the CliniMACS® System cells can be magnetically labeled in three different ways:

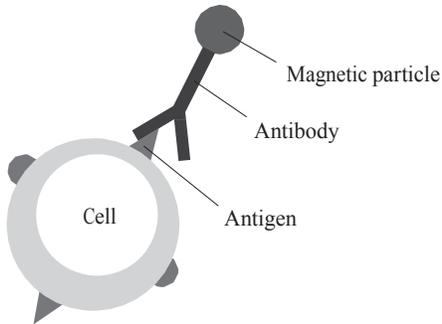


Fig 3.7: Magnetic labeling of cells.

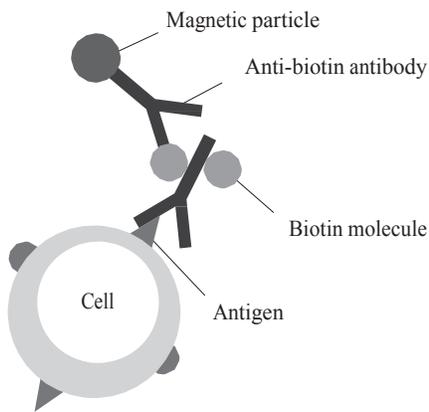


Fig 3.8: Flexible Labeling System.

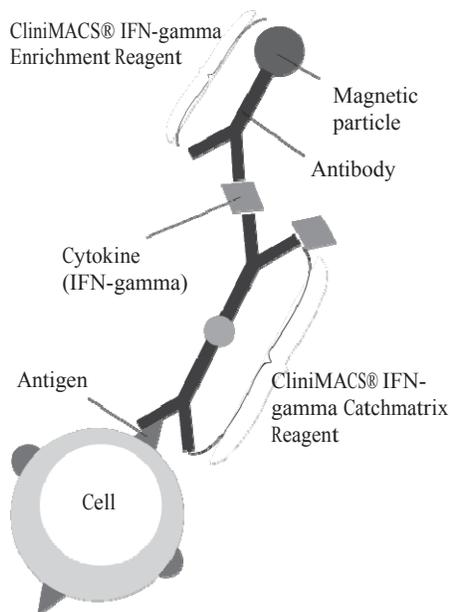


Fig 3.9: CliniMACS® Cytokine Capture System (IFN-gamma).

- Directly in a one-way labeling procedure by antigen-specific antibodies conjugated to super-paramagnetic iron-dextran beads (see Fig. 3.7).
- Indirectly in a two-step labeling procedure called Flexible Labeling System. In a first step cells are labeled with antigen-specific antibodies conjugated to biotin. In a second step these antibodies are labeled with biotin-specific antibodies conjugated to super-paramagnetic iron-dextran beads (see Fig. 3.8).
- Indirectly in a two-step labeling procedure called CliniMACS® Cytokine Capture System (IFN-gamma). First of all cells are *in vitro* restimulated to secrete the cytokine (e.g. IFN-gamma). In a first labeling step the re-stimulated cells are labeled by a hybrid-molecule consisting of a leucocyte-specific antibody and a cytokine-specific antibody, called the CliniMACS® IFN-gamma Catchmatrix Reagent. The cells are then incubated to allow secretion of the cytokine, which is “captured” by the catchmatrix on the cell surface. Subsequently, in a second labeling step the cytokine secreting cells are labeled with cytokine-specific antibodies conjugated to super-paramagnetic iron-dextran beads (CliniMACS® IFN-gamma Enrichment Reagent), (see Fig. 3.9).

3.10 High-gradient magnetic cell separation

The magnetically labeled cell suspension is loaded onto the CliniMACS® plus Instrument prepared with a tubing set. This high-gradient magnetic cell separation unit consists of a specifically developed, powerful permanent magnet and a separation column with a ferromagnetic matrix.

The high-gradient field allows the generation of strong magnetic forces and a rapid demagnetization. When small ferromagnetic structures, such as the column matrix, are placed within the magnetic field they disrupt the homogeneity of the field. This results in the generation of high magnetic gradients. In their immediate surrounding the ferromagnetic structures generate magnetic forces 10,000-fold stronger than in conventional geometries. The high-gradient field attracts labeled cells to the matrix and effectively retains them. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells.

3.11 CliniMACS® Separation strategies

The CliniMACS® System provides the user with a variety of separation programs. The separation programs can generally be divided into enrichment strategies (CD34 SELECTION 1 and 2, CD133 SELECTION 1 and 2, ENRICHMENT 1.1, and ENRICHMENT 3.2) and depletion strategies (DEPLETION 2.1 and DEPLETION 3.1).

Enrichment of magnetically labeled cells

When choosing an enrichment strategy, the magnetically labeled cells (primary labeled with a CliniMACS® Reagent) are retained in the separation column and the non-labeled cells pass through. The **labeled cells** (target cells) are collected in the **Cell Collection Bag** and the **non-labeled cells** (non-target cells) in the **Negative Fraction Bag** (see Fig. 3.10).

Depletion of magnetically labeled cells

The MACS® Technology is also very efficient for depleting specific cell populations. Unwanted cells are specifically labeled with superparamagnetic particles and separated from target cells upon passage through the high-gradient magnetic column. In contrast to the enrichment strategy, the **non-labeled cells** (target cells) are collected **in the Cell Collection Bag** and the **labeled cells** (non-target cells) are collected in the **Negative Fraction Bag** (see Fig. 3.11) or in the **Non-Target Cell Bag** (see Fig. 3.12 and Fig. 3.13) respectively.

Enrichment strategy

Enrichment of cells using the CliniMACS® Tubing Set or CliniMACS® Tubing Set LS

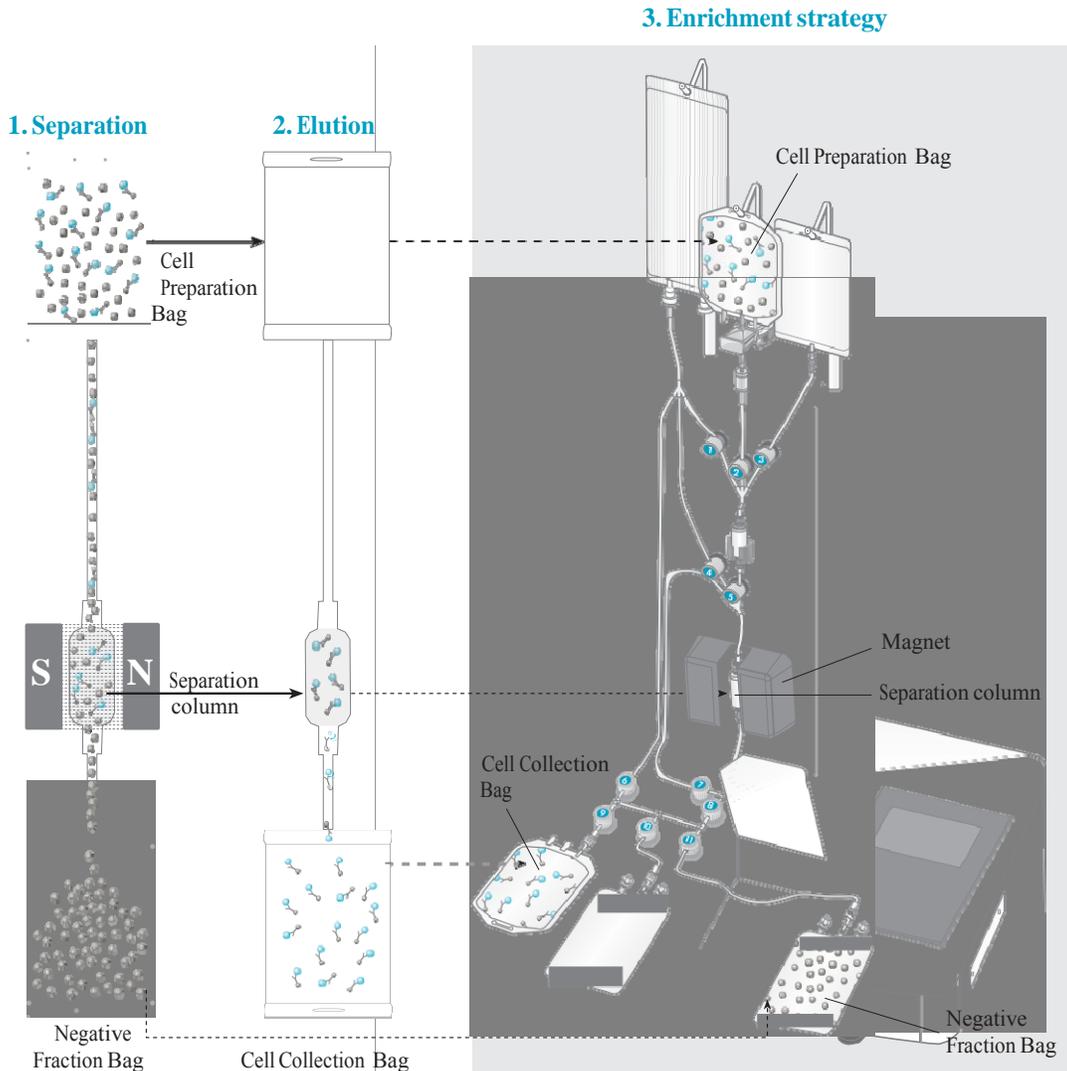


Fig 3.10: Strategy of enrichment programs using the CliniMACS® Tubing Set or CliniMACS® Tubing Set LS

1. The magnet is in the “ON”-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag.
2. The magnet is in the “OFF”-position. The magnetically labeled cells (target cells) are released from the separation column and collected in the Cell Collection Bag.

3. The **enrichment program** retains the magnetically labeled cells in the separation column, the **non-labeled cells** (non-target cells) flow through the column and are collected in the **Negative Fraction Bag**. When the magnet is moved into the “OFF”-position, the **magnetically labeled cells** (target cells) are released from the column and collected in the **Cell Collection Bag**.

Note

- The target cell fraction is always collected in the Cell Collection Bag.

Depletion strategy

Depletion of cells using the CliniMACS® Tubing Set LS - DEPLETION 2.1

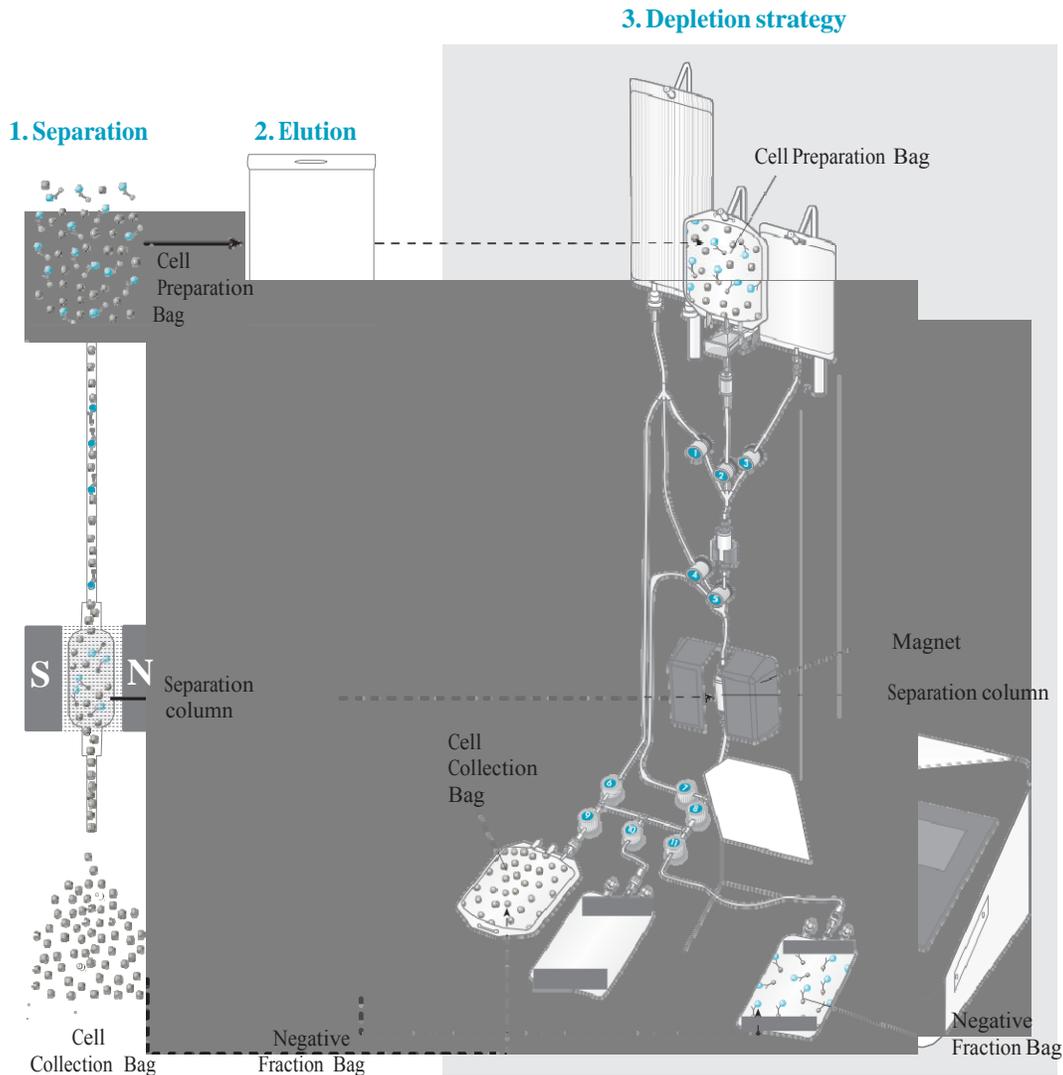


Fig 3.11: Strategy of the depletion program DEPLETION 2.1 using the CliniMACS® Tubing Set LS

1. The magnet is in the “ON”-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (target cells) flow through the column and are collected in the Cell Collection Bag.
 2. The magnet is in the “OFF”-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the Negative Fraction Bag.
 3. The **depletion program** (DEPLETION 2.1) retains the magnetically labeled cells in the separation column, the **non-labeled target cells** (target cells) flow through and are collected in the **Cell Collection Bag**. When the magnet is moved into the “OFF”-position, the **magnetically labeled cells** (non-target cells) are released from the column and collected in the **Negative Fraction Bag**.
- Note**
- The target cell fraction is always collected in the Cell Collection Bag.

**Depletion of cells using the CliniMACS® Depletion Tubing Set - DEPLETION 3.1
(Part 1: BULKLOADING STAGE)**

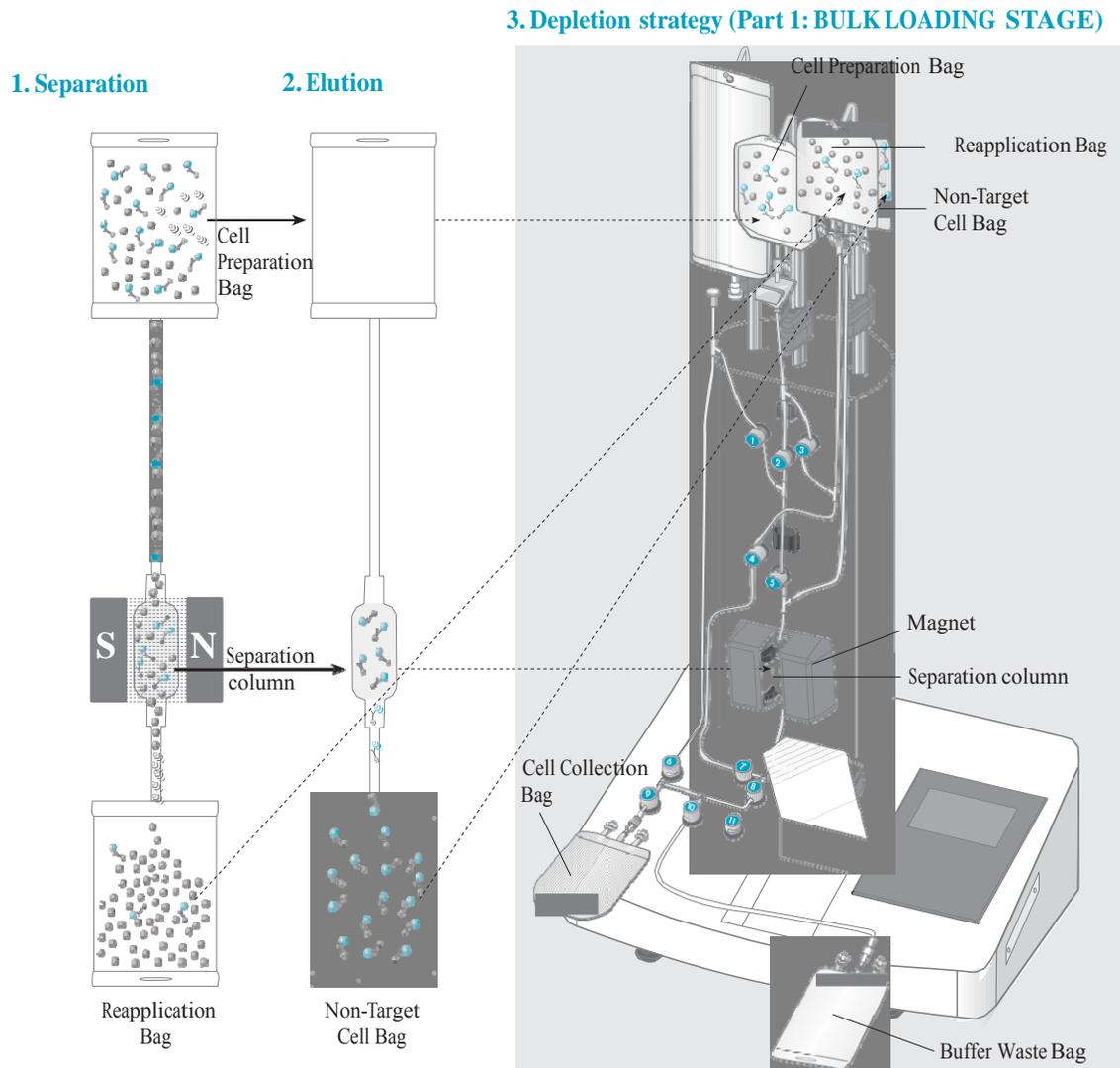


Fig 3.12: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS® Depletion Tubing Set (Part 1: BULKLOADING STAGE)

1. **BULK LOADING STAGE:** The magnet is in the “ON”-position. Magnetically labeled cells are held in the separation column, while other non-labeled cells (target cells) flow through and are collected in the **Reapplication Bag**.
2. The magnet is in the “OFF”-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the **Non-Target Cell Bag**.
3. The **depletion program** (DEPLETION 3.1) retains the magnetically labeled cells in the separation column, the **non-labeled target cells** (target cells) flow through the column and are collected in the **Reapplication Bag** in order to be reloaded onto the separation column in a second SENSITIVE LOADING STAGE (see Fig. 3.13). When the magnet is moved into the “OFF”-position, the **magnetically labeled cells** (non-target cells) are released from the column and collected in the **Non-Target Cell Bag**.

Depletion of cells using the CliniMACS® Depletion Tubing Set - DEPLETION 3.1
(Part 2: SENSITIVE LOADING STAGE)

3. Depletion strategy (Part 2: SENSITIVE LOADING STAGE)

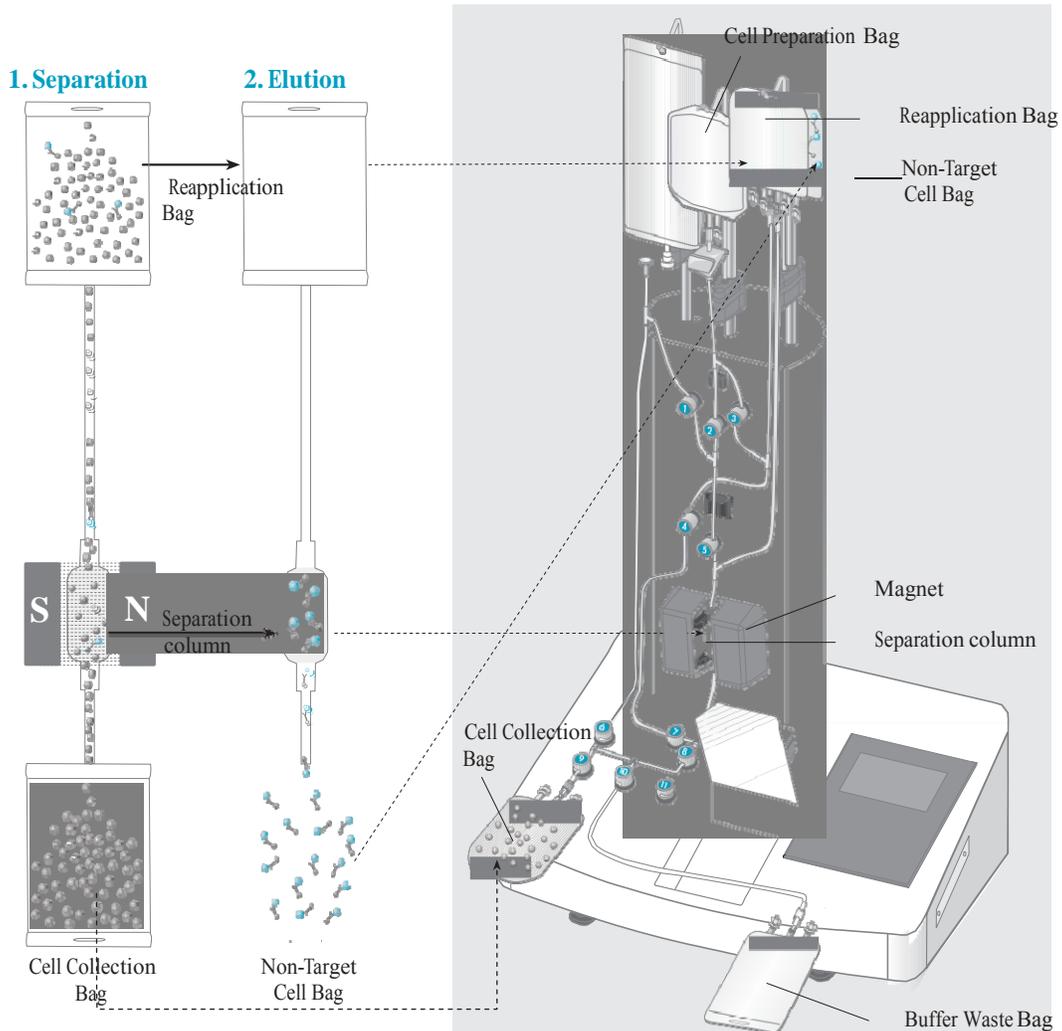


Fig 3.13: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS® Depletion Tubing Set (Part 2: SENSITIVE LOADING STAGE)

4. SENSITIVE LOADING STAGE: The magnet is in the “ON”-position. The cells from the Reapplication Bag are reloaded on the separation column. The few remaining magnetically labeled cells are held in the separation column, while the non-labeled cells (target cells) flow through the column and are collected in the Cell Collection Bag.
5. The magnet is in the “OFF”-position. The magnetically labeled cells (non-target cells) are released from the separation column and collected in the Non-Target Cell Bag.
6. At the end of the separation the **labeled cells** (non-target cells) are in the **Non-Target Cell Bag** and the **unlabeled cells** (target cells) are in the **Cell Collection Bag**.

Note

- The target cell fraction is always collected in the Cell Collection Bag.

4 Four STEPS to your target cells

In the following chapter an overview of the CliniMACS® Separation is presented. The CliniMACS® Separation is carried out in Four STEPS and the User Manual is divided into sections to describe each step. Before starting the separation, the applicable chapters of the User Manual must be chosen by the operator by using the overview table beginning on page “Four STEPS -Start-2”. This table summarizes information regarding specific chapters based on the type and amount of reagents used, the number of cells, the type of tubing set utilized and the relevant separation program required.

STEP 1 - Located in the Green Section of the CliniMACS® User Manual

STEP 1 describes the preparation of the cell product and the magnetic labeling of the specific cells, expressing the respective antigen. Follow the instructions of the applicable section (e.g. for enrichment of CD133 positive cells, proceed to section “CliniMACS® CD133 Reagent”). Continue with STEP 2.

STEP 2 - Located in the Orange Section of the CliniMACS® User Manual

STEP 2 describes the selection of a separation program of the CliniMACS® plus Instrument. Depending on cell type and cell number to be separated, different programs must be selected. To find out which program is applicable, go to the overview table on page “Four STEPS-Start-2”. Read the chapter indicated for the selected separation program (e.g. for CD133 normal scale enrichment the applicable separation program is CD133 SELECTION 1). Follow the instructions given in the applicable section. Continue with STEP 3.

STEP 3 - Located in the Brown Section of the CliniMACS® User Manual

STEP 3 explains the installation of the tubing set onto the CliniMACS® plus Instrument. Follow the instructions in the applicable chapter (“CliniMACS® Tubing Set (REF 161-01) and CliniMACS® Tubing Set LS (REF 162-01)” or “CliniMACS® Depletion Tubing Set (REF 261-01)”). Continue with STEP 4.

STEP 4 - Located in the Red Section of the CliniMACS® User Manual

STEP 4 describes the automated CliniMACS® Separation. The CliniMACS® plus Instrument performs the automated cell separation procedure (chosen in STEP 2). Follow the instructions of the applicable chapter (e.g. for the enrichment of CD14 positive cells, choose chapter “CliniMACS® Run - ENRICHMENT 1.1”).

Important

- The overview table on the following pages summarizes information on **typical applications** of the CliniMACS® Reagents. When separation of a different number of target cells is to be performed, refer to the detailed instructions in STEP 1 to find information on the maximum performance of the respective application.
- The color coding represents the “Four STEPS”. Follow the instructions of the **applicable** chapters. See overview table.

Reading example of the overview table

- For the separation of up to 0.6×10^9 CD133 positive cells from 60×10^9 total cells (small scale enrichment) one vial of CliniMACS® CD133 Reagent (REF 172-01) and a CliniMACS® Tubing Set (REF 161-01) are needed. For the procedure follow the subchapters:
 - › STEP 1: CliniMACS® CD133 Reagent,
 - › STEP 2: CD133 SELECTION 1/2,
 - › STEP 3: CliniMACS® TS/TS LS and
 - › STEP 4: CD133 SELECTION 1/2.

Antigen/ Reagent	CD34 Reagent	CD34 Reagent
Application	enrichment	enrichment (large scale)
Application capacity	0.6×10 ⁹ CD34 ⁺ cells from 60×10 ⁹ total cells	1.2×10 ⁹ CD34 ⁺ cells from 120×10 ⁹ total cells
No. of vials	1	2
Tubing set (TS)	TS (REF 161-01)	TS LS (REF 162-01)
No. of TS required for application	1	1
Software (Separation program)	CD34 SELECTION 1	CD34 SELECTION 2

Regulatory Statement

- Humanitarian Device:** Authorized by U.S. Federal law for processing hematopoietic progenitor cells- apheresis (HPC-A) to obtain a CD34 positive cell enriched population intended to provide for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft-vs-host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first or second morphologic complete remission. The effectiveness of the device for this indication has not been demonstrated
- CAUTION:** Federal law restricts this device to sale by or on the order of physician.

A p p l i c a b l e s e c t i o n s

STEP 1 Magnetic labeling	CliniMACS® CD34 Reagent	CliniMACS® CD34 Reagent
STEP 2 Choice of program	CD34 SELECTION 1/2	CD34 SELECTION 1/2
STEP 3 Installation of tubing set	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® Tubing Set / Tubing Set LS
STEP 4 CliniMACS® Separation	CD34 SELECTION 1/2	CD34 SELECTION 1/2

Antigen/ Reagent	CD34 Reagent	CD34 Reagent	CD133 Reagent	CD133 Reagent	CD133 Reagent
Application	enrichment	enrichment (large scale)	enrichment	enrichment (large scale)	enrichment (bone marrow)
Application capacity	0.6 × 10 ⁹ CD34 ⁺ cells from 60 × 10 ⁹ total cells	1.2 × 10 ⁹ CD34 ⁺ cells from 120 × 10 ⁹ total cells	0.6 × 10 ⁹ CD133 ⁺ cells from 60 × 10 ⁹ total cells	1.2 × 10 ⁹ CD133 ⁺ cells from 120 × 10 ⁹ total cells	50 mL aspirate and max 120 × 10 ⁹ total cells
No. of vials	1	2	1	2	1 or 2
Tubing set (TS)	TS (REF 161-01)	TS LS (REF 162-01)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	CD34 SELECTION 1	CD34 SELECTION 2	CD133 SELECTION 1	CD133 SELECTION 2	CD133 SELECTION 2

A p p l i c a b l e s e c t i o n s

STEP 1 Magnetic labeling	CliniMACS® CD34 Reagent	CliniMACS® CD34 Reagent	CliniMACS® CD133 Reagent	CliniMACS® CD133 Reagent	CliniMACS® CD133 Reagent (Bone Marrow)
STEP 2 Choice of program	CD34 SELECTION 1/2	CD34 SELECTION 1/2	CD133 SELECTION 1/2	CD133 SELECTION 1/2	CD133 SELECTION 1/2
STEP 3 Installation of tubing set	CliniMACS® Tubing Set / Tubing Set LS				
STEP 4 CliniMACS® Separation	CD34 SELECTION 1/2	CD34 SELECTION 1/2	CD133 SELECTION 1/2	CD133 SELECTION 1/2	CD133 SELECTION 1/2

Antigen/ Reagent	CD14 Reagent	Anti-Biotin Reagent	Anti-Biotin Reagent	Anti-Biotin Reagent	CD56 Reagent
Application	enrichment	enrichment	depletion	depletion (large scale)	enrichment
Application capacity	4×10 ⁹ CD14 ⁺ cells from 20×10 ⁹ total cells	max 40×10 ⁹ total cells	max 40×10 ⁹ total cells	max 80×10 ⁹ total cells	5×10 ⁹ CD56 ⁺ cells from 40×10 ⁹ total cells
No. of vials	1	1	1	2	1
Tubing set (TS)	TS (REF 161-01)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)	TS (REF 161-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	ENRICHMENT 1.1	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 1.1

A p p l i c a b l e s e c t i o n s

STEP 1 Magnetic labeling	CliniMACS® CD14 Reagent	CliniMACS® Anti-Biotin Reagent	CliniMACS® Anti-Biotin Reagent	CliniMACS® Anti-Biotin Reagent	CliniMACS® CD56 Reagent
STEP 2 Choice of program	ENRICHMENT 1.1	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 1.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® Tubing Set / Tub- ing Set LS			
STEP 4 CliniMACS® Separation	ENRICHMENT 1.1	ENRICHMENT 1.1	DEPLETION 2.1	DEPLETION 2.1	ENRICHMENT 1.1

Antigen/ Reagent	CCS Reagent	CD3 Reagent	CD3 Reagent	CD3 Reagent	CD3 Reagent
Application	enrichment	depletion	depletion (large scale)	depletion	depletion (large scale)
Application capacity	IFN-secreting cells from 1×10^9 total cells	15×10^9 CD3 ⁺ cells from 40×10^9 total cells	20×10^9 CD3 ⁺ cells from 80×10^9 total cells	15×10^9 CD3 ⁺ cells from 40×10^9 total cells	30×10^9 CD3 ⁺ cells from 80×10^9 total cells
No. of vials	1 kit	1	2	1	2
Tubing set (TS)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)	DTS (REF 261-01)	DTS (REF 261-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	ENRICHMENT 3.2	DEPLETION 2.1	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1

A p p l i c a b l e s e c t i o n s

STEP 1 Magnetic labeling	CliniMACS® Cytokine Capture System (IFN-gamma)	CliniMACS® CD3 Reagent	CliniMACS® CD3 Reagent	CliniMACS® CD3 Reagent	CliniMACS® CD3 Reagent
STEP 2 Choice of program	ENRICHMENT 3.2	DEPLETION 2.1	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® Depletion Tubing Set	CliniMACS® Depletion Tubing Set
STEP 4 CliniMACS® Separation	ENRICHMENT 3.2	DEPLETION 2.1	DEPLETION 2.1	DEPLETION 3.1	DEPLETION 3.1

Regulatory Statement

□ CAUTION: Limited by Federal (or United States) Law to Investigational Use.

Antigen/ Reagent	CD19 Reagent	CD19 Reagent	CD4 Reagent	CD4 Reagent	CD4 Reagent
Application	depletion	depletion (large scale)	enrichment	depletion	depletion (large scale)
Application capacity	5×10 ⁹ CD19 ⁺ cells from 40×10 ⁹ total cells	10×10 ⁹ CD19 ⁺ cells from 80×10 ⁹ total cells	5×10 ⁹ CD4 ⁺ cells from 40×10 ⁹ total cells	12×10 ⁹ CD4 ⁺ cells from 40×10 ⁹ total cells	20×10 ⁹ CD4 ⁺ cells from 80×10 ⁹ total cells
No. of vials	1	2	1	1	2
Tubing set (TS)	TS LS (REF 162-01)	TS LS (REF 162-01)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	DEPLETION 2 1	DEPLETION 2 1	ENRICHMENT 1 1	DEPLETION 2 1	DEPLETION 2 1

A p p l i c a b l e s e c t i o n s

STEP 1 Magnetic labeling	CliniMACS® CD19 Reagent	CliniMACS® CD19 Reagent	CliniMACS® CD4 Reagent	CliniMACS® CD4 Reagent	CliniMACS® CD4 Reagent
STEP 2 Choice of program	DEPLETION 2 1	DEPLETION 2 1	ENRICHMENT 1 1	DEPLETION 2 1	DEPLETION 2 1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set / Tubing Set LS				
STEP 4 CliniMACS® Separation	DEPLETION 2 1	DEPLETION 2 1	ENRICHMENT 1 1	DEPLETION 2 1	DEPLETION 2 1

Antigen/ Reagent	CD8 Reagent	CD8 Reagent	CD8 Reagent	CD8 Reagent	CD25 Reagent
Application	enrichment	enrichment (large scale)	depletion	depletion (large scale)	enrichment frequent CD25 highly expressing cells >10% of WBC
Application capacity	4×10 ⁹ CD8 ⁺ cells from 40×10 ⁹ total cells	8×10 ⁹ CD8 ⁺ cells from 80×10 ⁹ total cells	4×10 ⁹ CD8 ⁺ cells from 40×10 ⁹ total cells	8×10 ⁹ CD8 ⁺ cells from 80×10 ⁹ total cells	6×10 ⁹ highly expressing CD25 ⁺ cells from 40×10 ⁹ total cells (WBC)
No. of vials	1	2	1	2	1
Tubing set (TS)	TS (REF 161-01)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)	TS (REF 161-01)
No. of TS required for application	1	1	1	1	1
Software (Separation program)	ENRICHMENT 11	ENRICHMENT 11	DEPLETION 2 1	DEPLETION 2 1	ENRICHMENT 11

A p p l i c a b l e s e c t i o n s

STEP 1 Magnetic labeling	CliniMACS® CD8 Reagent	CliniMACS® CD8 Reagent	CliniMACS® CD8 Reagent	CliniMACS® CD8 Reagent	CliniMACS® CD25 Reagent
STEP 2 Choice of program	ENRICHMENT 11	ENRICHMENT 11	DEPLETION 2 1	DEPLETION 2 1	ENRICHMENT 11
STEP 3 Installation of tubing set	CliniMACS® Tubing Set / Tubing Set LS				
STEP 4 CliniMACS® Separation	ENRICHMENT 11	ENRICHMENT 11	DEPLETION 2 1	DEPLETION 2 1	ENRICHMENT 11

Antigen/ Reagent	CD25 Reagent	CD25 Reagent	CD25 Reagent	CD45RA Reagent
Application	enrichment rare CD25 highly expressing cells <10% of WBC	depletion	depletion (large scale)	depletion
Application capacity	0.6 × 10 ⁹ highly expressing CD25 ⁺ cells from 40 × 10 ⁹ total cells (WBC)	6 × 10 ⁹ CD25 ⁺ cells from 40 × 10 ⁹ total cells (WBC)	6-12 × 10 ⁹ CD25 ⁺ cells from 80 × 10 ⁹ total cells (WBC)	20 × 10 ⁹ CD45RA positive cells from 50 × 10 ⁹ total cells
No. of vials	1	1	2	1
Tubing set (TS)	TS (REF 161-01)	TS LS (REF 162-01)	TS LS (REF 162-01)	DTS (REF 261-01)
No. of TS required for application	1	1	1	1
Software (Separation program)	ENRICHMENT 3.2	DEPLETION 2.1	DEPLETION 2.1	DEPLETION 3.1

A p p l i c a b l e s e c t i o n s

STEP 1 Magnetic labeling	CliniMACS® CD25 Reagent	CliniMACS® CD25 Reagent	CliniMACS® CD25 Reagent	CliniMACS® CD45RA Reagent
STEP 2 Choice of program	ENRICHMENT 3.2	DEPLETION 2.1	DEPLETION 2.1	DEPLETION 3.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® Tubing Set / Tubing Set LS	CliniMACS® DTS
STEP 4 CliniMACS® Separation	ENRICHMENT 3.2	DEPLETION 2.1	DEPLETION 2.1	DEPLETION 3.1

Enrichment of CD34 positive cells

STEP 1

Cell preparation and magnetic labeling

I. General information, warnings and precautions

Read “General information, warnings and precautions” in the CliniMACS® User Manual: General information, CliniMACS® CD34 Reagent, Handling of biohazardous material, Leukapheresis product, CliniMACS® Tubing Sets, CliniMACS® PBS/EDTA Buffer

II. First actions required

- Material Enrichment:**
- Normal scale:** Up to 0.6×10^9 labeled cells out of 60×10^9 total cells (WBC):
1 vial of CliniMACS CD34 Reagent (REF 171-01), 1 CliniMACS Tubing Set (REF 161- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)
 - Large scale:** 0.6 to 1.2×10^9 labeled cells out of 120×10^9 total cells (WBC):
2 vials of CliniMACS CD34 Reagent (REF 171-01), 1 CliniMACS Tubing Set LS (REF 162- 01), CliniMACS PBS/EDTA Buffer (REF 700-25)

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement buffer with HSA to a final concentration of 0.5% (w/v)

Labeling and preparation of bags

- Label bags as: **Cell Collection, Cell Preparation, Plasma Waste and Wash Waste.**
- Determine the weight of the empty Cell Collection and Cell Preparation Bag

III. Leukapheresis product

Analysis of leukapheresis product (LP) Transfer of LP into Cell Preparation Bag

Dilution of LP Add buffer: Amount of buffer to be added = Weight of LP \times 2

Centrifugation 200 \times g, 15 min, without brake, room temperature (RT)

Volume adjustment **Normal scale:** Volume to remove = Volume of LP + Volume of buffer - 95 g **Large scale:** Volume to remove = Volume of LP + Volume of buffer - 190 g Remove supernatant completely taking care not to resuspend the cell pellet

IV. Magnetic labeling of cells

Incubation with CliniMACS® CD34 Reagent

Add 1 or 2 vial(s) of CliniMACS CD34 Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT

Removal of excess reagent

- Fill Cell Preparation Bag with buffer
- Centrifuge (200 \times g, without brake, 15 min, RT)
- Removal of supernatant
- Resuspension of cell pellet
- Fill Cell Preparation Bag with buffer
- Centrifuge (200 \times g, without brake, 15 min, RT)
- Removal of supernatant
- Resuspension of cell pellet
- Adjust volume: **Normal scale:** 100 g

Enrichment of CD34 positive cells

STEP 2 Start the CliniMACS® plus Instrument

Switch on the CliniMACS® plus Instrument
Choose CD34 Selection 1/2
ample Parameter Input



STEP 3 Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation
Attach Cell Collection Bag
Attach Priming Waste Bag and insert pre-column
Insert selection column and load valve no. 5
Load valves nos. 1, 2, 3, and 4
Load pump tubing
Load valves nos. 7 and 8
Load valves nos. 6, 9, 10, and 11
Recheck all tubing and attachments
Seating of valves
Attach CliniMACS® PBS/EDTA Buffer
Start priming
Check during the priming
Final check of all tubing and attachments
Integrity test
Connect Cell Preparation Bag
Final check of the liquid sensor



STEP 4 CliniMACS® Separation

Separation procedure
Disconnect bags and record process code
Unload tubing set and shutdown



STEP 1:

CliniMACS® CD34 Reagent

I. General information, warnings and precautions

HUMANITARIAN DEVICE: Authorized by U.S. Federal law for processing hematopoietic progenitor cells-apheresis (HPC-A) to obtain a CD34 positive cell enriched population intended to provide for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft-vs-host disease (GVHD) prophylaxis in patients with Acute Myeloid Leukemia (AML) in first or second morphologic complete remission. The effectiveness of the device for this indication has not been demonstrated

CAUTION: Federal law restricts this device to sale by or on the order of a physician.

General information

The CliniMACS® CD34 System including the CliniMACS® plus Instrument, the CliniMACS® CD34 Reagent, the CliniMACS® Tubing Set or CliniMACS® Tubing Set LS, and CliniMACS® PBS/EDTA Buffer is available as a humanitarian device, authorized by U.S. Federal law for processing hematopoietic progenitor cells-apheresis (HPC-A) to obtain a CD34 positive cell enriched population intended to provide for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft-vs-host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first or second morphologic complete remission. The effectiveness of the device for this indication has not been demonstrated

The CD34 antigen is a highly glycosylated 115 kD type 1 integral membrane protein of unknown function which is expressed on 1% to 4% of normal bone marrow cells and less than 0.2% of normal peripheral blood leukocytes, on subsets of bone marrow stromal cells, and on small vessel endothelium of various tissues

The CliniMACS® CD34 System uses selective CD34 monoclonal antibodies conjugated to super-paramagnetic particles. The CD34 positive cells are specifically labeled by incubation with the CliniMACS® CD34 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the enrichment in an automated continuous flow separation process

Important

- The CliniMACS® CD34 Reagent is intended for the *in vitro* enrichment of human CD34 positive cells from heterogeneous hematologic cell populations
- The CliniMACS® CD34 Reagent must only be used in combination with a CliniMACS® plus Instrument, a CliniMACS® Tubing Set or CliniMACS® Tubing Set LS, and CliniMACS® PBS/EDTA Buffer

The CliniMACS® CD34 System passes the antibody-labeled suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD34 positive target cells while unwanted cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled CD34 positive cells are released from the column by removing the column from the magnetic field and eluting the target cells into the Cell Collection Bag.

Important

- Please read and observe the warnings and precautions given in chapter 3 and in the CliniMACS CD34 Reagent Package Insert carefully

Warnings and precautions

For warnings and precautions concerning the handling of biohazardous material, leukapheresis product, CliniMACS® Tubing Sets, and CliniMACS® PBS/EDTA Buffer, please refer to the applicable section in chapter 3: “3.6 Warnings and precautions”

CliniMACS® CD34 Reagent

Intended purpose

HUMANITARIAN DEVICE: The CliniMACS® CD34 Reagent System is intended for processing hematopoietic progenitor cell-apheresis (HPCA) to obtain a CD34 positive cell enriched population intended to provide for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft-vs-host disease (GVHD) prophylaxis in patients with Acute Myeloid Leukemia (AML) in first or second morphologic complete remission. The effectiveness of the device for this indication has not been demonstrated

CAUTION: Federal law restricts this device to sale by or on the order of a physician.

Important

- Use undamaged and sealed vials only
The CliniMACS® CD34 Reagent is for single use only and not for direct infusion.

- The sterile CliniMACS® CD34 Reagent consists of murine monoclonal CD34 antibodies conjugated to iron-dextran particles. The CliniMACS® CD34 Reagent is intended for the *in vitro* enrichment of human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations
- The CliniMACS® CD34 Reagent must only be used in combination with the CliniMACS® plus Instrument, for the “small scale application” preparation with a CliniMACS® Tubing Set, and for the “large scale application” preparation with a CliniMACS® Tubing Set LS, and CliniMACS® PBS/EDTA Buffer

Side-effects

- When the enriched CD34 positive cells are reinjected into a patient, patients may receive traces of murine antibody and iron-dextran. Iron-dextran beads and/or murine antibodies may cause allergic or anaphylactic reactions in patients. Intensive care equipment and medication should be available
- Iron-dextran solutions without associated antibodies are clinically used for the treatment of iron-deficiency syndromes. Hypersensitivity reactions, including anaphylactoid reactions, have been reported following their use. The most common symptoms are myalgia and fever, though lymphadenopathy, general malaise, raised ESR (Erythrocyte Sedimentation Rate) and splenomegaly have also been noted (Bielory, 1990). Individuals with a history of allergies, asthma or active inflammatory disease appear to be highly susceptible to iron-dextran reactions

Precautions

- Before human applications, the suitability of the target cells must be demonstrated regarding indication, quality and quantity
- For the manufacturing and use of target cells in humans, the national legislation and regulations must be followed
- Any clinical application of the separated cells is exclusively within the responsibility of the user
- The enrichment of CD34 positive cells using the CliniMACS® CD34 Reagent must be performed by trained operators only
- Clinicians using the CliniMACS® CD34 Reagent should have experience in the separation of cells from bone marrow or peripheral blood
- All materials which have come into contact with blood or blood products must be treated as infectious material. Regulations for the treatment of infectious material must be observed

Warnings

- Do not inject or infuse the reagent directly into the patient. For *in vitro* use only. Not for parenteral application.
- The CliniMACS® CD34 Reagent is not for use with patients known or suspected to have sensitivity against mouse immunoglobulins or iron-dextran.
- Sensitive patients may develop human anti-mouse antibodies (HAMA).

Further information

- The CliniMACS® CD34 Reagent is for single use only (see Fig 4.1-1).
- Do not use after the use-by date printed on the vial label (see Fig. 4.1-2).
- Store at +2 °C to +8 °C (see Fig. 4.1-3). Do not freeze
- Do not use the CliniMACS® CD34 Reagent if package is damaged (see Fig. 4.1-4). Use undamaged and sealed vials only
- The CliniMACS® CD34 Reagent is sterile (see Fig. 4.1-5). It is manufactured aseptically, sterile filtered and filled aseptically

Important

- This protocol requires the use of components which are not part of the CliniMACS® CD34 System (e. g. HSA). Therefore either materials of pharmaceutical grade must be used or all risks arising from these materials must be evaluated by the user



Fig 4 1-1: Do not reuse.



Fig 4 1-2: Use-by date.



Fig 4 1-3: Temperature limitation +2 °C to +8 °C



Fig 4 1-4: Do not use if package is damaged.



Fig 4 1-5: Sterile. Manufactured aseptically, sterile filtered, filled aseptically.

Note

- The application capacity for the enrichment of CD34 positive cells using the CliniMACS® CD34 System is 0.6×10^9 CD34 positive cells out of a total cell number not exceeding 60×10^9 cells

For the enrichment of up to 1.2×10^9 CD34 positive cells out of a total cell number of 120×10^9 cells (large scale application), two vials of the CliniMACS® CD34 Reagent are needed

Note

- For detailed information on additional materials suggested for a CliniMACS® Separation, please refer to chapter 3 “3.4 Additional material required”

II. First actions required

CliniMACS® Materials required

- CliniMACS® CD34 Reagent, REF 171-01.
- CliniMACS® Tubing Set, REF 161-01
or
CliniMACS® Tubing Set LS, REF 162-01.
- CliniMACS® PBS/EDTA Buffer, REF 700-25.
CliniMACS® PBS/EDTA Buffer must be used for the cell preparation and the CliniMACS® Separation. For the preparation procedure, two liters of buffer are required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Additional materials required

- *Cell Preparation Bag:*
One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- *Plasma Waste Bag and Wash Waste Bags:*
Three 600 mL transfer bags, suitable for centrifugation.
- *Cell Collection Bag:*
One 150 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set.
- Pre-system filter
- Locking forceps
- Appropriate syringes (2×1 mL, 1×10 mL or 1×20 mL) and hypodermic 20 gauge needles
- Human serum albumin (HSA) to be added to the CliniMACS® PBS/EDTA Buffer to a final concentration of 0.5% (w/v).
- Sample tubes

Equipment required

Please find information on equipment required for a CliniMACS® Separation in chapter 3: “3.5 Equipment required”

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement three liters of CliniMACS® PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i.e., add 5 g HSA per liter buffer

Labeling and preparation of bags

A master copy of a worksheet is provided with the user manual to record important information and data generated during sample handling and preparation.

- Label one 150 mL transfer bag as: **Cell Collection** (This should include patient identification, date and time of run, and operator identification.) Insert a Luer/Spike Interconnector into the port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (C₅).
- Label one 600 mL transfer bag as: **Cell Preparation** (This should include patient identification, date and time of run, and operator identification.) Insert a sampling site coupler into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight on the worksheet (A₂).
- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run and operator identification.)
- Label two 600 mL transfer bag as: **Wash Waste No. 1** and **Wash Waste No. 2** (This should include patient identification, date and time of run, operator identification.)

Important

- Before use, CliniMACS® PBS/EDTA Buffer must be supplemented with HSA to a final concentration of 0.5% (w/v). Note that **HSA is not a component of the CliniMACS® System**. Use only FDA licensed HSA. Carefully read the package insert of the HSA used
- Keep the buffer for cell preparation at +19 °C to +25 °C. Lower or higher ambient temperature will result in less purity and yield of the target cells
- Due to the fact that the length of the coupler can vary during the preparation procedure be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading make sure the locking forceps is always positioned close to the bag and is lying on the balance and the rest of the tubing is lying on the table next to the balance

Important

- All bag handling should be done in a sterile environment (e.g., laminar flow hood) using aseptic techniques. Connecting tubes with the help of the TSCD® may be done outside the laminar flow hood.
- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C). Lower or higher ambient temperature will result in less purity and yield of the target cells.

III. Leukapheresis product

The following sections describe the recommended procedure for the preparation of the leukapheresis product using the Terumo Sterile Connection Device (TSCD®).

- The operator has to be familiar with the operation and use of the TSCD®.
- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available.

Analysis of leukapheresis product

Before starting the preparation of the leukapheresis product the following parameters must be determined:

- Total number of leukocytes,
- Percentage of CD34 positive cells,
- Total number of CD34 positive cells,
- Viability

Other tests might be required depending on the intended use of the cells (e.g. T cell enumeration). Record all data on the work- sheet.

Transfer of leukapheresis product into Cell Preparation Bag

1. Record the date and the start time on the worksheet before beginning to prepare the leukapheresis product (A_1).
2. Determine the volume of the original leukapheresis product by estimating 1 mL of leukapheresis product as equivalent to 1 g (1 g = 1 mL).
3. Holding the leukapheresis product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.
4. Using the TSCD®, connect the Cell Preparation Bag to the original leukapheresis product bag
5. Open the locking forceps to transfer the leukapheresis product material into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps
6. Heat seal the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see Fig 4 1-6) Retain the original leukapheresis bag until cell separation and final cell analysis have been completed
7. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the leukapheresis product. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS PRODUCT (This should include patient identification) and retain for cell analysis
8. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight on the worksheet (A_3).
9. Determine the actual weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the Cell Preparation Bag filled with leukapheresis product (A_3). Record the weight on the worksheet (A_4).
10. If the weight of the leukapheresis product (A_4) is more than 200 g, but the number of cells is less than 120×10^9 total cells and 1.2×10^9 CD34 positive cells, centrifuge the sample to reduce the volume to 200 g at the most and proceed with "Dilution of leukapheresis product" Note the reduced volume on the worksheet ($A_{4 \text{ reduced}}$).

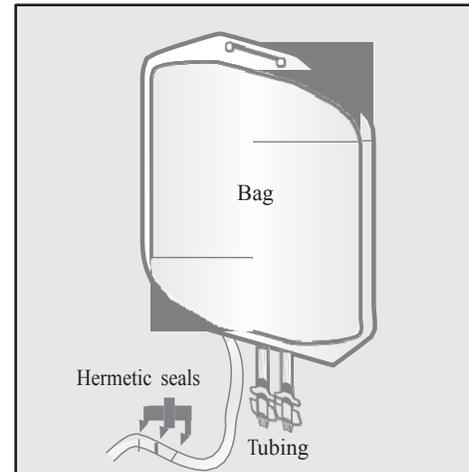


Fig 4 1-6: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Worksheet equation:

$$A_4 = A_3 - A_2$$

Dilution of leukapheresis product

The leukapheresis product should be diluted with CliniMACS® PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the following equation and record it on the worksheet (A₅).

Worksheet equation:

$$A_5 = A_4 \times 2$$
 Weight of buffer to be added (g) = Weight of leukapheresis product (g) × 2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag
2. Using the TSCD®, connect the buffer bag to the Cell Preparation Bag.
3. Place the Cell Preparation Bag on the balance and tare the balance. Hang the buffer bag on the bag hanger. Open the locking forceps next to the Cell Preparation Bag.
4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (A₅) to the Cell Preparation Bag.
5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the actual amount of buffer added on the worksheet (A₆).
6. Make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag. Disconnect the buffer bag.
7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.
8. Tare the balance and weigh the Cell Preparation Bag. Record the weight on the worksheet (A₇).
9. Determine the weight of diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag (A₂) from the weight of the filled Cell Preparation Bag (A₇). Note the weight on the worksheet (A₈).

Worksheet equation:

$$A_8 = A_7 - A_2$$

Centrifugation

- Using the TSCD®, connect the empty Plasma Waste Bag to the Cell Preparation Bag.
- Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.
- Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is calibrated accurately.
- Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Note centrifugation conditions on the worksheet (A₉).
- Remove the bag from the centrifuge, taking care not to resuspend the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

- For magnetic labeling of CD34 positive cells, the optimal weight of the cell sample is a) 95 g (± 5 g), if one vial of CliniMACS® CD34 Reagent is sufficient, or b) 190 g (± 5 g), if two reagent vials are needed (see table).

Calculate the amount of supernatant to be removed to adjust the sample to 95 g (or 190 g) using the equation below:

$$\text{a) Weight of supernatant to be removed (g)} = \text{Weight of diluted leukapheresis product (g)} - 95 \text{ g}$$

$$\text{b) Weight of supernatant to be removed (g)} = \text{Weight of diluted leukapheresis product (g)} - 190 \text{ g}$$

Record the amount of supernatant to be removed on the worksheet (A₁₀).

- Place the empty Plasma Waste Bag on the balance and tare the balance.
- Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Carefully press out excess supernatant using the plasma extractor. Continue until the calculated amount of supernatant (A₁₀) has been transferred into the Plasma Waste Bag so that a) 95 g or b) 190 g remain in the Cell Preparation Bag.

	Normal scale preparation	Large scale preparation
Labeled cells	0.6×10 ⁹	0.6–1.2×10 ⁹
Total cells	60×10 ⁹ WBC	60-120×10 ⁹ WBC
Number of CD34 Reagent vials	1	2
Optimal labeling weight	95 g (± 5 g)	190 g (± 5 g)

Table 4.1-1: Optimal labeling volume for the enrichment of CD34 positive cells

Worksheet equation:

$$\text{a) } A_{10} = A_8 - 95 \text{ g or b) } A_{10} = A_8 - 190 \text{ g}$$

Important

- Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is in the open position before beginning the transfer. Release the extractor handle slowly.
- During removal of supernatant be careful not to lose cells.

4. When the appropriate amount of supernatant has been transferred, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the actual weight of the supernatant removed on the worksheet (A_{11}).
5. Seal off the tubing to disconnect the Plasma Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.
6. Weigh the Cell Preparation Bag and record the weight on the worksheet (A_{12}).
7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (A_{12}). Note the calculated weight on the worksheet (A_{13}).
8. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been accomplished.

Worksheet equation:

$$A_{13} = A_{12} - A_2$$

IV. Magnetic labeling of cells

One vial (7.5 mL) CliniMACS® CD34 Reagent is ready to use and sufficient for one application as described below. The reagent is not for parenteral administration.

Store the reagent at +2 °C to +8 °C. DO NOT freeze. The reagent is to be used cold directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD34 Reagent

1. Record the lot number (B_1) and use-by date (B_2) of the CliniMACS® CD34 Reagent on the worksheet.
2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one vial CliniMACS® CD34 Reagent (7.5 mL). A 10 mL syringe is sufficient to take the contents of one vial, or respectively, a 20 mL syringe is sufficient to take the contents of two vials. The syringe should be equipped with a 20 gauge needle.
3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately start counting the incubation time of 30 min.
4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Note the incubation start time on the worksheet (B_3).
5. Place the Cell Preparation Bag flat on the orbital rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C). Record the incubation stop time on the worksheet (B_4).

Removal of excess reagent

Wash no. 1

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least one liter of buffer. Make sure the clamp on the plasma transfer set is closed.
2. With the help of the TSCD®, connect the buffer bag to the Cell Preparation Bag.
3. Place the Cell Preparation Bag on the balance. Hang the buffer bag on the bag hanger. Tare the balance.
4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e. add 400 g to 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B₃).
5. Close the locking forceps next to the Cell Preparation Bag. Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.
6. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.
7. Using the TSCD®, connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag.
8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 1 securely to the centrifuge bucket.
9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is calibrated accurately.
10. Centrifuge at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Note the centrifugation conditions on the worksheet (B₆).
11. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.
12. Carefully hang the Cell Preparation Bag on the plasma extractor.

13. Place the Wash Waste Bag No 1 on the balance Tare the balance
14. Open the locking forceps next to the Cell Preparation Bag.
Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Note the amount of removed supernatant on the worksheet (B₇).
15. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag No 1 leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections
16. Keep the Wash Waste Bag No.1 until the separation and final analysis of all cells has been accomplished
17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended

Wash no. 2

18. With the help of the TSCD®, connect the plasma transfer set, inserted into a buffer bag containing at least 500 mL of buffer, to the Cell Preparation Bag. Make sure the clamp on the plasma transfer set is closed
19. Place the Cell Preparation Bag on the balance Hang the buffer bag on the bag hanger Tare the balance
20. Open the locking forceps next to the Cell Preparation Bag.
Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e., add approximately 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag on the worksheet (B₈).
21. Close the locking forceps next to the Cell Preparation Bag Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections Disconnect the buffer bag
22. Holding the Cell Preparation Bag with both hands, mix the contents (leukapheresis product and buffer) thoroughly by using a gentle rotating motion.
23. Using the TSCD®, connect the empty Wash Waste Bag No 2 to the Cell Preparation Bag.
24. Fold any loose parts of the bags or tubing downwards
Transfer Cell Preparation Bag and Wash Waste Bag No 2 securely to the centrifuge bucket.

Important

- For the normal scale preparation (one vial of CliniMACS® CD34 Reagent), the volume of supernatant removed should be at least 500 mL.
- For the large scale preparation (two vials of CliniMACS® CD34 Reagent), the volume of supernatant removed should be at least 450 mL.
- If the supernatant removed is less than the value listed above a total of three washing steps (instead of only two) is recommended Otherwise the removal of unbound reagent may be insufficient.

25. Centrifuge at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C). Note the centrifugation conditions on the worksheet (B₉).
26. Taking care not to disturb the cell pellet, remove the bags from the centrifuge. Carefully hang the Cell Preparation Bag on the plasma extractor.
27. Place the Wash Waste Bag No 2 on the balance. Tare the balance.
28. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much excess supernatant as possible from the Cell Preparation Bag. Note the amount of removed supernatant on the worksheet (B₁₀).
29. Close the locking forceps and seal off the tubing to disconnect the Wash Waste Bag No 2.
30. Keep the Wash Waste Bag No.2 until the separation and final analysis of all cells has been accomplished.
31. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.
32. Weigh the Cell Preparation Bag and record the weight on the worksheet (B₁₁). Determine the weight of the leukapheresis product after the washes by subtracting the weight of the empty Cell Preparation Bag (A₂) from the weight of the filled Cell Preparation Bag (B₁₁). Note the weight on the worksheet (B₁₂).
33. Adjust sample loading volume: Calculate the amount of buffer necessary to adjust the weight of the cell suspension to approximately a) 150 g (normal scale preparation) or b) 275 g (large scale preparation), (B₁₃).
34. Using the TSCD®, connect the buffer bag to the Cell Preparation Bag.
35. Place the Cell Preparation Bag on the balance and tare the balance. Hang the buffer bag on the bag hanger. Open the locking forceps next to the Cell Preparation Bag.
36. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer (B₁₃) to the Cell Preparation Bag.

Worksheet equation:

$$B_{12} = B_{11} - A_2$$

Worksheet equation: a)

$$B_{13} = 150 \text{ g} - B_{12} \text{ or b)}$$

$$B_{13} = 275 \text{ g} - B_{12}$$

37. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.
38. Make three hermetic seals between both clamps. Disconnect the buffer bag.
39. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (This should include patient identification.) and retain for cell analysis.
40. Weigh the Cell Preparation Bag and record the weight on the worksheet (B_{14}). Determine the weight of the leukapheresis product (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag (A_2) from the weight of the filled Cell Preparation Bag (B_{14}). Note the weight on the worksheet (B_{15}).

Worksheet equation:

$$B_{15} = B_{14} - A_2$$

Proceed to STEP 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed to do so by the instrument display.

Patient Data _____

Operator ID _____

Transfer of leukapheresis product into Cell Preparation Bag

Starting: Date _____ Time _____ (A₁)

Remove leukapheresis product sample (if yes)

Weight of empty Cell Preparation Bag
(with locking forceps and
sampling site coupler) _____ g (A₂)

Weight of Cell Preparation Bag
filled with leukapheresis product _____ g (A₃)

Weight of leukapheresis product (difference
between filled and empty Cell Preparation Bag) A₄
= A₃ - A₂
Should not exceed 200 g _____ g (A₄)

Weight of leukapheresis product after volume reduction A_{4 reduced}
(difference between filled bag and empty Cell Preparation Bag,
should not exceed 200 g, see below) _____ g (A_{4 reduced})

Dilution of leukapheresis product

The leukapheresis product should be diluted with CliniMACS® PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Calculate the amount of buffer to be added using the equation below

Weight of buffer to be added = Weight of leukapheresis product × 2

Weight of buffer to be added
A₅ = A₄ × 2 _____ g (A₅)

Actual amount of buffer added _____ g (A₆)

Weight of filled Cell Preparation
Bag after dilution _____ g (A₇)

Weight of diluted leukapheresis product
A₈ = A₇ - A₂ _____ g (A₈)

Adjusting the leukapheresis product to 95 g or 190 g

The optimal weight of the leukapheresis product for magnetic labeling of the CD34 positive cells depends on the number of CliniMACS® CD34 Reagent vials to be used for labeling. The contents of one CliniMACS® CD34 Reagent vial is capable of labeling up to 0.6×10^9 CD34 positive cells out of a total leukocyte number of up to 60×10^9 cells. In this case the optimal labeling weight is 95 g. For large scale preparations (cell numbers in the range of 0.6 to 1.2×10^9 CD34 positive cells out of 60 – 120×10^9 total cells) use two vials of CliniMACS® CD34 Reagent and adjust the labeling weight to 190 g. After centrifugation follow (a) or (b) respectively.

Centrifugation conditions

Time:

g Force:

Temperature:

(A₉)

- a) Calculate the amount of supernatant to be removed to give a final volume of 95 g. Use equation below.

$$\text{Amount of supernatant to be removed} = \text{Weight of diluted leukapheresis product} - 95 \text{ g}$$

Amount of supernatant to be removed
 $A_{10} = A_8 - 95 \text{ g}$ _____ g (A₁₀)

Actual amount of supernatant removed _____ g (A₁₁)

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A₁₂)

Weight of leukapheresis product after volume adjustment
 $A_{13} = A_{12} - A_2$ _____ g (A₁₃)

- b) Calculate the amount of supernatant to be removed to give a final volume of 190 g. Use equation below.

$$\text{Amount of supernatant to be removed} = \text{Weight of diluted leukapheresis product} - 190 \text{ g}$$

Amount of supernatant to be removed
 $A_{10} = A_8 - 190 \text{ g}$ _____ g (A₁₀)

Actual amount of supernatant removed _____ g (A₁₁)

Weight of filled Cell Preparation Bag after volume adjustment _____ g (A₁₂)

Weight of leukapheresis product after volume adjustment
 $A_{13} = A_{12} - A_2$ _____ g (A₁₃)

CliniMACS[®]

Worksheet

for preparation and enrichment of CD34 positive cells

Magnetic labeling of CD34 positive cells

Lot number of CD34 Reagent _____ (B₁) Second CD34 Reagent _____ (B₁)
(optional)

Use-by date of CD34 Reagent _____ (B₂) Second CD34 Reagent _____ (B₂)
(optional)

Incubation

Incubation start time _____ (B₃)

Incubation stop time _____ (B₄)

Wash no. 1

Amount of buffer transferred
into the Cell Preparation Bag _____ g (B₅)

Centrifugation conditions

Time:

g Force:

Temperature:

(B₆)

Amount of removed supernatant _____ g (B₇)

Wash no. 2

Amount of buffer transferred
into the Cell Preparation Bag _____ g (B₈)

Centrifugation conditions

Time:

g Force:

Temperature:

(B₉)

Amount of removed supernatant _____ g (B₁₀)

Weight of filled Cell Preparation
Bag after washing procedure _____ g (B₁₁)

Weight of leukapheresis product
after washing procedure
 $B_{12} = B_{11} - A_2$ _____ g (B₁₂)

Final sample preparation

Adjust the volume of the leukapheresis product for loading on the CliniMACS®^{plus} Instrument to 150 g (normal scale preparation) or 275 g (large scale preparation) - sample loading volume. Calculate the amount of buffer to be added:

Amount of buffer to be added

a) $B_{13} = 150 \text{ g} - B_{12}$ (normal scale)

b) $B_{13} = 275 \text{ g} - B_{12}$ (large scale) _____ g (B_{13})

Optional: Remove leukapheresis product sample (ORIGINAL)

(if yes)

Weight of filled Cell Preparation Bag after buffer addition

_____ g (B_{14})

Weight of leukapheresis product after buffer addition (sample loading volume)

$B_{15} = B_{14} - A_2$ _____ g (B_{15})

CliniMACS® Separation (Run)

Date, time started run: Date _____ Time _____ (C_1)

Lot number of tubing set _____ (C_2)

Use-by date of tubing set _____ (C_3)

Calculate the amount of the target cell fraction

Weight of filled Cell Collection Bag after the run has been finished

_____ g (C_4)

Weight of empty Cell Collection Bag (with locking forceps)

_____ g (C_5)

Weight of target cell fraction

$C_6 = C_4 - C_5$ _____ g (C_6)

Process code

_____ (C_7)



Worksheet

for preparation and enrichment of CD34 positive cells

Analysis data

This list is an example of analysis data acquired. Further analysis of additional parameters should be included based on the intended use of the target cells.

Leukapheresis product

(before preparation procedure, sample LEUKAPHERESIS PRODUCT) Total

number of leukocytes _____

Viability _____

Total number of CD34 positive cells _____

Percentage of CD34 positive cells _____

Leukapheresis product

(after magnetic labeling, sample ORIGINAL)

Total number of leukocytes _____

Viability _____

Total number of CD34 positive cells _____

Target cell fraction of CD34 positive cells

(Cell Collection Bag)

Total number of leukocytes _____

Viability _____

Total number of CD34 positive cells _____

Purity of CD34 positive cells Recovery _____

of CD34 positive cells _____

Non-target cell fraction

(Negative Fraction Bag) Total

number of leukocytes _____

Viability _____

STEP 2:

CD34 SELECTION 1/2

Switch-on the CliniMACS[®] plus Instrument

Switch on the CliniMACS[®] plus Instrument by using the ON/OFF switch located on the back panel of the instrument Record the date and time on the worksheet (C₁) when the instrument run has been started.

The window will display screens no 1.1 and no 1.2 as shown in chapter 1.8.

To proceed to the program menu, press

ENT

Choice of separation program CD34 SELECTION 1/2

The window will display screen no 4.2-1 as shown.

Depending on the application, choose CD34 SELECTION 1 or CD34 SELECTION 2

To choose the separation program, highlight the name of the program with the black bar Move the bar up and down by using the keys '0' and '8'

To proceed with the highlighted program, press

ENT

Confirmation of program choice

The window will display screen no 4.2-2 as shown.

Make sure the correct program has been chosen.

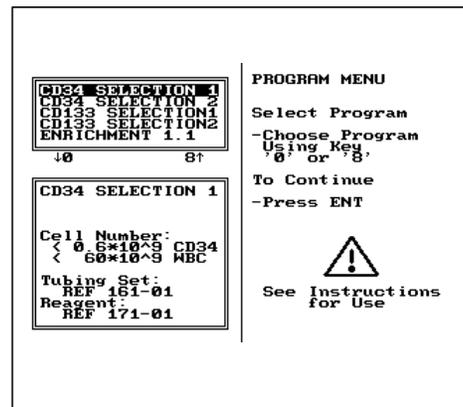
If not, press the „Undo“ key (17, Fig. 1.13) to return to the previous step in order to amend the choice

To confirm and proceed, press

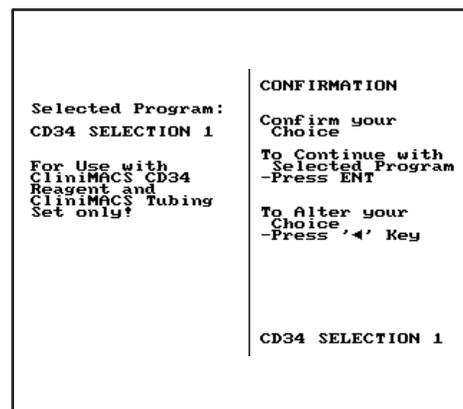
ENT

Note

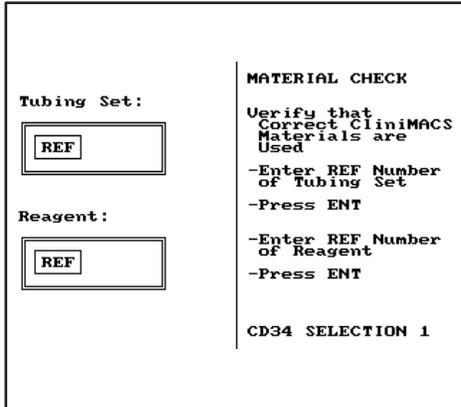
- Screen prompts and diagrams serving as procedure guides will appear in the display window Perform and check each step according to the manual instructions before proceeding to the next step



Screen no. 4.2-1



Screen no. 4.2-2



Screen no. 4.2-3

Note

- CD34 SELECTION 1 must only be used in combination with the CliniMACS® Tubing Set (REF 161-01), while CD34 SELECTION 2 must only be used in combination with the CliniMACS® Tubing Set LS (REF 162-01) Carefully check the tubing set you want to install
- To correct a mistake during data input, press the „Undo“ key (17, Fig 1.13).

Material check

The window will show screen no 4.2-3 as shown.

CD34 SELECTION 1 and CD34 SELECTION 2 are optimized for the enrichment of CD34 positive cells

To make sure the suitable tubing set is available and the proper reagent has been used for cell labeling, enter respective reference number (REF) in the query box The instrument will check whether the materials can be used in combination with the chosen program.

1. Enter reference number of the tubing set to be used for automated cell separation.

To confirm and proceed, press

2. Enter reference number of the reagent that has been used for cell labeling

To confirm and proceed, press

If the reference number of a tubing set or a reagent not specified for the chosen separation program has been entered, a message appears Press ‘ENT’ to confirm and enter the correct reference number again. If the reference number entered is still incorrect, the message will appear a second time After pressing ‘ENT’ the program will return to the program menu (see screen no 4.2-1).

If the material check has been successful, the program continues automatically with the instructions to install the tubing set.

Proceed to STEP 3.

STEP 3:

CliniMACS® Tubing Set (REF 161-01) and CliniMACS® Tubing Set LS (REF 162-01)

Preparation for tubing set installation

The window will display screen no 4.3-1 as shown.

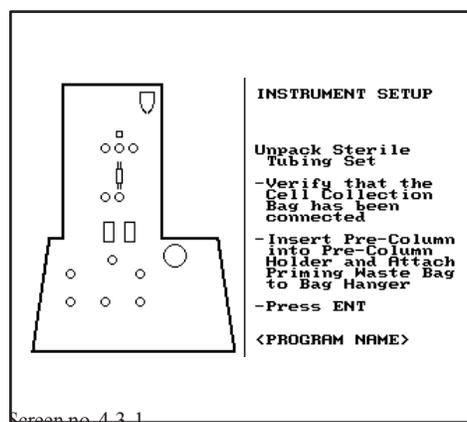
The instruction is on the right, and a diagram corresponding to the instruction is displayed on the left. The blinking features on the screen indicate the areas of attention.

The CliniMACS® Tubing Set and the CliniMACS® Tubing Set LS are provided in a sealed, sterilized package. Each tubing set contains preassembled tubing and columns for one cell separation (see Fig. 4.3-2). When the packaging is intact, a sterile fluid path is provided.

1. Record the lot number and use-by date of the tubing set on the worksheet. Unpack the sterile tubing set under sterile conditions (e.g. laminar flow hood).
2. Check luer lock connections to bags. Luer lock must be closed tightly.

Attach Cell Collection Bag

1. Record the weight of the empty Cell Collection Bag on the worksheet.
2. In an aseptic environment, remove caps and attach the sterile Cell Collection Bag to the luer connector on the tubing set before loading the tubing set onto the CliniMACS®^{plus} Instrument.
3. Make sure that unrestricted flow to the Cell Collection Bag is possible.

**Note**

- The CliniMACS®^{plus} Instrument shows the chosen program name, e.g. CD34 SELECTION 1, in the bottom line of the instrument screen.
- At any step during the tubing set installation the Undo Key (17, Fig. 1.13) can be pushed to return to the previous step.

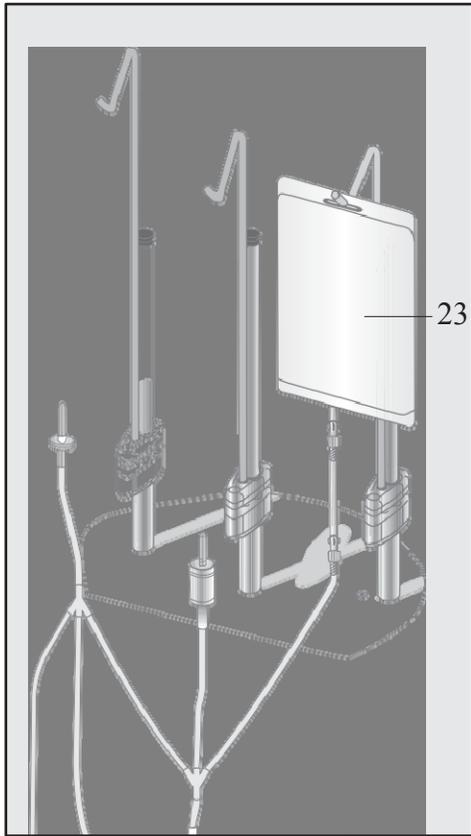


Fig 4.3-1: Attach Priming Waste Bag (23) to bag hanger.

Note

- The bag hangers are made for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.
- When the pre-column is placed into the pre-column holder, ensure that the plastic projections found at the bottom of the column are facing you.

Attach Priming Waste Bag and insert pre-column

The window will display screen no 4.3-1 as shown.

1. Attach the Priming Waste Bag (23) to the right hand bag hanger on the instrument as shown (see Fig. 4.3-1).
2. Place the pre-column into the holder as shown (see Fig 4.3-5).
3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the Priming Waste Bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow, and that it is low enough to avoid the tubing or connections being stretched.

To proceed, press

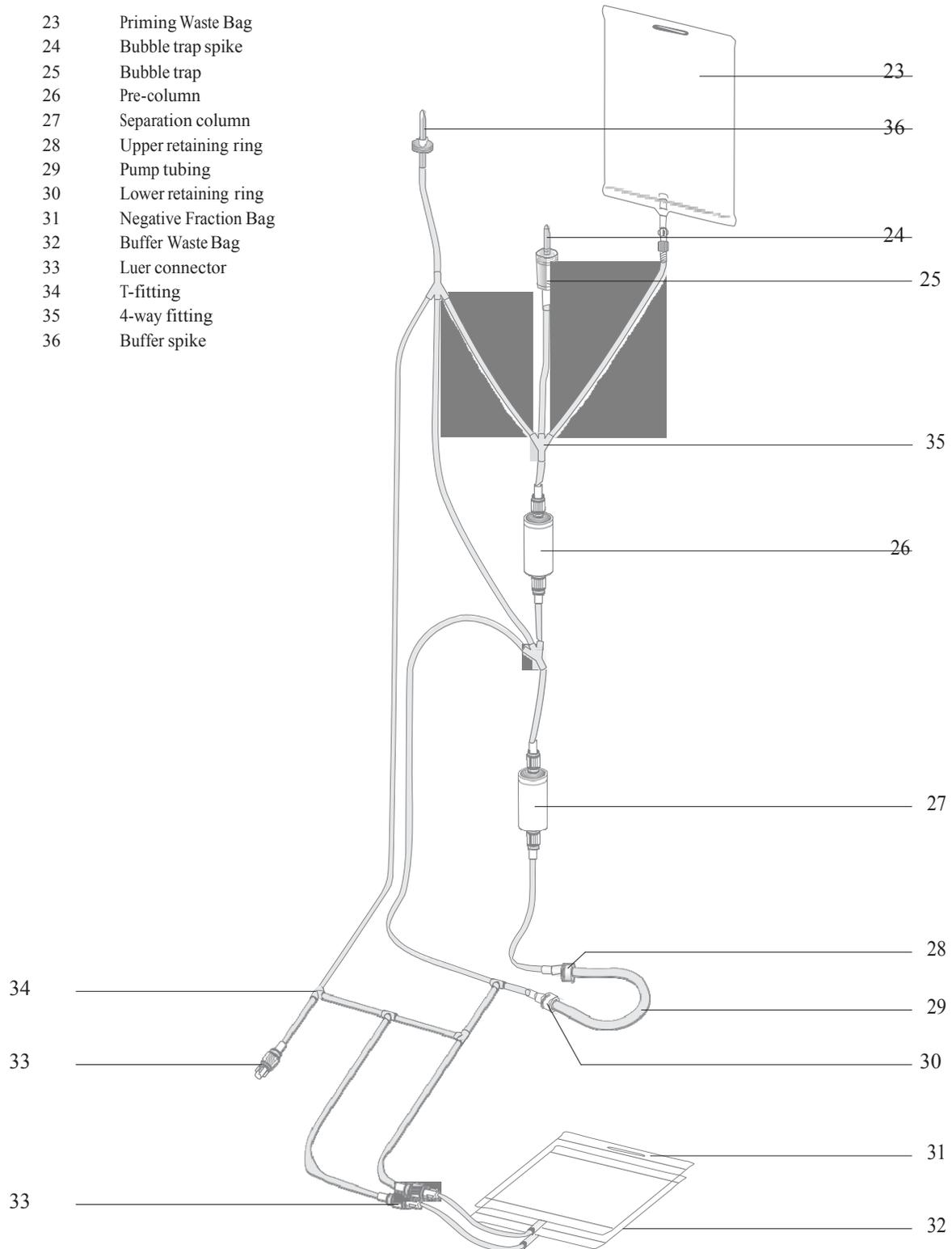
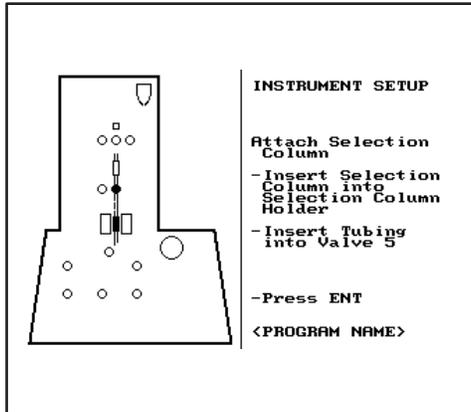


Fig 4 3-2: General construction of a CliniMACS® Tubing Set (e.g. CliniMACS® Tubing Set, REF 161-01).



Screen no. 4.3-2

Insert separation column and load valve no. 5

The window will display screen no 4.3-2 as shown.

The valves shown on the screen will be opened automatically

1. Insert the separation column into the separation column holder as shown (see Fig 4.3-3).

Note

- To avoid possible pinch injury, insert the separation column as follows: Hold the top and bottom of the column between thumb and index finger, then carefully insert the separation column into the separation column holder

2. Load the tubing into valve no 5.

Note

- As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly

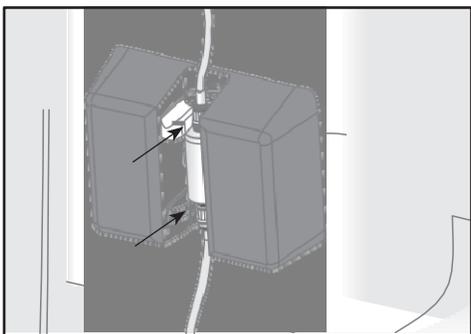


Fig 4 3-3: Separation column in separation column holder.

- Only insert the tubing set into open valves (when button is pushed inwards). The tubing will not fit correctly if inserted into a closed valve

- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve (see Fig. 4.3-4).

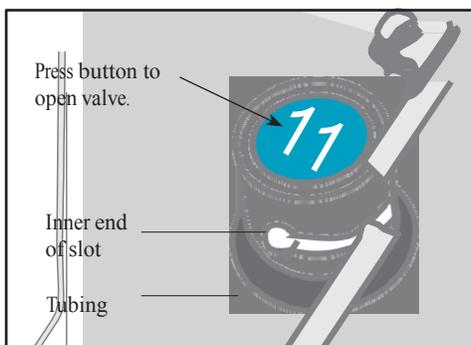


Fig 4 3-4: Correctly inserted tubing.

To proceed, press

ENT



Load valves nos. 1, 2, 3, and 4

The window will display screen no 4.3-3 as shown.

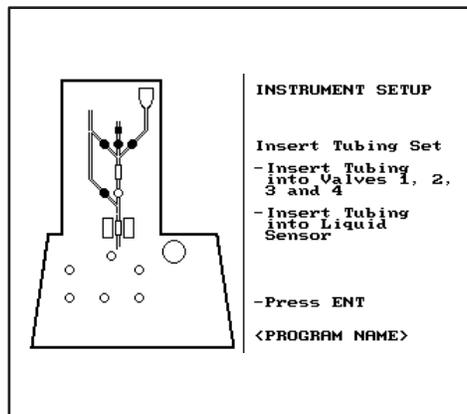
1. Load the tubing into valve no 4. Confirm that the tubing is placed securely in the valve opening (see Fig. 4.3-4). Pay particular attention to the area between valves nos 4 and 5 (37, Fig. 4.3-5).
2. Insert the tubing into valve no 1.
3. Position the 4-way fitting just below valve no 2 Pay particular attention to the area below valve no 2 (37, Fig. 4.3-5).
4. Insert the tubing into valve nos 2 and 3.
5. Mount the tubing between valve no 2 and the bubble trap into the liquid sensor (2, Fig. 4.3-5). Confirm that the tubing is placed correctly into the sensor fitting

Note

- To assure proper operation, both the liquid sensor and the tubing being inserted MUST BE DRY. Carefully inspect both. If any liquid is present, dry the area with a soft, lint-free cloth.

To proceed, press

ENT



Screen no. 4.3-3

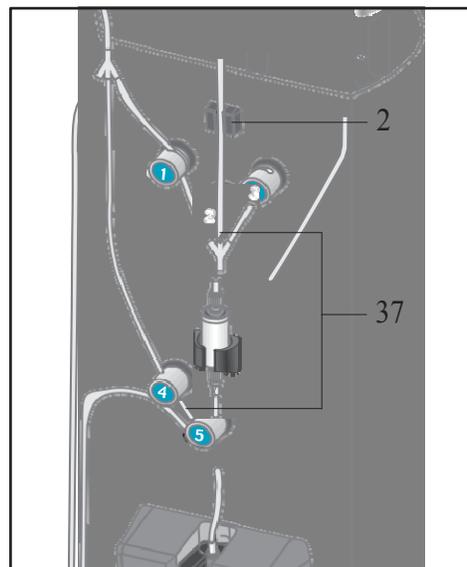
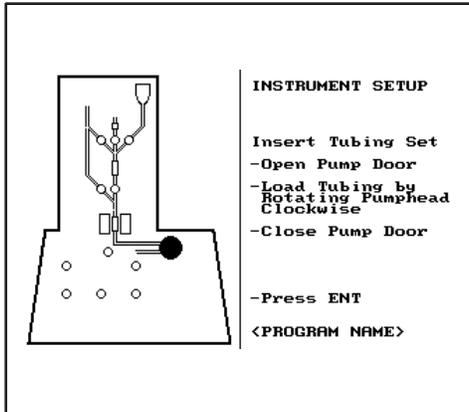


Fig 4 3-5: Tubing in valves.

- 2 Liquid sensor
- 37 Important areas between valves nos. 4 and 5 and below valve no. 2.



Screen no. 4.3-4

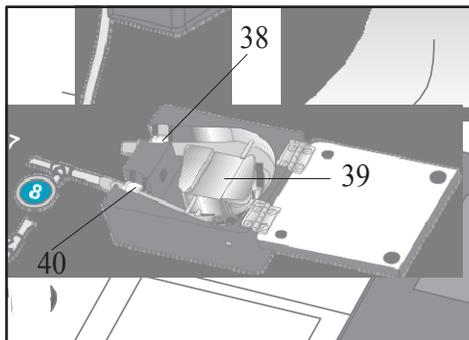


Fig 4 3-6: Loading of pump tubing.

- 38 Upper retaining ring
- 39 Pump roller
- 40 Lower retaining ring

Load pump tubing

The window will display screen no 4.3-4 as shown.

1. Open the pump door by lifting up at the left hand edge
2. Insert the upper retaining ring on the pump tubing into the retaining ring groove (38, Fig 4.3-6) on the pump housing
3. Rotate the pump roller clockwise (39, Fig. 4.3-6) until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins. If adjustment of the tubing inside the pump is necessary, the tubing can be unloaded by lifting the lower end and turning the pump roller anti-clockwise
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove (40, Fig. 4.3-6) on the pump housing
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely
6. Close the pump door

Note

- During the cell separation program the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

To proceed, press

ENT

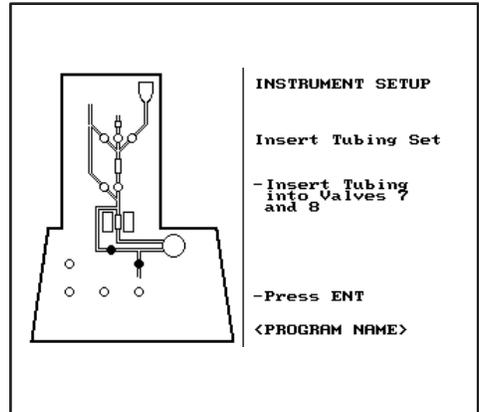
Load valves nos. 7 and 8

The window will display screen no 4.3-5.

1. Load the tubing into valve no 7.
2. Load the tubing into valve no 8.

To proceed, press

ENT



Screen no. 4.3-5

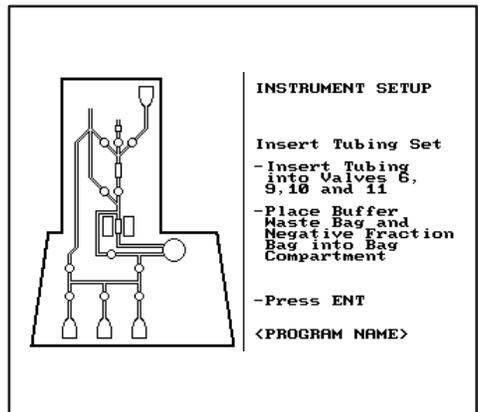
Load valves nos. 6, 9, 10, and 11

The window will display screen no 4.3-6.

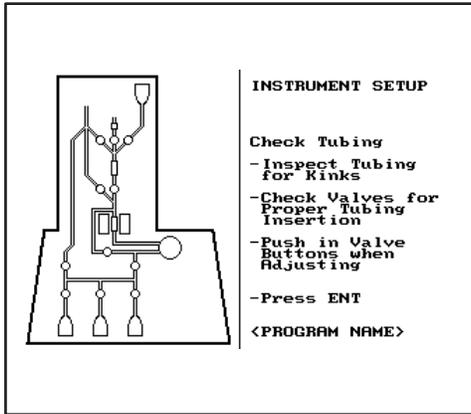
1. Load the tubing into valves nos 6, 9, 10, and 11.
2. Place the Negative Fraction Bag and the Buffer Waste Bag in the bag compartment. Make sure the tubing is not compressed under the bag compartment lid

To proceed, press

ENT



Screen no. 4.3-6



Screen no. 4 3-7

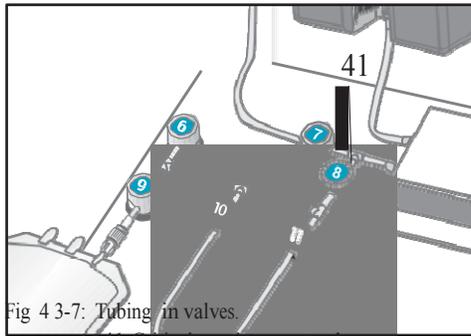
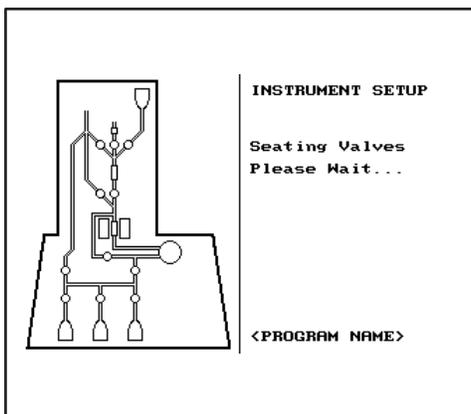


Fig 4 3-7: Tubing in valves

41 Critical area between valve nos. 7 and 8



Screen no. 4.3-8

Recheck all tubing and attachments

The window will display screen no 4.3-7.

Before beginning the run, recheck all tubing and attachments

Note

- Check all valves for proper tubing insertion. Make sure that the tubing is spaced uniformly, and that there are no kinks or stretched areas in the tubing. Pay particular attention to the pre-column area, as well as the area between the pump and valves nos 7 and 8 (41, Fig. 4.3-7), and between valves nos 4 and 5 (37, Fig. 4.3-5).
- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve. If a tubing has been adjusted, it is absolutely necessary to press the corresponding valves firmly two times.

To proceed, press

ENT

Seating of valves

The window will display screen no 4.3-8.

In order to ensure the proper fit of tubing in the valves, the CliniMACS® plus Instrument will operate all of the valves in sequence, twice. Watch and listen to make sure all valves are working properly. If any valve does not operate correctly, see troubleshooting section. This step can be repeated by using the Undo Key (17) followed by the Enter Key (18, Fig. 1.13).

The magnet drive will also be tested during this check sequence.

Attach CliniMACS® PBS/EDTA Buffer

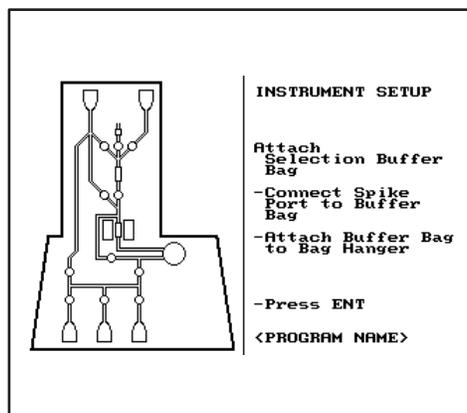
The window will display screen no 4.3-9.

The prescribed buffer for CliniMACS® Separations is CliniMACS® PBS/EDTA Buffer supplemented with HSA to a final concentration of 0.5% (w/v).

1. Using aseptic techniques, remove the cap from the buffer spike on the tubing set (36, Fig. 4.3-2) and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.
2. Attach the buffer bag (43) to the buffer bag hook (42) on the bag hanger (15, Fig. 4.3-8).
3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow, and that it is low enough to avoid the tubing or connections being stretched (see Fig. 4.3-8).

To proceed, press

ENT



Screen no. 4.3-9

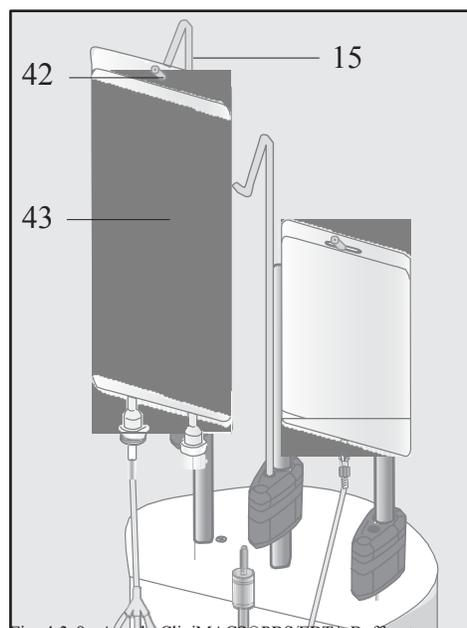
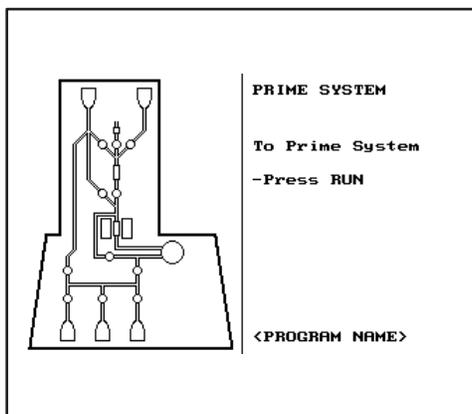


Fig 4.3-8. Attach CliniMACS® PBS/EDTA Buffer to bag hanger.

- 15 Bag hanger for buffer bag
- 42 Buffer bag hook
- 43 CliniMACS® PBS/EDTA Buffer



Screen no. 4.3-10

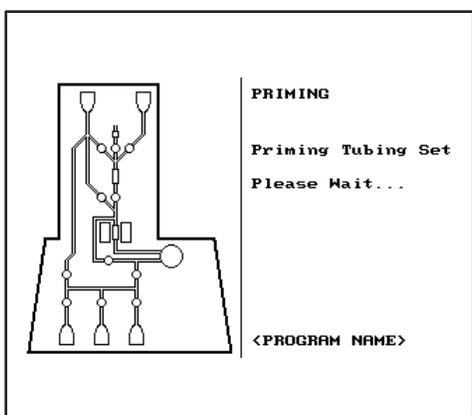
Start priming

The window will display screen no 4.3-10.

To start priming, press

RUN

The window will display screen no 4.3-11. During the priming phase the tubing set is filled with buffer. The buffer will be circulated through the tubing set including both the pre-column and the separation column. Priming waste is collected in the Priming Waste Bag (23) and the Buffer Waste Bag (32, Fig. 4.3-2). The priming cycles will continue, repeating a series of steps. The priming phase will take approximately 1 minute. Priming status will be updated on the display.



Screen no. 4.3-11

Check during the priming

During the priming phase, check all tubing, fittings, valves, and columns for the appearance of any leaks or the presence of any folds that may block fluid flow.

If leaks or malfunctions are observed, stop run by pressing 'STOP'. You will have 600 seconds to resolve the problem. Restart the process by pressing the 'RUN'.

After 600 seconds, the separation will be aborted. If you cannot resolve the problem or if the tubing set is defective, remove the tubing set and replace it with a new one.

Note

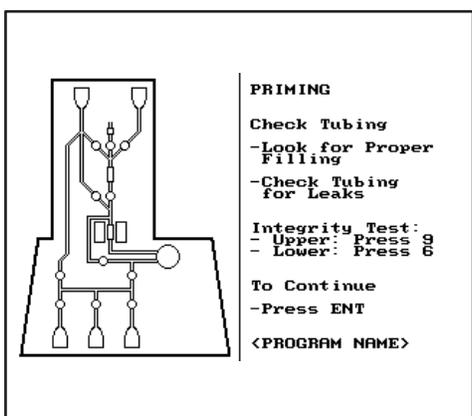
- Once priming has started, it is not possible to return to the instrument set-up procedure.

Final check of all tubing and attachments

The window will display screen no 4.3-12. Before

beginning the run, check the following:

- fluid in all parts of tubing set,
- **no** excess air in tubing set,
- fluid in the Priming Waste Bag and the Buffer Waste Bag,
- **no** fluid in the Negative Fraction Bag or in the Cell Collection Bag.



Screen no. 4.3-12

Integrity test

For additional safety, an integrity test must be performed. Therewith, the tubing set is tested for leakages. The test sequence consists of two automated sequence parts, which allows the upper and the lower part of the tubing set to be tested separately.

Integrity test for the upper part of the tubing set

1. When the operator performs “Final check of all tubing and attachments” the window displays screen no. 4.3-12.
2. After finalizing “Final check of all tubing and attachments”, DO NOT press ENT
3. To enter the integrity test for the upper part, press 9
4. The window will display screen no. 4.3-13.
5. To start the test sequence, press RUN
 To go back to screen no. 4.3-12, press 3
6. Once the RUN button has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

The window will display screen no. 4.3-14.

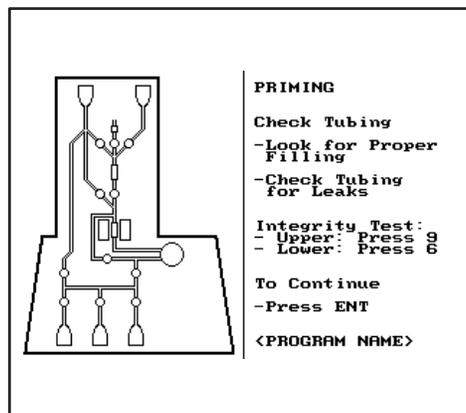
Overpressure will be created and held for 2 minutes. During this time the operator should watch the connections above and under the pre-column and separation column, and the upper pump tube connection.

At each point the test sequence can be finished by pressing ‘ENT’

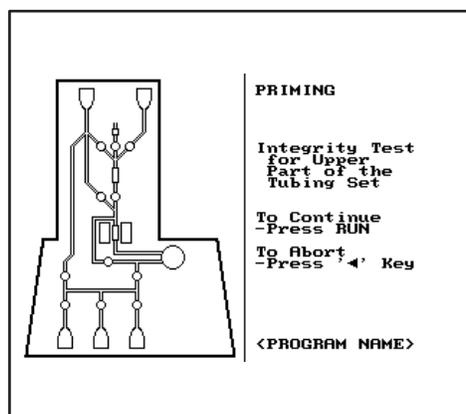
7. After 2 minutes the pressure is automatically released, and the window displays screen no. 4.3-14.

Using tissue the operator should now check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Send the defective tubing set back to your Miltenyi Biotec office or to your CliniMACS® distributor.

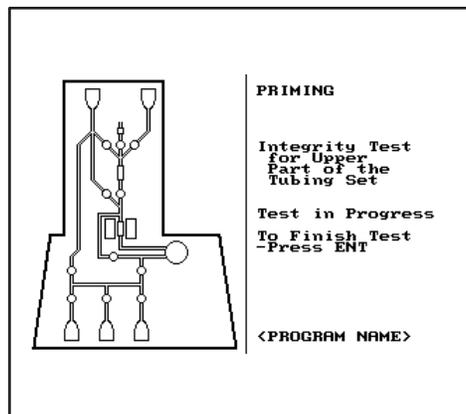
8. If no leaks are observed, continue with the integrity test of the lower part of the tubing set.



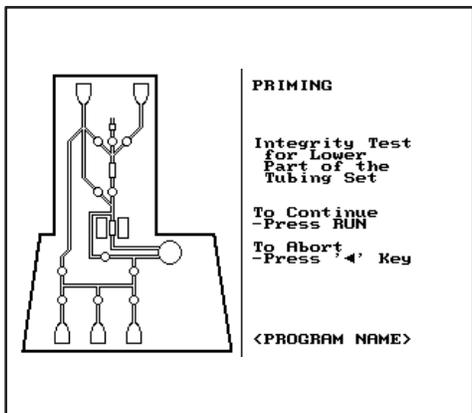
Screen no. 4.3-12



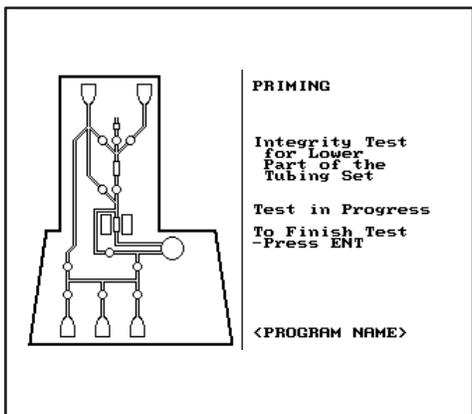
Screen no. 4.3-13



Screen no. 4.3-14



Screen no. 4.3-15



Screen no. 4.3-16

Integrity test for the lower part of the tubing set

1. The window displays screen no 4.3-12.
2. To enter the integrity test for the lower part, press DO NOT press 'ENT'
3. The window will display screen 4.3-15.
4. To start the test sequence, press To go back to screen no 4.3-12, press
5. Once the RUN button has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set.

The window will display screen no 4.3-16.

Overpressure will be created and held for 30 seconds. During this time the operator should watch the lower pump tube connection and the T-fittings between valves nos 6, 8, 9, 10, and 11.

At each point the test sequence can be finished by pressing 'ENT'

6. After 30 seconds the pressure is automatically released, and the window displays screen no 4.3-12.

Using tissue the operator should now check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Send the defective tubing set back to your Miltenyi Biotec office or to your CliniMACS® distributor.

7. If no leaks are observed the operator can now continue with the next step by pressing ENT

Connect Cell Preparation Bag

The window will display screen no 4.3-17.

After the priming phase has been completed and no leaks or malfunctions are observed, the Cell Preparation Bag can be attached (see Fig. 4.3-9). Use aseptic techniques for all steps

Connect the Cell Preparation Bag containing the magnetically labeled and washed cells with the pre-system filter:

1. Remove the cap from the bubble trap spike (24) of the bubble trap (25, Fig. 4.3-2).
2. Remove the cap from the lower opening of the pre-system filter (44, Fig. 4.3-9). **Firmly** insert the spike into the pre-system filter DO NOT remove the top cap of the pre-system filter
3. Remove the cap from the pre-system filter spike (47, Fig. 4.3-9).
4. Spike the Cell Preparation Bag (46) with the pre-system filter (44) ensuring that the septum is punctured, allowing free flow of liquid Gently squeeze the bag to confirm that the spike has penetrated the bag
5. Check the connection between the pre-system filter and the tubing set to confirm that the connection is secure
6. Hang the Cell Preparation Bag on the bag hanger (14, Fig. 4.3-9).
7. Adjust the bag hanger for the Cell Preparation Bag (14, Fig. 4.3-9) to hold the Cell Preparation Bag in an upright position.

To proceed, press

ENT

Final check of the liquid sensor

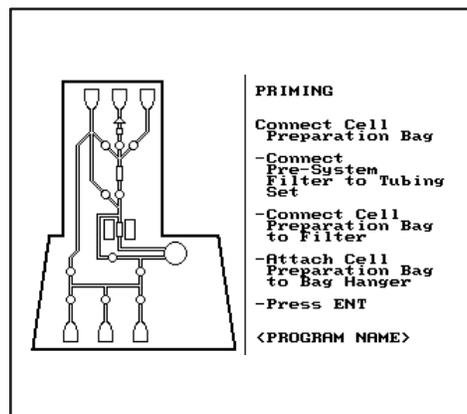
The window will display screen no 4.3-18.

1. Check the liquid sensor tubing Ensure the tubing has been properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure
2. Make sure that unrestricted flow to the Cell Collection Bag is possible

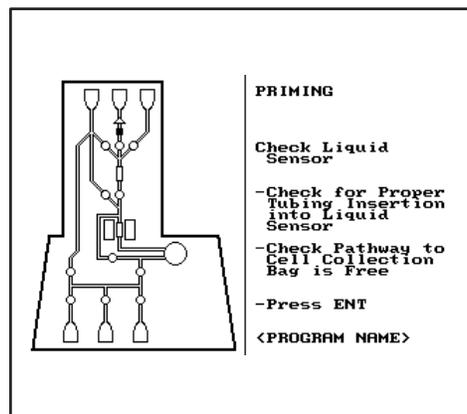
To proceed, press

ENT

Proceed to STEP 4.



Screen no. 4.3-17



Screen no. 4.3-18

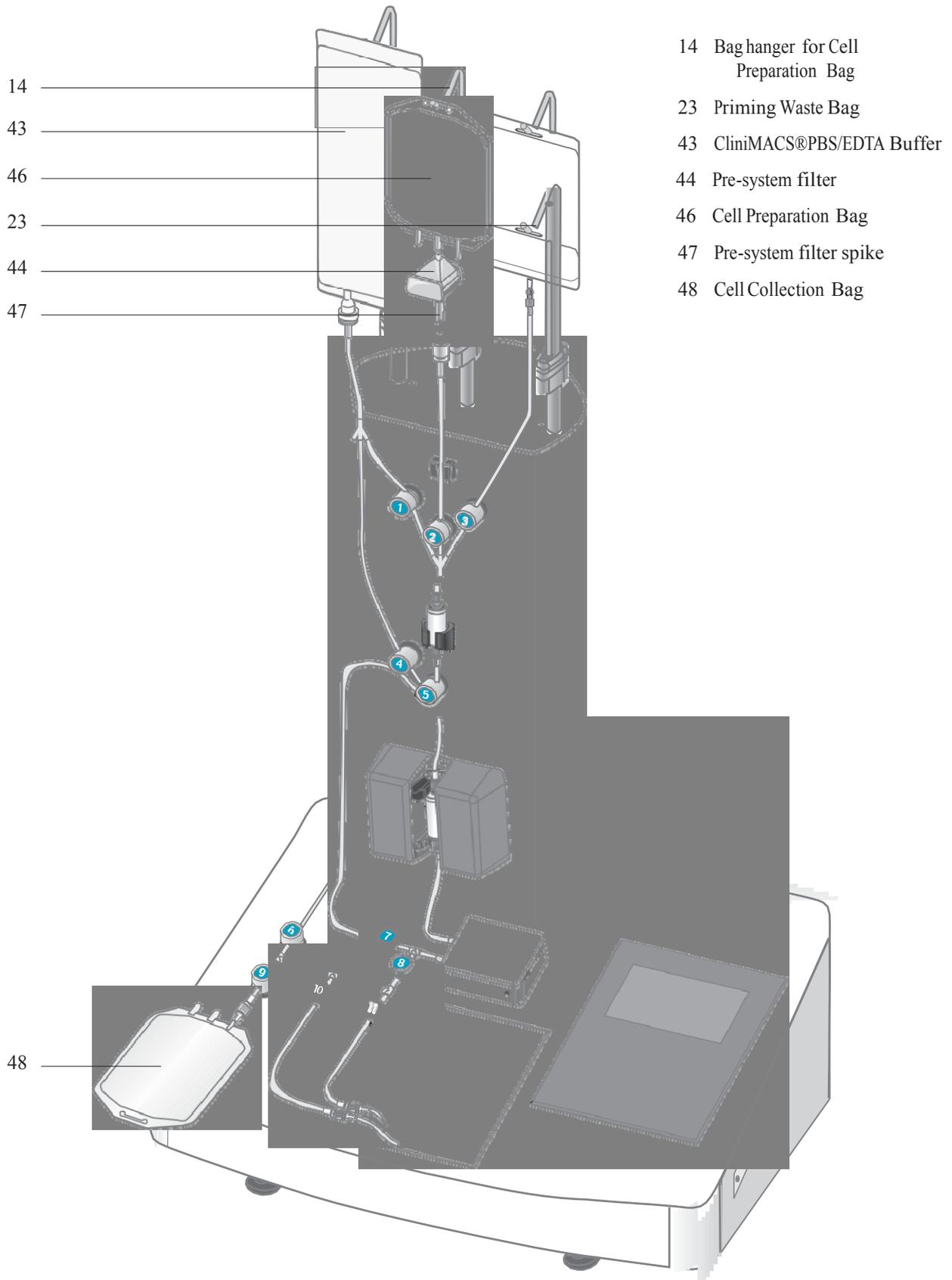


Fig 4 3-9: CliniMACS® plus Instrument with CliniMACS® Tubing Set, CliniMACS® PBS/EDTA Buffer, Cell Preparation Bag, and Cell Collection Bag.

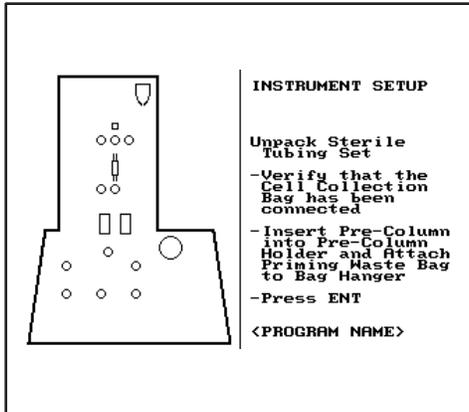
Alternative installation of CliniMACS® Tubing Sets

The instructions in STEP 3 and the screens displayed by the CliniMACS® ^{plus} Instrument describe the installation of the CliniMACS Tubing Sets under sterile conditions (clean room).

The CliniMACS System itself is a closed system which does not necessarily need to be operated in a clean room. However, if operated outside a clean room, the installation procedure of the tubing set needs to be adapted, in order to ensure that the sterility of the cell separation process is guaranteed.

The sterility of the cell separation process is jeopardized during the attachment of the Cell Collection Bag, the CliniMACS PBS/EDTA Buffer, the pre-system filter, and the Cell Preparation Bag. In order to ensure that the system remains sterile, these components need to be attached to the tubing set under sterile conditions (e.g. laminar flow hood). When the components are attached to the tubing set before installation onto the CliniMACS ^{plus} Instrument, the order of the instructions provided by the instrument and the instructions in STEP 3 needs to be changed and further actions taken.

When operating the CliniMACS ^{plus} Instrument outside a clean room, follow the following additional instructions, altering the instructions in STEP 3.



Screen no. 4.3-1

Preparation for tubing set installation

The window will display screen no 4.3-1.

As described, the Cell Collection Bag, the CliniMACS® PBS/EDTA Buffer, the pre-system filter and the Cell Preparation Bag need to be attached to the CliniMACS Tubing Sets before installing the tubing set onto the instrument under sterile conditions

Unpack the tubing set under sterile conditions and attach the following components under sterile conditions:

1. Attachment of Cell Collection Bag

Follow the instructions:

□ Attach Cell Collection Bag

2. Attachment of CliniMACS PBS/EDTA Buffer

Clamp the tubing just below the buffer spike with a clamp in order to prevent the buffer from flowing into the tubing set during its installation (see (1), Fig. 4.3-10). Using aseptic techniques remove the cap from the buffer spike on the tubing set and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

3. Attachment of pre-system filter

Remove the cap from the spike of the bubble trap. Remove the cap from the lower opening of the pre-system filter. Firmly insert the spike into the pre-system filter. DO NOT remove the top cap of the pre-system filter. Close the tubing just below the bubble trap using a clamp (see (2), Fig. 4.3-10). This prevents the prepared cell suspension in the Cell Preparation Bag from entering the pre-system filter.

4. Attachment of Cell Preparation Bag

Connect Cell Preparation Bag containing the magnetically labeled and washed cells to the tubing set. Spike the Cell Preparation Bag with the pre-system filter ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

5. Installation on the CliniMACS^{plus} Instrument

Attach the buffer bag to the left bag hanger, the Cell Preparation Bag to the middle bag hanger and the Priming Waste Bag to the right bag hanger on the instrument.

To proceed, press

ENT

Follow the instructions:

- Attach Priming Waste Bag and insert pre-column
- Insert selection column and load valve no. 5
- Load valves nos. 1, 2, 3, and 4
- Load pump tubing
- Load valves nos. 7 and 8
- Load valves nos. 6, 9, 10, and 11
- Recheck all tubing and attachments
- Seating of valves

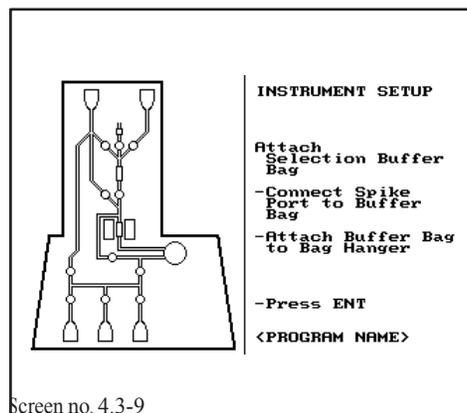
Attach CliniMACS® PBS/EDTA Buffer

The window will display screen no 4.3-9.

1. The buffer bag has been attached during „Preparation for tubing set installation“ Therefore only the height of the buffer bag hanger has to be adjusted Raise or lower the hanger to accomodate the size of the buffer bag, ensuring that the height allotted is high enough to prevent the tubing from severe bending that could restrict the flow, and low enough to avoid stretching the tubing or connections
2. Remove the clamp from the tubing just below the buffer spike

To proceed, press

ENT



Screen no. 4.3-9

Follow the instructions:

- Start priming
- Check during priming
- Final check of all tubing and attachments
- Integrity test

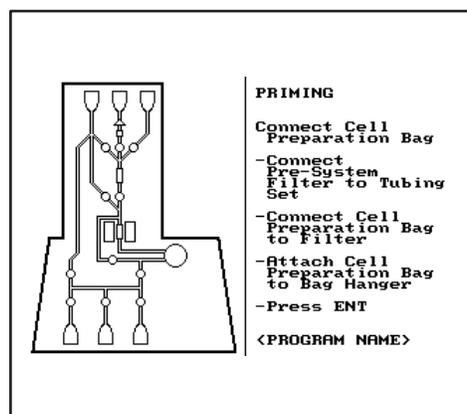
Connect Cell Preparation Bag (& pre-system filter)

The window will display screen no 4.3-17.

1. The Cell Preparation Bag and the pre-system filter have been attached during „Preparation for tubing set installation“
2. Remove the clamp below the bubble trap

To proceed, press

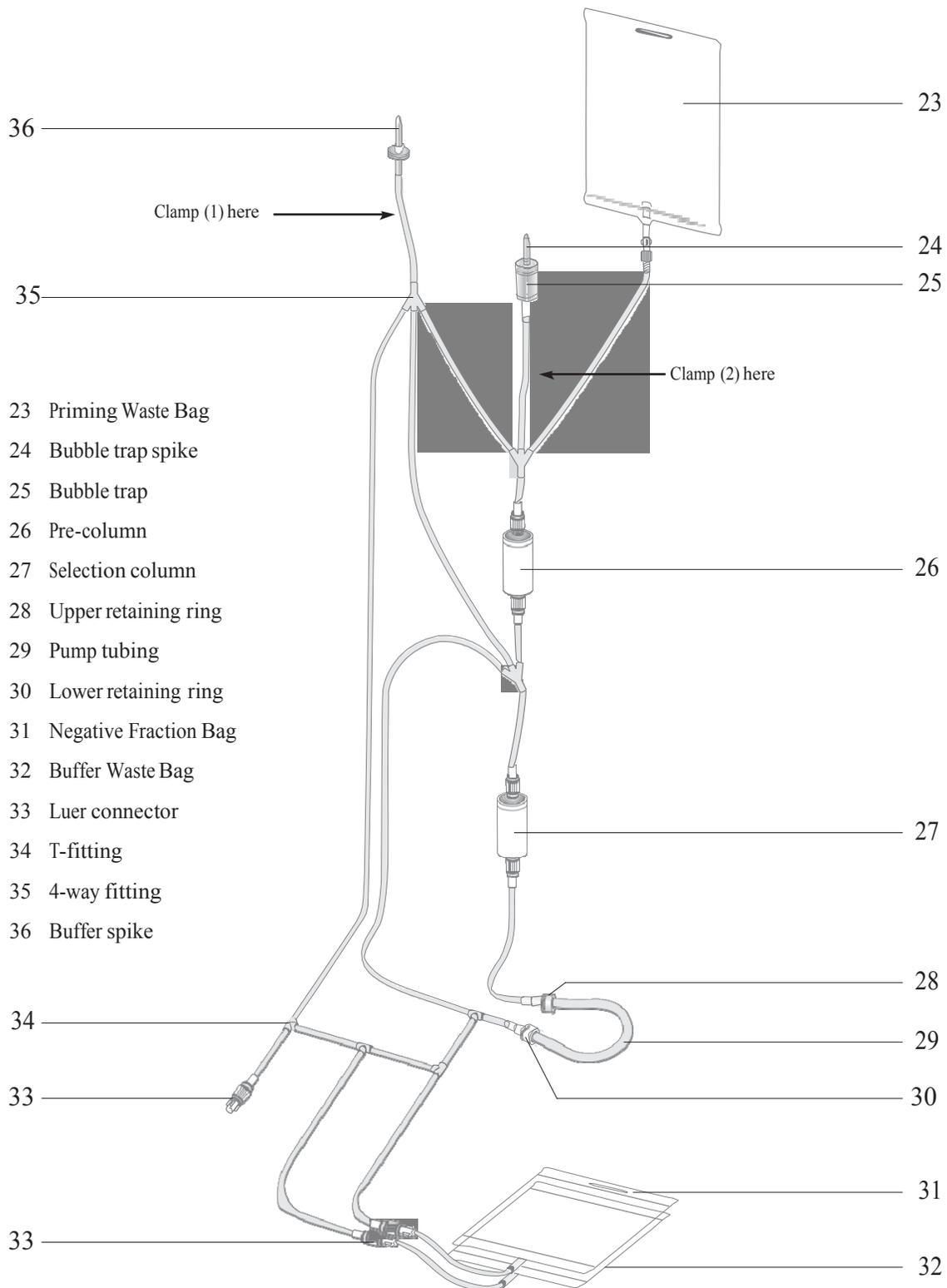
ENT



Screen no. 4.3-17

Follow the instructions:

- Final check of the liquid sensor



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Clamp (1) here
Clamp (2) here

- 23 Priming Waste Bag
- 24 Bubble trap spike
- 25 Bubble trap
- 26 Pre-column
- 27 Selection column
- 28 Upper retaining ring
- 29 Pump tubing
- 30 Lower retaining ring
- 31 Negative Fraction Bag
- 32 Buffer Waste Bag
- 33 Luer connector
- 34 T-fitting
- 35 4-way fitting
- 36 Buffer spike

Fig 4 3-10: General construction of a CliniMACS® Tubing Set (e.g. CliniMACS Tubing Set, REF 161-01).

STEP 4:

CD34 SELECTION 1/2

Once the final check has been completed

When the CliniMACS® plus Instrument is ready to begin the separation, the window will display screen no 4.4-1 as shown.

Make a final check of all tubing and attachments

To proceed, press

RUN

Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to screen no 4.4-2 will be displayed to show the status of the separation procedure

The magnet position indicator is displayed as two black boxes next to the separation column when the magnet is "ON"; i.e. it has been moved to the front to magnetize the separation column. If the magnet position indicator is transparent (e.g. screen no 4.4-1), the magnet is "OFF", i.e. it has been moved to the rear of the instrument. With the magnet withdrawn the separation column is outside the magnetic field and is not magnetized.

Separation procedure

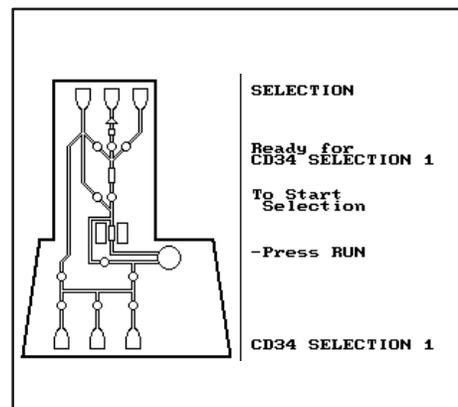
The general steps of the separation procedure are the same for both CD34 separation programs (CD34 SELECTION 1 and 2).

Loading cells

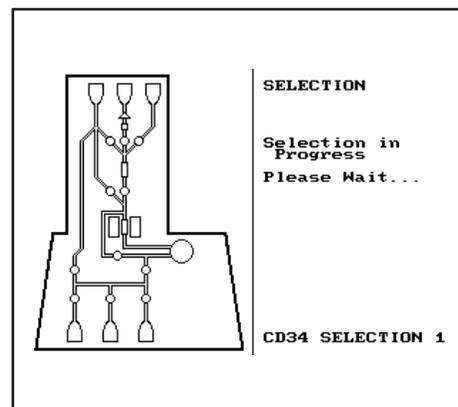
The separation procedure starts with the filling of the pre-system filter to complete the priming of the system. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and collected in the Negative Fraction Bag. When the Cell Preparation Bag is empty (detected automatically by the liquid sensor) the pre-system filter is rinsed twice with buffer.

Column wash I

Pre-column and separation column are washed extensively to remove all unlabeled cells. Wash buffer is collected in the Buffer Waste Bag. When 'Column Wash I' starts, the total remaining time until the end of the separation procedure is shown.



Screen no. 4.4-1



Screen no. 4.4-2

Note

- At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter. Tap the side of the filter several times to remove any bubbles which might be trapped in the filter.
- At each phase of the operation, the status of the separation procedure is shown on the screen.

Release of cells I

The magnet is moved to the rear of the instrument (“OFF” position). The retained cells are released at a high speed flow, but the cells remain within an internal tubing cycle.

Reloading of cells I

The magnet is moved into the “ON” position again to magnetize the separation column and the cells are reapplied on the separation column.

Column wash II

Reloading of the cells is followed by a second washing step to remove remaining unlabeled cells. Also all tubing is rinsed several times.

Release of cells II, reloading of cells II, column wash III

The cells are released and reapplied on the separation column for a second time in order to remove any unlabeled cells that only stick to the column matrix. Afterwards the separation column is washed again.

Release of cells III, reloading of cells III, column wash IV

Additionally, the separation program CD34 SELECTION 2 includes a third release and reapplication step.

Final elution of the cells

The magnet is moved into the “OFF” position and the magnetically labeled CD34 positive cells are released from the separation column and collected in the attached Cell Collection Bag.

Disconnect bags and record process code

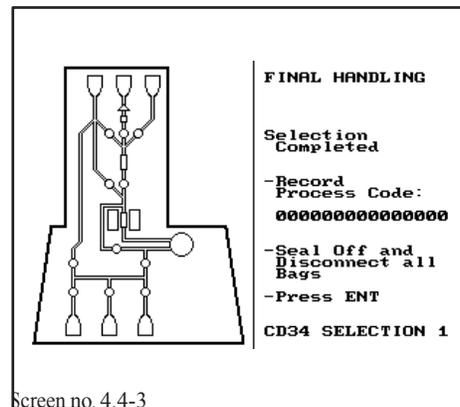
When the run has been completed, the CliniMACS®^{plus} Instrument will display screen no 4.4-3 as shown.

1. Record the process code on the worksheet (C₇).
2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (49, Fig 4.4-1) Make three hermetic seals in the tubing directly below valve no 9 Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set
3. Weigh the filled Cell Collection Bag. Record the weight on the worksheet (C₄). Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag (C₅) from the weight of the Cell Collection Bag containing the target cells (C₄) Record the weight on the worksheet (C₆).
4. Mix the target cell suspension thoroughly by rotating the bag Take an aliquot of 0.5 mL and retain for analysis
5. Heat seal the tubing above the luer lock of the Negative Fraction Bag (50, Fig 4.4-1). Make three hermetic seals in the tubing Sever the center seal to disconnect the Negative Fraction Bag.
6. Disconnect the Buffer Waste Bag in the same way (51, Fig 4.4-1).
7. Remove the Negative Fraction Bag and Buffer Waste Bag. The

target cells can now be processed in accordance with clinical protocols

To proceed, press

ENT



Screen no. 4.4-3

Worksheet equation: C₆
= C₄ - C₅

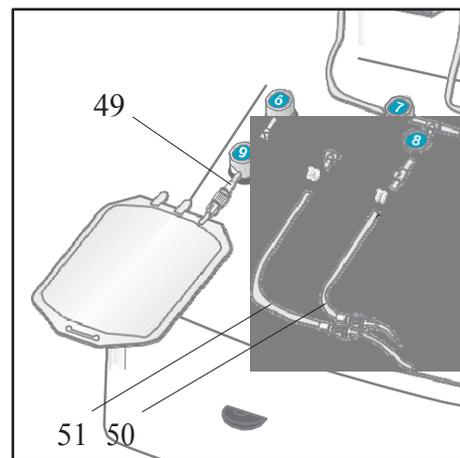
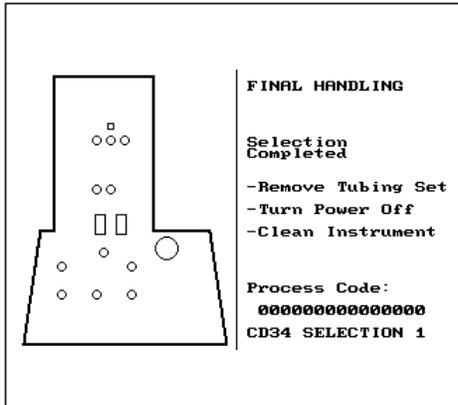


Fig 4.4-1: Where to disconnect the tubing set after the run has been completed. Seal off above luer lock.

- 49 Seal off Cell Collection Bag
- 50 Seal off Negative Fraction Bag
- 51 Seal off Buffer Waste Bag



Screen no. 4.4-4

Important

- Prior to target cell infusion the CliniMACS® PBS/EDTA Buffer contained in the target fraction must be exchanged to a medium suitable for applications in humans. The user of the CliniMACS® System is responsible for the selection of an appropriate medium for infusion.

Unload tubing set and shutdown

The window will display screen no 4.4-4 as shown.

1. Remove the tubing set:
Beginning with valve nos 6, 9, 10 and 11, and working upwards, release the tubing from the liquid sensor and from the valves, by pressing on the valves. Release the columns from the column holders. Dispose the tubing set as a biohazard, according to standard hospital procedures.
2. Switch off the CliniMACS®^{plus} Instrument.
3. Clean the instrument according to cleaning instructions, see chapter 16. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

The target cells must be examined regarding quality and quantity in view of their intended use.

This must include the following parameters:

- Total number of leukocytes,
- Viability and total number of CD34 positive cells,
- Purity and recovery of CD34 positive cells.

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction.

The list is an example and other tests should be included based on the intended use and clinical protocols.

Record the analysis data on the worksheet.

5 Troubleshooting

This section is intended as a reference to provide information about possible unexpected events that might occur and to suggest appropriate corrective action. For information not covered in the following section, please contact our Technical Service as soon as possible.

Please note that the order of this chapter follows the actual sequence of a separation.

5.1 Preparation of the leukapheresis product

The sample received is diluted

Ideally, magnetic labeling is performed in diluted leukapheresis product. Adjust the leukapheresis product to a final dilution of approximately 1:3. If the sample received is more diluted than 1:3, or if you do not exactly know the concentration of plasma in your sample, add immunoglobulin to the sample prior to the addition of the reagent (recommended concentration of immunoglobulin in the labeling volume: 1.5 mg/mL). It is important to have a certain amount of immunoglobulin in the sample during the labeling in order to minimize non-specific binding of the reagent.

The number of target cells is low in the leukapheresis product.

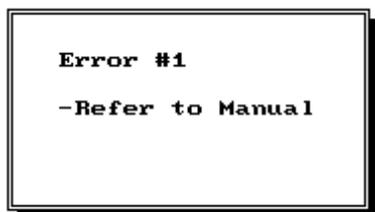
The mobilization of stem cells was insufficient. Check analysis of the leukapheresis product.

Poor viability of cells in the leukapheresis product.

The leukapheresis product may have been harvested, stored or transported inappropriately. To ensure better sample quality, the preparation and separation of the leukapheresis product should be performed immediately after leukapheresis. Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis product with autologous plasma. The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure. If the leukapheresis product has to be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C).

5.2 CliniMACS® plus Instrument and CliniMACS® Tubing Sets

Error messages



Error message no. 1

There are a number of possible instrument or software malfunctions. These are marked as such and will be displayed on the screen. They refer to internal errors that **cannot** be corrected by the operator. Record the displayed error number and contact our Technical Service.

One possible error message is shown in the illustration opposite (Error message no. 1).

Other than error messages, malfunctions that can be corrected by the operator are marked "Warning messages". These are described in section 5.3.

Loading and priming of the tubing set

Valve does not open when operator is instructed to insert tubing into a particular valve

The valves are designed to work properly once the tubing has been inserted. Press the valve manually to open it. Watch the valve carefully during the valve exercise sequence. If the valve does not depress during the valve exercise sequence, see section “Valve does not depress during valve exercise sequence”.

Valve does not depress during valve exercise sequence

Make sure that tubing is correctly inserted. Check whether valves have been cleaned thoroughly. Any valve that has been contaminated by fluid has to be exchanged. Please contact our Technical Service.

Buffer is leaking from tubing set during priming

Tubing set is defective. Turn off the CliniMACS® plus Instrument and restart priming with a new tubing set installed and sufficient new buffer.

Excessive air occurs in tubing set after priming

Buffer bag is not properly spiked. Use a new tubing set and sufficient new buffer and restart the CliniMACS run. Make sure that the septum of the buffer bag is properly punctured.

Unexpected volume of buffer in bags after priming. After priming, liquid should only be in the Priming Waste Bag and Buffer Waste Bag.

Tubing set is not mounted correctly. Liquid can leak behind the valves if the tubing set is not installed correctly or the valves are not functioning properly. Remove the tubing set and replace it with a new one. Restart the CliniMACS priming procedure with sufficient new buffer. Poor CliniMACS separation performance may result if the tubing set is not inserted properly.

Pump motor stalls during priming

Pump tubing has not been inserted correctly. Press ‘STOP’ to interrupt the priming and turn the power “OFF” and then “ON” again. Clamp the buffer line with a haemostat during the installation procedure and remove the clamp before restarting the priming sequence.

5.3 Automated cell separation

Warning messages

Unlike error messages (see section 5.2), warning messages are displayed on the screen when the internal control system of the CliniMACS® plus Instrument recognizes a malfunction which **can** be corrected by the operator. Usually, a warning message appears in combination with a sound (“beep”). If a warning message appears during the CliniMACS run, follow the instructions on the screen to proceed with the cell separation. Generally speaking, warning messages appear when the ‘STOP’ key is pressed, when the pump door is opened, when the pump stalls or when the liquid sensor detects an error.

Since different kinds of unexpected events can occur during different separation programs, the following section has been subdivided accordingly.

Unexpected events

- All separation programs

Error detected by liquid sensor



Warning message no. 1

Warning message No 1 will appear during the starting phase of the cell loading process if the liquid sensor is not able to detect liquid in the tubing. As the pre-system filter is rinsed with buffer prior to the cell loading, there must be liquid in the tubing at this point.

Check the following points:

1. Has the tubing been inserted correctly? If not, do so.
2. Is the tubing filled with buffer? If not, see point 3.
3. If the tubing is not filled with buffer, inspect the tubing for kinks blocking the buffer flow upwards into the pre-system filter and Cell Preparation Bag. Adjust the position of the tubing set. If necessary, raise or lower the bag hangers using the bag hanger clamps. Adjust the position of the tubing in the valves. To alter the position of the tubing, open the valve by manually pressing the button. Make sure that the tubing is not kinked, twisted or taut.
4. Is the Cell Preparation Bag spiked properly? Make sure the pre-system filter spike has penetrated the septum of the Cell Preparation Bag port.
5. If the tubing set has not been completely filled with buffer: Has the buffer spike of the tubing set penetrated the buffer bag? For correction see section “Excessive air occurs in tubing set after priming” (section 5.2).

After the corrective action, continue with the separation in progress and press ‘5’.

If warning message no 1 appears again after each of the possible causes listed above have been ruled out, the liquid sensor may be defect. Please contact our Technical Service.

Loading stopped before complete sample has been loaded onto the columns

Liquid sensor defective or not filled correctly Check liquid sensor by running the instrument check, taking care to insert the liquid filled tubing correctly into the liquid sensor. If the instrument check fails, contact our Technical Service

Cells move to wrong part of tubing set. Liquid is leaking past valve(s).

- Tubing set has not been properly inserted If the run is ongoing, press the 'STOP' and clamp the line with a hemostat. Adjust the tubing by first depressing the appropriate valve Remove the clamp and press 'RUN' to resume separation. The cell separation will be aborted if 'RUN' is not pressed within 600 seconds
- Valve is not functioning properly Press 'STOP'. Clamp the line with a hemostat Depress the valve manually several times to un-stick the stuck valve Remove the clamp and press 'RUN' to continue The cell separation will be aborted if the 'RUN' key is not pressed within 600 seconds If you cannot un-stick the valve, contact our Technical Service
- Wrong software program used Check display for name of program currently used Abort run by pressing the STOP key and immediately contact our Technical Service

Magnet does not move

Magnet drive does not work

- Due to an ongoing power failure, the magnet cannot be moved by the magnet drive The viability of the cells trapped in the tubing set may be compromised Contact our Technical Service for help
- A magnet drive failure has occurred An error message will be displayed (see section 5.2, "Error messages"). Record the number of the error message and contact our Technical Service

Pump motor stalls during cell separation.

Pump tubing has not been inserted correctly, so the pump might be unable to rotate In this case warning message no 2 will appear on the screen window. You then have 600 seconds to correct the position of the pump tubing



Warning message no. 2

1. Carefully remove the pump tubing from the pump
2. Make sure that the pump tubing has not been damaged by the incorrect insertion. If the pump tubing is leaking, clamp the tubing above and below the selection column to save the cells retained on the column and contact our Technical Service for help
3. If the pump tubing has not been damaged, it can be reinserted into the pump housing
4. Press 'RUN' to restart the separation within 600 seconds or the separation will be aborted

Sample loading does not stop although the Cell Preparation Bag and the pre-system filter are empty

Liquid sensor is not working properly because the surface of the tubing in the liquid sensor maybe is wet. Press 'STOP' to interrupt the separation. Remove tubing from liquid sensor. Dry the tubing and the contact area using a paper towel or absorbent material. Replace the tubing in the liquid sensor and press 'RUN'. Do not interrupt the sequence for more than 600 seconds or the cell separation will be terminated

If it is not possible to activate the liquid sensor in this way, press 'STOP', then '2' and confirm with 'ENT' to skip sample loading and to continue the separation.

Unexpected events - CD34 SELECTION 1/2 and CD133 SELECTION 1/2

Loading stopped before complete sample has been loaded onto the columns

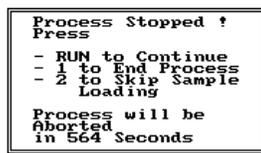
Pre-system filter is clogged due to large amount of cell debris or due to incomplete filling of the filter. Due to continued pumping, a vacuum has been created which has led to the generation of air bubbles activating the liquid sensor. Therefore, the separation process has been continued with the column washes before all of the sample could be loaded. It is not possible to restart the sample loading once the loading sequence has stopped

Allow the CliniMACS® plus Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag. Immediately perform a second separation with a new tubing set and sufficient new buffer

Pump tubing collapsed and/or excessive air appeared in tubing set below pre-column during cell loading

Pre-column is clogged due to large amount of cell debris in the Cell Preparation Bag. It is necessary to skip the loading of the remaining sample manually and to continue the separation with the cells that have already been loaded onto the system.

1. Press 'STOP' to interrupt cell loading. Warning message no. 3 will appear



Warning message no. 3

2. Press '2' to skip the cell loading process

Warning message no 4 will appear and will give you the opportunity to confirm or amend the decision you have made because skipping of sample loading is not reversible



Warning message no. 4

3. Press 'ENT' and the separation program will stop the sample loading and continue with column washes

Clamp the tubing below the pre-system filter to prevent cells from leaking out of the Cell Preparation Bag into the tubing set. After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new transfer bag. Immediately perform a second separation with a new tubing set and sufficient new buffer.

Unexpected events**- Enrichment programs only**

Run is aborted before completion of cell separation program.



Warning message no. 5

- 'RUN' has not been pressed within 600 seconds after interrupting the procedure by pressing the 'STOP' key The cell separation will not be completed Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM (see page 5 - 9).
- 'RUN' has not been pressed within 600 seconds after interrupting the procedure by opening the pump door For safety reasons, the separation in progress will automatically stop during the instrument run if the pump door is opened Message no 5 will appear on the screen window

Close the pump door and press 'RUN'

If, as in this case, 'RUN' is not pressed within 600 seconds, the separation will be aborted and the cell separation will not be completed Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM.

- Power failure results in the termination of the CliniMACS® Separation. The cell separation will **not** be completed once the power supply has been restored Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM.

Unexpected events**- ENRICHMENT 1.1**

Loading stopped before complete sample has been loaded onto the columns

Pre-system filter is clogged due to large amount of cell debris or due to incomplete filing of the filter Due to continued pumping, a vacuum has been created which has led to the generation of air bubbles These have activated the liquid sensor and the separation process has been continued directly with the final stage of sample loading A part of the sample may remain in the Cell Preparation Bag after separation is finished

Allow the CliniMACS^{plus} Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss

Determine the total cell number, percentage of target cells, and the remaining sample loading volume in the Cell Preparation Bag, and enter actual sample parameters during set-up of the instrument After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new Transfer Bag. Immediately perform a second separation with a new tubing set and sufficient new buffer

Wrong sample parameter values were entered

The sample loading volume entered was lower than the actual volume in the attached Cell Preparation Bag. It is not possible to restart the sample loading once it has stopped

Allow the CliniMACS®^{plus} Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss

Determine the total cell number, percentage of target cells, and the remaining sample loading volume in the Cell Preparation Bag and enter actual sample parameters during set-up of the instrument. Process remaining sample with a new tubing set, a new pre-system filter and sufficient new buffer

Pump motor stalls during elution of target cells into Cell Collection Bag.

The clamp to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked. Press 'STOP' and open the clamp to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and press 'RUN' to restart the elution sequence within 600 seconds

Unexpected events - ENRICHMENT 3.2

Loading stopped before complete sample has been loaded onto the columns

Pre-system filter is clogged due to large amount of cell debris or due to incomplete filling of the filter. Due to continued pumping, a vacuum has been created which has led to the generation of air bubbles. These have activated the liquid sensor and the separation process has been continued with the column washes before all of the sample has been loaded. It is not possible to restart the sample loading once the loading sequence has stopped

Allow the CliniMACS^{plus} Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. After the separation procedure has been finished, filter the remaining sample with a 200 µm in-line blood filter and transfer it into a new Transfer Bag. Immediately perform a second separation with a new tubing set and sufficient new buffer

Pump motor stalls during elution of target cells into Cell Collection Bag.

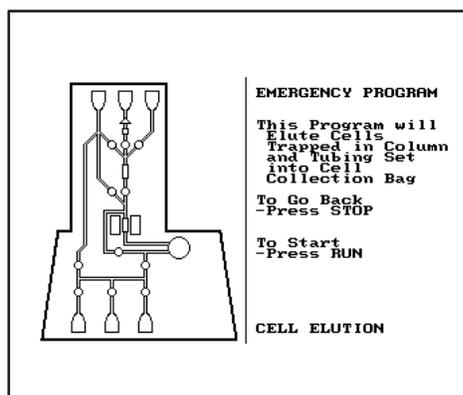
The clamp to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked. Press 'STOP' and open the clamp to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and press 'RUN' to restart the elution sequence within 600 seconds

EMERGENCY PROGRAM (to be used with enrichment programs only)

If, for any reason, a run has irreversibly terminated prior to the target cells' being eluted from the selection column, the EMERGENCY PROGRAM can be run to elute the cells from the selection column. This program has been designed only for use with all enrichment programs together with either a CliniMACS® Tubing Set (REF 161-01) or a CliniMACS Tubing Set LS (REF 162-01). **The Emergency Program must not be used with any depletion programs**

Note

- The emergency program will elute approximately 75 mL of fluid. Make sure that a suitable Cell Collection Bag is attached to the tubing set.
1. To make sure that the selection column is not magnetized, turn the instrument off, wait 5 seconds, then turn the instrument on again. The magnet will be withdrawn from the magnetic separation unit (4, Fig. 1.14, chapter 1.7). Check this by holding a small magnetizable item to the magnetic separation unit. If the magnet has not been withdrawn, or if there is an ongoing power failure, please contact our Technical Service for help.
 2. Wait until screen no. 1.2 appears in the window (see chapter 1.8).
 3. To call up the EMERGENCY PROGRAM, press '4'.
 4. Screen no. 5.1 appears:



Screen no. 5.1

To continue with the elution of the trapped cells, press 'RUN'

5. Transfer the eluted cells collected in the Cell Collection Bag to a new 600 mL transfer bag. Eventually pool the remaining cells in the Cell Preparation Bag with the cells eluted by the Emergency Program. Start a new separation procedure using a new tubing set and sufficient new buffer.

Note

- To leave the EMERGENCY PROGRAM without starting the elution of the trapped cells, press 'STOP'

Unexpected events

- DEPLETION 2.1 (with TS LS)

Target cells do not reach the Cell Collection Bag.

Pump is unable to load the product from the Cell Preparation Bag because the clamp to the Cell Collection Bag is not open during the cell loading sequence. Press 'STOP' and open clamp to Cell Collection Bag. Continue the depletion process by pressing 'RUN'

Allow the CliniMACS® plus Instrument to finish the separation program. Do not abort current run, this may result in unnecessary cell loss. If a part of the product remains in the Cell Preparation Bag after depletion procedure has been finished, process the remaining cells with a new tubing set, a new pre-system filter and sufficient new buffer

Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameters during the set-up of the instrument (STEP 2) and start the new depletion.

Loading stopped before complete sample has been loaded onto the columns

Air bubbles from the sample and/or pre-system filter activated the liquid sensor before all of the sample had been loaded. Abort the current run and check total cell number and depletion efficiency of the cells in the Cell Collection Bag. If necessary, process the remaining sample with a new tubing set, a new pre-system filter and sufficient new buffer

Determine the total cell number, percentage of labeled cells and sample loading volume and enter actual sample parameters during the set-up of the instrument (STEP 2) and start the new depletion.

Unexpected events

- DEPLETION 3.1 (with DTS)

Loading stopped during **bulk loading stage** before complete sample has been loaded onto the columns

Pre-system filter inserted wrong way around. Therefore, the drip chamber function is not available and air bubbles may pass the liquid sensor directly causing the termination of the sample loading before the complete sample has been applied. The instrument continues the depletion process by directly beginning the next step. It is not possible to restart the sample loading once it has stopped.

Abort the current run and pool the contents of the Reapplication Bag and the Cell Preparation Bag. Process the remaining cells with a new tubing set, a new pre-system filter and sufficient new buffer

Loading stopped during **sensitive loading stage**

The Reapplication Bag is hanging lower than the Cell Preparation Bag. During sensitive loading step, Cell Preparation Bag runs empty before Reapplication Bag volume is completely loaded. It is therefore possible that an air bubble activates the liquid sensor before the sample is completely loaded from the Reapplication Bag after the depletion procedure is finished. It is not possible to restart sample loading once it has stopped.

Allow the CliniMACS® ^{plus} Instrument to finish the separation program. Do not abort current run, this may result in unnecessary cell loss. Process remaining sample with a new depletion tubing set, pre-system filter and sufficient new buffer.

Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameters during the set-up of the instrument (STEP 2). Consider the obligatory need of three large bag hangers for the program DEPLETION 3.1 and refer to installation instructions for appropriate bag heights.

Target cells (unlabeled cells) do not reach Reapplication Bag during bulk loading step

Wrong tubing is inserted in valve no 3 (right branch of Reapplication Bag tubing instead of left branch (Y-fitting) of tubing). Press 'STOP' and remove wrong tubing from valve no 3. Manually insert correct tubing (left branch of Reapplication Bag tubing) into valve no 3 as described in the installation instructions. Press 'RUN' to continue with the depletion procedure within 600 seconds.

Allow the CliniMACS ^{plus} Instrument to finish the separation program. Do not abort the current run, this may result in unnecessary cell loss. Check depletion efficiency of the target fraction in the Cell Collection Bag. If depletion efficiency is not sufficient, repeat depletion procedure with a new tubing set, a new pre-system filter and sufficient new buffer.

Determine the total cell number, percentage of labeled cells, and sample loading volume and enter actual sample parameter values during set-up of the instrument (STEP 2) and start the new depletion.

Cells flow into buffer bag during gravimetric rinsing steps

Buffer bag is hanging too low (lower than Cell Preparation Bag). Press 'STOP' and adjust buffer bag hanger to correct position. Make sure that three large bag hangers are installed for the program DEPLETION 3.1 and refer to installation instructions for appropriate bag heights (see chapter "STEP 3: CliniMACS® Depletion Tubing Set").

- If cells have not reached the buffer bag, it is sufficient to manually open valves nos 1 and 2 to allow short backflushing of cells into Cell Preparation Bag. After the buffer bag tube is clear again, close valves nos 1 and 2 and press 'RUN' to continue with the depletion procedure within 600 seconds
- If cells have already reached the buffer bag, exchange buffer bag, manually open valves nos 1 and 2 for a short flushing of the buffer bag tube and press 'RUN' to continue with the depletion procedure within 600 seconds Process the remaining sample in the buffer bag if necessary (if volume exceeds 300 mL, transfer the sample into a centrifugable bag for volume reduction first) using a new tubing set, a new pre-system filter, and sufficient new buffer

Pump motor stalls during first elution of labeled cells into Non-Target Cell Bag.

- Hemostat has not been removed after integrity test. Press 'STOP' and remove hemostat Open pump door and check whether pump tubing is correctly inserted Close pump door and press 'RUN' to restart elution sequence within 600 seconds
 - Wrong tubing is inserted in valve no 3 (Non-Target Cell Bag tubing instead of left branch (Y-fitting) of Reapplication Bag tubing). Press 'STOP' and remove Non-Target Cell Bag tubing from valve no 3 Manually insert correct tubing (left branch of Reapplication Bag tubing) into valve no 3 as described in the installation instructions Press 'RUN' to restart elution sequence within 600 seconds
-

5.4 Cell separation performance

Unexpected events

- Enrichment programs only

The yield of target cells is low

- Target cell content was over-estimated in the leukapheresis product. During analysis, target cells were incorrectly counted or an error occurred during counting of leukocytes. Repeat the analysis of leukapheresis product for starting target cell content.
- Target cells were poorly labeled with the reagent.
 - Reagent has expired. Check use-by date. Do not use any reagent after the use-by date
 - Reagent has not been stored properly. Check storage temperature. Do not use any reagent that has been stored improperly (see reagent package insert).
 - Recommended labeling procedure has not been followed. Refer to sample preparation and separation procedure chapters in the manual
- Cells were lost during the preparation steps
 - Cells were removed with the supernatant into Plasma Waste Bag and Wash Waste Bags due to incomplete sedimentation or too early resuspension of the cells, e.g. when the bag was removed from the centrifuge. Compare leukocyte content of the unlabeled leukapheresis product and the labeled leukapheresis product. Check centrifugation settings for proper centrifugation. Determine cell counts from all waste bags
 - Buffer did not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5 % (w/v), (see chapter “Preparation of CliniMACS® PBS/EDTA Buffer”; STEP 1).
 - Centrifuge settings were suboptimal. Check centrifugation settings
 - Centrifuge imbalance or use of brake or asymmetrical loading of centrifuge
- Cell viability decreased during preparation. See section “Viability of the positive fraction is less than 90% or the color of the supernatant during the washing steps was red” below
- Analysis was incorrect.
 - Sampling error occurred. Check cell suspension for clumped or settled cells. Make sure that representative samples have been taken and repeat analysis
 - Staining error occurred. Check flow cytometry reagents. Repeat staining
 - Flow cytometer settings were improper. Check instrument settings

The purity of target cells is low

- The leukapheresis product has been stored inappropriately. Preparation and separation of the leukapheresis product should be performed immediately after leukapheresis. Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis product with autologous plasma. The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure. If the leukapheresis product has to be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C).
- The magnetic labeling protocol has not been followed (e.g., incorrect volumes during magnetic labeling). Please follow the instructions given for the magnetic labeling (STEP 1).

For troubleshooting purposes determine the leukocyte subsets (B cells, T cells, monocytes, granulocytes as well as platelets) contaminating the target fraction and contact our Technical Service for advice

- High numbers of granulocytes contaminated the start product (sub-optimal apheresis setting). Dying granulocytes will then bind the CliniMACS® Reagent non-specifically which may lead to decreased purity of the target cells
- Valve malfunction occurred. Eluted target cell fraction has been contaminated by part of the negative fraction or buffer waste fraction. Inspect tubing placement within the valves to ensure proper functioning. Assess target cell content of the negative fraction and buffer waste. If necessary, pool the target and negative fraction, reduce to suitable volume and repeat the run with a new tubing set and sufficient new buffer.

Viability of the positive fraction is less than 90% or the color of the supernatant during the washing steps was red

Cell lysis occurred due to incorrect osmolarity of the buffer. Check buffer and use recommended buffer (see CliniMACS PBS/EDTA Buffer, 1.4 Glossary).

Non-specific retention of dead cells from leukapheresis product or high non-specific cell losses throughout the procedure

- Buffer does not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5 % (w/v), (see “Preparation of CliniMACS PBS/EDTA Buffer”, STEP 1).
- The leukapheresis product may have been stored inappropriately. Preparation and separation of the leukapheresis product should be performed immediately after leukapheresis. Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis with autologous plasma. The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure. If the leukapheresis product has to be stored, e.g. overnight, it should be kept at controlled room temperature (+19 °C to +25 °C).
- Incomplete sample loading due to clogging of selection column, pre-system filter, or pre-column. Check total cell number and depletion efficiency of the remaining cells.

Unexpected events**- CD34 SELECTION 1/2,
CD133 SELECTION 1/2
and ENRICHMENT 1.1**

The purity of the target cells is low

Elution from the selection column was incomplete

- Separation program has been aborted Check display screen for error message. Continue with section "Run is aborted before completion of cell separation program" (see section 5.3).
- Pump failure or valve failure occurred Recover cells from the tubing set following the EMERGENCY PROGRAM described in section 5.3. Check volumes of all fractions Assess target cell content of Buffer Waste Bag and Negative Fraction Bag.
- Tubing to Cell Collection Bag is blocked Check tubing set for closed clamps, occlusions or kinks
- Tubing has not been properly inserted Check all valves for proper tubing insertion.

Unexpected events**- CD34 SELECTION 1/2 and
CD133 SELECTION 1/2**

The purity of the target cells is low

Non-target cells were retained

- Mobilization of target cells was poor Very low number of target cells has occurred Therefore, a low number of contaminating non-target cells (e.g. granulocytes, monocytes, platelets) may lead to decreased purity
- Insufficient plasma or immunoglobulins were present during magnetic labeling Please follow the instructions given for the magnetic labeling (STEP 1).

If a final concentration of about 30% autologous plasma in the sample during magnetic labeling cannot be guaranteed, add immunoglobulin to the sample. A final concentration of 1.5 mg/mL is recommended for the efficient blocking of non-specific reagent binding during magnetic labeling.

Unexpected events**- ENRICHMENT 1.1**

The purity of the target cells is low

Incorrect sample parameter input Cross-check parameter input with analysis results

Unexpected events

- DEPLETION 2.1 and DEPLETION 3.1

The depletion efficiency is low

If the depletion efficiency is insufficient, process the remaining sample with a new depletion tubing set and sufficient new buffer. A new labeling of the cells to be depleted should be considered. Determine the cell count and percentage of cells to be depleted and enter actual sample parameters during set-up of the instrument (STEP 2). If visible clumps occur, it may be helpful to filter the cells prior to a new run. Take into account that cells may be lost due to clumping and additional filtration.

- Capacity of reagent and/or tubing set has been exceeded. Refer to capacity limits of the relevant products.
- Incorrect determination of cells to be labeled by the reagent.
- Incorrect sample parameter input. Cross-check parameter input with analysis results.
- Reduced viability of the cells. Dead cells can bind non-specifically to selection column thereby reducing the labeling capacity of the column, potentially resulting in lower depletion efficiency. Ensure better leukapheresis product quality and process product as fresh as possible. Stored products that are older than 24 hours should not be used. If storage is necessary, leukapheresis product should be kept at controlled room temperature (+19 °C to +25 °C). The cell concentration should not exceed $0.2 \times 10^9/\text{mL}$. If necessary, dilute the leukapheresis product with autologous plasma to achieve optimal cell concentration.