Revision history form

Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________
Delivery no. ________	Exchange of pages:	Date ____________	Signature ____________

CliniMACS® Plus System

User Manual (Canadian edition)

Software 2.4x

MD

CE 0123

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany

Miltenyi Biotec Technical Support:
+49 2204 8306-8484
+49 2204 8306-80 (hotline)
+49 2204 8306-89
macsde@miltenyi.com

www.miltenyibiotec.com

37091/04 – ch20 (Issued: 2020-06)

The CliniMACS System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485. In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Set TS and CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. All other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE). In Australia, the following components of the CliniMACS Plus System are included in the Australian Register of Therapeutic Goods (ARTG) and are therefore approved for supply: CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Set, CliniMACS Tubing Set LS, CliniMACS Depletion Tubing Set, and CliniMACS PBS/EDTA Buffer. Only those products which are included in the ARTG may be used in Australia. CliniMACS MicroBeads are for research use only and not for human therapeutic or diagnostic use.

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

Table of contents

4 STEP 1: Cell preparation and magnetic labeling

4 STEP 1: Cell preparation and magnetic labeling (continued)

4 STEP 2: Start of the CliniMACS® Plus Instrument and choice of separation program

4 STEP 3: Installation of CliniMACS® Tubing Sets

4 STEP 4: CliniMACS® Plus Separation

1 General

1.1 Introduction

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

Miltenyi Biotec as the manufacturer of the CliniMACS Plus System does not give any recommendations regarding the use of separated cells for therapeutic purposes and does not make any claims regarding a clinical benefit.

For the manufacturing and use of target cells in humans the national legislation and regulations –e.g., for the EU the Directive 2004/23/EC (human tissues and cells) or the Directive 2002/98/EC (human blood and blood components)– must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS Plus System.

Before using the CliniMACS Plus System or any components outside the European Economic Community, the regulatory approval of the CliniMACS Plus System or any CliniMACS Component in the country must be confirmed.

In Canada, any clinical application of the output product must be performed in accordance with applicable Canadian legislation and regulations that pertain to cellular therapies (e.g. for advanced cellular therapies, the applicable sections of the Food and Drugs Act and the Food and Drug Regulations).

Limited warranty

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

1.2 Service information

Miltenyi Biotec Technical Support (Clinical)

For any information regarding the CliniMACS Plus System and its components, contact the Miltenyi Biotec Technical Support:

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany

+49 2204 8306-8484
+49 2204 8306-80 (hotline)
+49 2204 8306-89
macsde@miltenyi.com
www.miltenyibiotec.com

CliniMACS Plus Instrument information

Record below the model and serial number located on the back of the CliniMACS Plus Instrument. The operator should refer to these numbers when calling to obtain information or request service on the instrument.

Model no.: _____

Serial no.: _____

Software version: 2.41

1.3 The CliniMACS® Plus Instrument

1.3.1 Regulatory information

The CliniMACS Plus Instrument conforms to the Medical Device Directive MDD 93/42/EEC:

CE 0123

The CliniMACS Plus Instrument complies with the following standards:

IEC/EN/SN EN 61010-1
UL 61010-1
CAN/CSA-C22.2 No. 61010-1
IEC 60601-1-2
EN 60601-1-2

For applied standard version refer to the respective Certificate of Conformance.

The CliniMACS Plus Instrument is in conformity with Directive 2011/65/EU of the European Parliament and of the Council of 8 June 2011 on the restriction of the use of certain hazardous substances in electrical and electronic equipment.

The CliniMACS Plus Instrument is UL Listed (see Figure 1.1).

For regulatory status in countries outside Europe, contact the authorized local Miltenyi Biotec Service Provider.

c UL us
LISTED
LABORATORY EQUIPMENT

Figure 1.1: UL listing mark, listed as laboratory equipment

1.3.2 Intended purpose

The CliniMACS Plus Instrument including its software is intended for the *in vitro* separation of human cells from heterogeneous cell populations in combination with a CliniMACS Tubing Set, CliniMACS Reagents and CliniMACS PBS/EDTA Buffer only.

Figure 1.2: Consult instructions for use.

Figure 1.3: Strong magnetic field

1.3.3 Warnings and precautions

1. Read and observe all operating instructions carefully (see Figure 1.2).

2. Equipment safety will be compromised if it is not used according to the manufacturer's instructions.

3. The CliniMACS Plus Instrument is equipped with an extremely strong permanent magnet generating a strong magnetic field. There is a risk of severe personal injury for persons carrying pacemakers, brain shunts, or electronic medical implants.

   Keep any magnetic information carriers (such as credit cards or magnetic tapes), electronic equipment (such as hearings aids, measuring and control instruments, computers and watches), and magnetizable tools and objects at a distance of at least 30 cm from the magnetic cover. These items may be affected or damaged by the magnetic field (see Figure 1.3).

4. Column insertion: Do not try to insert the separation column into the separation column holder or remove the separation from there in case the magnet unit is switched on. Contact Miltenyi Biotec Technical Support.

5. The 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 instrument, the power cord cable should be disconnected.

7. To disconnect the instrument from the power supply unplug the power cord. Only use the originally supplied power cord. Ensure that the main switch as well as the connector for the power cable are easily accessible and located as close to the operator of the instrument as possible.

8. To prevent the risk of an electric shock, do not remove the back cover of the instrument. The instrument may be opened and any spare parts may be exchanged by authorized personnel only.

9. Movement or vibration may affect the instrument. Do not place the instrument next to any equipment that vibrates or can cause the instrument to move.

10. There are no components which can be serviced or calibrated by the operator.

11. Never leave the instrument unattended during a run. If an error occurs, the cell separation can be interrupted by the user 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.

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 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 instrument should be treated as a biohazard.

16. The instrument may be used repeatedly. It is not intended for disposal after single use. It must be returned to Miltenyi Biotec for final disposal (see section 1.3.11).

17. Only components (e.g. CliniMACS Reagents, CliniMACS Tubing Sets) recommended by the manufacturer must be used.

18. Allow sufficient air circulation around the instrument –at least 15 cm on all sides– during operation to ensure adequate cooling. In the absence of adequate circulation, ambient air may not cool the instrument to acceptable operating temperatures.

Caution

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

1.3.4 Specifications

The technical data of the CliniMACS Plus Instrument are listed in Table 1.1. **WARNING! The instrument shall not be used outside its specifications.**

Technical data	
Model	CS3
REF	151-01
Dimensions	Width: 70 cm Height: 90–140 cm Depth: 60 cm
Weight	35 kg
Input voltage	100–240 VAC (Single phase alternating current)
Power consumption	350 VA
Power source	An uninterruptible power source is recommended (reliable, noise free utility). Recommended UPS: APC Smart-UPS 1500 VA USB & Serial 230 V, manufactured by APC (American Power Conversion) or equivalent.
Instrument power	Instrument power inlet IEC-320-C13 A country specific power cord is supplied with the CliniMACS Plus Instrument.
Frequency	50/60 Hz
Fuses	2× T4A/250V, 5×20 mm Use only fuses with UL and European approvals, acc. to IEC 127-2/III, EN 60127-2/III, DIN 41662.
Operation conditions	+10 °C to +30 °C (+50 °F to +86 °F) with 0% to 85% humidity at an altitude of max. 2000 m. Supply voltage fluctuations up to ±10% of the nominal voltage. Transient over-voltages present on the mains supply: category II. The instrument is suitable for rated pollution degree 2. The instrument is intended for indoor use only.
Storage conditions	–10 °C to +60 °C (+14 °F to +140 °F) with 0% to 85% humidity, when contained and sealed in the outer packaging provided by the manufacturer

Table 1.1: Technical data of the CliniMACS Plus Instrument

- **Protection class:**
  The 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.

- **Interferences:**
  This equipment has been tested and found to comply with the limits for a class A 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.

1.3.5 Guidance and manufacturer's declaration on electromagnetic compatibility

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.

Note: The emissions characteristics of this instrument make it suitable for use in industrial areas and hospitals (CISPR 11 class A). If it is used in a residential environment (for which CISPR 11 class B is normally required) this instrument might not offer adequate protection to radio-frequency communication services. The user might need to take mitigation measures, such as relocating or re-orienting the instrument.

WARNING! Portable RF communications equipment (including peripherals such as antenna cables and external antennas) should be used no closer than 30 cm (12 inches) to any part of the CliniMACS Plus Instrument, including cables specified by the manufacturer. Otherwise, degradation of the performance of this equipment could result.

EMC compliance with IEC 60601-1-2:2014 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, contact Miltenyi Biotec for information on a replacement part.

Guidance and manufacturer's declaration – Electromagnetic emissions	
The CliniMACS Plus Instrument is intended for the use in the professional facility healthcare environment. The instrument is not intended to be used near active HF surgical equipment. The customer or user of the instrument should assure that it is used in such an environment.	
Emissions test	**Compliance**
RF Emissions CISPR 11	Group 1
RF Emissions CISPR 11	Class A
Harmonic emissions IEC 61000-3-2	Class A
Voltage fluctuations/Flicker emissions IEC 61000-3-3	Complies

Table 1.2: Guidance and manufacturer's declaration – Electromagnetic emissions

WARNING! Use of this equipment adjacent to or stacked with other equipment should be avoided because it could result in improper operation. If such use is necessary, this equipment and the other equipment should be observed to verify that they are operating normally.

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 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 the use in the professional facility healthcare environment. The instrument is not intended to be used near active HF surgical equipment. The customer or user of the instrument should assure that it is used in such an environment.		
Immunity test	**IEC 60601-1-2 Test level**	**Compliance level**
Electrostatic discharge (ESD) IEC 61000-4-2	±8 kV contact discharge ±2 kV, ±4 kV, ±8 kV, ±15 kV air discharge	±8 kV contact discharge ±2 kV, ±4 kV, ±8 kV, ±15 kV air discharge
Electrical fast transients (Bursts) IEC 61000-4-4	±2 kV 100 kHz repetition frequency Power supply lines ±1 kV 100 kHz repetition frequency Input/output lines	±2 kV 100 kHz repetition frequency Power supply lines ±1 kV 100 kHz repetition frequency Input/output lines
Surges IEC 61000-4-5	±0.5 kV, ±1 kV line to line ±0.5 kV, ±1 kV, ±2 kV line to ground	±0.5 kV, ±1 kV line to line ±0.5 kV, ±1 kV, ±2 kV line to ground
Voltage dips, interruptions, and variations IEC 61000-4-11	0% U_T during 0.5 cycle @ 0°, 45°, 90°, 135°, 180°, 225°, 270°, 315° 0% U_T during 1 cycle and 70% U_T during 25/30 cycles (single phase) @ 0° 0% U_T during 250/300 cycle	0% U_T during 0.5 cycle @ 0°, 45°, 90°, 135°, 180°, 225°, 270°, 315° 0% U_T during 1 cycle and 70% U_T during 25/30 cycles (single phase) @ 0°
Rated power frequency magnetic field IEC 61000-4-8	30 A/m 50Hz or 60Hz	30 A/m 50 Hz or 60 Hz
Conducted disturbances induced by RF fields IEC 1000-4-6	3 V (0.15 MHz to 80 MHz) 6 V in ISM bands between 0.15 MHz and 80 MHz 80% AM @ 1kHz	3 V (0.15 MHz to 80 MHz) 6 V in ISM bands between 0.15 MHz and 80 MHz 80% AM @ 1 kHz
Radiated RF EM fields IEC 61000-4-3	3 V/m (80 MHz–2.7 GHz) 80% AM @ 1kHz	3 V/m (80 MHz–2.7 GHz) 80% AM @ 1 kHz
Proximity fields from RF wireless communication equipment IEC 61000-4-3	See table below: Specifications for immunity to RF wireless communication equipment	See table below: Specifications for immunity to RF wireless communication equipment
NOTE U_T is the a.c. mains voltage prior to application of the test level.		

Table 1.3: Guidance and manufacturer's declaration – Electromagnetic immunity

The performance of the device that was determined to be essential performance:

1. Establishing and maintaining the intended flow path.
2. Delivering the specified flow.
3. Setting the necessary magnetic field in the separation column.
4. Displaying user information on progress of the process.
5. Running the sequence as intended.

Guidance and manufacturer's declaration – Electromagnetic immunity to RF wireless communication equipment							
Test Frequency (MHz)	**Band (MHz)**	**Service**	**Modulation**	**Maximum Power (W)**	**Distance (m)**	**Immunity Test Level (V/m)**	**Compliance Level (V/m)**
385	380 – 390	TETRA 400	Pulse modulation 18 Hz	1.8	0.3	27	27
450	430 – 470	GMRS 460, FRS 460	FM ±5 kHz deviation 1 kHz sine	2	0.3	28	28
710 745 780	704 – 787	LTE Band 13, 17	Pulse modulation 217 Hz	0.2	0.3	9	9
810 870 930	800 – 960	GSM 800/900, TETRA 800, iDEN 820, CDMA 850, LTE Band 5	Pulse modulation 18 Hz	2	0.3	28	28
1720 1845 1970	1700 – 1990	GSM 1800; CDMA 1900; GSM 1900; DECT; LTE Band 1, 3, 4, 25; UMTS	Pulse modulation 217 Hz	2	0.3	28	28
2450	2400 – 2570	Bluetooth, WLAN, 802.11 b/g/n, RFID 2450, LTE Band 7	Pulse modulation 217 Hz	2	0.3	28	28
5240 5500 5785	5100 – 5800	WLAN 802.11 a/n	Pulse modulation 217 Hz	0.2	0.3	9	9

Table 1.4: Guidance and manufacturer's declaration – Electromagnetic immunity to RF wireless communication equipment

1.3.6 Components of the CliniMACS® Plus Instrument

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 Figure 1.4).

The magnetic separation unit includes the movable permanent magnet and the separation column holder for the separation column. During the separation, the peristaltic pump controls the flow rate through the 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 Software offers the operator the choice between various separation programs. For further details see chapter 4. The CliniMACS Plus Instrument and CliniMACS Tubing Sets allow the operator to perform cell separations in a closed and sterile system.

The power connection module (see Figure 1.5) is located at the rear of the instrument. Viewed from behind, the connection consists of two sections. The left section is the recessed male 3-pin connector to which the power cord is attached. The right section is the main ON/OFF switch. When positioned to the left, the switch is 'OFF' (O). When positioned to the right, the switch is 'ON' (I).

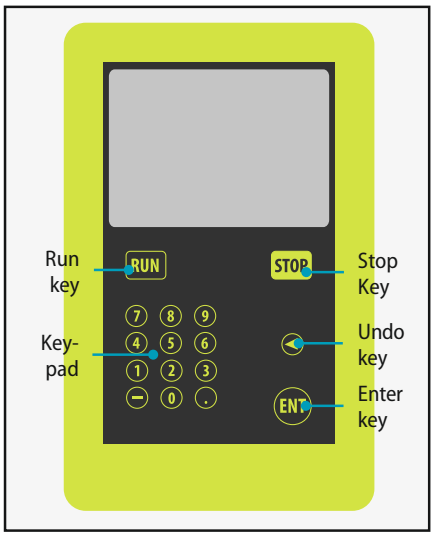


Figure 1.4: Display with touchscreen

Important

This CliniMACS Plus System User Manual only contains the instructions for **clinical applications** using the **CliniMACS Reagents.**

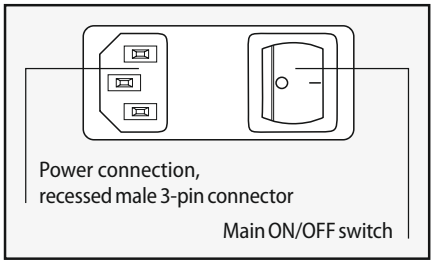


Figure 1.5: Power connection

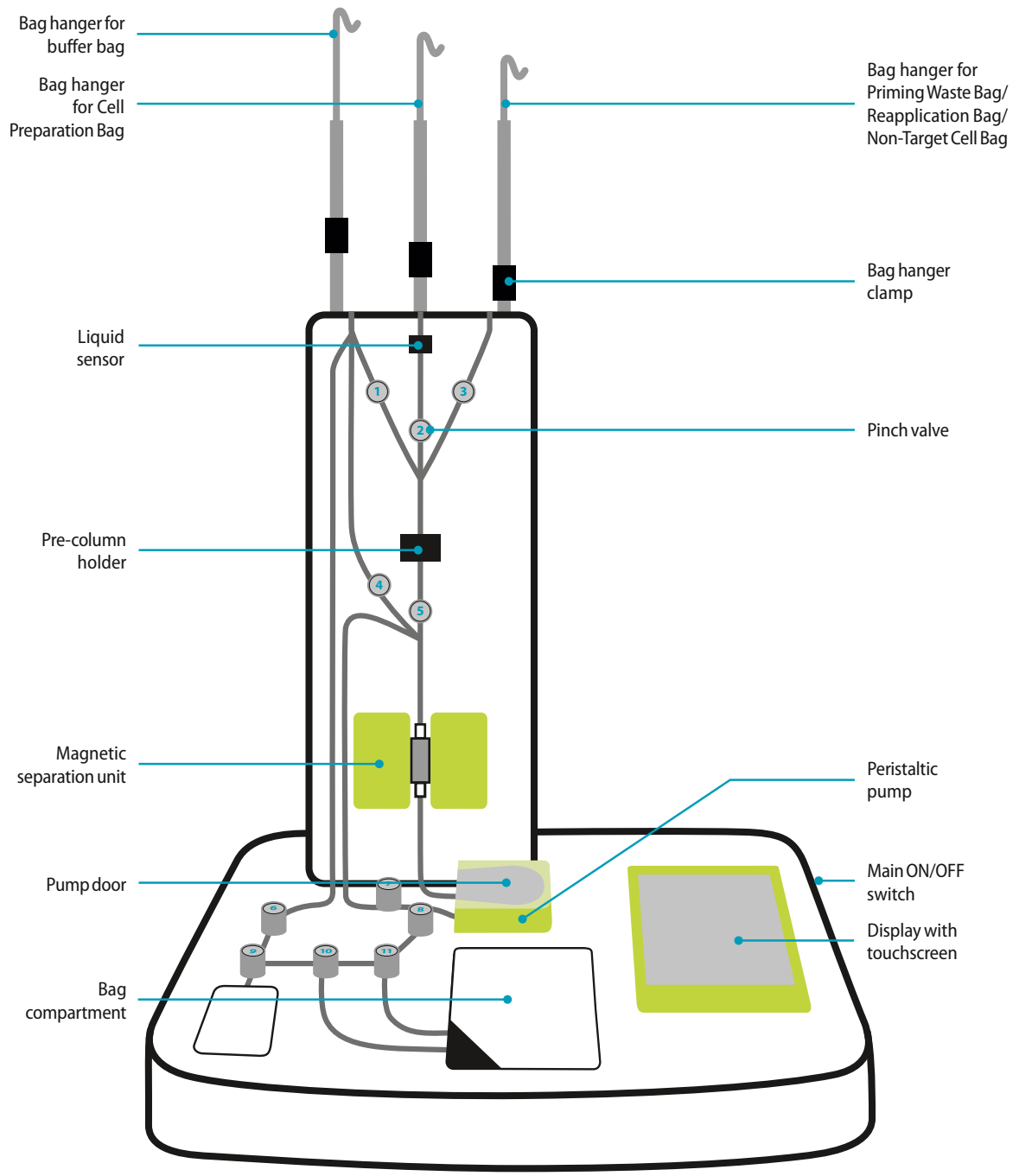


Figure 1.6: The CliniMACS Plus Instrument

1.3.7 Unpacking and installation

WARNING! Medical electrical equipment requires special precautions regarding electromagnetic compatibility (EMC) and must be installed and placed in service according to the EMC information. Portable and mobile RF communications equipment can affect medical electrical equipment. Unpacking and installation of the CliniMACS Plus Instrument must only be performed by an authorized local Miltenyi Biotec Service Provider.

Unpacking

Unpacking the instrument should be performed by two people, according to the following instructions.

Note

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

1. Cut the plastic straps using a pair of scissors (see Figure 1.7).

   #### Caution

   Wear safety glasses. The straps are wrapped under tension.

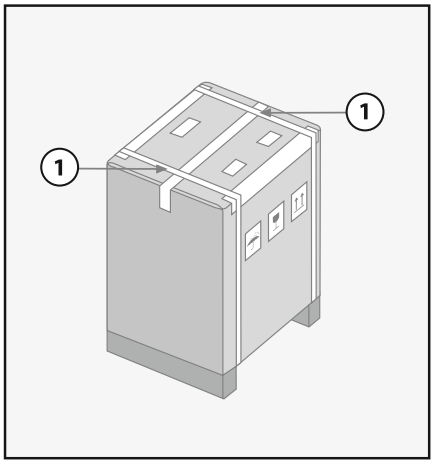


Figure 1.7: Cut plastic straps.

2. Open the top carton by cutting the adhesive tape (see Figure 1.8).

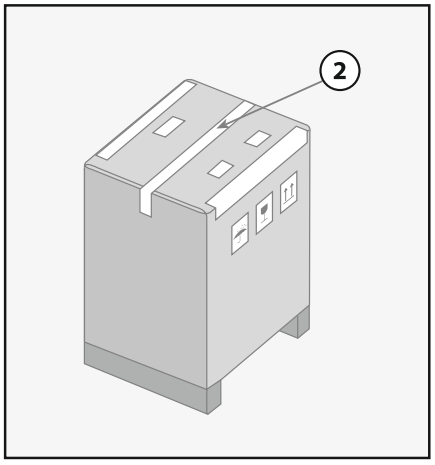


Figure 1.8: Open top carton.

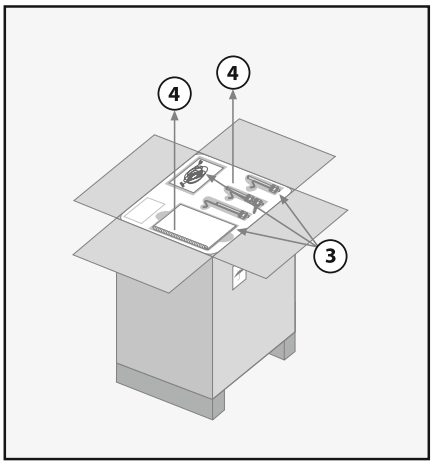


Figure 1.9: Remove the parts and the protective foam.

3. Open the carton and remove the parts (power cord, bag hangers) from the protective foam (see Figure 1.9).

4. Remove the protective foam.

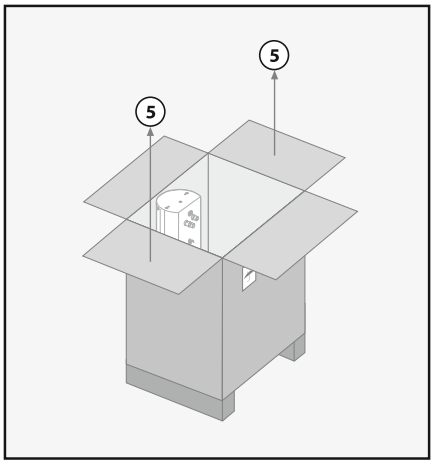


Figure 1.10: Lift the top carton.

5. Lift the top carton vertically off the pallet (see Figure 1.10).

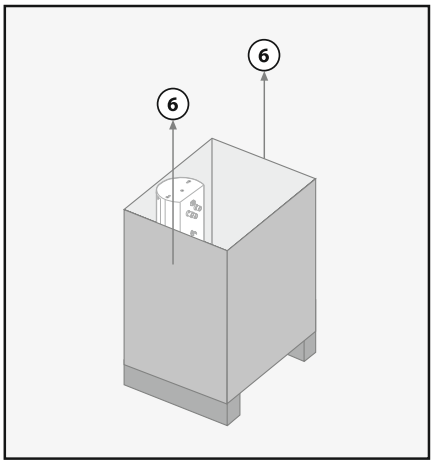


Figure 1.11: Remove the inner carton.

6. Remove the inner carton (see Figure 1.11).

7. Unwrap the large shipping bag (see Figure 1.12).

Caution

Two people should carefully lift the 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. The instrument is heavier at the back and should be stabilized while lifting it. Take care to avoid personal strain or injury.

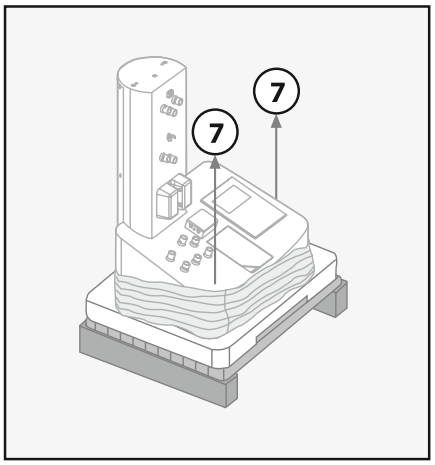


Figure 1.12: Lift the instrument.

8. To maintain ventilation, place the instrument at least 10 cm away from the wall.

Note

Do not locate the instrument next to any vibrating equipment which might cause movement during operation.

9. Attach bag hangers. Tighten rods with clockwise twists until hand tight. The height of the bag hangers can be adjusted by pressing the bag hanger clamps (see Figure 1.13).

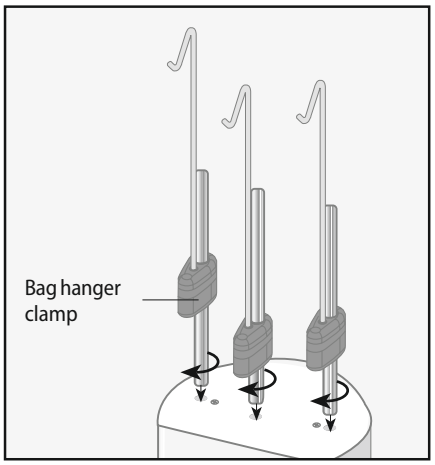


Figure 1.13: Attach bag hangers.

10. A stabilization foot (see Figure 1.14) is included with the delivery. The foot has to be installed at the back of the instrument.

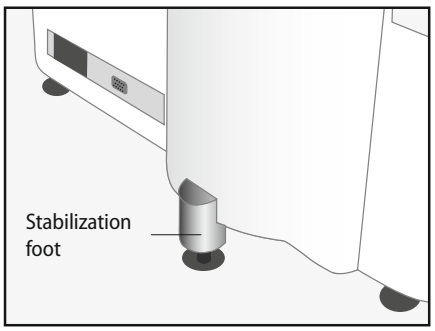
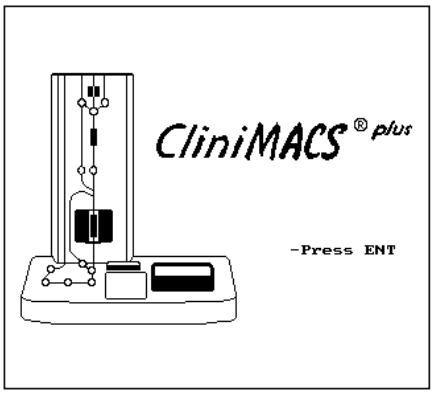


Figure 1.14: Rear of instrument with installed stabilization foot

Connect and switch on the instrument

Connect the instrument to an uninterruptible power supply using the supplied power cord. Switch on the instrument by using the main ON/OFF switch (see Figure 1.5) located on the right hand back panel of the instrument.

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



Screen 1.1: Main screen

Upon start up, the program will automatically be loaded and Screen 1.1 will appear in the display window.

If the 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. Then switch on the instrument again.

If the instrument does not start correctly or the window displays an error message, note the error message number, switch the instrument off and contact the Miltenyi Biotec Technical Support.

Proper training is required to operate the instrument. Read the instructions carefully. Further training by Miltenyi Biotec authorized representative may be required. This instruction includes details on instrument operation, sample handling, and troubleshooting.

Language selection and service menu

The instument provides a menu to set-up the language and a service menu.

To enter these menus wait until Screen 1.1 appears in the display window and DO NOT press 'ENT' as shown on the window display.

To start the general menus, refer to section 1.3.8.

1.3.8 General set-up menus

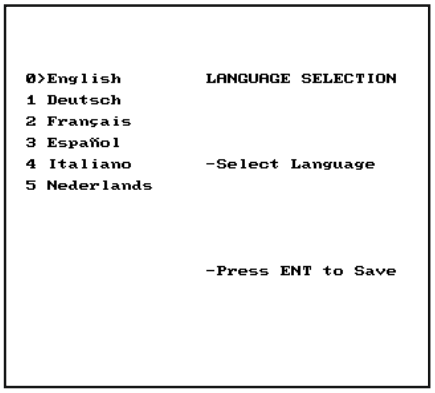
Language selection

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 1.1 and **DO NOT** press **'ENT'** then.

To start language selection, press [2]

The window will display Screen 1.2 as shown. To select a language, press the corresponding number.

To save the language, press [ENT]



Screen 1.2: Language selection

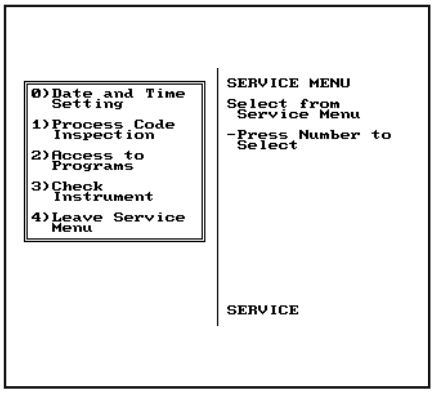
Service menu

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

To open the service menu folder, wait until the window displays Screen 1.1 and **DO NOT** press **'ENT'** then.

Then press [5]

The window will display Screen 1.3 as shown.



Screen 1.3: Service menu

DATE AND TIME SETTING

To set date and time, press [0]

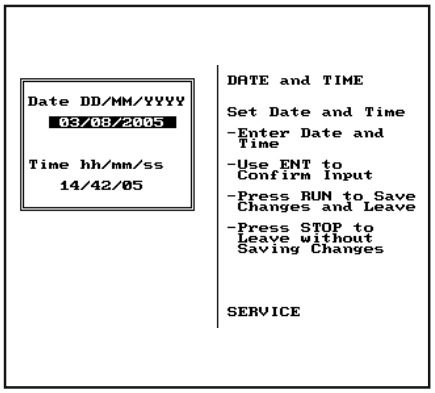
Follow the instructions shown on Screen 1.4. Date or time can be changed, when the respective field is highlighted by the black bar.

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

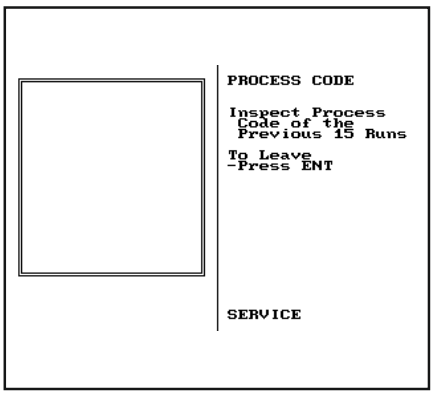
Enter the current date (order: day/month/year) and time (order: hours/minutes/seconds). A wrong input can be amended by pressing "Undo" (see Figure 1.4).

To save the data and leave, press [RUN]

If the operator 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.



Screen 1.4: Sercive menu (Date and time)



Screen 1.5: Service menu (Process code)

Note

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

PROCESS CODE INSPECTION

The operator is able to call up the process codes of the last 15 separations. A process code is saved when the operator has started the installation of the tubing set or when the EMERGENCY PROGRAM (see chapter 5) has been used. Saving a process code is independent from whether the separation has been completed or interrupted.

To call up a process code, press 1

The process codes from the last 15 separations are listed chronologically. The list begins with the most recent procedure.

To return to the service menu, press ENT

ACCESS TO PROGRAMS

This program allows for the activation of additional separation programs by Miltenyi Biotec authorized personnel. Contact the Miltenyi Biotec Technical Support for further information and instructions to continue.

If an operator had entered this program by mistake, the operator may leave the program by pressing 'STOP'.

CHECK INSTRUMENT

In case of a suspected malfunction of the instrument contact the Miltenyi Biotec Technical Support. If an instrument check is indicated the specialist will assist the operator in performing the instrument check sequence.

If an operator had e entered this program by mistake, the operator may leave the program by pressing 'STOP'.

To leave the service menu, press 4

.

1.3.9 Cleaning

The surface of the CliniMACS Plus Instrument should be cleaned at regular intervals and after each application with an antiseptic solution, e.g. [1]Bacillol® plus or [2]Meliseptol®, 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.

Caution

- **Clean the instrument only when it is switched off and the power cord is unplugged.**
- **Avoid ingress of any liquid into the valves.**

1.3.10 Maintenance

The CliniMACS Plus Instrument does not contain operator serviceable 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.

1.3.11 Instrument disposal

The CliniMACS Plus Instrument must be separately collected according to the European directive of waste of electrical and electronic equipment (WEEE). For final disposal, the instrument must be returned to Miltenyi Biotec. Clean the instrument according to the instructions given in section 1.3.9.

Contact Miltenyi Biotec Instrument Service for assistance prior to disposal of the instrument.

Figure 1.15: Separate collection for waste of electrical and electronic equipment

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

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

2 Glossary

2.1 Glossary of symbols

2.1.1 Safety symbols

General warning sign

Warning: Magnetic field

2.1.2 Symbols used for labeling of products

MD

Medical device

CE 0123

European conformity approval with ID number 0123 (ID number of Notified Body: "TÜV SÜD Product Service GmbH, Munich").

c UL us
LISTED
LABORATORY EQUIPMENT

UL listing mark, listed as laboratory equipment

Consult instructions for use.

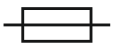
Caution

Manufacturer

Date of manufacture

Non-ionizing radiation

Separate collection for waste of electrical and electronic equipment



Fuse

Keep dry.

Fragile, handle with care.

This way up.

"OFF" (power)

"ON" (power)

Do not re-use.

Do not use if package is damaged.

Packaging PVC free

	Use-by date
	Temperature limit
	Non-pyrogenic fluid path
LOT	Batch code
P/N	Part number
QTY	Contents of the packaging
REF	Catalogue number (REF)
SN	Serial number
UDI	Unique Device Identifier
STERILE A	Sterilized using aseptic processing techniques
STERILE EO	Sterilized using ethylene oxide
STERILE 	Sterilized using steam or dry heat

Single sterile barrier system

Single sterile barrier system with protective packaging outside

Phone

Fax

E-mail

Website

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
Anti-biotin antibody	This antibody recognizes cells which have previously been labeled with an appropriate biotinylated antibody or ligand
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
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.
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.
Cell Collection Bag	Bag in which the purified target cells are accumulated after separation
Cell Preparation Bag	Bag into which cellular product is transferred and in which magnetic labeling and washing of cells are performed
Cellular starting product	Cell-containing product used as starting material for the CliniMACS Plus Separation process, e.g., leukapheresis harvest, PBMCs, or bone marrow
CliniMACS Anti-Biotin Reagent	Reagent for magnetic labeling of cells primarily labeled with biotinylated antibodies or ligands
CliniMACS CD19 Reagent	Reagent for magnetic labeling of cells expressing the CD19 antigen
CliniMACS CD34 Reagent	Reagent for magnetic labeling of cells expressing the CD34 antigen

CliniMACS Depletion Tubing Set	Set of tubing, 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 PBS/EDTA Buffer	Buffer used for cell preparation and cell separation with the CliniMACS Plus 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 Plus Instrument	Magnetic cell separation instrument based on the MACS Technology
CliniMACS TCRα/β-Biotin	Biotinylated antibody for labeling of human cells expressing the TCRα/β antigen prior to the magnetic labeling with the CliniMACS Anti-Biotin Reagent
CliniMACS Tubing Set, CliniMACS Tubing Set LS	Set of tubing, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place
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. Pharmaceutical grade HSA approved in your country is necessary as a buffer supplement when used with the CliniMACS Plus System.
IgG	Immunoglobulin G
Labeling	Reaction of cells with magnetic labeling reagent, e.g., CliniMACS CD34 Reagent to CD34 positive cells
Leukapheresis	Apheresis collecting leukocytes
Liquid sensor	Component of the CliniMACS Plus Instrument that detects liquid in the tubing
Luer connector	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
PBMC	Peripheral blood mononuclear cell
Peristaltic pump	Tubing 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 applicated 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.
Selection column	See separation column.
Selection column holder	See separation column holder.
Separation column	Column in which magnetically labeled cells are separated when exposed to the magnetic field
Separation column holder	Molded guides in the magnet housing that holds the separation column in place
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 tubing meet
TCRα/β antigen	The TCRα/β is the T cell receptor heterodimer composed of two transmembrane glycoprotein chains, α and β. Both chains are members of the Ig superfamily and consist of a constant and a polymorphic variable region. The variable region of the TCRα/β is involved in recognition of antigenic peptides presented by the MHC complex of antigen presenting cells. The TCRα/β antigen is expressed on the majority of peripheral blood T cells.
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 is a well proven 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.

3.2 The CliniMACS® Plus System

The different applications run on the CliniMACS® Plus Instrument require the use of specific CliniMACS Materials as well as additional materials and equipment as described in the instructions for use for the respective application (chapter 4, STEP 1).

The following CliniMACS Materials may be part of a CliniMACS Plus System:

- The CliniMACS Plus Instrument

- The CliniMACS Reagents and Biotin Conjugates are intended for *in vitro* magnetic labeling of human cells to enable the separation of specific human cells with a CliniMACS System for clinical applications. The CliniMACS Reagents are dark colored, non-viscous, colloidal solutions, containing the cell specific antibody conjugates in buffer. The reagents consist of the antibody chemically coupled to super-paramagnetic 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.

- CliniMACS Tubing Sets are intended for *in vitro* enrichment or depletion of human cells from heterogeneous haematologic cell populations in combination with the CliniMACS Plus System only. The different tubing sets have been developed for the special needs of the respective application for use in combination with the CliniMACS Plus System only. They consist of pre-assembled, tubing, pre-assembled bags, and other components as required.

- The CliniMACS PBS/EDTA Buffer is intended as wash and transport fluid to enable the *in vitro* separation of human cells with a CliniMACS System only. It is used as process buffer during cell separation and is provided in 1000 mL sterilized plastic bags, individually packed.

Important

Instructions, warnings, precautions, and other important information for the use of the CliniMACS Plus Instrument are described in chapter 1. For instructions for use, e.g., warnings and precautions, concerning the specific CliniMACS Plus System components, refer to the instructions for use provided for the respective component.

The procedures may require the use of components which are not part of the CliniMACS Plus System. Therefore either materials of pharmaceutical grade must be used or the user has to evaluate all risks arising from these materials.

3.3 Additional materials required

Note

For information on the amount of materials required for an application or further specific materials, refer to the instructions of the respective application given in chapter 4, STEP 1.

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

- **Transfer bags, suitable for centrifugation**
  Transfer Bag 150 mL, Terumo GmbH, or equivalent
  Transfer Bag 600 mL, Terumo GmbH, or equivalent
  Transfer Bag 1000 mL, Terumo GmbH, or equivalent

- **Sampling site coupler**
  Sampling Site Coupler, Terumo GmbH, or equivalent

- **Plasma transfer set**
  Transfer Set Coupler/Coupler, Fenwal, or equivalent

- **Luer/Spike Interconnector**
  Luer/Spike Interconnector, Charter Medical, or equivalent

- **Pre-system filter**
  Blood Transfusion Filter, [1]Haemonetics®

- **Syringes and needles**
  Appropriate syringes (1 mL, 10 mL, 20 mL, 50 mL) and hypodermic 20 gauge needles

- Locking forceps

- Sample tubes

- **Clinical grade immunoglobulin G**
  Use IgG of pharmceutical grade quality only, which is available as approved drug in your country.

- **Human serum albumin (HSA)**
  Use HSA of pharmaceutical grade quality only, which is available as approved product in your country.

- **Application specific materials:**
  Individual biotinylated cell specific antibody or ligand; AB serum or, alternatively, autologous serum

1 Haemonetics is a registered trademark of Haemonetics Corporation, Braintree, USA.

3.4 Equipment required

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

- Laminar flow hood

- **Sterile tubing connector**
  Terumo Sterile Connection Device, [2]TSCD®, or equivalent

- **Orbital rotator**
  Lab-Line or equivalent

- **Centrifuge**
  Sorvall or equivalent, buckets for centrifugation with aerosol containment caps

- **Plasma extractor**
  Plasma Separation Stand, Terumo Equipment, or equivalent

- **Table top balance**
  Mettler Toledo or equivalent, with 1 kg capacity;
  resolution to 0.1 g

- **Tubing heat sealer**
  Hematron III, Baxter, or equivalent

- **Tubing stripper**
  Tube Stripper, Baxter, or equivalent

- Biohazard waste containers

2 TSCD is a registered trademark of Terumo Corporation, Tokyo, Japan.

Limited warranty

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

Regulatory and legal note

In Canada, any clinical application of the output product must be performed in accordance with applicable Canadian legislation and regulations that pertain to cellular therapies (e.g. for advanced cellular therapies, the applicable sections of the Food and Drugs Act and the Food and Drug Regulations).

3.5 General warnings and precautions

Separation procedure

- All separation procedures must be performed by trained operators only. The operator training will be provided by Miltenyi Biotec Technical Support.

- For the manufacturing and use of target cells in humans the national legislation and regulations –e.g., for the EU the Directive 2004/23/EC (human tissues and cells) or the Directive 2002/98/EC (human blood and blood components)– must be followed. Thus, any clinical application of the target cells is exclusively within the responsibility of the user of a CliniMACS System.

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

- All cell preparation and labeling procedures must be performed at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]) unless otherwise stated. Higher ambient temperature results in less purity and yield of the target cells.

- All tubing, fittings, valves, the pre-column, and the separation column should be checked thoroughly for leaks during the priming step.

- 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.

Handling of biohazardous material

- To avoid contamination of the cellular starting product, 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 patient samples 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.

- 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 section 1.3.9 „Cleaning“) according to standard hospital or institutional requirements.

- Disposable materials must be treated as biohazardous materials according to standard hospital or institutional requirements.

Important

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

Cellular starting product

– The cellular starting product (e.g. leukapheresis product, buffy coat, bone marrow aspirate) should be collected according to standard hospital or institutional procedures in standard collection bags. Bone marrow aspirate should be collected in heparin-coated containers (e.g. 5 mL syringes). Prior to the cell labeling procedure, no additional anticoagulants or blood additives (heparin etc.) should be included beyond those normally used during leukapheresis or during bone marrow aspiration.

– The container containing 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 of leukapheresis product or buffy coat, the cellular starting product should be packed in insulated containers and should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]) 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.

– For transportation of bone marrow aspirate, the product should be packed in insulated containers and should be kept at controlled temperature (+4 °C [+39 °F]).

– 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 [+66 °F to +77 °F]). Bone marrow aspirate should be kept at controlled temperature (+4 °C [+39 °F]). 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.

3.6 Labeling of cells with CliniMACS® Reagents

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

– Directly in a one-way labeling procedure by antigen-specific anti-bodies conjugated to super-paramagnetic iron-dextran beads (see Figure 3.1).

– 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 Figure 3.2).

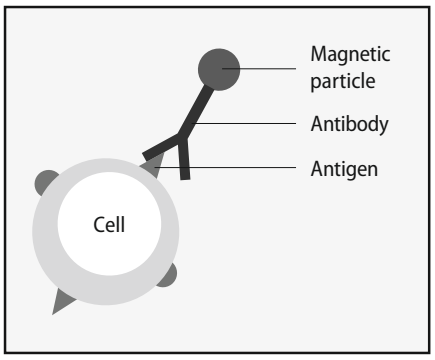


Figure 3.1: Magnetic labeling of cells

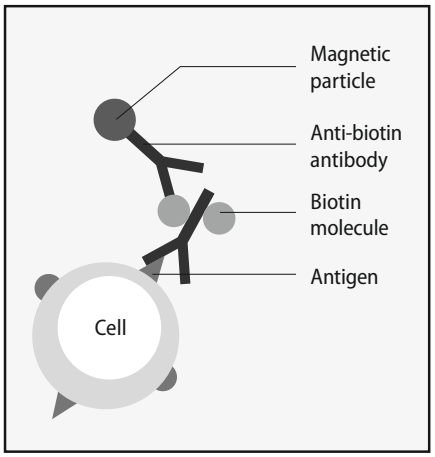


Figure 3.2: Flexible Labeling System

3.7 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.

3.8 CliniMACS® Plus Separation strategies

The CliniMACS® Plus System provides the user with a variety of separation programs. The separation programs can generally be divided into enrichment strategies (CD34 SELECTION 1/2) 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 Figure 3.3).

Depletion of magnetically labeled cells

The MACS Technology is also very efficient for depleting specific cell populations. Unwanted cells are specifically labeled with super-paramagnetic 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 Figure 3.4) or in the **Non-Target Cell Bag** (see Figure 3.5 and Figure 3.6) respectively.

3.8.1 Enrichment strategy

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

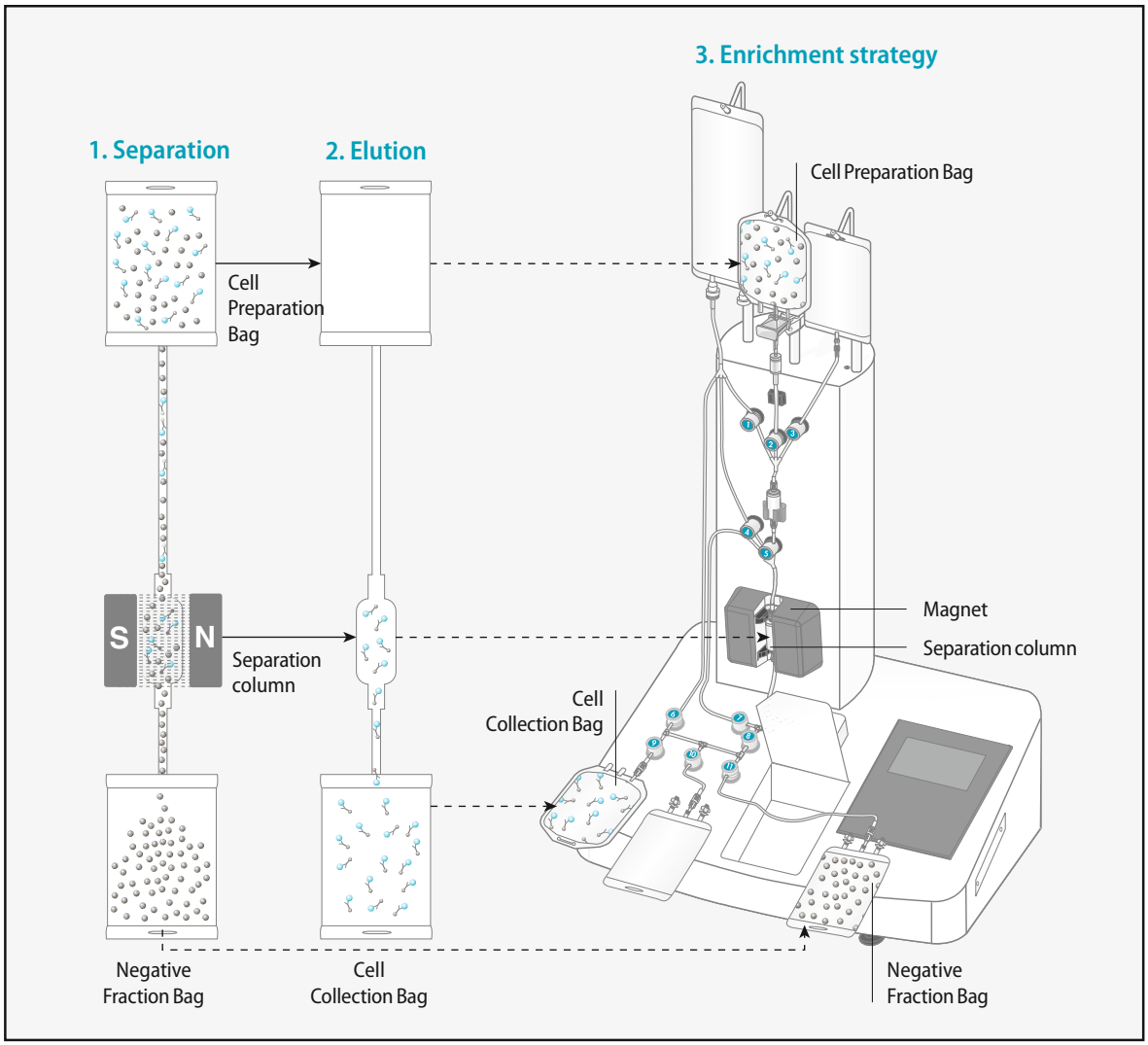


Figure 3.3: Strategy of enrichment programs using the CliniMACS Tubing Set or CliniMACS Tubing Set LS

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.

3.8.2 Depletion strategy

Depletion of cells using the CliniMACS Tubing Set LS – DEPLETION 2.1

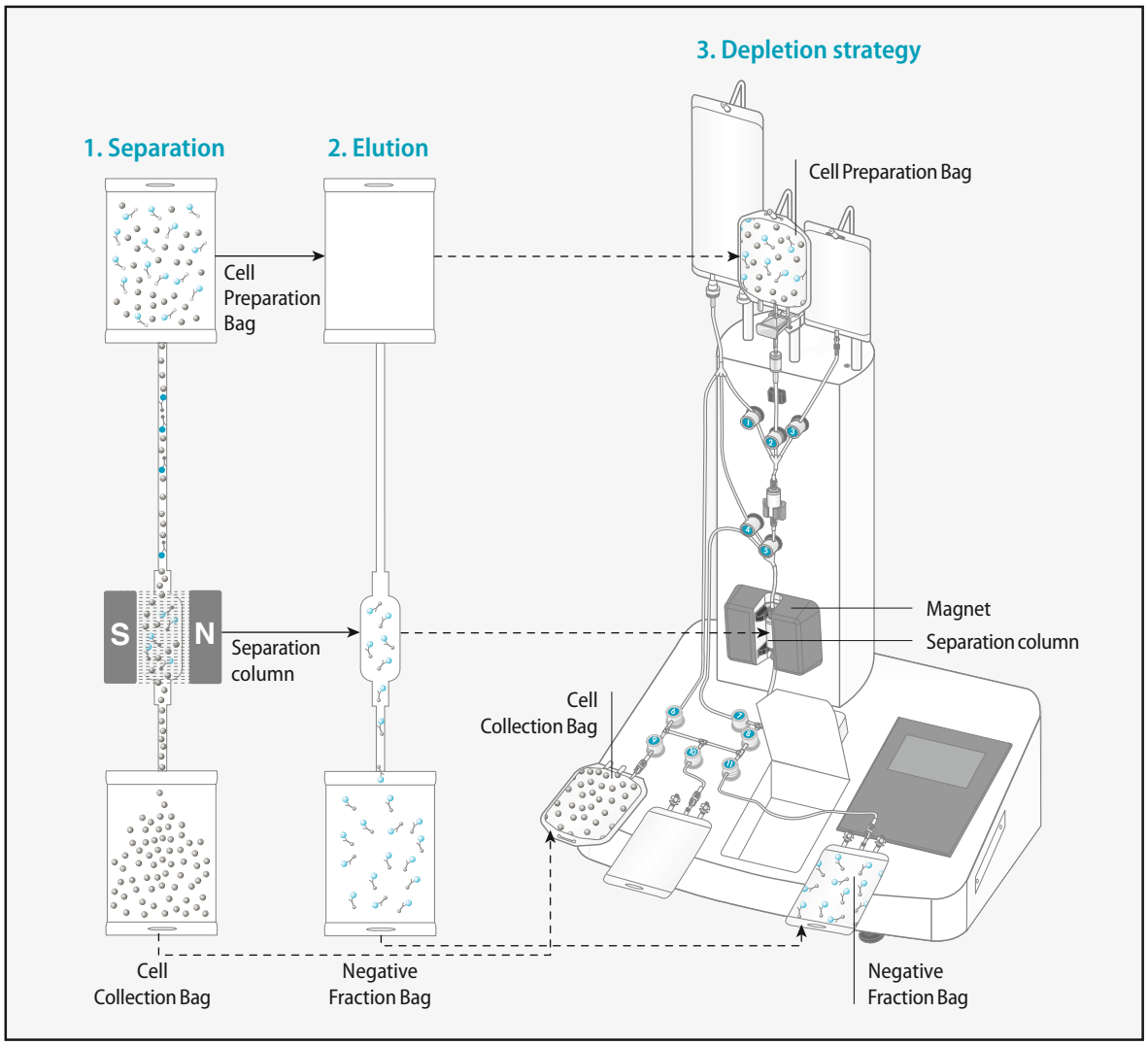


Figure 3.4: Strategy of the depletion program DEPLETION 2.1 using the CliniMACS Tubing Set LS

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: BULK LOADING STAGE)

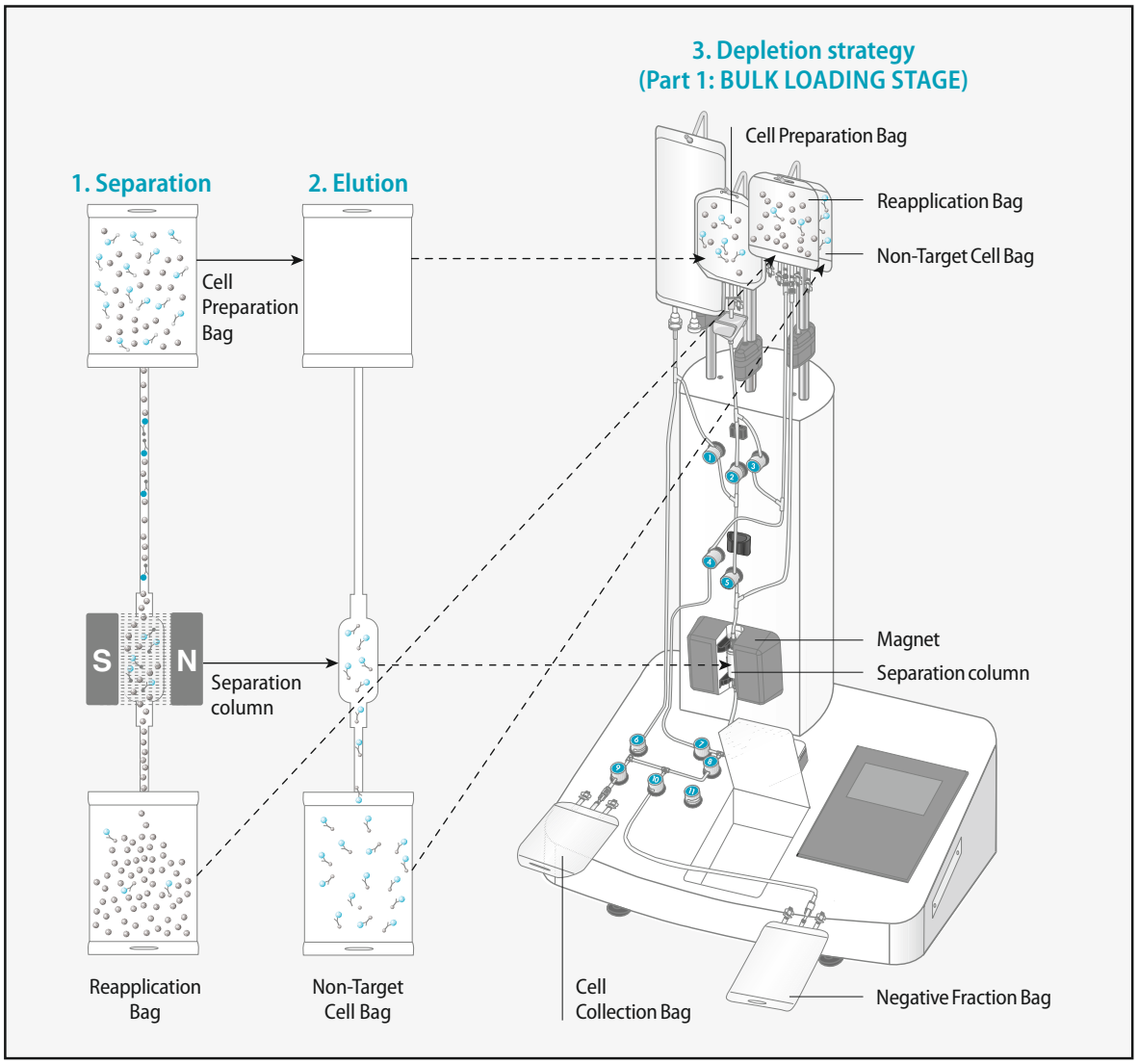


Figure 3.5: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS Depletion Tubing Set (Part 1: BULK LOADING STAGE)

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 ccells) 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 Figure 3.6). 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)

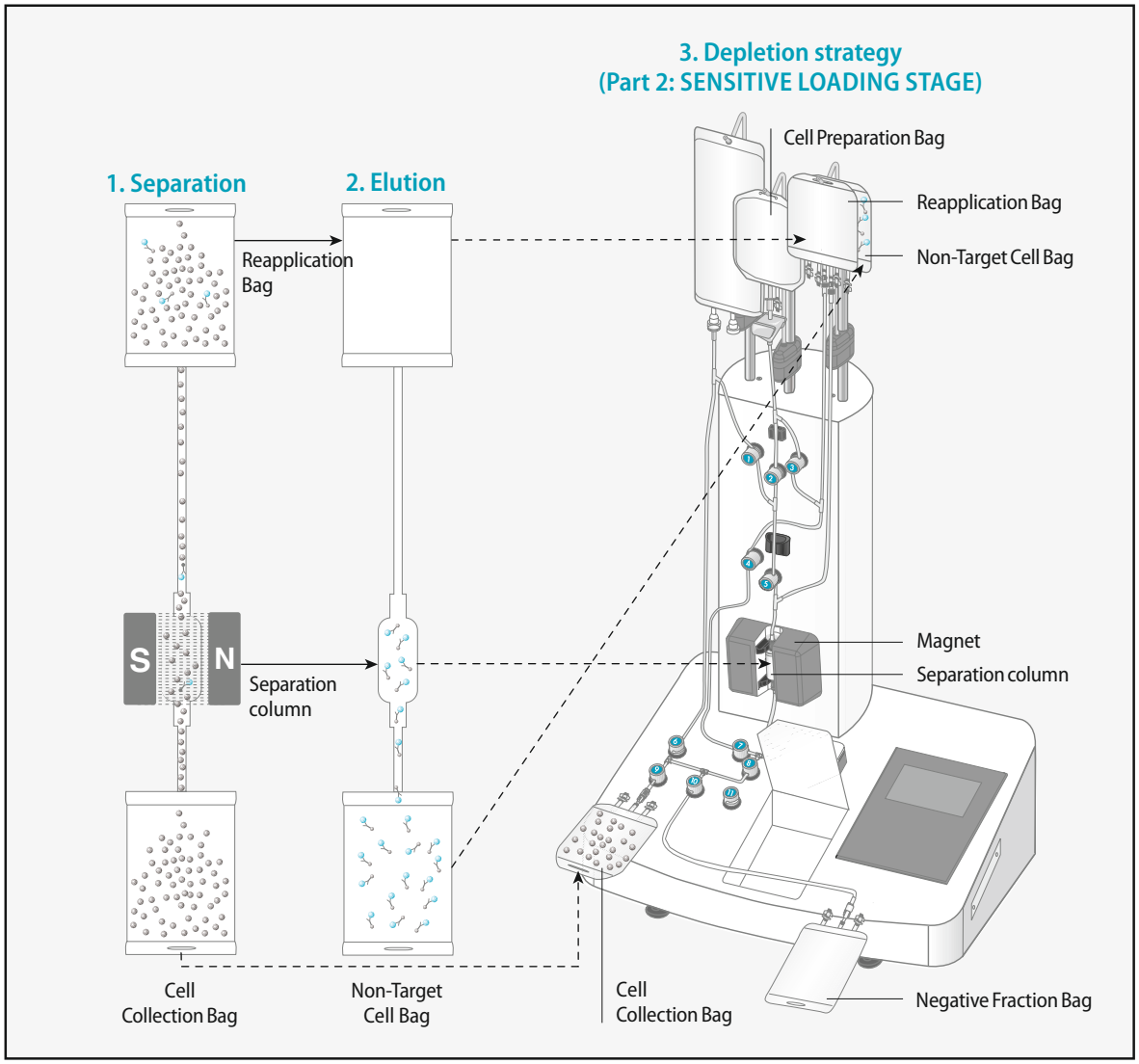


Figure 3.6: Strategy of the depletion program DEPLETION 3.1 using the CliniMACS Depletion Tubing Set (Part 2: SENSITIVE LOADING STAGE)

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 Plus Separations is presented. The CliniMACS Plus Separation is carried out in four STEPS. In a first step the cells are prepared and magnetically labeled, in a second step a cell type specific separation program is chosen, in a third step the tubing set is installed onto the CliniMACS Plus Instrument and in the fourth step cells are separated automatically. Recording of application-related process data may be required. For supporting worksheets, contact Miltenyi Biotec Technical Support.

The CliniMACS Plus System User Manual comprises different options for each step (different reagents, tubing sets, and programs). Before starting the separation, the applicable chapters must be chosen by using the overview table beginning on the next page. The overview table contains information on the applicable chapters of the CliniMACS Plus System User Manual on a basis of the kind and number of reagents, the number of cells, the type of tubing set and the relevant separation program needed for the separation.

STEP 1

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. Continue with STEP 2.

STEP 2

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 determine which program is applicable, go to the overview table on the next page. Read the chapter indicated for the selected separation program. Follow the instructions given in the applicable section. Continue with STEP 3.

STEP 3

STEP 3 explains the installation of the tubing set onto the CliniMACS Plus Instrument. Follow the instructions in the applicable chapter. Continue with STEP 4.

STEP 4

STEP 4 describes the automated CliniMACS Plus Separation. The CliniMACS Plus Instrument performs the automated cell separation procedure (chosen in STEP 2). Follow the instructions of the applicable chapter.

Important

The overview table on the following page summarizes information on applications of the CliniMACS Reagents. The color coding represents the “Four STEPS”. Follow the instructions of the applicable chapters.

Reading example of overview table

For the separation of up to 0.6×10^9 CD34 positive cells from 60×10^9 total cells (normal scale application), one vial of CliniMACS CD34 Reagent (REF 171-01) and a CliniMACS Tubing Set (REF 161-01) are needed. For the procedure follow the subchapters:

- STEP 1: CliniMACS CD34 Reagent,
- STEP 2: CD34 SELECTION 1/2,
- STEP 3: CliniMACS Tubing Set and CliniMACS Tubing Set LS,
- STEP 4: CD34 SELECTION 1/2.

Antigen/ Reagent	CD19 REF 179-01	CD34 REF 171-01	CD34 REF 171-01	TCRα/ß-Biotin REF 701-48
Application	Depletion	Enrichment	Enrichment (large scale)	Depletion
Application capacity	5×10^9 CD19 positive cells from 40×10^9 total cells	0.6×10^9 CD34 positive cells from 60×10^9 total cells	1.2×10^9 CD34 positive cells from 120×10^9 total cells	24×10^9 TCRα/β positive cells from 60×10^9 total cells
Reagent vial(s)	1× CD19 Reagent	1× CD34 Reagent	2× CD34 Reagent	1× TCRα/β-Biotin 2× Anti-Biotin Reagent
Tubing set	1× CliniMACS Tubing Set LS (REF 162-01)	1× CliniMACS Tubing Set (REF 161-01)	1× CliniMACS Tubing Set LS (REF 162-01)	1× CliniMACS Depletion Tubing Set (REF 261-01)
Separation program	DEPLETION 2.1	CD34 SELECTION 1	CD34 SELECTION 2	DEPLETION 3.1

Applicable sections

STEP 1 Magnetic labeling	CliniMACS® CD19 Reagent	CliniMACS® CD34 Reagent	CliniMACS® CD34 Reagent	CliniMACS® TCRα/β-Biotin
STEP 2 Choice of program	DEPLETION 2.1	CD34 SELECTION 1/2	CD34 SELECTION 1/2	DEPLETION 3.1
STEP 3 Installation of tubing set	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Tubing Set and CliniMACS Tubing Set LS	CliniMACS® Depletion Tubing Set
STEP 4 CliniMACS Plus Separation	DEPLETION 2.1	CD34 SELECTION 1/2	CD34 SELECTION 1/2	DEPLETION 3.1

Enrichment of CD34 positive cells

STEP 1
Cell preparation and magnetic labeling

I. General information

Read the instructions for use provided for the respective component and material:
CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Sets, CliniMACS PBS/EDTA Buffer, handling of biohazardous material, and cellular starting product.

II. Materials required

- **Normal scale application:** 0.6×10^9 CD34 positive cells out of 60×10^9 total cells (WBC):
  1 vial of CliniMACS CD34 Reagent (REF 171-01), CliniMACS Tubing Set (REF 161-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- **Large scale application:** 0.6×10^9 to 1.2×10^9 CD34 positive cells out of 120×10^9 total cells (WBC):
  2 vials of CliniMACS CD34 Reagent (REF 171-01), CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- Refer to STEP 1 for additional materials and equipment required.

III. Preparative steps

Preparation of 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, Wash Waste No. 1,** and **Wash Waste No. 2.**
- Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag.

IV. Preparation of leukapheresis product (LP)

Analysis

Transfer into Cell Preparation Bag

Dilution

Add buffer: **Weight of buffer to be added = Weight of leukapheresis product × 2**

Centrifugation

200×g, without brake, 15 min, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F])

Volume adjustment

Labeling volume: **95 g (normal scale application)** or
190 g (large scale application)

- Remove supernatant to adjust the sample using the following equation:
  Weight of supernatant to be removed = Weight of diluted LP – Labeling volume
- Resuspend the cell pellet.

V. Magnetic labeling of the cells

Incubation

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

Removal of excess reagent

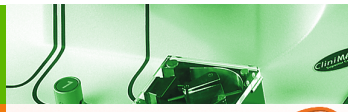
- Fill Cell Preparation Bag with buffer.
- Centrifuge (200×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend the cell pellet.
- Fill Cell Preparation Bag with buffer.
- Centrifuge (200×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend the cell pellet.
- Adjust sample loading volume to **150 g (Normale scale application)** or **275 g (Large scale application).**

Four STEPS to your target cells

37091/04
ch20
(Issued:
2020-06)

Four STEPS to your target cells

Enrichment of CD34 positive cells



STEP 2
Start the CliniMACS® Plus Instrument

Switch-on of the CliniMACS® Plus Instrument

Choice of separation program CD34 SELECTION 1/2
(using 1 vial: CD34 SELECTION 1, or using 2 vials: CD34 SELECTION 2)

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 separation 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® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37091/04
ch20
(Issued:
2020-06)

Depletion of CD19 positive cells

STEP 1
Cell preparation and magnetic labeling

I. General information

Read the instructions for use provided for the respective component and material:
CliniMACS Plus Instrument, CliniMACS CD19 Reagent, CliniMACS Tubing Sets, CliniMACS PBS/EDTA Buffer, handling of biohazardous material, and cellular starting product.

II. Materials required

- Up to 5×10^9 CD19 positive cells out of 40×10^9 total cells:
  1 vial of CliniMACS CD19 Reagent (REF 179-01), CliniMACS Tubing Set LS (REF 162-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- Refer to STEP 1 for additional materials and equipment required.

III. Preparative steps

Preparation of 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 Bag and Cell Preparation Bag.

IV. Preparation of leukapheresis product

Analysis

Transfer into Cell Preparation Bag

Dilution

Add buffer:
Weight of buffer to be added = 600 g – Weight of leukapheresis product

Centrifugation

200×g, without brake, 15 min, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F])

Volume adjustment

Labeling volume: **95 g**

- Remove supernatant completely taking care not to resuspend the cell pellet.
- Resuspend the cell pellet carefully after removal of supernatant.
- Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation:
  Target weight of filled CPB = 95 g + Weight of empty CPB
- Resuspend the cell pellet.

V. Magnetic labeling of the cells

Incubation

Add **1 vial** of CliniMACS® CD19 Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess reagent

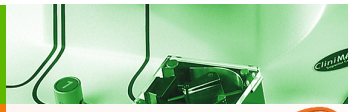
- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend the cell pellet.
- Adjust sample loading volume to **100 g.**

Four STEPS to your target cells

37091/04
ch20
(Issued:
2020-06)

Four STEPS to your target cells

Depletion of CD19 positive cells



STEP 2
Start the CliniMACS® Plus Instrument

Switch-on of the CliniMACS® Plus Instrument

Choice of separation program DEPLETION 2.1

Sample 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 separation 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® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37091/04
ch20
(Issued:
2020-06)

Depletion of TCRα/β positive cells

STEP 1
Cell preparation and magnetic labeling

I. General information, warnings, and precautions

Read the instructions for use provided for the respective component and material:
CliniMACS Plus Instrument, CliniMACS TCRα/β-Biotin, CliniMACS Tubing Sets, CliniMACS PBS/EDTA Buffer, handling of biohazardous material, and cellular starting product.

II. Materials required

- Up to 24×10^9 TCRα/β positive cells out of 60×10^9 total cells (WBC):
  1 vial of CliniMACS TCRα/β-Biotin (REF 701-48), **2 vials** of CliniMACS Anti-Biotin Reagent (REF 173-01), CliniMACS Depletion Tubing Set (REF 261-01), CliniMACS PBS/EDTA Buffer (REF 700-25)
- Refer to STEP 1 for additional materials and equipment required.

III. Preparative steps

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

Labeling and preparation of bags
- Label bags as: **Cell Preparation**, **Plasma Waste**, **Wash Waste No. 1, Wash Waste No. 2,** and **Wash Waste No. 3**.
- Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag.

IV. Preparation of leukapheresis product

Analysis

Transfer into Cell Preparation Bag

Dilution
Add buffer: **Weight of buffer to be added = Weight of leukapheresis product × 2**

Centrifugation
200×g, without brake, 15 min, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F])

Volume adjustment
Labeling volume: **95 g**
- Resuspend the cell pellet.

V. Labeling of the cells with the CliniMACS® TCRα/β-Biotin

Incubation
Add **1 vial** of CliniMACS® TCRα/β-Biotin, incubate on orbital rotator (25 rpm) for 30 min at RT.

Removal of excess conjugate: Repeat this step.
- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend cell pellet and adjust the weight to 190 g after second wash.

VI. Magnetic labeling of the cells with the CliniMACS® Anti-Biotin Reagent

Incubation
Add **2 vials** of CliniMACS® Anti-Biotin Reagent, incubate on orbital rotator (25 rpm) for 30 min at RT.

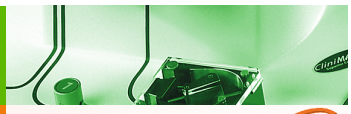
Removal of excess reagent
- Fill Cell Preparation Bag with buffer.
- Centrifuge (300×g, without brake, 15 min, RT).
- Remove supernatant as much as possible.
- Resuspend the cell pellet.
- Adjust sample loading volume to **150 g.**
  (A maximum WBC concentration of 0.4×10^9 WBCs/mL is recommended.)

Four STEPS to your target cells

37091/04
ch20
(Issued:
2020-06)

Four STEPS to your target cells

Depletion of TCRα/β positive cells



STEP 2
Start the CliniMACS® Plus Instrument

Switch-on of the CliniMACS® Plus Instrument

Choice of separation program DEPLETION 3.1

Sample parameter input

STEP 3
Installation of CliniMACS® Tubing Sets

Preparation for tubing set installation

Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

Load valves nos. 1, 2, 3, 4, and 5

Load pump tubing

Load valves nos. 6, 7, 8, 9, and 10

Recheck all tubings 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® Plus Separation

Separation procedure

Disconnect bags and record process code

Unload tubing set and shutdown

Analysis of cells

37091/04
ch20
(Issued:
2020-06)

STEP 1:

CliniMACS® CD34 Reagent

I. General information

The CliniMACS® Plus CD34 System including the CliniMACS Plus Instrument, the CliniMACS CD34 Reagent, the CliniMACS Tubing Set or the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* enrichment of human CD34 positive hematopoietic progenitor cells from heterogeneous hematologic cell populations.

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 Plus 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 *in vitro* enrichment in an automated continuous flow separation process.

The CliniMACS Plus 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 cells (target cells) while the non-labeled cells (non-target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

Important

- Instructions, warnings, precautions, and other important information for the use of the CliniMACS Plus Instrument as well as warnings and precautions concerning the handling of biohazardous materials and cellular starting product are described in chapter 1 of this user manual. For instructions for use, e.g., warnings and precautions, concerning the specific CliniMACS Plus CD34 System components, refer to the instructions for use provided for the respective component.
- The procedures may require the use of components which are not part of the CliniMACS Plus CD34 System. Therefore either materials of pharmaceutical grade must be used or the user has to evaluate all risks arising from these materials.

II. Materials required

Important

The application capacity for the *in vitro* enrichment of CD34 positive cells using the CliniMACS Plus CD34 System amounts to 0.6×10^9 CD34 positive cells out of a total cell number not exceeding 60×10^9 cells. For the *in vitro* 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.

CliniMACS® Plus CD34 System components

- **CliniMACS® CD34 Reagent (REF 171-01)**
  Using the CliniMACS Plus CD34 System, one vial is required for the normal scale application and two vials for the large scale application.

- **CliniMACS Tubing Set (REF 161-01)**
  One CliniMACS Tubing Set must be used for the normal scale application.
  o r
  CliniMACS Tubing Set LS (REF 162-01)
  One CliniMACS Tubing Set LS must be used for the large scale application.

- **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).

Note

Refer to chapter 3 for detailed information regarding additional materials and equipment required.

Additional materials

- **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 (1 mL, 10 mL, 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

III. Preparative steps

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

- 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 lying on the table next to the balance. Record the weight.

- 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.

– 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, and operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS System**. Use only pharmaceutical grade HSA approved in your country. 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.

- Store the buffer for cell preparation at +19 °C to +25 °C (+66 °F to +77 °F). Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are 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. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Lower or higher ambient temperature will result in less purity and yield of the target cells.

IV. Preparation of 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 must 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

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.

Transfer into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

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 next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate bags, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see Figure 4-1.1). Keep the original leukapheresis bag until the separation and final analysis of all cells have been accomplished.

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.

9. Determine the weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the calculated weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. If the weight of the leukapheresis product 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 weight to 200 g at the most and proceed with "Dilution". Record the reduced weight.

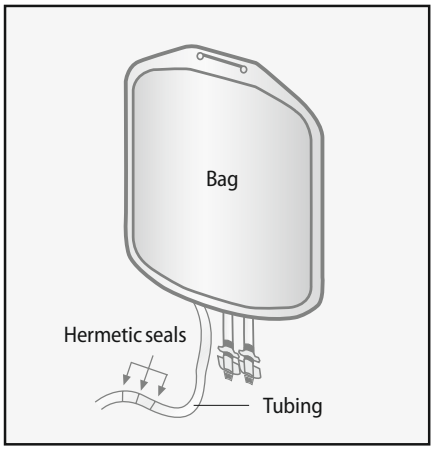


Figure 4-1.1: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Dilution

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

Weight of buffer to be added (g)	=	Weight of leukapheresis product (g)	×	2

1. Take a plasma transfer set and confirm 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. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

5. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of buffer added.

6. Using the heat sealer, 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.

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 filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Record the centrifugation conditions.

5. 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

1. For magnetic labeling of CD34 positive cells, the optimal weight of the cell sample is a) 95 g (±5 g), if one CliniMACS CD34 Reagent vial is sufficient, or b) 190 g (±5 g), if two reagent vials are needed (see Table 4-1.1).

   Calculate the weight of supernatant to be removed to adjust the sample to a) 95 g or b) 190 g using the equation below:

   a) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 95 g

   b) Weight of supernatant to be removed (g) = Weight of diluted leukapheresis product (g) – 190 g

   Record the weight of supernatant to be removed.

2. Place the empty Plasma Waste Bag on the balance and tare the balance.

3. Open the locking forceps next to the Cell Preparation Bag. By visually monitoring the scale on the balance, remove the supernatant using the plasma extractor. Carefully press out excess supernatant until the calculated "weight of supernatant to be removed" is reached.

4. When the appropriate weight of supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed.

	Normal scale application	Large scale application
Labeled cells	0.6×10^9	$0.6–1.2\times10^9$
Total cells	60×10^9 WBCs	$60–120\times10^9$ WBCs
Number of CD34 Reagent vials	1	2
Optimal labeling weight	95 g (±5 g)	190 g (±5 g)

Table 4-1.1: Optimal labeling weight for the *in vitro* enrichment of CD34 positive cells

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 open before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

5. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag.

6. Resuspend the cells in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

7. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

8. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag . Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

9. Keep the Plasma Waste Bag until the separation and final analysis of all cells have been accomplished.

V. Magnetic labeling of cells

The CliniMACS CD34 Reagent vials (7.5 mL each) are ready to use and sufficient for one application as described below. The reagents are not for parenteral administration.

Store the reagents at +2 °C to +8 °C (+36 °F to +46 °F). DO NOT freeze. The reagents must be used cold directly from the refrigerator. DO NOT warm up before use. The lot number and use-by date of the reagents are printed on the vial label. DO NOT use the reagents after the use-by date.

Incubation with the CliniMACS® CD34 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD34 Reagent(s).

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one vial or b) two reagent vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial, or respectively, a 20 mL syringe is sufficient to remove the contents of two reagent 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 minutes.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of 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 [+66 °F to +77 °F]). Record the incubation stop time.

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. Confirm the clamp on the plasma transfer set is in the closed position.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Completely fill the Cell Preparation Bag with buffer (i.e., add 400 g to 500 g of buffer). Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of buffer transferred into the Cell Preparation Bag.

5. Using the heat sealer, 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 the 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 balanced accurately.

10. Centrifuge at 200×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Record the centrifugation conditions.

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 and 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. Close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of removed supernatant.

15. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag No. 1.

16. Keep the Wash Waste Bag No. 1 until the separation and final analysis of all cells have 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. Using the TSCD, connect a buffer bag containing at least 500 mL of buffer to the Cell Preparation Bag. Confirm the clamp on the plasma transfer set is in the closed position. Hang the buffer bag on a bag hanger.

19. Place the Cell Preparation Bag on the balance and tare the balance.

20. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Completely fill the Cell Preparation Bag with buffer (i.e., add approximately 500 g of buffer). Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of buffer transferred into the Cell Preparation Bag.

21. Using the heat sealer, 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.

Important

- For the normal scale application (one CliniMACS CD34 Reagent vial), the weight of supernatant removed should be at least 500 g.
- For the large scale application (two CliniMACS CD34 Reagent vials), the weight of supernatant removed should be at least 450 g.
- 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.

24. Fold any loose parts of the bags or tubing downwards. Transfer the Cell Preparation Bag and Wash Waste Bag No. 2 securely to the centrifuge bucket.

25. Centrifuge at 200×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Record the centrifugation conditions.

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 and 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. Close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of removed supernatant.

29. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag No. 2.

30. Keep the Wash Waste Bag No. 2 until the separation and final analysis of all cells have 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. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

33. Determine the weight of the leukapheresis product after the washes by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after the washes (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

34. Adjust sample loading volume: Calculate the weight of buffer necessary to adjust the weight of the cell suspension to approximately a) 150 g (normal scale application) or b) 275 g (large scale application).

a)	Weight of buffer to be added (g)	=	150 g	–	Weight of leuka-pheresis product after the washes (g)
b)	Weight of buffer to be added (g)	=	275 g	–	Weight of leuka-pheresis product after the washes (g)

35. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

36. Place the Cell Preparation Bag on the balance and tare the balance.

37. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

38. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

39. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

40. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

41. 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.

42. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

43. Determine the weight of the leukapheresis product after addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after addition of buffer (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

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

STEP 1:

CliniMACS® CD19 Reagent

I. General information

The CliniMACS® Plus CD19 System including the CliniMACS Plus Instrument,the CliniMACS CD19 Reagent, the CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human CD19 positive cells from heterogeneous hematologic cell populations.

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.

The CliniMACS Plus CD19 System uses of selective CD19 monoclonal antibodies conjugated to super-paramagnetic particles. The CD19 positive cells are specifically labeled by incubation with the CliniMACS CD19 Reagent. After unbound reagent has been removed from the suspension, the cells are ready for the *in vitro* depletion in an automated continuous flow separation process.

The CliniMACS Plus CD19 System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD19 positive cells (non-target cells) while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

Important

- Instructions, warnings, precautions, and other important information for the use of the CliniMACS Plus Instrument as well as warnings and precautions concerning the handling of biohazardous materials and cellular starting product are described in chapter 1 of this user manual. For instructions for use, e.g., warnings and precautions, concerning the specific CliniMACS Plus CD19 System components, refer to the instructions for use provided for the respective component.
- The procedures may require the use of components which are not part of the CliniMACS Plus CD19 System. Therefore either materials of pharmaceutical grade must be used or the user has to evaluate all risks arising from these materials.

II. Materials required

CliniMACS® Plus CD19 System components

Important

The application capacity for the *in vitro* depletion of CD19 positive cells using the CliniMACS Plus CD19 System amounts to 5×10^9 CD19 positive cells out of a total cell number not exceeding 40×10^9.

- **CliniMACS® CD19 Reagent (REF 179-01)**
  One vial is required for the application using the CliniMACS Plus CD19 System.

- **CliniMACS Tubing Set LS (REF 162-01)**
  One CliniMACS Tubing Set LS must be used for the depletion of CD19 positive cells.

- **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, one liter of buffer is required. For the separation, one liter of buffer are required. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Additional materials

Note

Refer to chapter 3 for detailed information regarding additional materials and equipment required.

- **Cell Preparation Bag**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure

- **Plasma Waste Bag and Wash Waste Bag**
  Two 600 mL transfer bags, suitable for centrifugation

Important

After input of sample parameters (STEP 2), the CliniMACS Plus Software calculates the volumes that will be collected in the Priming Waste Bag, Cell Collection Bag, Negative Fraction Bag, and Buffer Waste Bag. If the volume of the calculated liquid exceeds the standard volume of 500 mL, replacement of bags according to the following overview of materials required is necessary.

- **Cell Collection Bag**
  One 600 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 (1 mL, 10 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

III. Preparative steps

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter 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

- Label one 600 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 outside 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 lying on the table next to the balance. Record the weight.

- 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 lying on the table next to the balance. Record the weight.

- Label one 600 mL transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run, and operator identification.)

- Label one 600 mL transfer bag as: **Wash Waste**. (This should include patient identification, date and time of run, and operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS System**. Use only pharmaceutical grade HSA approved in your country. 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.

- Store the buffer for cell preparation at +19 °C to +25 °C (+66 °F to +77 °F). Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are 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. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Lower or higher ambient temperature will result in less purity and yield of the target cells.

IV. Preparation of 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 must 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

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

- Total number of leukocytes
- Percentage of CD19 positive cells
- Total number of CD19 positive cells
- Viability

Recheck the parameters after the labeling procedure. This is necessary because the following three sample parameters have to be entered in the software during the setup of the CliniMACS Plus Instrument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL
- Percentage of CD19 positive cells
- Final volume of the cell sample (sample loading volume)

Other tests might be required depending on the intended use of the cells. Record all data.

Transfer into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

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 next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing and separate bags, leaving at least 15 cm of tubing on the Cell Preparation for further connections (see Figure 4-1.1). Keep the original leukapheresis bag until the separation and final analysis of all cells have been accomplished.

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 of the filled Cell Preparation Bag.

9. Determine the weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the calculated weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

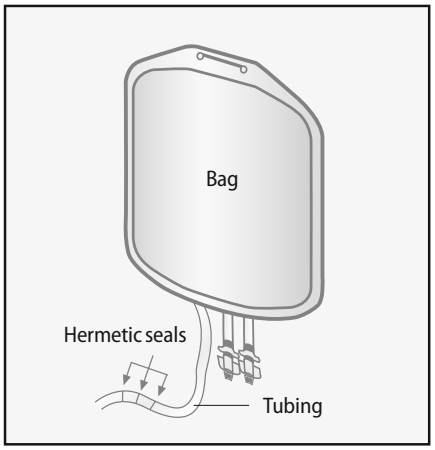


Figure 4-1.1: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Dilution

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product with buffer to a total weight of 600 g. Calculate the weight of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	600 g	–	Weight of leukapheresis product (g)

1. Take a plasma transfer set and confirm 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. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

5. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, 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.

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 filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD19 positive cells, the optimal weight of the cell sample must be adjusted to 95 g (±5 g) (see Table 4-1.1).

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Carefully resuspend the cell pellet.

   Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the weight of the Cell Preparation Bag filled with diluted leukapheresis product (LP) using the equation below:

Target weight of Cell Preparation Bag filled with diluted LP (g)	=	95 g	+	Weight of empty Cell Preparation Bag (g)

2. Insert the spike of a transfer set to a port of a buffer bag containing at least one liter of buffer. Confirm the clamp on the plasma transfer set is in the closed position.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

Volume adjustment	
Total cell number	≤40×10⁹ WBC
Total CD19 positive cells	≤5×10⁹
Number of CD19 Reagent vials	1
Optimal labeling weight	95 g (±5 g)
CliniMACS PBS/EDTA Buffer	1 liter

Table 4-1.1: Reagent and buffer needed for labeling

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 open before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

5. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, 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.

7. Resuspend the cells in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of product empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the final separation and analysis of all cells have been accomplished.

V. Magnetic labeling of the cells

The CliniMACS CD19 Reagent vial (7.5 mL) 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 (+36 °F to +46 °F). DO NOT freeze. The reagent must 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 label. DO NOT use the reagent after the use-by date.

Incubation with the CliniMACS® CD19 Reagent

1. Record the lot number and use-by date of the CliniMACS® CD19 Reagent.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one reagent vial (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one reagent vial. 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 minutes.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of 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 [+66 °F to +77 °F]). Record the incubation stop time.

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least one liter of buffer. Confirm the clamp on the plasma transfer set is in the closed position.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Tare the balance. Place the Cell Preparation Bag on the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Completely fill the Cell Preparation Bag with buffer. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.

5. Using the heal sealer, 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 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer the Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

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 on the balance and 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. Close locking forceps next to the Cell Preparation Bag.

15. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag.

16. Keep the Wash Waste Bag until the final separation and analysis of all cells of interest have been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

19. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

20. Adjust sample loading volume: Calculate the weight of buffer necessary to adjust the weight of the cell suspension to approximately 100 g. For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

Weight of buffer to be to be added (g)	=	100 g	–	Weight of leukapheresis product after the wash (g)

21. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

22. Place the Cell Preparation Bag on the balance and tare the balance.

23. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

Important

If the supernatant removed is less than 500 mL, a total of two washing steps (instead of only one) is recommended. Otherwise the removal of unbound reagent may be insufficient.

24. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

25. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

26. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

27. 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.

28. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

29. Determine the weight of the leukapheresis product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after addition of buffer (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2.

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

STEP 1:

CliniMACS® TCRα/β-Biotin

I. General information

The CliniMACS® Plus TCRα/β-Biotin System including the CliniMACS Plus Instrument, the CliniMACS TCRα/β-Biotin, the CliniMACS Anti-Biotin Reagent, the CliniMACS Depletion Tubing Set, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human TCRα/β positive cells from heterogeneous hematologic cell populations.

The TCRα/β is the T cell receptor heterodimer composed of two transmembrane glycoprotein chains, α and β. Both chains are members of the Ig superfamily and consist of a constant and a polymorphic variable region. The variable region of the TCRα/β receptor is involved in recognition of antigenic peptides presented by the MHC complex of antigen presenting cells. The TCRα/β is expressed on the majority of peripheral blood T cells.

The CliniMACS Plus TCRα/β-Biotin System uses murine monoclonal antibodies specific for the TCRα/β antigen conjugated to biotin in combination with the CliniMACS Anti-Biotin Reagent. The TCRα/β positive cells are labeled for separation by incubation with the CliniMACS TCRα/β-Biotin. After unbound conjugate is removed from the suspension, the cells are magnetically labeled with the CliniMACS Anti-Biotin Reagent. After excess reagent is removed from the suspension, the cells are ready for the *in vitro* depletion in an automated separation process.

The CliniMACS Plus TCRα/β-Biotin System passes the antibody-labeled cell suspension through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled TCRα/β positive cells (non-target cells) while the unlabeled cells (target cells) flow through the column. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically labeled cells are released from the column when the magnet is disengaged and the magnetic field is removed. The target cells are collected in the Cell Collection Bag.

Important

- Instructions, warnings, precautions, and other important information for the use of the CliniMACS Plus Instrument as well as warnings and precautions concerning the handling of biohazardous materials and cellular starting product are described in chapter 1 of this user manual. For instructions for use, e.g., warnings and precautions, concerning the specific CliniMACS Plus TCRα/β-Biotin System components, refer to the instructions for use provided for the respective component.
- The procedures may require the use of components which are not part of the CliniMACS Plus TCRα/β-Biotin System. Therefore either materials of pharmaceutical grade must be used or the user has to evaluate all risks arising from these materials.

II. Materials required

Important

The application capacity for the *in vitro* depletion of TCRα/β positive cells using the CliniMACS Plus TCRα/β-Biotin System amounts to 24×10^9 TCRα/β positive cells out of a total cell number not exceeding 60×10^9 cells (WBC).

CliniMACS® Plus TCRα/β-Biotin System components

- **CliniMACS® TCRα/β-Biotin (REF 701-48)**
  One vial is required for the application using the CliniMACS Plus TCRα/β-Biotin System.

- **CliniMACS Anti-Biotin Reagent (REF 173-01)**
  Two vials are required for the application using the CliniMACS Plus TCRα/β-Biotin System.

- **CliniMACS Depletion Tubing Set (REF 261-01)**
  One CliniMACS Depletion Tubing Set must be used for the depletion of TCRα/β positive cells.

- **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, up to three 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).

Note

Refer to chapter 3 for detailed information regarding additional materials and equipment required.

Additional materials

- **Cell Preparation Bag**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets for the cell preparation procedure

- **Plasma Waste Bag and Wash Waste Bags**
  Four 600 mL (or 1000 mL) transfer bags, suitable for centrifugation

- **Cell Collection Bag**
  One 600 mL Cell Collection Bag is already assembled to the CliniMACS Depletion Tubing Set.

- Pre-system filter

- Locking forceps

- Appropriate syringes (5 mL, 10 mL, 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

III. Preparative steps

Preparation of the CliniMACS® PBS/EDTA Buffer

Supplement each required liter 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

- One 600 mL transfer bag is already assembled to the CliniMACS Depletion Tubing Set and labeled as: **Cell Collection**. (Labeling should include patient identification, date and time of run, and operator identification.) Weigh the empty Cell Collection Bag and record the weight.

- 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 lying on the table next to the balance. Record the weight.

- Label one 600 mL (or 1000 mL) transfer bag as: **Plasma Waste**. (This should include patient identification, date, time of run, and operator identification.)

- Label three 600 mL (or 1000 mL) transfer bags as: W**ash Waste No. 1**, **Wash Waste No. 2**, and **Wash Waste No. 3**. (This should include patient identification, date, time of run, and operator identification.)

Important

- Note that **HSA is not a component of the CliniMACS System**. Use only pharmaceutical grade HSA approved in your country. 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.

- Store the buffer for cell preparation at +19 °C to +25 °C (+66 °F to +77 °F). Lower or higher ambient temperature will result in less purity and yield of the target cells.

- Since the length of the tubing can vary during the preparation procedure, be careful when determining the weight of the Cell Preparation Bag. To acquire an accurate reading confirm the locking forceps are always positioned close to the bag and are 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. The connection of tubing using the TSCD may be performed outside the laminar flow hood.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Lower or higher ambient temperature will result in less purity and yield of the target cells.

IV. Preparation of leukapheresis product

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

- The operator must 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

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

- Volume and cell concentration to determine the total number of leukocytes
- Percentage of TCRα/β positive cells
- Total number of TCRα/β positive cells
- Viability

Determine the percentage/total number of all TCRα/β-labeled cells (including unspecific TCRα/β binding, not only real TCRα/β positive cells). Consider this number for the application specifications and for sample parameter input during the setup of the CliniMACS Plus Instrument (see STEP 2).

Other tests might be required depending on the intended use of the cells. Record all data.

Transfer into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the leukapheresis product.

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 content 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 next to the Cell Preparation Bag.

6. Usgint the heat sealer, seal off the tubing and separate bags leaving at least 15 cm of tubing on the Cell Preparation for further connections (see Figure 4-1.1). Keep the original leukapheresis bag until the separation and final analysis of cells have been accomplished.

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 of the filled Cell Preparation Bag.

9. Determine the weight of the leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with leukapheresis product. Record the calculated weight.

Weight of leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

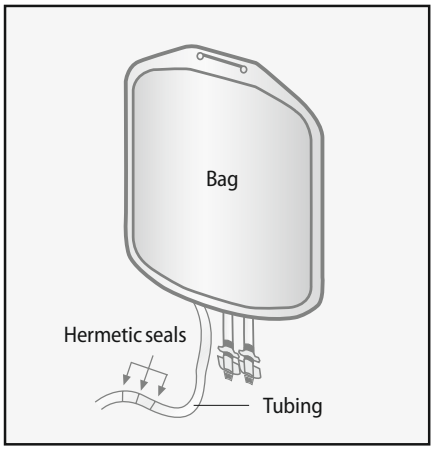


Figure 4-1.1: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

Dilution

The leukapheresis product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% (w/v)) before magnetic labeling. Dilute the leukapheresis product to a total weight of three times the weight of the leukapheresis product, but not exceeding a total weight of 600 g. Calculate the weight of buffer to be added using the following equation and record it.

Weight of buffer to be added (g)	=	Weight of leukapheresis product (g)	×	2

1. Take a plasma transfer set and ensure that 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. Hang the buffer bag on a bag hanger.

3. Place the Cell Preparation Bag on the balance and tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

5. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, 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.

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 weight the filled Cell Prepration Bag. Record the weight.

9. Determine the weight of the diluted leukapheresis product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of diluted leukapheresis product (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Centrifugation

1. Using the TSCD, connect the empty Plasma Waste Bag to the Cell Preparation Bag.

2. Fold any loose parts of the Cell Preparation Bag or tubing downwards. Place the two bags securely in the centrifuge bucket.

3. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at 200×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

6. Carefully remove the supernatant taking care not to disturb the cell pellet.

Volume adjustment

1. For labeling of TCRα/β positive cells, the optimal weight of the cell sample is 95 g (±5 g).

   Remove supernatant completely, taking care not to resuspend the cell pellet during removal of supernatant. When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag. Carefully resuspend the cell pellet.

   Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag filled with diluted leukapheresis product using the equation below:

Target weight of Cell Preparation Bag filled with diluted leukapheresis product (g)	=	95 g	+	Weight of empty Cell Preparation Bag (g)

2. Insert the spike of a transfer set to a port of a buffer bag containing one liter of buffer. Confirm the clamp on the plasma transfer set is in the closed position.

3. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

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 open before beginning the transfer. Release the extractor handle slowly.

- During removal of supernatant be careful not to lose cells.

4. Tare the balance. Place the Cell Preparation Bag on the balance.

5. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with diluted leukapheresis product" is reached. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

6. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Resuspend the cells in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

9. Determine the weight of the leukapheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after volume adjustment (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

10. Keep the Plasma Waste Bag until the separation and final analysis of all cells have been accomplished.

V. Labeling of the cells with the CliniMACS® TCRα/β-Biotin

One CliniMACS® TCRα/β-Biotin vial (7.5 mL) is ready to use and sufficient for one application as described below. The conjugate is not for parenteral administration.

Store the conjugate at +2 °C to +8 °C (+36 °F to +46 °F). DO NOT freeze. The conjugate is to be used cold directly from the refrigerator. DO NOT warm up before use. The lot number and use-by date of the conjugate are printed on the vial label. DO NOT use the conjugate after the use-by date.

Incubation with the CliniMACS® TCRα/β-Biotin

1. Record the lot number and use-by date of the CliniMACS® TCRα/β-Biotin.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one vial (7.5 mL). A 10 mL syringe is sufficient to remove the content of one vial. 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 conjugate into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately start counting the incubation time of 30 minutes.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of 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 [+66 °F to +77 °F]). Record the incubation stop time.

Removal of excess conjugate

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is in the closed position.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Tare the balance. Place the Cell Preparation Bag on the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Completely fill the Cell Preparation Bag with buffer. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.

5. Using the heat sealer, 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 the 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 balanced accurately.

10. Centrifuge at 300×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

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 and 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. Close the locking forceps next to the Cell Preparation Bag to stop the liquid flow.

15. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag No. 1.

16. Keep the Wash Waste Bag No. 1 until the separation and final analysis of cells have been accomplished.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Repeat "washing procedure" (step 1 to 17) using the Wash Waste Bag No. 2 and continue with step 19.

19. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

20. Determine the weight of the leukapheresis product after the second wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after the second wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

21. Calculate the weight of buffer necessary to adjust the weight of the cell suspension to approximately 190 g, for magnetic labeling of the TCRα/β-Biotin labeled cells with the CliniMACS Anti-Biotin Reagent.

Weight of buffer to be added (g)	=	190 g	–	Weight of leukapheresis product after the second wash (g)

22. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

23. Place the Cell Preparation Bag on the balance and tare the balance.

24. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

25. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

26. Using the TSCD, seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag. Disconnect the buffer bag.

27. Resuspend the cell pellet in the Cell Preparation Bag. Avoid too intensive mixing of the cells. Ensure that all cells are resuspended.

VI. Magnetic labeling of the cells with the CliniMACS® Anti-Biotin Reagent

The CliniMACS® Anti-Biotin Reagent vials (7.5 mL each) are ready to use and sufficient for one application as described below. The reagents are not for parenteral administration.

Store the reagents at +2 °C to +8 °C (+36 °F to +46 °F). DO NOT freeze. The reagents must be used cold directly from the refrigerator. DO NOT warm up before use. The lot number and use-by date of the reagents are printed on the vials. DO NOT use the reagents after the use-by date.

Incubation with the CliniMACS® Anti-Biotin Reagent

1. Record the lot number and use-by date of the CliniMACS® Anti-Biotin Reagent vials.

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from each of the two CliniMACS Anti-Biotin Reagent vials (7.5 mL each). A 20 mL syringe is sufficient to remove 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 both reagent vials into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately start counting the incubation time of 30 minutes.

4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.

5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of 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 [+66 °F to +77 °F]). Record the incubation stop time.

Removal of excess reagent

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is in the closed position.

2. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

3. Tare the balance. Place the Cell Preparation Bag on the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Completely fill the Cell Preparation Bag with buffer. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.

5. Using the heat sealer, 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. 3 to the Cell Preparation Bag.

8. Fold any loose parts of the bags or tubing downwards. Transfer the Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.

9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is balanced accurately.

10. Centrifuge at 300×g (without brake) for 15 minutes at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

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. 3 on the balance and tare the balance.

14. Open the locking forceps next to the Cell Preparation Bag. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag. Close the locking forceps next to the Cell Preparation Bag to stop the liquid flow.

15. Using the heat sealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag No. 3.

16. Keep the Wash Waste Bag No. 3 until the separation and final analysis of all cells has been completed.

17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

18. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

19. Determine the weight of the leukapheresis product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after the wash (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

20. Adjust sample loading volume: Calculate the weight of buffer necessary to adjust the weight of the cell suspension to approximately 150 g. For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.

Weight of buffer to be to be added (g)	=	150 g	–	Weight of leukapheresis product after the wash (g)

21. Using the TSCD, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.

22. Place the Cell Preparation Bag on the balance and tare the balance.

23. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.

24. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.

25. Using the heat sealer, seal off the tubing between both clamps. Disconnect the buffer bag.

26. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.

27. 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 sample 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.

28. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.

29. Determine the weight of the leukapheresis product after addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

Weight of leukapheresis product after addition of buffer (g)	=	Weight of filled Cell Preparation Bag (g)	–	Weight of empty Cell Preparation Bag (g)

Proceed to STEP 2

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

STEP 2:

CD34 SELECTION 1/2

Switch-on of 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 start time of the instrument run.

The window will display Screen 1.1 as shown in chapter 1.

To proceed to the program menu, 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.

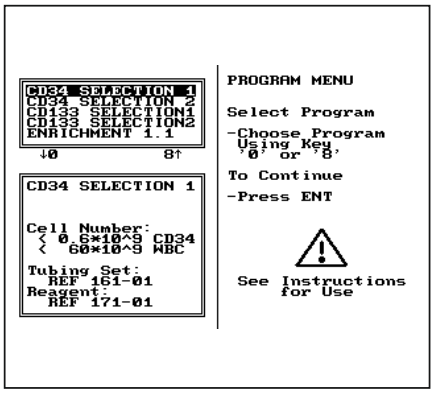
Choice of separation program CD34 SELECTION 1/2

The window will display Screen 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



Screen 4-2.1: Choice of separation program

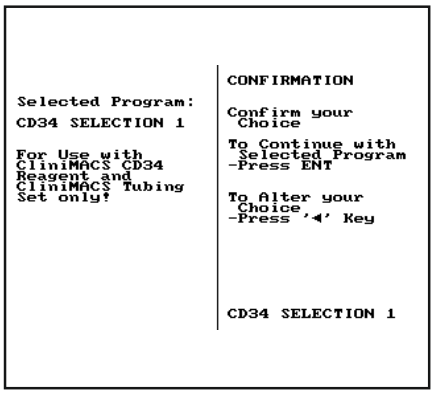
Confirmation

The window will display Screen 4-2.2 as shown.

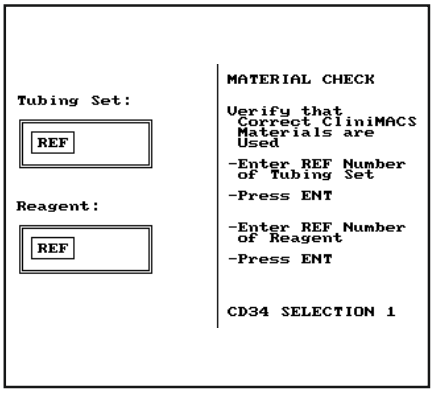
Confirm the correct program has been chosen.

If not, press the "Undo" key (see Figure 1.4) to return to the previous step in order to amend the choice.

To confirm and proceed, press ENT



Screen 4-2.2: Confirmation



Screen 4-2.3: Material check

Material check

The window will show Screen 4-2.3 as shown.

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

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

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

   To confirm and proceed, press ENT

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

   To confirm and proceed, press ENT

If the catalogue 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 catalogue number again. If the catalogue 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 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.

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 prior to installation.
- To correct a mistake during data input, press the "Undo" key (see Figure 1.4).

STEP 2:

DEPLETION 2.1

Switch-on of 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 start time of the instrument run.

The window will display Screen 1.1 as shown in chapter 1.

To proceed to the program menu, press ENT

Important

- The separation program DEPLETION 2.1 must only be used with the CliniMACS Tubing Set LS (REF 162-01).
- 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.

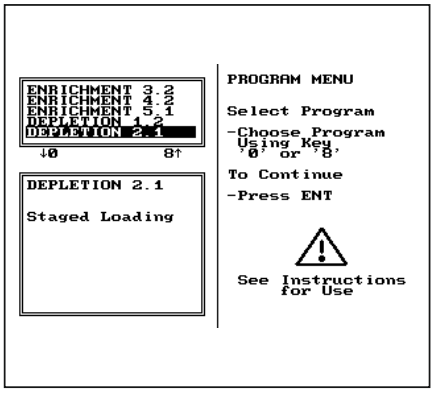
Choice of separation program DEPLETION 2.1

The window will display Screen 4-2.1 as shown.

Choose DEPLETION 2.1.

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



Screen 4-2.1: Choice of separation program

Selection of the tubing set

The window will display Screen 4-2.2 as shown.

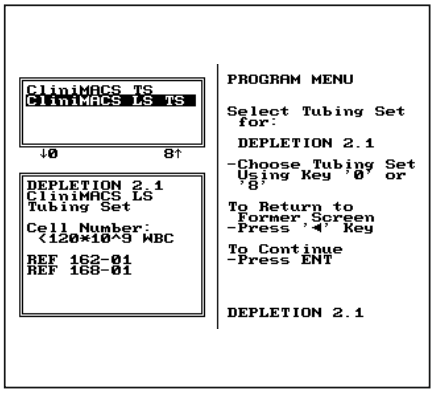
Confirm that the correct program has been chosen.

If not, press the "Undo" key (see Figure 1.4) to return to the previous step in order to amend the choice.

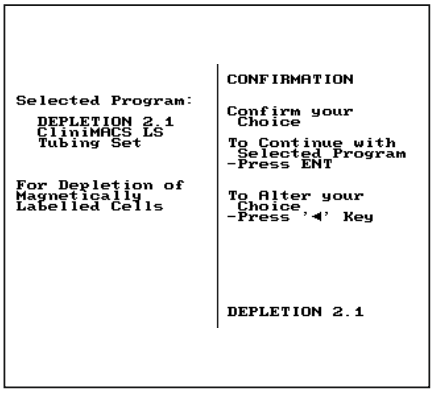
Select the tubing set by highlighting it in the box at the top left hand side with the black bar. Move the bar up and down by using the keys '0' and '8'.

The window below displays the relevant data like capacity and catalogue number (REF) of the selected tubing set.

To continue with the selected combination of separation program and tubing set, press ENT



Screen 4-2.2: Selection of the tubing set

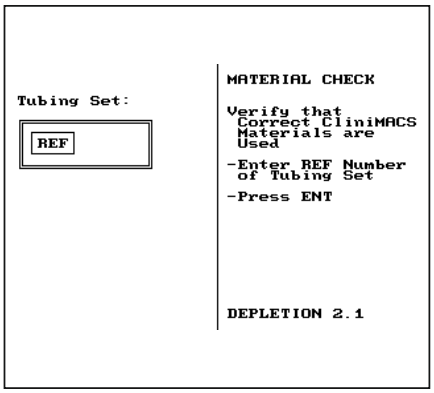


Screen 4-2.3: Confirmation

Confirmation

The window will display Screen 4-2.3 as shown.

To confirm the combination of separation program and tubing set and to proceed, press ENT



Screen 4-2.4: Material check

Material check

The window will show Screen 4-2.4 as shown.

The query of the catalogue number of the tubing set serves as a security check to ensure that the tubing set the operator selected can be used.

Enter catalogue number of the selected tubing set and proceed with pressing ENT

If a non-corresponding catalogue number has been entered, a message appears. To confirm press 'ENT' and enter the correct catalogue number. If the entered catalogue number is still wrong, the message appears a second time. After pressing 'ENT' the program will return to the program menu (see Screen 4-2.2).

Sample parameter input

If the material check has been successful, the program continues automatically with the query for different sample parameters that are necessary to adjust the separation to each individual sample and to provide the operator important information for required buffer and bag volumes.

The window will display Screen 4-2.5 as shown.

1. Start by entering the WBC concentration of the sample. The acceptable range is shown in the box on the bottom left hand side. Press the "Undo" key to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see "Important").

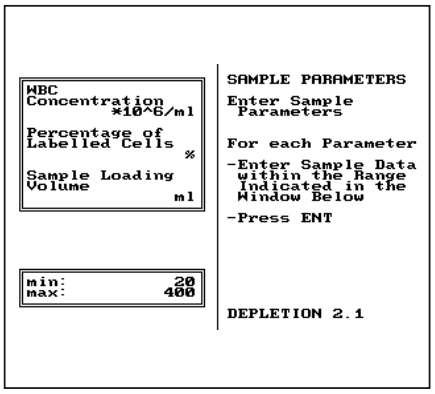
   To continue with the next input, press ENT

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see "Important").

   To continue with the next input, press ENT

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see "Important").

To continue, press ENT



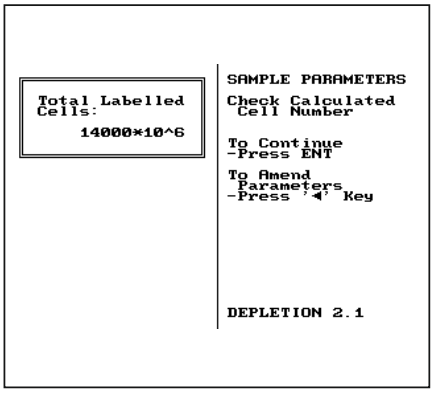
Screen 4-2.5: Sample parameter input

Important

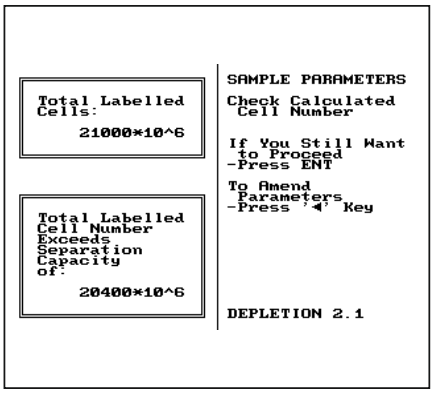
- All sample parameters required refer to the sample **post** cell labeling and washing procedure.

- The acceptable range shown in the box on the bottom left hand side always takes account of the previous input. After each input the software calculates and adjusts the accepted range of the next query to ensure the separation capacity of the system will not be exceeded.

- **Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of target cell loss.** Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may yield in cell loss due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading or even to incomplete sample processing. If the specification of any of the three parameters is too high, this will result in increased processing time and enlarged volume of the target cell fraction.



Screen 4-2.6: Calculation



Screen 4-2.7: Calculation: Cell number exceeds separation capacity

Calculation

The CliniMACS Plus Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens.

- The result of the calculation is shown on a screen similar to Screen 4-2.6. Ensure the calculated number of labeled cells is correct.

  To continue, press ENT

  If the calculated number of labeled cells is not correct, a correction of the data input is possible. Press the "Undo" key to return to the previous Screen 4-2.5 for correction.

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS Plus System a screen similar to Screen 4-2.7 is shown. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press the "Undo" key to return to the previous Screen 4-2.5 and to correct the sample parameters.

  Consider that depletion conditions outside the specifications for tubing set and separation program result in lower depletion efficiency and target cell recovery.

  If the operator do not want to overload the system, split the sample and determine the volume of each portion. Return to the previous Screen 4-2.5 by pressing the "Undo" key. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

To proceed with this sample, press ENT

Volume information

From the total number of labeled cells (see previous page) the software calculates the number of separation stages, the amount of buffer needed for the entire separation and the liquid volumes that will be collected in the Cell Collection Bag, Negative Fraction Bag, Buffer Waste Bag, and Priming Waste Bag.

Standard values are the following:

- CliniMACS PBS/EDTA Buffer 1000 mL,
- Cell Collection Bag 160 mL
- Negative Fraction Bag 500 mL,
- Buffer Waste Bag 500 mL,
- Priming Waste Bag 500 mL.

If one of the calculated values exceeds these standard values, Screen 4-2.8 appears to inform about the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set (capacity approximately 500 mL) with alternative bags of the appropriate size, if replacement is necessary. The bags originally attached to the tubing set are connected by luer connections. Bags that replace the original bags should have a female luer connector. Standard bags can be connected by a Luer/Spike Interconnector.

1. Confirm the requested amount of buffer is available. Do not attach more than three liters of buffer on the bag hanger (see "Important", bullet 2).

2. Open the tray of the tubing set under sterile conditions and replace the original bags by larger ones if necessary. Ensure unrestricted flow to these bags.

3. Attach a Cell Collection Bag of sufficient size to the free luer connector. Confirm that unrestricted flow to the Cell Collection Bag is possible.

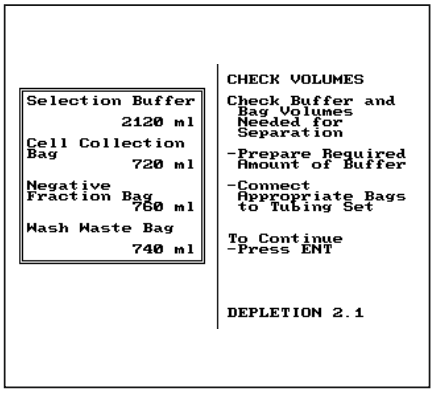
4. Check luer lock connections on the columns. Luer locks must be closed tightly.

5. If the replacement of the Priming Waste Bag is necessary, use the TSCD to replace the bag with a transfer bag of sufficient size.

To continue, press ENT

The program automatically continues with the installation instructions to install the tubing set on the instrument.

Proceed to STEP 3.



Screen 4-2.8: Check volumes

Important

- Ensure the required amount of buffer is available and the volume of the bags mentioned on Screen 4-2.8 is sufficient. Otherwise the performance of the depletion will be compromised.

- The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

- Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g., in the laminar flow hood.

- For further analysis or cell processing note that the volumes listed on Screen 4-2.8 are not the exact volumes but volumes within the safety requirements.

STEP 2:

DEPLETION 3.1

Switch-on of 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 start time of the instrument run.

The window will display Screen 1.1 as shown in chapter 1.

To proceed to the program menu, press ENT

Choice of separation program DEPLETION 3.1

The window will display Screen 4-2.1 as shown.

Choose DEPLETION 3.1.

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

Selection of the tubing set

The window will display Screen 4-2.2 as shown.

Confirm that the correct program has been chosen.

If not, press the "Undo" key (see Figure 1.4) to return to the previous step in order to amend the choice.

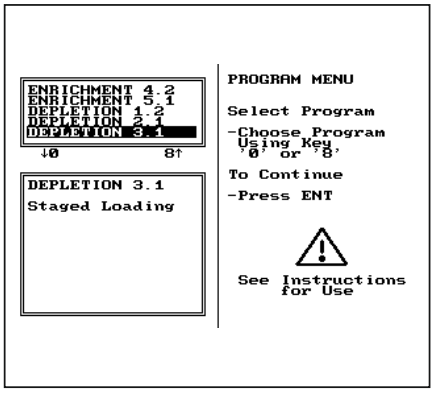
Select the tubing set by highlighting it in the box at the top left hand side with the black bar. Move the bar up and down by using the keys '0' and '8'.

The window below displays the relevant data like capacity and catalogue number (REF) of the selected tubing set.

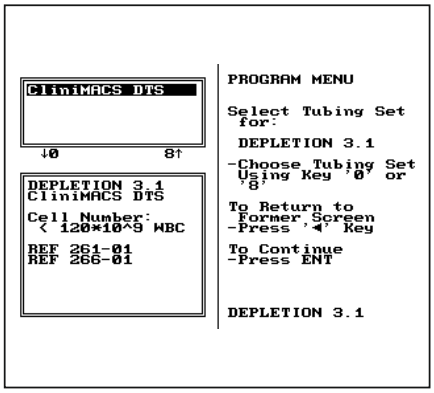
To continue with the selected combination of separation program and tubing set, press ENT

Important

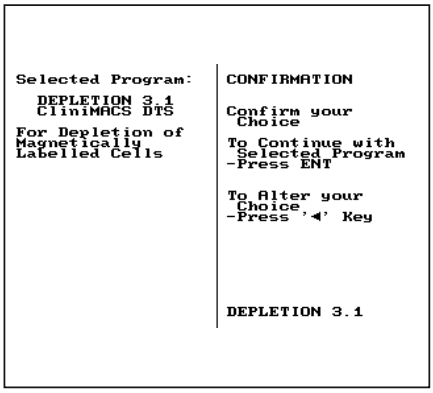
- The separation program DEPLETION 3.1 must only be used with the CliniMACS Depletion Tubing Set (REF 261-01).
- Screen prompts and diagrams serving as procedure guides will appear in the display window. Perform and check each step according to the manual instructions before proceeding to the next step.



Screen 4-2.1: Choice of separation program



Screen 4-2.2: Selection of the tubing set

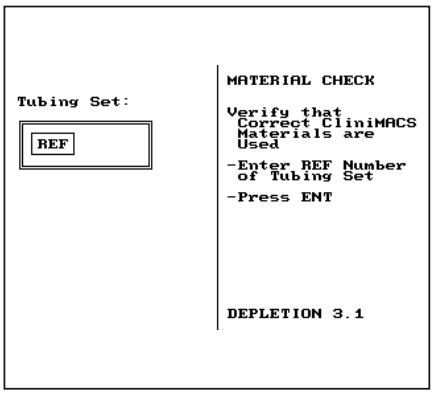


Screen 4-2.3: Confirmation

Confirmation

The window will display Screen 4-2.3 as shown.

To confirm the combination of separation program and tubing set and to proceed, press ENT



Screen 4-2.4: Material check

Material check

The window will show Screen 4-2.4 as shown.

The query of the catalogue number of the tubing set serves as a security check to ensure that the tubing set the operator selected can be used.

Enter the catalogue number of the selected tubing set and proceed with pressing ENT

If a non-corresponding catalogue number has been entered, a message appears. To confirm press 'ENT' and enter the correct catalogue number. If the entered catalogue number is still wrong, the message appears a second time. After pressing 'ENT' the program will return to the program menu (see Screen 4-2.2).

Sample parameter input

If the material check has been successful, the program continues automatically with the query for different sample parameters that are necessary to adjust the separation to each individual sample and to provide the operator important information for required buffer and bag volumes.

The window will display Screen 4-2.5 as shown.

1. Start by entering the WBC concentration of the sample. The acceptable range is shown in the box on the bottom left hand side. Press the "Undo" key to correct a wrong input.

   Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see "Important").

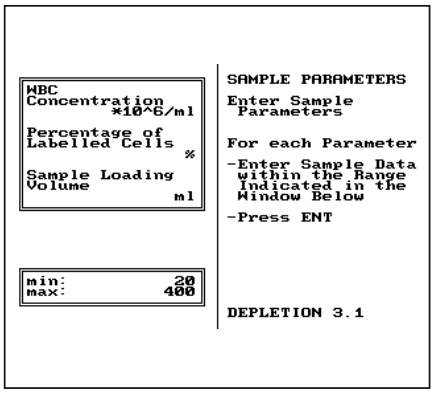
   To continue with the next input, press ENT

2. Enter the percentage of labeled cells. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see "Important").

   To continue with the next input, press ENT

3. Enter the final volume of the cell sample. Confirm that the value entered reflects the value of the sample. Otherwise sample processing may not be optimal (see "Important").

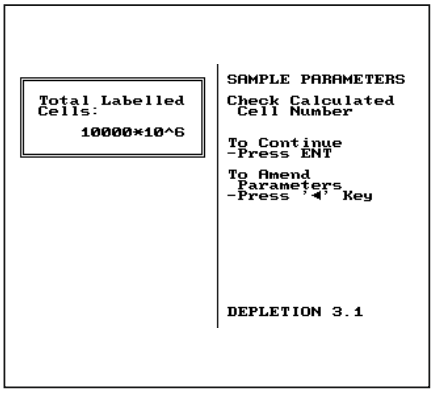
   To continue, press ENT



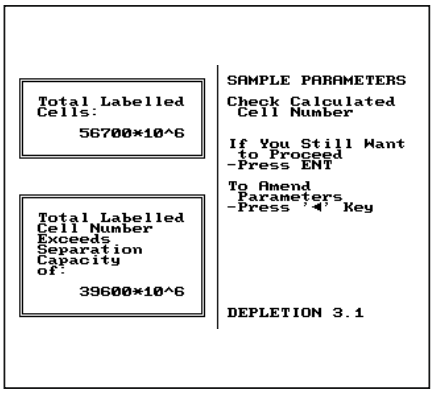
Screen 4-2.5: Sample parameter input

Important

- All sample parameters required refer to the sample **post** cell labeling and washing procedure.
- The acceptable range shown in the box on the bottom left hand side always takes account of the previous input. After each input the software calculates and adjusts the accepted range of the next query to ensure the separation capacity of the system will not be exceeded.
- **Specifying wrong parameters may result in non-optimal sample processing and may increase the risk of lower depletion efficiency.** Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual sample values may result in lower depletion efficiency due to the system being overloaded. Entering a lower sample volume may result in cell loss due to overloading or even to incomplete sample processing. If the specification of any of the three parameters is too high, processing time will increase and the volume of the target cell fraction will enlarge.



Screen 4-2.6: Calculation



Screen 4-2.7: Calculation: Cell number exceeds separation capacity

Calculation

The CliniMACS Plus Software calculates the total number of labeled cells in order to check whether the separation capacity is sufficient.

The result is shown on one of two possible screens.

- The result of the calculation is shown on a screen similar to Screen 4-2.6. Ensure the calculated number of labeled cells is correct.

  To continue, press ENT

  If the calculated number of labeled cells is not correct, a correction of the data input is possible. Press the "Undo" key to return to the previous Screen 4-2.5 for correction.

- If the calculated number of labeled cells exceeds the separation capacity of the CliniMACS Plus System a screen similar to Screen 4-2.7 is shown. Ensure the correct sample parameters have been entered and the calculated number of cells is correct. Press the "Undo" key to return to the previous Screen 4-2.5 and to correct the sample parameters.

  Consider that depletion conditions outside the specifications for tubing set and separation program result in lower depletion efficiency.

  If the operator do not want to overload the system, split the sample and determine the volume of each portion. Return to the previous Screen 4-2.5 by pressing the "Undo" key. Enter the sample parameters for the first aliquot of the sample and continue with the separation of this portion. After the separation has been finished, start a second separation with the second aliquot using a new tubing set.

  To proceed with this sample, press ENT

Volume information

From the total number of labeled cells (see previous page) the software calculates the number of separation stages, the amount of buffer needed for the entire depletion and the liquid volumes that will be collected in the Cell Collection Bag, Non-Target Cell Bag, Buffer Waste Bag, and Reapplication Bag.

If one of the calculated values exceeds the standard values, a screen similar to Screen 4-2.8 appears to inform about the amount of liquid that will be required and accumulated during the separation process. Replace the bags delivered with the tubing set with alternative bags of the appropriate size, if replacement is necessary. The bags originally attached to the tubing set are connected by luer connections. Bags that replace the original bags should have a female luer connector. Standard bags can be connected by a Luer/Spike Interconnector.

1. Confirm the requested amount of buffer is available. Do not attach more than three liters of buffer on the bag hanger (see "Important", bullet 2).

2. Open the tray of the tubing set under sterile conditions and replace the original bags by larger ones or attach aditional bags if necessary. Ensure unrestricted flow to these bags.

3. If replacement is neccessary, replace the Cell Collection Bag of the tubing set with a transfer bag of sufficient size. Determine the weight of the new, empty Cell Collection Bag and record it. Confirm that unrestricted flow to the Cell Collection Bag is possible. Alternatively attach an additional 600 mL transfer bag to the first Cell Collection Bag, using a plasma transfer set.

4. Check luer lock connections on the columns. Luer locks must be closed tightly.

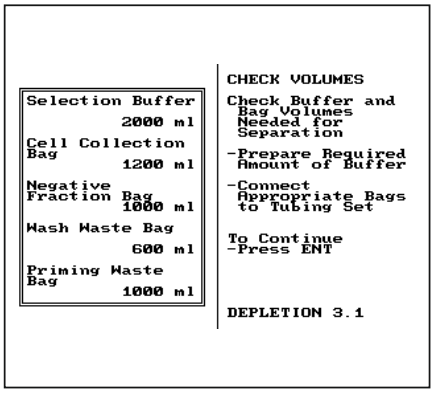
To continue, press ENT

The program continues with the installation instructions to install the tubing set on the instrument.

Proceed to STEP 3.

Important

- Negative Fraction Bag corresponds to Non-Target Cell Bag.
- Priming Waste Bag corresponds to Reapplication Bag.



Screen 4-2.8: Check volumes

Important

- Ensure the required amount of buffer is available and the volume of the bags mentioned on Screen 4-2.8 is sufficient. Otherwise the performance of the separation will be compromised.
- The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.
- Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, e.g., in the laminar flow hood.
- For further analysis or cell processing note that the volumes listed on Screen 4-2.8 are not the exact volumes but maximal volumes.

STEP 3:

CliniMACS® Tubing Set and CliniMACS Tubing Set LS

Preparation for tubing set installation

The window will display Screen 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 each provided in sealed, sterilized packages. Each tubing set contains preassembled tubing and columns for one cell separation (see Figure 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. Unpack the sterile tubing set under sterile conditions (e.g. laminar flow hood). Remove the pinch clamp used for packaging of the CliniMACS Tubing Set LS before usage of the tubing set. The clamp is labeled with "Remove before assembly to CliniMACS® Plus Instrument".

2. Check luer lock connections to bags. Luer lock must be closed tightly.

Attach Cell Collection Bag

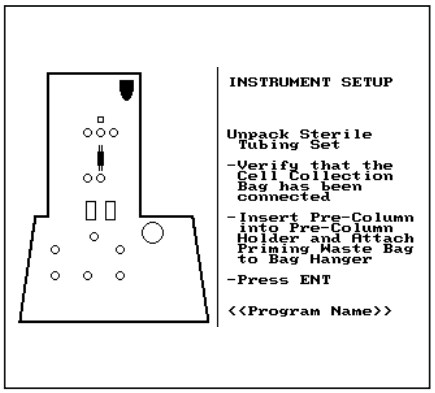
1. Note the weight of the empty Cell Collection Bag.

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.

   If more than one Cell Collection Bag is necessary for a separation, connect the bags using a plasma transfer set. Make sure that all connections are closed tightly.

3. Confirm that unrestricted flow to the Cell Collection Bag is possible.

4. Proceed with the installation of the tubing set using either the clean room procedure described below, or the "Alternative installation for the CliniMACS Tubing Sets".



Screen 4-3.1: Unpack tubing set

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 (see Figure 1.4) can be pushed to return to the previous step.

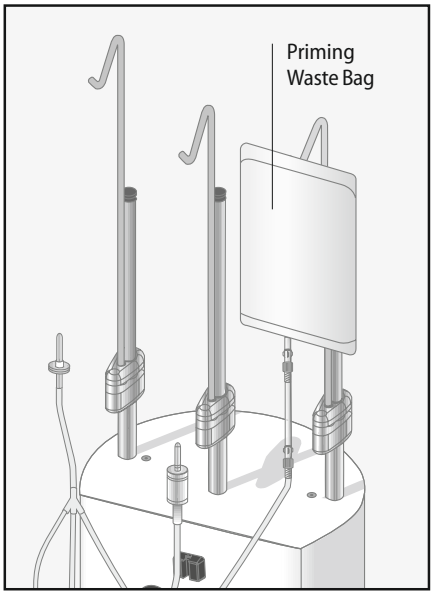


Figure 4-3.1: Attach Priming Waste Bag to bag hanger

Note

- The bag hangers are designed 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 the operator.

Attach Priming Waste Bag and insert pre-column

The window will display Screen 4-3.1 as shown.

1. Attach the Priming Waste Bag to the right hand bag hanger on the instrument as shown (see Figure 4-3.1).

2. Place the pre-column into the holder as shown (see Figure 4-3.5).

3. Adjust the height of the bag hanger. Raise or lower the bag hanger to accommodate the height to 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 ENT

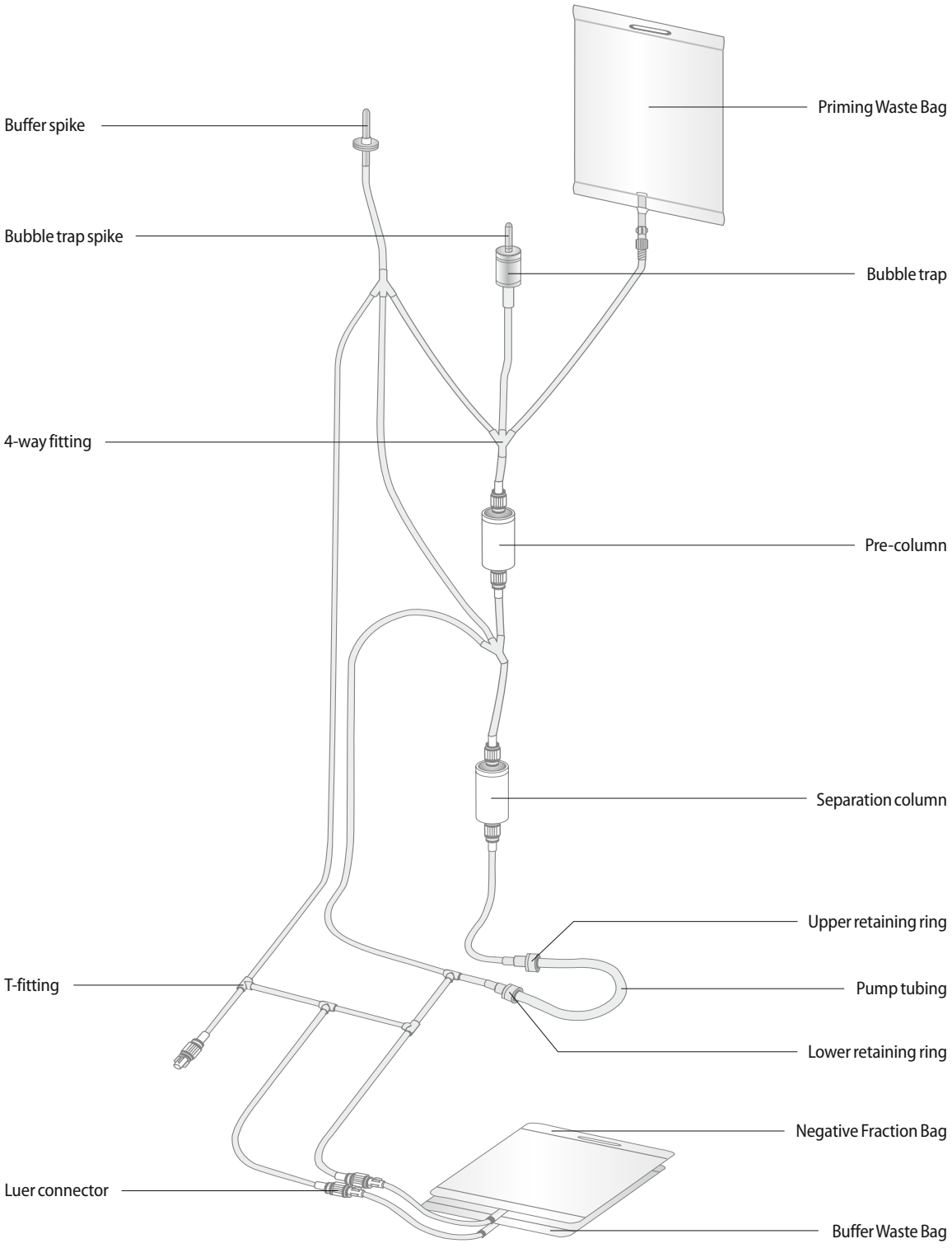
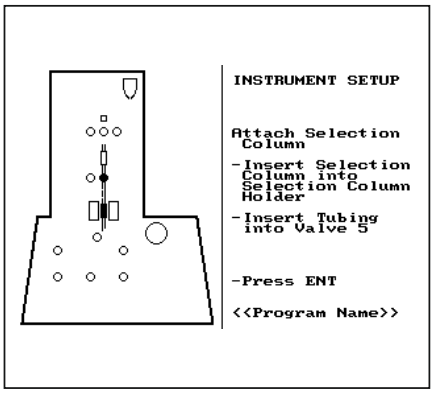


Figure 4-3.2: General construction of a CliniMACS Tubing Set (e.g. CliniMACS Tubing Set, REF 161-01)

Insert separation column and load valve no. 5



Screen 4-3.2: Insert separation column and load valve no. 5

The window will display Screen 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 Figure 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.

To proceed, press ENT

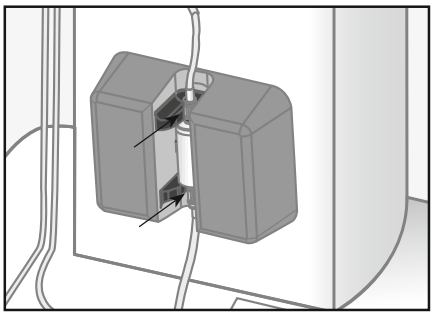


Figure 4-3.3: Separation column in separation column holder

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.
- 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 Figure 4-3.4).

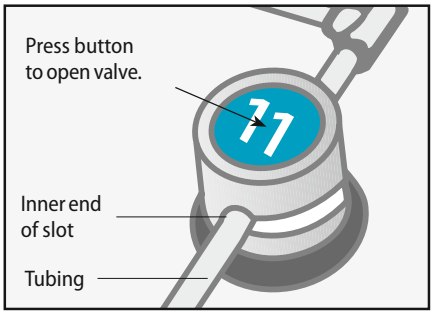
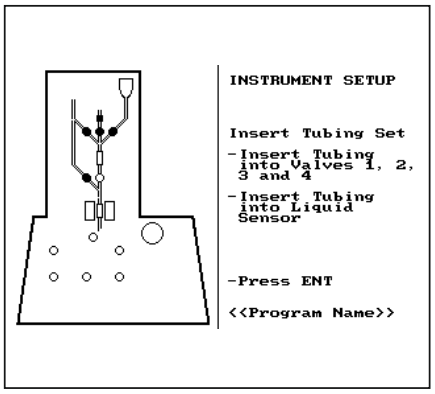


Figure 4-3.4: Correctly inserted tubing

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

The window will display Screen 4-3.3 as shown.



Screen 4-3.3: Load valves nos. 1, 2, 3, 4, and liquid sensor

1. Load the tubing into valve no. 4. Confirm that the tubing is placed securely in the valve opening (see Figure 4-3.4). Pay particular attention to the area between valves nos. 4 and 5 (see Figure 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 (see Figure 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 (see Figure 4-3.5). Confirm that the tubing is placed correctly into the sensor fitting.

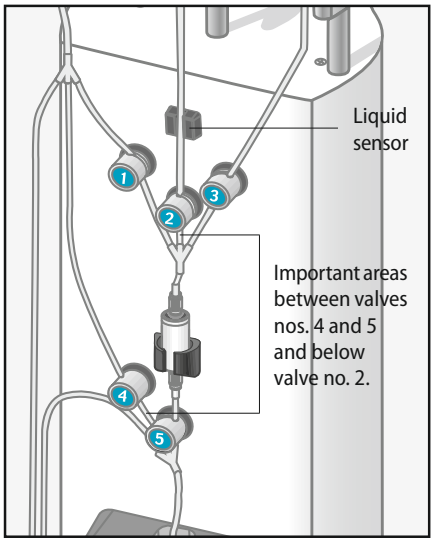
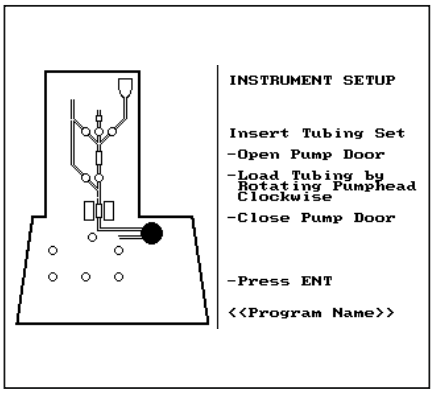


Figure 4-3.5: Tubing in valves

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 4-3.4: Load pump tubing

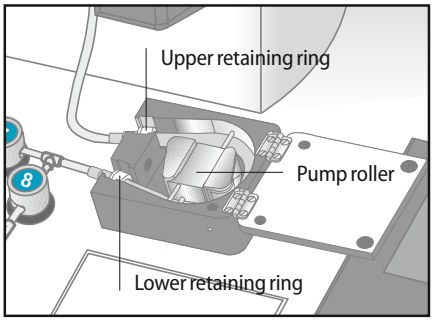


Figure 4-3.6: Load pump tubing

Load pump tubing

The window will display Screen 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 on the pump housing (see Figure 4-3.6).
3. Rotate the pump roller clockwise (see Figure 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 neccessary, 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 on the pump housing (see Figure 4-3.6).
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely.
6. Close the pump door.

To proceed, press ENT

Note

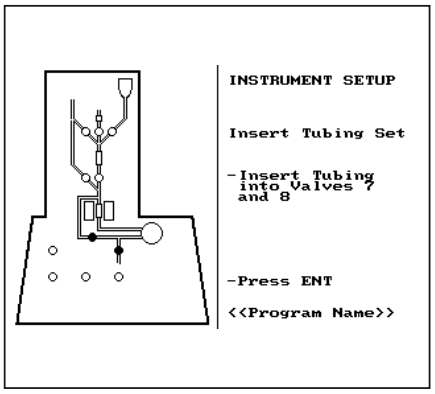
During the cell separation program the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

Load valves nos. 7 and 8

The window will display Screen 4-3.5 as shown.

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

To proceed, press ENT



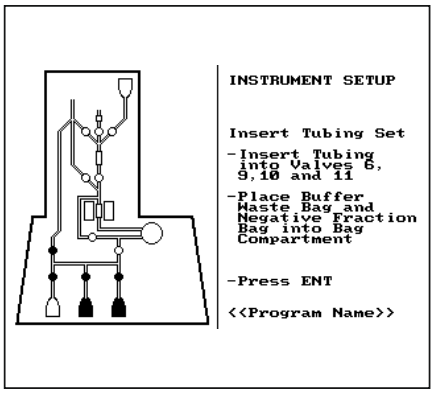
Screen 4-3.5: Load valves nos. 7 and 8

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

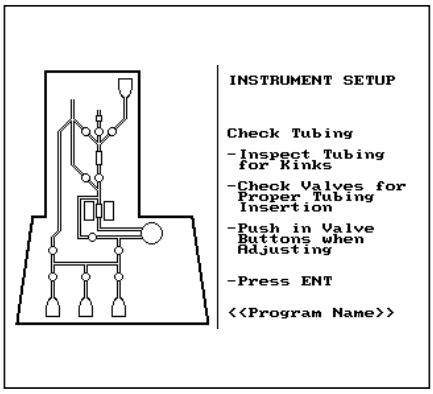
The window will display Screen 4-3.6 as shown.

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 4-3.6: Load valves nos. 6, 9, 10, and 11



Screen 4-3.7: Recheck all tubing and attachments

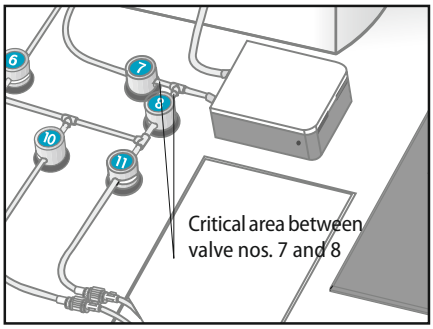
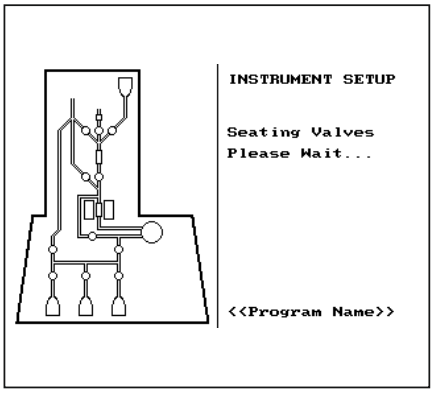


Figure 4-3.7: Tubing in valves



Screen 4-3.8: Seating of valves

Recheck all tubing and attachments

The window will display Screen 4-3.7 as shown.

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 (see Figure 4-3.7), and between valves nos. 4 and 5 (see Figure 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 4-3.8 as shown.

In order to ensure the proper fit of tubing in the valves, the 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 (chapter 5). This step can be repeated by using the "Undo" key followed by the "Enter" key (see Figure 1.4).

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

Attach CliniMACS® PBS/EDTA Buffer

The window will display Screen 4-3.9 as shown.

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

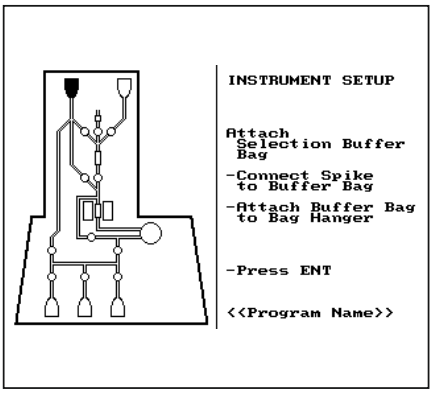
1. Using aseptic techniques, remove the cap from the buffer spike on the tubing set (see Figure 4-3.2) and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.

   If more than one liter buffer is necessary for a separation, connect two buffer bags using a plasma transfer set.

2. Attach the buffer bag to the buffer bag hook on the bag hanger (see Figure 4-3.8).

3. Adjust the height of the buffer bag hanger. Raise or lower the bag hanger to accommodate the height to 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 Figure 4-3.8).

To proceed, press ENT



Screen 4-3.9: Attach buffer bag

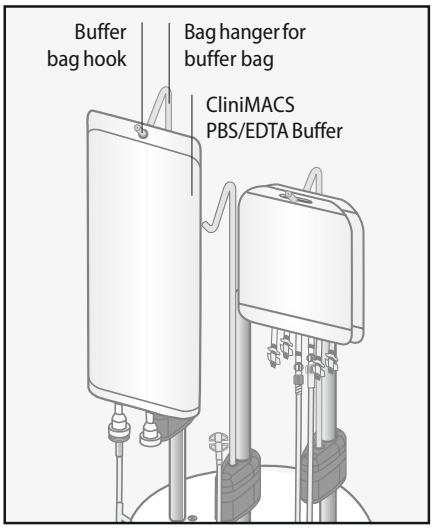
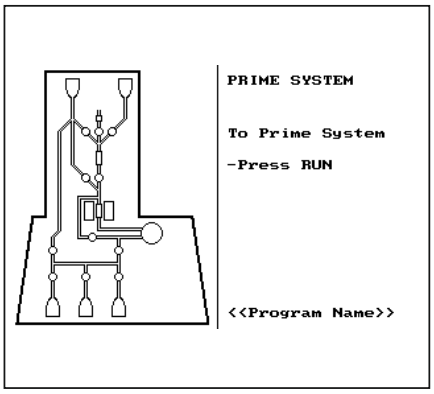


Figure 4-3.8: Attach buffer bag

Note

The bag hangers are made for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

Start priming



Screen 4-3.10: Start priming

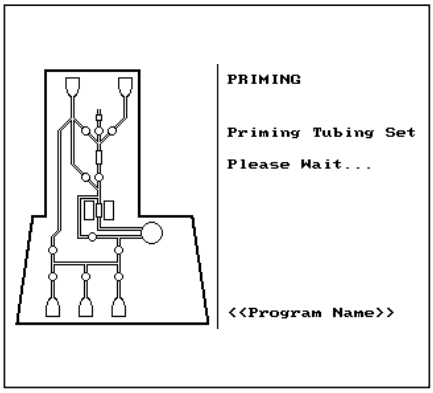
The window will display Screen 4-3.10 as shown.

To start priming, press RUN

The window will display Screen 4-3.11 as shown.

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 and the Buffer Waste Bag (see Figure 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.

Check during the priming



Screen 4-3.11: Priming in process

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.

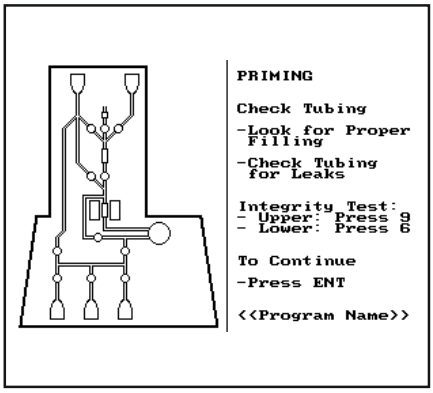
If leaks or malfunctions are observed, stop run by pressing 'STOP'. The operator will have 600 seconds to resolve the problem. Restart the process by pressing the 'RUN'.

After 600 seconds, the separation will be aborted. If the operator 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



Screen 4-3.12: Final check of tubing

The window will display Screen 4-3.12 as shown.

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.

DO NOT press **'ENT'** yet.

Integrity test

For additional safety, an integrity test must be performed to test the tubing set for leaks. The test sequence consists of two automated sequences, which allow both the upper and the lower parts of the tubing set to be over pressurized and 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 4-3.12.

2. After performing the "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 4-3.13 as shown.

5. To start the test sequence, press RUN

   To return to Screen 4-3.12, press ◀

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 4-3.14 as shown.

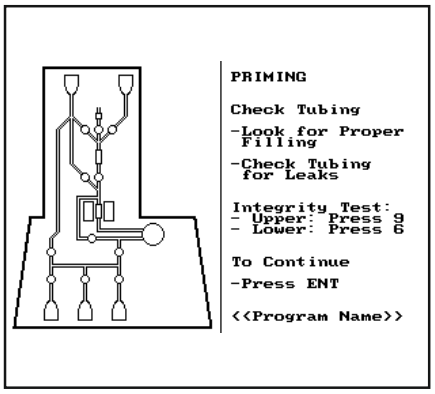
   Overpressure will be created and held for two minutes. During this time the operator should watch the connections above and under the pre-column and separation column, and the upper pump tube connection.

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

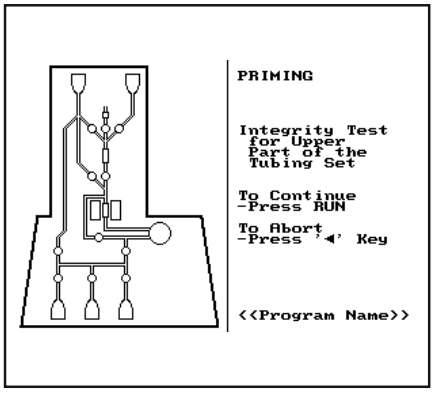
7. After 2 minutes the pressure is automatically released, and the window displays Screen 4-3.12.

   Using tissue the operator should check to determine 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. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

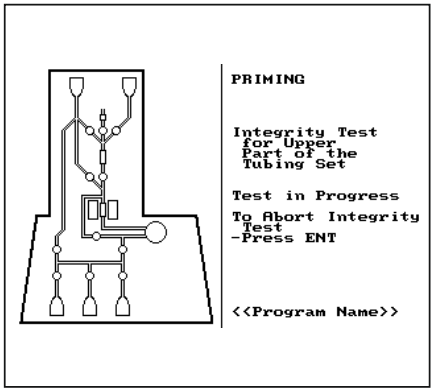
8. If no leaks are observed, continue with the integrity test of the lower part of the tubing set.



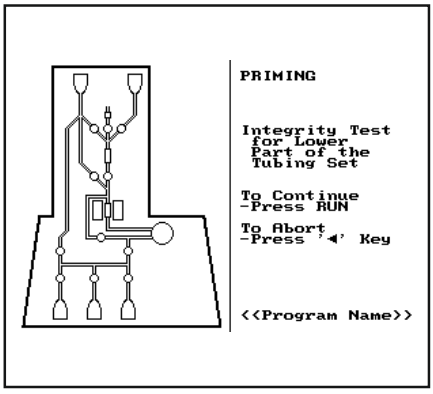
Screen 4-3.12: Final check of tubing



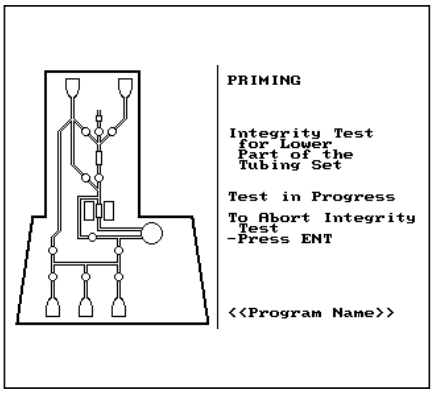
Screen 4-3.13: Start integrity test for upper part



Screen 4-3.14: Integrity test for upper part in process



Screen 4-3.15: Start integrity test for lower part



Screen 4-3.16: Integrity test for lower part in progress

Integrity test for the lower part of the tubing set

1. The window displays Screen 4-3.12.

2. To enter the integrity testfor the lower part, press [6]
   DO NOT press 'ENT'.

3. The window will display Screen 4-3.15 as shown.

4. To start the test sequence, press [RUN]

   To return to Screen 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 4-3.16 as shown.

   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 4-3.12.

   Using tissue the operator should check to determine 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. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

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 4-3.17 as shown.

After the priming phase has been completed and no leaks or malfunctions are observed, the Cell Preparation Bag can be attached (see Figure 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 of the bubble trap (see Figure 4-3.2).

2. Remove the cap from the lower opening of the pre-system filter (see Figure 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 (see Figure 4-3.9).

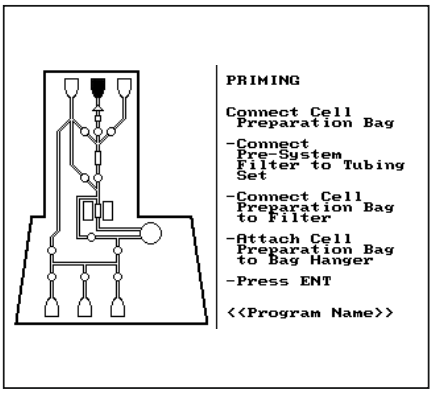
4. Spike the Cell Preparation Bag with the pre-system filter (see Figure 4-3.9) ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.

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.

7. Adjust the bag hanger for the Cell Preparation Bag to hold the Cell Preparation Bag in an upright position.

To proceed, press ENT



Screen 4-3.17: Connect Cell Preparation Bag

Final check of the liquid sensor

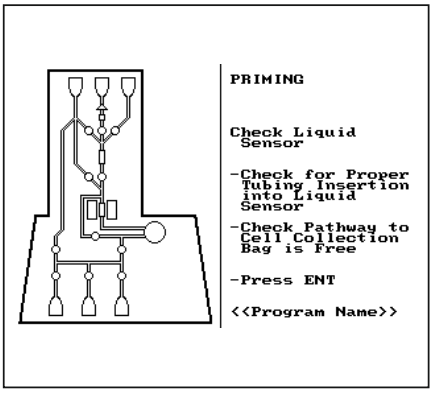
The window will display Screen 4-3.18 as shown.

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. Confirm that the unrestricted flow to the Cell Collection Bag is possible.

To proceed, press ENT

Proceed to STEP 4.



Screen 4-3.18: Final check of the liquid sensor

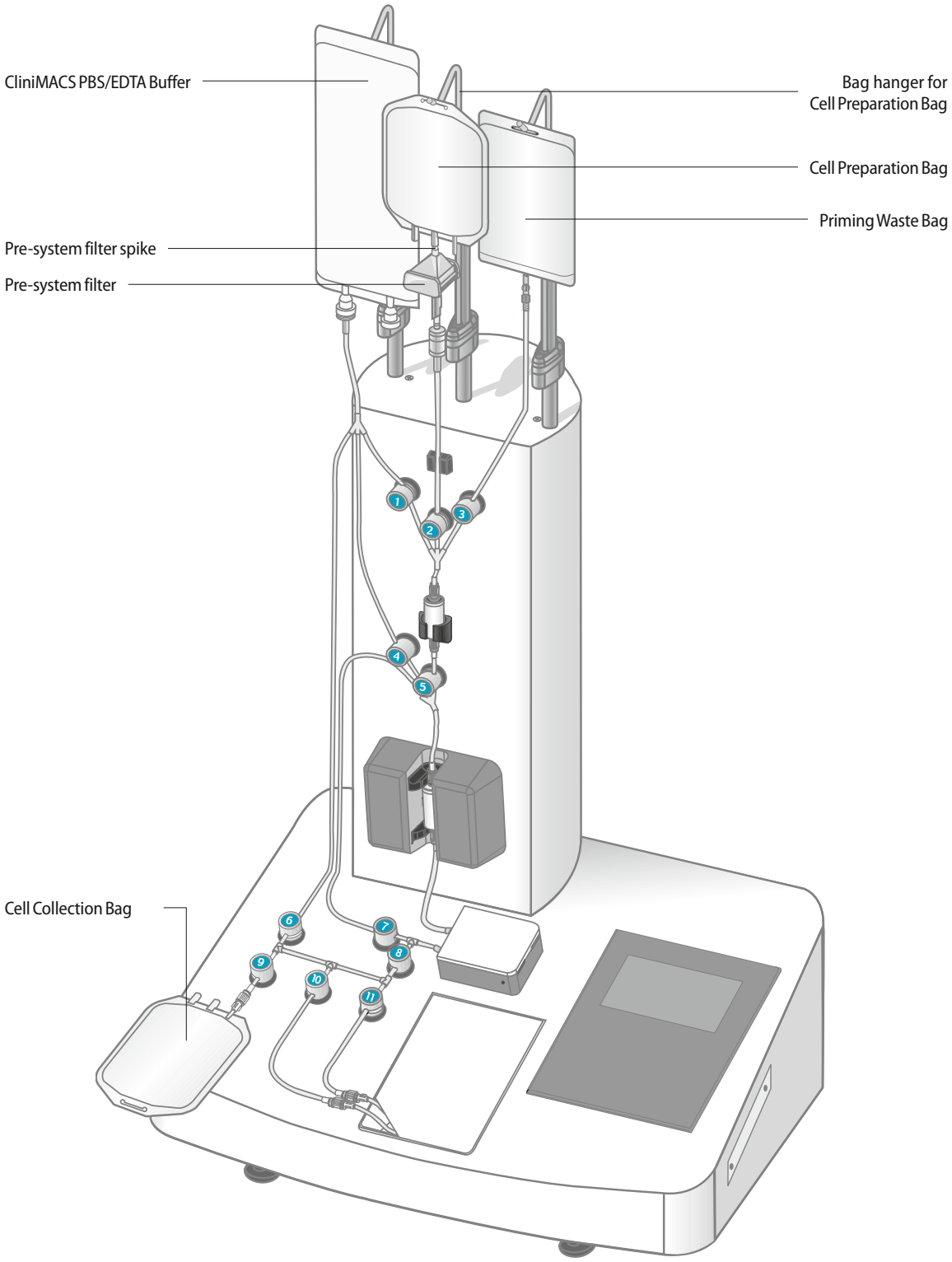


Figure 4-3.9: CliniMACS Plus Instrument with CliniMACS Tubing Set, CliniMACS PBS/EDTA Buffer, Cell Preparation Bag, and Cell Collection Bag

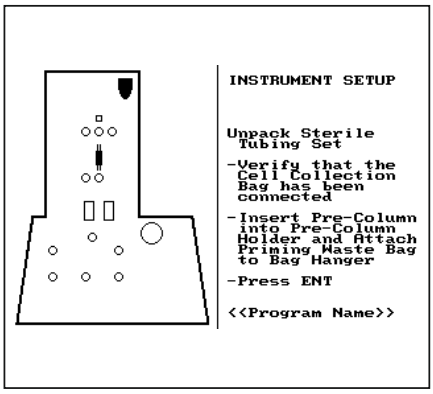
Alternative installation of CliniMACS® Tubing Sets

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 Plus 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 may be compromised during the attachment of the Cell Collection Bag, the CliniMACS PBS/EDTA Buffer, the pre-system filter, and the Cell Preparation Bag. To ensure that the system remains sterile, these components must 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 must 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 4-3.1: Unpack tubing set

Preparation for tubing set installation

The window will display Screen 4-3.1 as shown.

As described, the Cell Collection Bag, the CliniMACS PBS/EDTA Buffer, the pre-system filter, and the Cell Preparation Bag must be attached to the tubing set 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 locking forceps in order to prevent the buffer from flowing into the tubing set during its installation (see (1), Figure 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 locking forceps (see (2), Figure 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**

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

To proceed, press ENT

Follow the instructions:

- **Attach Priming Waste Bag and insert pre-column**
- **Insert separation 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**

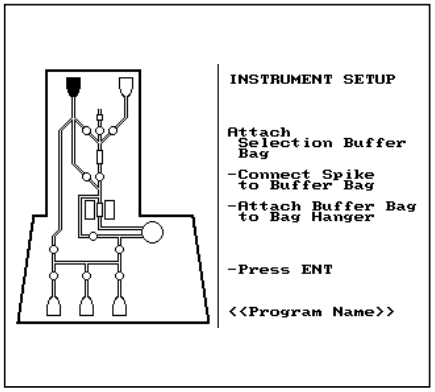
Attach CliniMACS® PBS/EDTA Buffer

The window will display Screen 4-3.9.

1. The buffer bag was attached during „Preparation for tubing set installation". Therefore only the height of the buffer bag hanger may need 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 liquid flow, and low enough to avoid stretching the tubing or connections.

2. Remove the locking forceps from the tubing just below the buffer spike.

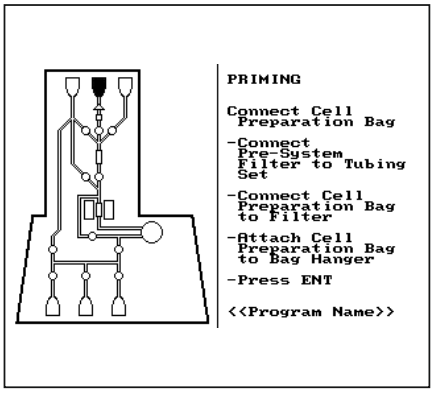
To proceed, press ENT



Screen 4-3.9: Attach buffer bag

Follow the instructions:

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



Screen 4-3.17: Connect Cell Preparation Bag

Connect Cell Preparation Bag (& pre-system filter)

The window will display Screen 4-3.17 as shown.

1. The Cell Preparation Bag and the pre-system filter were attached during „Preparation for tubing set installation".

2. Remove the locking forceps below the bubble trap.

To proceed, press ENT

Follow the instructions:

Final check of the liquid sensor

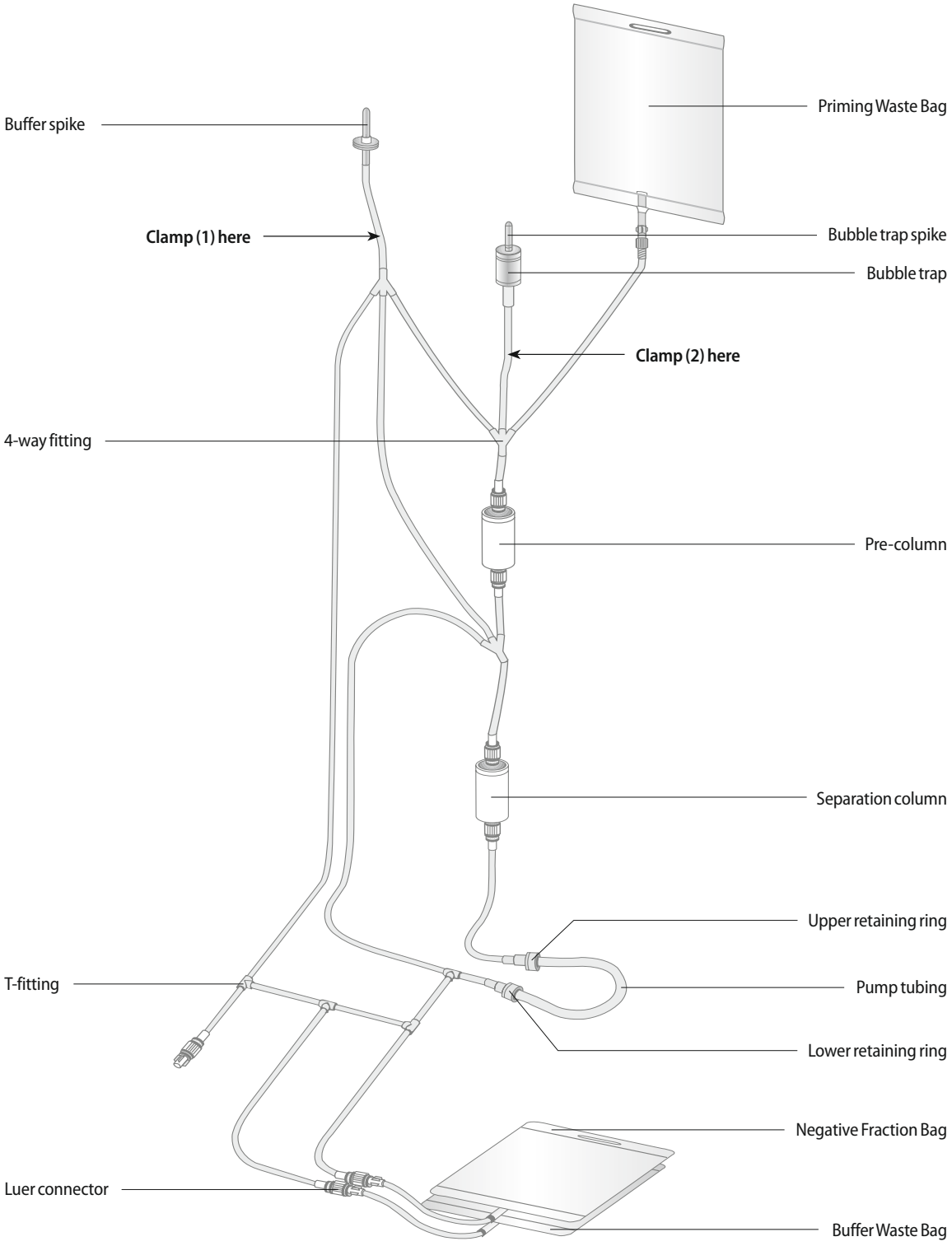


Figure 4.3-10: General construction of a CliniMACS Tubing Set (e.g. CliniMACS Tubing Set, REF 161-01)

STEP 3
Installation of the CliniMACS® Depletion Tubing Set

Follow the installation instructions given on the screens of the CliniMACS® Plus Instrument.

(1) Install upper part.
(2) Install lower part.

Proceed to the priming procedure.

(3) Perform integrity test.

WARNING:

- **To perform the integrity test, clamp the tubing below the Non-Target Cell Bag, before starting the integrity test.**
- **Remove the clamp after completion of the integrity test.**

(4) Connect the Cell Preparation Bag:

- Firmly insert the spike of the pre-system filter into the tubing set,
- Connect pre-system filter and spike connector,
- Spike Cell Preparation Bag,
- Ensure that the connections are secure and that free liquid flow is possible.

(5) Level bags:

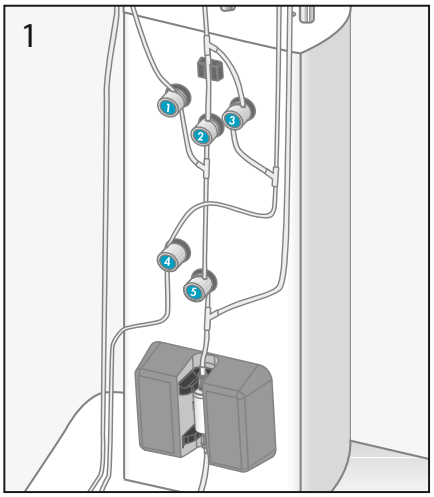
VERY IMPORTANT

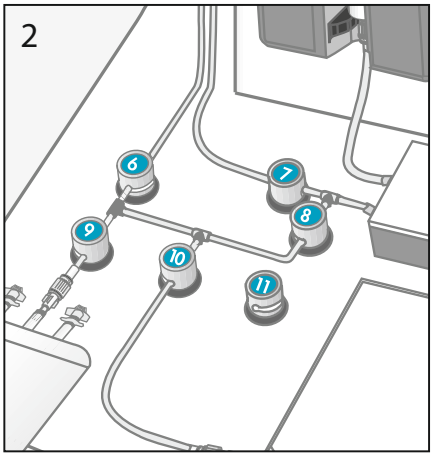
Ensure that the buffer bag, the Cell Preparation Bag, the Non-Target Cell Bag, and the Reapplication Bag are leveled correctly as displayed and that the bags and tubings are neither stretched nor bent.

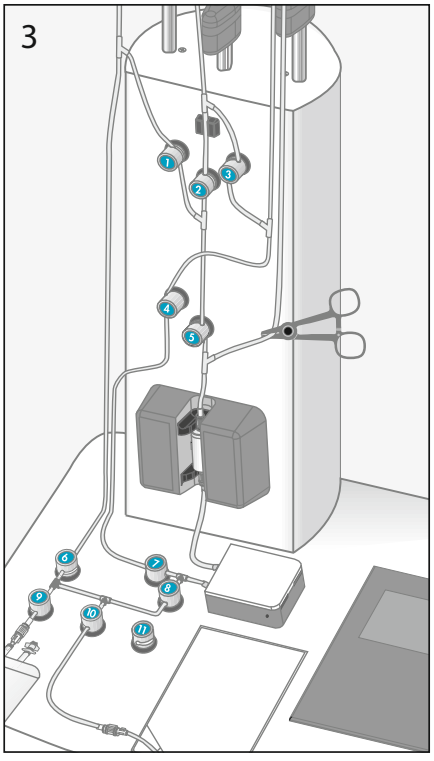
Highest position: Buffer bag

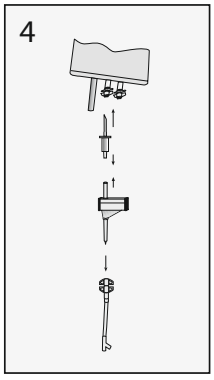
Middle Position: Reapplication Bag and Non-Target Cell Bag

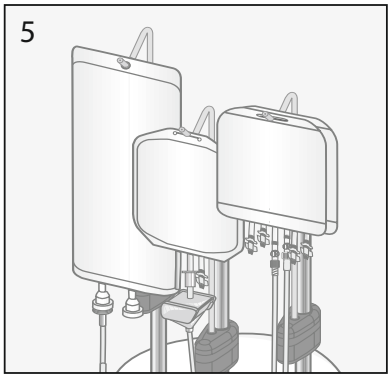
Lowest position: Cell Preparation Bag





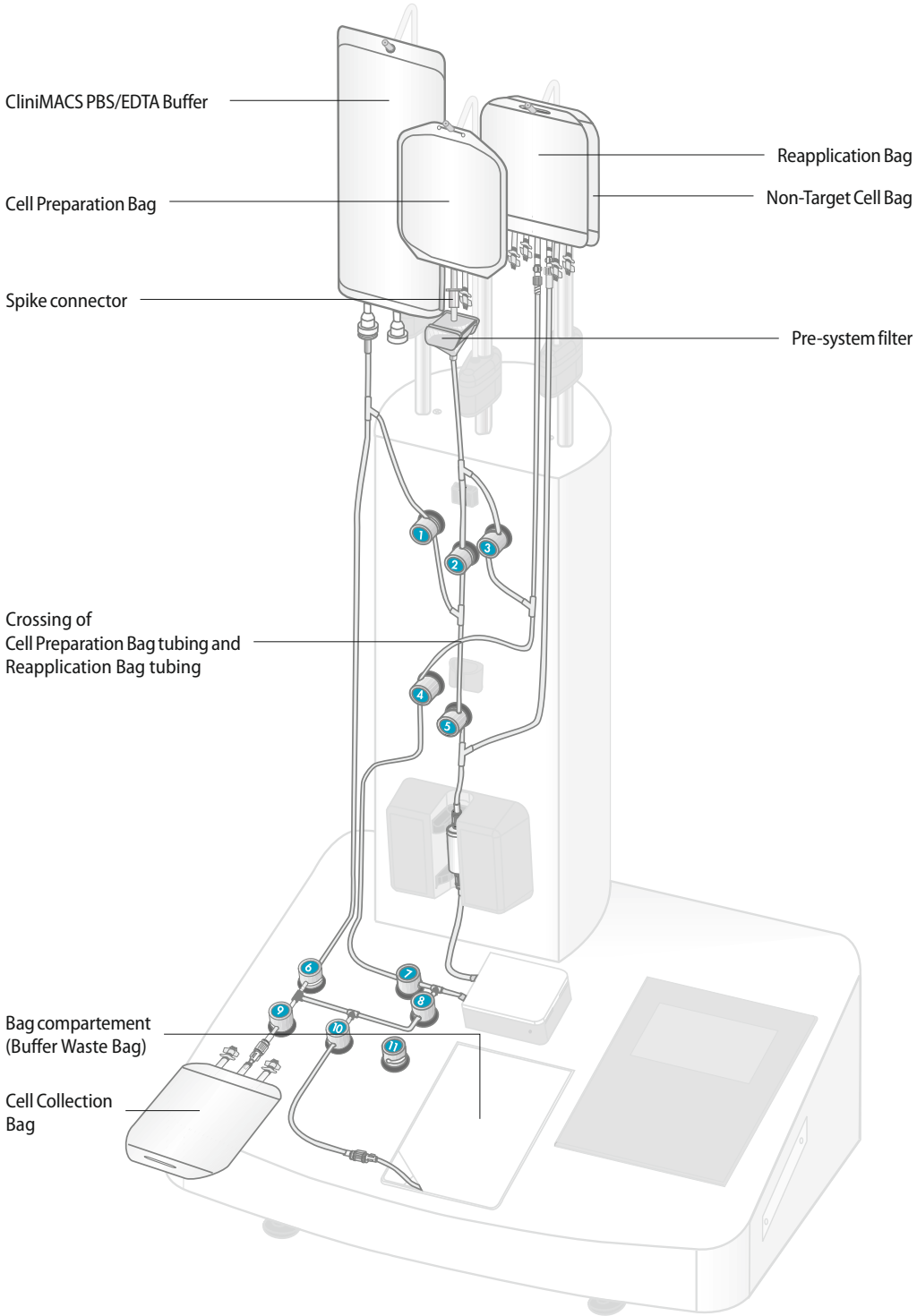






CliniMACS® Depletion Tubing Set

Installed on the CliniMACS® Plus Instrument



STEP 3:

CliniMACS® Depletion Tubing Set

Preparation for tubing set installation

The window will display Screen 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® Depletion Tubing Set is provided in a sealed, sterilized package. Each tubing set contains preassembled tubings, column and bags for one cell separation (see Figure 4-3.3). When the packaging is intact, a sterile fluid path is provided.

1. Record the lot number and use-by date of the tubing set. Unpack the sterile tubing set under sterile conditions (e.g. laminar flow hood). Remove the pinch clamp used for packaging before usage of the tubing set. The clamp is labeled with "Remove before assembly to CliniMACS® Plus Instrument".

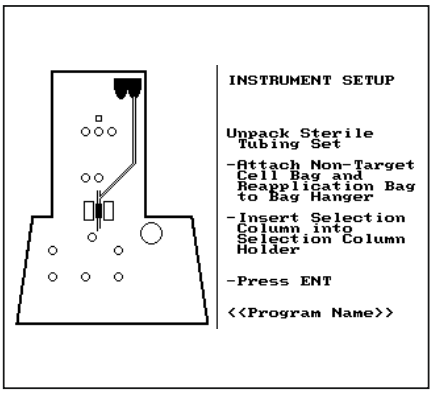
2. Check luer lock connections to bags. Luer lock must be closed tightly.

Cell Collection Bag

The CliniMACS Depletion Tubing Set is provided with an attached Cell Collection Bag.

The weight of the empty Cell Collection Bag attached is 32 g. In case of bag replacement, determine and note the weight of the new empty bag. Attach the sterile Cell Collection Bag to the luer connector on the tubing set before loading the tubing set onto the CliniMACS Plus Instrument.

If more than one Cell Collection Bag is necessary for a separation, connect the bags using a plasma transfer set. Make sure that all connections are closed tightly.



Screen 4-3.1: Unpack tubing set

Note

- The CliniMACS Plus Instrument shows the chosen program name, e.g., DEPLETION 3.1, in the bottom line.

- At any step during the tubing set installation the "Undo" key (see Figure 1.4) can be pushed to return to the previous step.

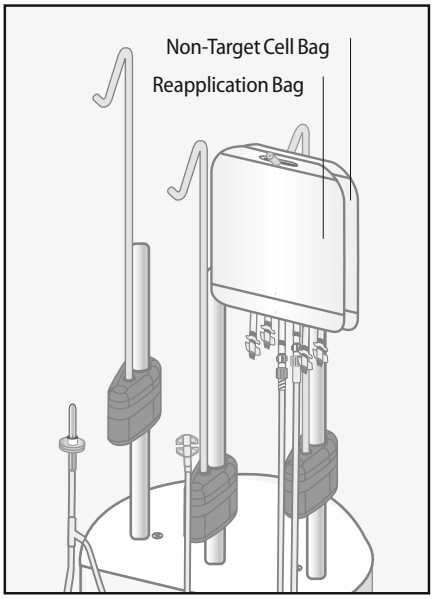


Figure 4-3.1: Attach Non-Target Cell Bag and Reapplication Bag to bag hanger

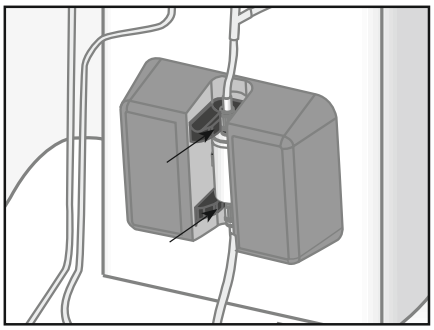


Figure 4-3.2: Separation column in separation column holder

Note

The bag hangers are designed for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

1. Attach the Non-Target Cell Bag and the Reapplication Bag to the right hand bag hanger on the instrument as shown (see Figure 4-3.1).

2. Adjust the height of the bag hangers. Raise or lower the bag hangers to accommodate the height to the size of the Non-Target Cell Bag and Reapplication Bag. Ensure that they are 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.

3. Insert the separation column into the separation column holder as shown (see Figure 4-3.2).

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.

To proceed, press ENT

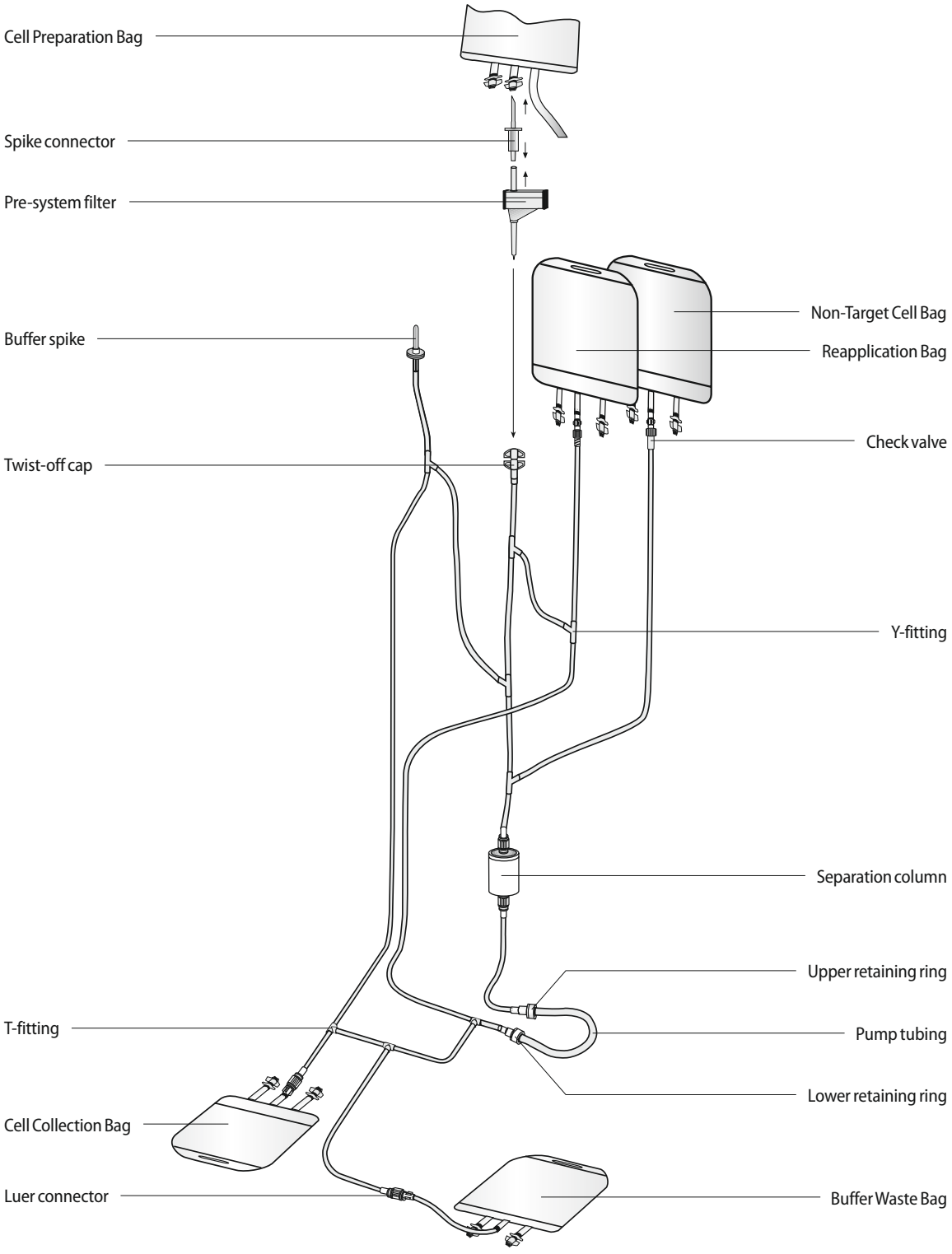
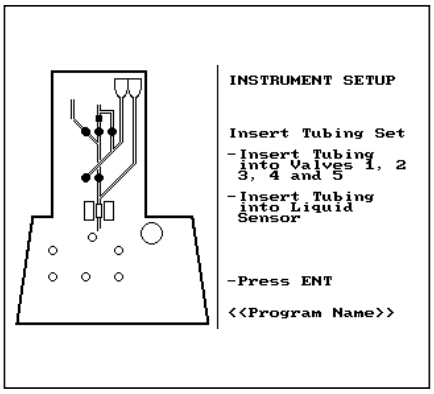


Figure 4-3.3: General construction of a CliniMACS Depletion Tubing Set (REF 261-01)



Screen 4-3.2: Load valves nos. 1, 2, 3, 4, and 5

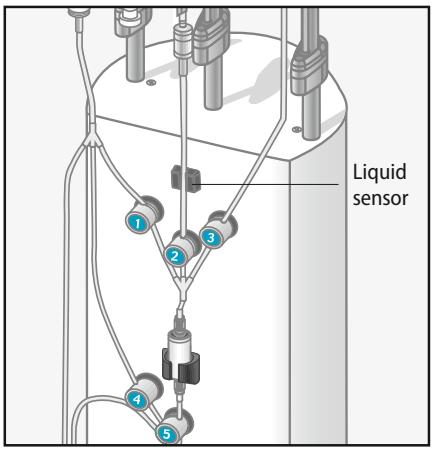


Figure 4-3.4: Tubing in valves

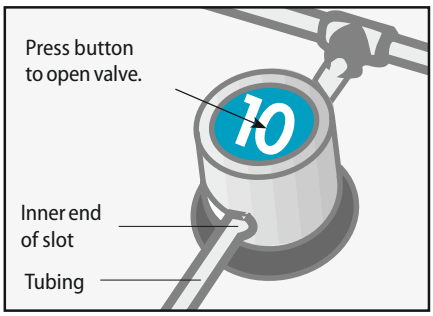


Figure 4-3.5: Correctly inserted tubing

Load valves nos. 1, 2, 3, 4, and 5

The window will display Screen 4-3.2 as shown.

The valves shown on the screen will be opened automatically.

1. Load the tubing into valves nos. 1, 2, 3, 4, and 5.

2. Mount the tubing between valve no. 2 and the twist-off cap into the liquid sensor (see Figure 4-3.4). Ascertain that the tubing is placed correctly into the sensor fitting.

Note

To ensure 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

Note

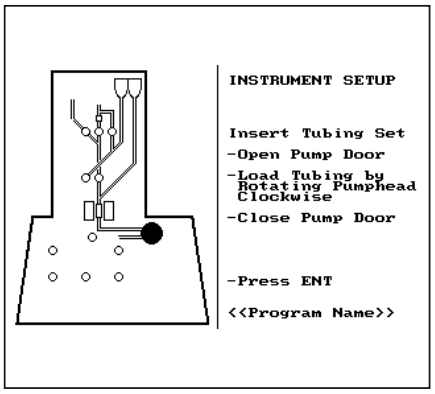
- As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly.

- 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 Figure 4-3.5).

Load pump tubing

The window will display Screen 4-3.3 as shown.



Screen 4-3.3: Load pump tubing

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 on the pump housing (see Figure 4-3.6).
3. Rotate the pump roller clockwise (see Figure 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 neccessary, the tubing can be unloaded by lifting the lower ending and turning the pump roller anti-clockwise.)
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove on the pump housing (see Figure 4-3.6).
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely.
6. Close the pump door.

To proceed, press ENT

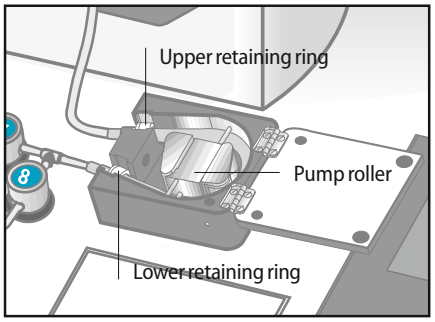
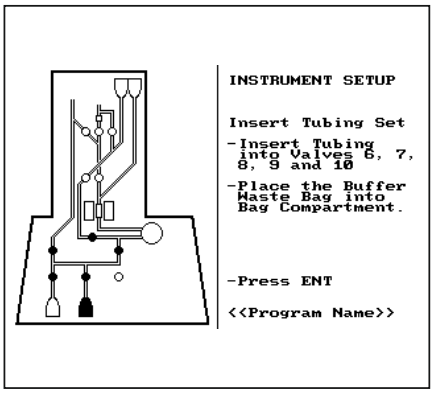


Figure 4-3.6: Load pump tubing

Note

During the cell separation 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.

Load valves nos. 6, 7, 8, 9, and 10



Screen 4-3.4: Load valves nos. 6, 7, 8, 9, and 10

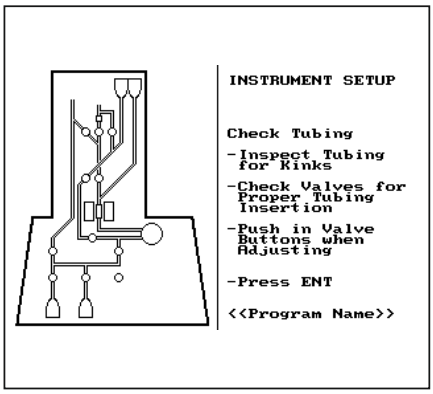
The window will display Screen 4-3.4 as shown.

1. Load the tubing into valve nos. 6, 7, 8, 9, and 10. Ascertain that the tubing is placed securely in the valve opening.

2. Place the Buffer Waste Bag in the bag compartment. Make sure the tubing is not compressed under the bag compartment lid.

To proceed, press ENT

Recheck all tubing and attachments



Screen 4-3.5: Recheck all tubing and attachments

The window will display Screen 4-3.5 as shown.

1. Beginning with valve no. 1, verify that the tubing fits properly and is positioned in each valve correctly.

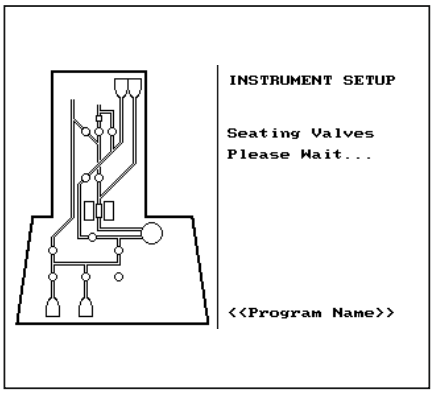
2. Reinspect the tubing in each valve. Be certain that the tubing enters and leaves each valve through the enlargement at the inner end of the slot and is positioned in the center of the jaws of the valve (see Figure 4-3.5). Check that the tubing is not kinked or twisted and does not show any tendency to move away from the center of the pinch valve.

Note

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.

To proceed, press ENT

Seating of valves



Screen 4-3.6: Seating of valves

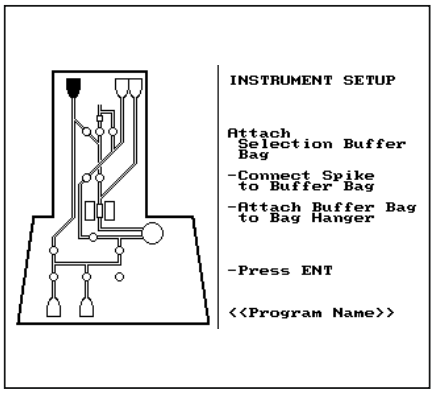
The window will display Screen 4-3.6 as shown.

In order to ensure the proper fitting of tubing in the valves, the 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 trouble-shooting (chapter 5). This step can be repeated by using the "Undo" key followed by the "Enter" key (see Figure 1.4).

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

Attach CliniMACS® PBS/EDTA Buffer

The window will display Screen 4-3.7 as shown.



Screen 4-3.7: Attach buffer bag

The prescribed buffer for CliniMACS® Plus 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 (see Figure 4-3.3) 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.

2. Attach the buffer bag to the buffer bag hook on the bag hanger (see Figure 4-3.7).

3. Adjust the height of the buffer bag hanger. Raise or lower the bag hanger to accommodate the height to 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 Figure 4-3.7).

To proceed, press ENT

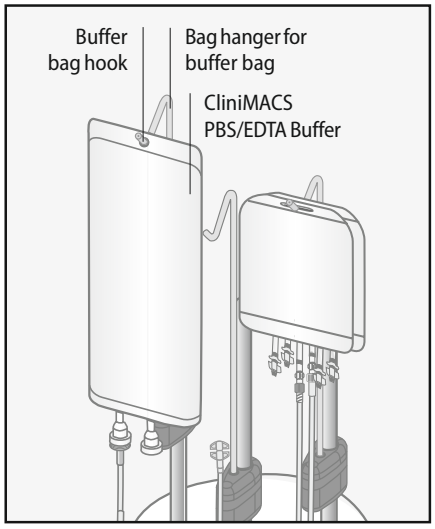
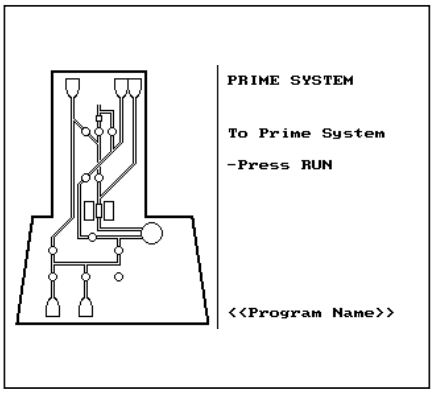


Figure 4-3.7: Attach buffer bag

Important

Due to the gravimetric rinsing steps, it is important that the buffer bag is positioned higher than the Reapplication Bag and the Non-Target Cell Bag (see Figure 4-3.8).

Start priming



Screen 4-3.8: Start priming

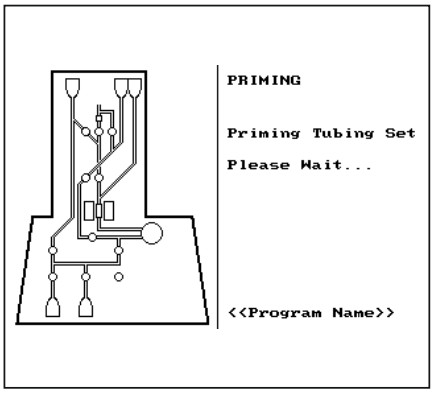
The window will display Screen 4-3.8 as shown.

To start priming, press RUN

The window will display Screen 4-3.9 as shown.

During the priming phase the tubing set is filled with CliniMACS PBS/EDTA Buffer. The buffer will be circulated through the tubing set including the separation column. Priming waste is collected in the Buffer Waste Bag, Reapplication Bag and the Non-Target Cell Bag (see Figure 4-3.3). The priming cycles will continue, repeating a series of steps. The priming phase will take approximately 2.5 minutes. Priming status will be updated on the display.

Check during the priming



Screen 4-3.9: Priming in process

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

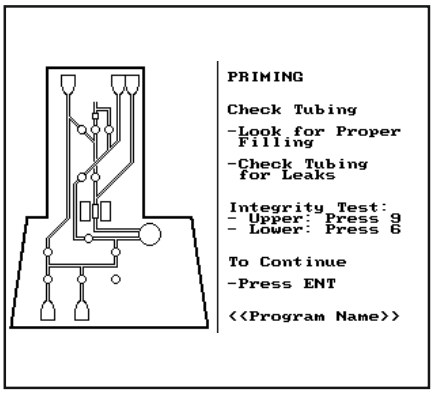
If leaks or malfunctions are observed, stop run by pressing 'STOP'. The operator will have 600 seconds to resolve the problem. Restart the process by pressing the 'RUN'.

If the operator 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



Screen 4-3.10: Final check of tubing and attachments

The window will display Screen 4-3.10 as shown.

Before beginning the run, check the following:

- fluid in all parts of tubing set except for tubing above valves nos. 2 and 3,
- **no** excess air in tubing set,
- fluid in Reapplication Bag, Buffer Waste Bag, and Non-Target Cell Bag,
- **no** fluid in the Cell Collection Bag.

Do not press 'ENT' yet.

Integrity test

For additional safety, an integrity test must be performed to test the tubing set for leaks. The test sequence consists of two automated sequences, which allow both the upper and the lower parts of the tubing set to be over pressurized and 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 4-3.10.

2. After performing the "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 4-3.11 as shown.

   #### Important

   Clamp the tubing underneath the check valve of the Non-Target Cell Bag.

5. To start the test sequence, press [RUN]

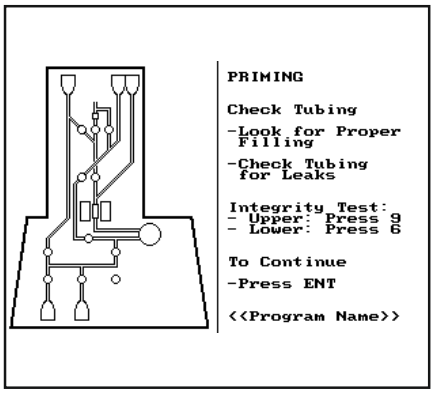
   To return to Screen 4-3.10, press [◀]

6. Once the 'RUN' button has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

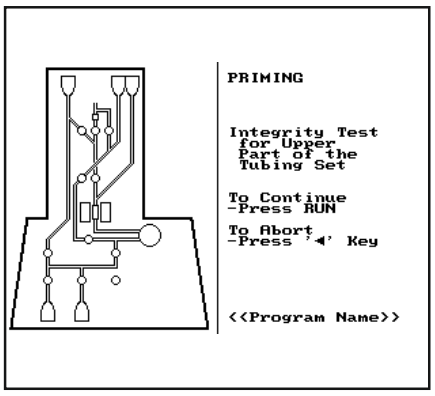
   The window will display Screen 4-3.12 as shown.

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

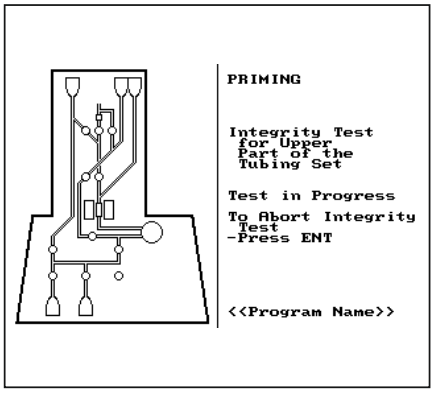
   At each point the test sequence can be finished by pressing 'ENT'.



Screen 4-3.10: Final check of tubing and attachments



Screen 4-3.11: Start integrity test for upper part



Screen 4-3.12: Integrity test for upper part in process

7. After two minutes the pressure is automatically released and the window displays Screen 4-3.10.

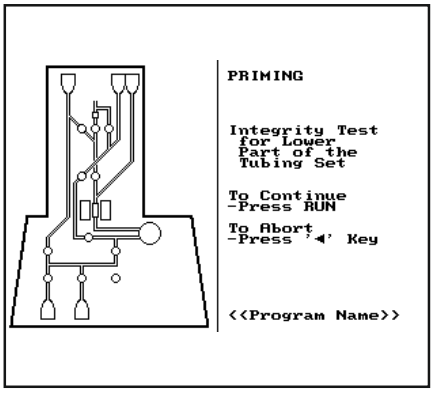
   Using tissue the operator should check to determine if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

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

Integrity test for the lower part of the tubing set

1. The window will display Screen 4-3.10.

2. To enter the integrity test, press 6
   Do not press 'ENT'.



Screen 4-3.13: Start integrity test for lower part

3. The window will display Screen 4-3.13 as shown.

4. To start the test sequence, press RUN

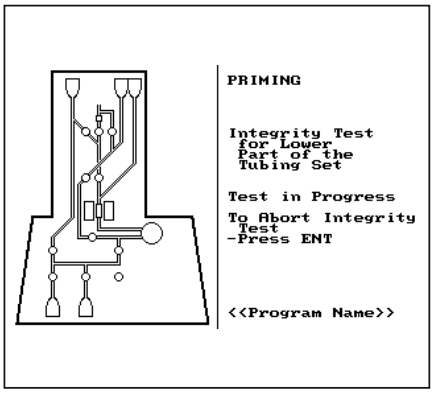
   To return to Screen 4-3.10, press ◄

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 4-3.14 as shown.

   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, and 10.

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



Screen 4-3.14: Integrity test for lower part in progress

6. After 30 seconds the pressure is automatically released and the window displays Screen 4-3.10.

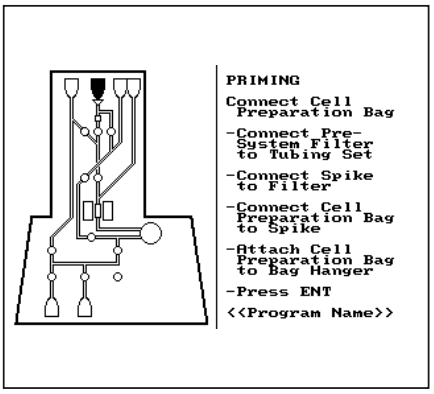
   Using tissue the operator should check to determine if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.

7. **Open the pathway to the Non-Target Cell Bag by removing the clamp underneath the check valve.**

8. If no leakages are observed, the operator can now continue with the next step by pressing ENT

Connect Cell Preparation Bag

The window will display Screen 4-3.15 as shown.



Screen 4-3.15: Connect Cell Preparation Bag

After the priming phase has been completed and no leaks or malfunctions are observed, the Cell Preparation Bag can be attached (see Figure 4-3.9). Use aseptic techniques for all steps.

Connect the Cell Preparation Bag containing the magnetically labeled and washed cells with the tubing set:

1. Remove the twist-off cap (see Figure 4-3.3) from the tubing set.

2. Remove the cap from the spike of the pre-system filter (see Figure 4-3.3). **Firmly** insert the spike of the pre-system filter into the tubing set, ensuring the septum is punctured.

3. Remove the caps from the pre-system filter and the blunt end of the spike connector (see Figure 4-3.3) and connect both parts.

4. Remove the other cap from the spike connector and connect the spike to the Cell Preparation Bag (see Figure 4-3.3) 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. Check the connection between the Cell Preparation Bag, spike connector, 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 (see Figure 4-3.9).

7. Make sure the bags and tubings attached to the bag hanger are neither streched nor bent.

   Due to the gravimetric rinsing steps performed by the instrument during the automated separation it is **very important** that the bag are leveled correctly (see Figure 4-3.8):

   - **Highest position**: buffer bag,
   - **Middle position:** Reapplication Bag and Non-Target Cell Bag,
   - **Lowest position:** Cell Preparation Bag.

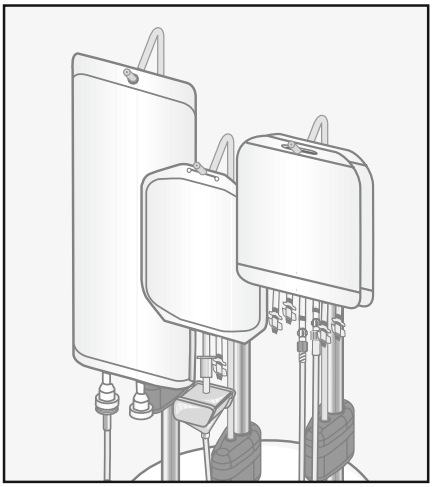
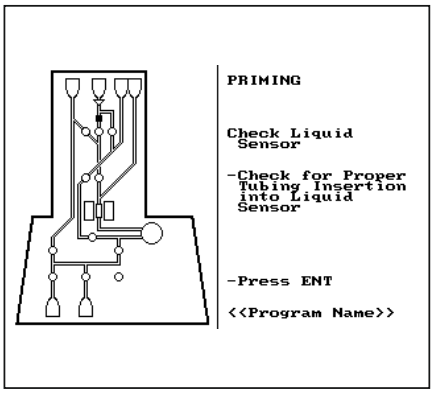


Figure 4-3.8: Correctly leveled bags

To proceed, press ENT



Screen 4-3.16: Final check of the liquid sensor

Final check of the liquid sensor

The window will display Screen 4-3.16 as shown.

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. Confirm that the unrestricted flow of fluid is possible to each bag.

To proceed, press ENT

Proceed to STEP 4.

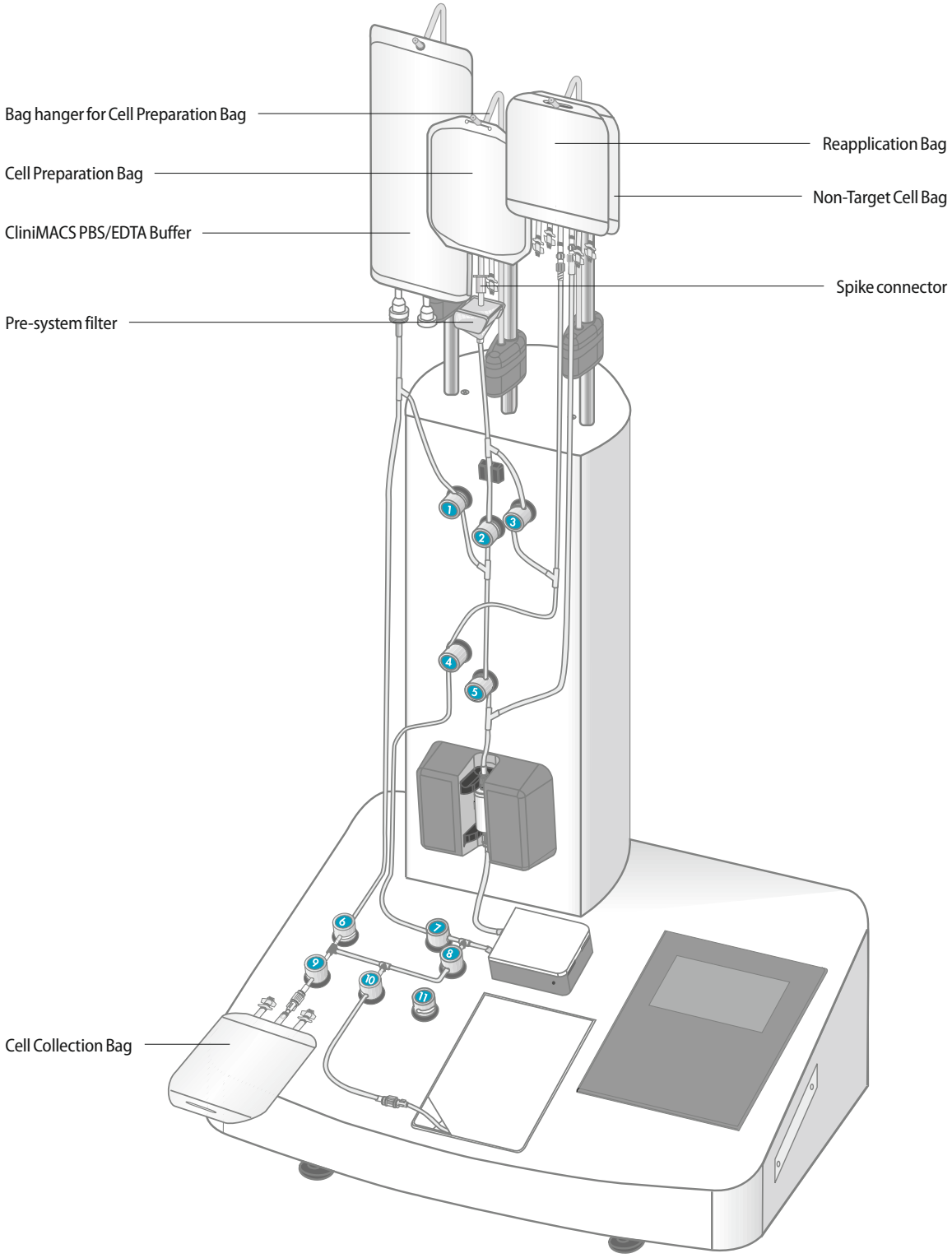


Figure 4-3.9: CliniMACS Plus Instrument with CliniMACS Depletion Tubing Set, CliniMACS PBS/EDTA Buffer, Cell Preparation Bag, and Cell Collection Bag

STEP 4:

CD34 SELECTION 1/2

Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window will display Screen 4-4.1 as shown.

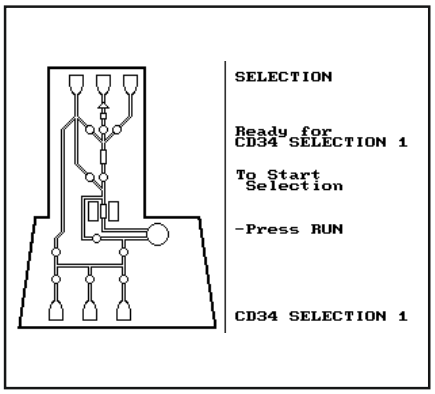
Make a final check of all tubing and attachments.

To proceed, press RUN

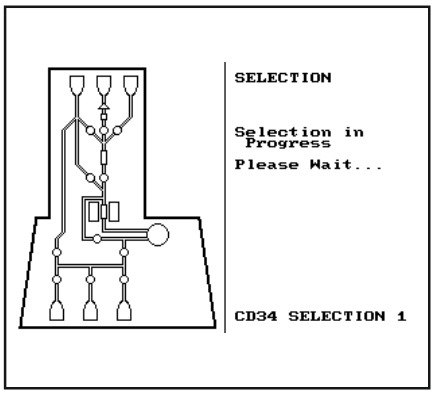
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 4-4.2 is 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 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.



Screen 4-4.1: Start separation



Screen 4-4.2: Separation in process

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 (target 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.

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.

Column wash I

The 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.

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 are 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

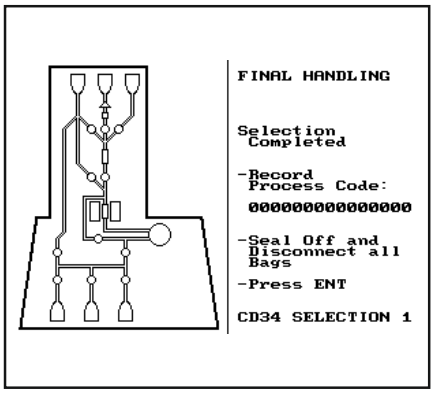
Additionally, the separation program CD34 SELECTION 2 includes a third release and reapplication step.

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 window will display Screen 4-4.3 as shown.



Screen 4-4.3: Disconnect bags and record process code

1. Record the process code.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 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. Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.

4. Mix the target cell suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag (see Figure 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 (see Figure 4-4.1).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

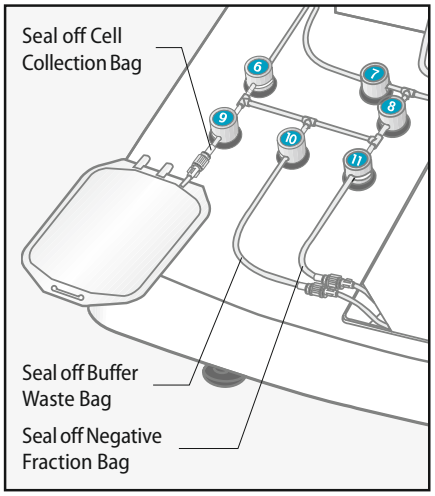
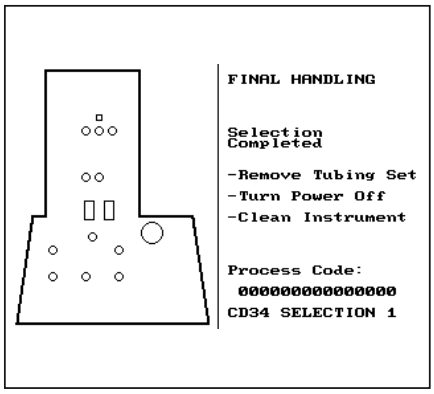


Figure 4-4.1: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT



Screen 4-4.4: Unload tubing set and shutdown

Unload tubing set and shutdown

The window will display Screen 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 section 1.3.9. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

⚠ CAUTION

Suitability of the target cells for clinical application may be compromised.

The target cells must be analysed, otherwise the suitability for clinical application may be compromised. 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.

Important

- The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.

- If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.

STEP 4:

DEPLETION 2.1

Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window will display Screen 4-4.1 as shown.

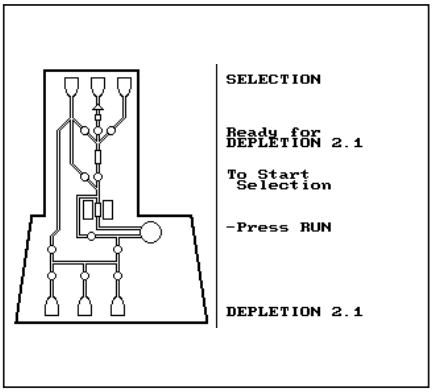
Make a final check of all tubing and attachments.

To proceed, press RUN

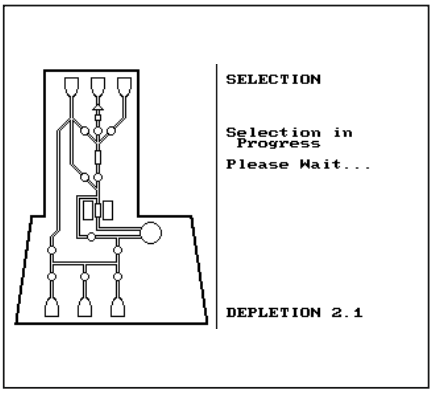
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 4-4.2 is 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 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.



Screen 4-4.1: Start separation



Screen 4-4.2: Separation in process

Separation procedure

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 (non-target cells) are retained in the separation column, placed in the magnetic field, while the unlabeled cells (target cells) are passed through and collected in the Cell Collection Bag.

Rinsing pre-system filter

After the cell sample has been completely loaded onto the tubing set, the pre-system filter is rinsed twice with buffer to reduce cell loss in the filter.

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.

Note

If the number of magnetically labeled cells (calculated by the CliniMACS Plus Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in smaller portions ("staged loading").

The loading will be stopped when the capacity of the separation column is reached and the separation program will proceed to the next step of the separation. After the last step (Final elution of the cells) has been completed, the next portion of the sample will be loaded onto the column.

Column wash

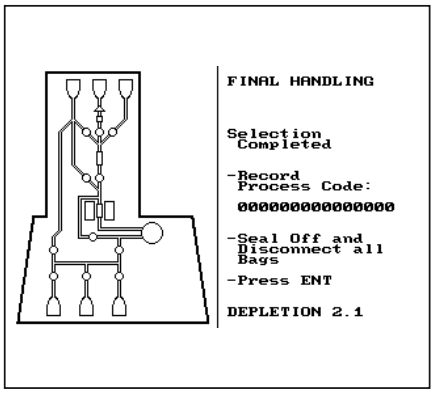
The pre-column and separation column are washed extensively to remove all unlabeled cells. Wash buffer is collected in the Buffer Waste Bag.

Final elution of the cells

The magnet is moved into the "OFF" position. At first the magnetically labeled cells are eluted to the Priming Waste Bag. Only after the last loading stage has been finished, all magenetically labeled cells (non-target cells) are released from the separation column and collected in the Negative Fraction Bag to reduce carryover with labeled cells in the pump tubing.

Disconnect bags and record process code

When the run has been completed, the window will display Screen 4-4.3 as shown.



Screen 4-4.3: Disconnect bags and record process code

1. Record the process code.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 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. Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.

4. Mix the target cells suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Negative Fraction Bag (see Figure 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 (see Figure 4-4.1).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

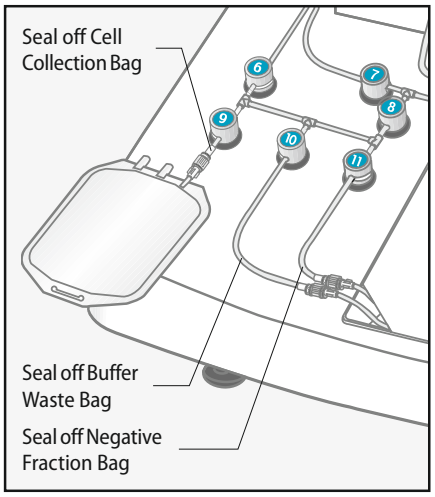
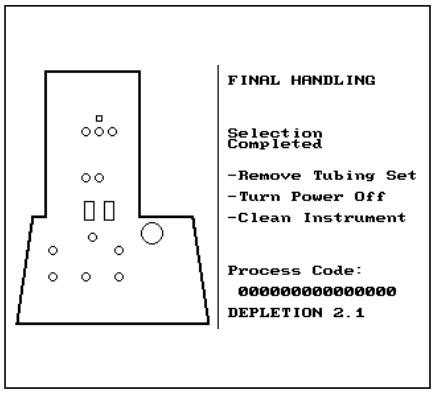


Figure 4-4.1: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT



Screen 4-4.4: Unload tubing set and shutdown

Unload tubing set and shutdown

The window will display Screen 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 section 1.3.9. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

⚠ CAUTION

Suitability of the target cells for clinical application may be compromised.

The target cells must be analysed, otherwise the suitability for clinical application may be compromised. 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 target cells
- Purity and recovery of target 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.

Important

- The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.

- If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.

STEP 4:

DEPLETION 3.1

Once the final check has been completed, the CliniMACS Plus Instrument is ready to begin the separation. The window will display Screen 4-4.1 as shown.

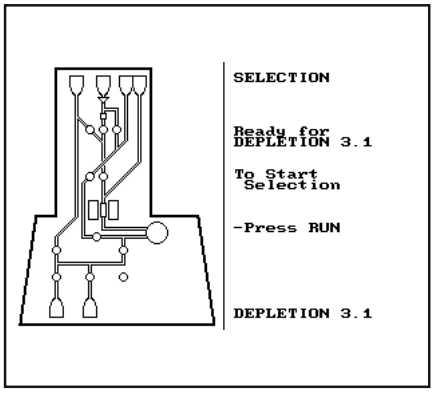
Make a final check of all tubing and attachments.

To proceed, press RUN

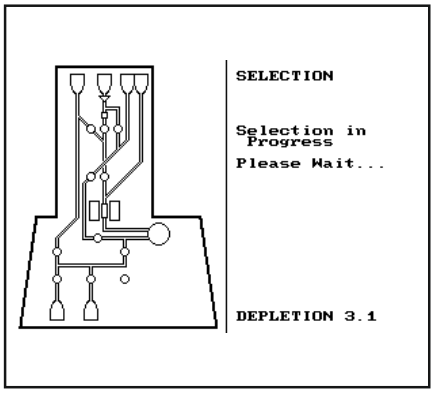
Once 'RUN' has been pressed, the instrument will automatically perform the separation procedure chosen.

At each phase of the operation, a screen similar to Screen 4-4.2 is 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 4-4.1), the magnet is "OFF", i.e., it has been moved to the rear of the instrument. With the magnet withdrawn the separationcolumn is outside the magnetic field and is not magnetized.



Screen 4-4.1: Start separation



Screen 4-4.2: Separation in process

Separation procedure

The cells are applicated onto the separation column twice. In the first loading stages the bulk of labeled cells is depleted ("BULK LOADING STAGE"). In the second sensitive loading step the remaining labeled cells are depleted ("SENSITIVE LOADING STAGE").

Bulk loading stage

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 (non-target cells) are retained in the separation column, placed in the magnetic field, while the unlabeled cells (target cells) are passed through and collected in the Reapplication Bag.

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.

Important

- If the number of magnetically labeled cells (calculated by the CliniMACS Plus Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell sample in smaller portions ("staged loading").

- Between the bulk loading and the sensitive loading stage, the separation column is washed to remove all labeled cells. The labeled cells will be eluted and held in the Non-Target Cell Bag. The wash buffer is collected in the Buffer Waste Bag.

Sensitive loading stage

For further depletion the cells hold in the Reapplication Bag are loaded onto the separation column again. The unlabeled cells (target cells) flow through the magnetic field and are collected in the Cell Collection Bag.

Rinsing pre-system filter and Reapplication Bag

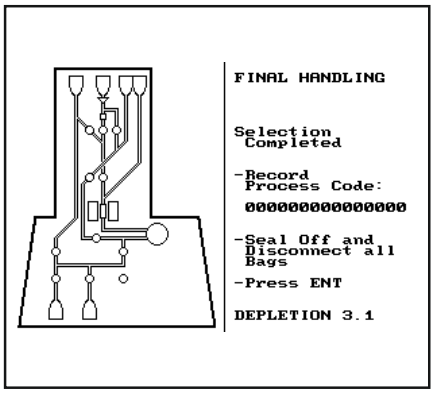
After the cell sample has been completely loaded onto the tubing set, the pre-system filter and the Reapplication Bag are rinsed with buffer twice to reduce cell loss in the system.

Final removal of non-target cells

The magnet is moved into the "OFF" position. The magnetically labeled cells (non-target cells) are released from the separation column and collected into the Non-Target Cell Bag.

Disconnect bags and record process code

When the run has been completed, the window will display Screen 4-4.3 as shown.



Screen 4-4.3: Disconnect bags and record process code

1. Record the process code.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 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. Determine the weight of the target cell fraction by subtracting the weight of the empty Cell Collection Bag from the weight of the Cell Collection Bag containing the target cells. Record the weight.

4. Mix the target cell suspension thoroughly by rotating the bag. Take an aliquot of 0.5 mL and retain for analysis.

5. Using the heat sealer, seal off the tubing above the luer lock of the Buffer Waste Bag (see Figure 4-4.1). Make three hermetic seals in the tubing. Sever the center seal to disconnect the Buffer Waste Bag.

6. Disconnect the Non-Target Cell Bag in the same way.

7. Remove the Non-Target Cell Bag and the Buffer Waste Bag. Keep all bags until final analysis of all cells has been accomplished.

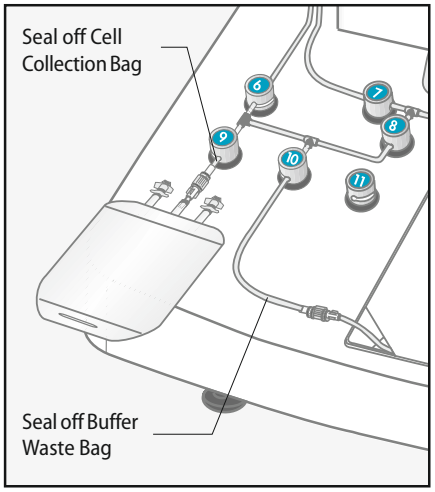
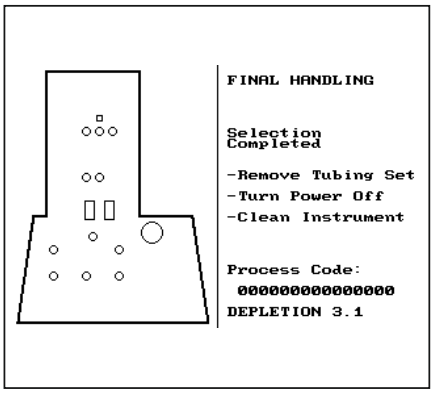


Figure 4-4.1: Disconnect bags

The target cells can now be processed in accordance with clinical protocols.

To proceed, press ENT



Screen 4-4.4: Unload tubing set and shutdown

Unload tubing set and shutdown

The window will display Screen 4-4.4 as shown.

1. Remove the tubing set: Beginning with valve nos. 6, 9, and 10 and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves. Release the column from the column holder. 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 section 1.3.9. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

⚠ CAUTION

Suitability of the target cells for clinical application may be compromised.

The target cells must be analysed, otherwise the suitability for clinical application may be compromised. 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 target cells
- Purity and recovery of target 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.

Important

- The CliniMACS PBS/EDTA Buffer does not have to be removed before administration of the separated target cells if the volume does not exceed 100 mL per transplant at appropriate infusion rates. The patient's renal status and effects on blood electrolytes need to be considered. Especially in children, infusion rates need to be adjusted accordingly.

- If the volume of the target cell fraction exceeds 100 mL, it must be reduced by appropriate means, e.g., through centrifugation or buffer exchange, to an infusion solution approved in the country of the user.

5 Troubleshooting

This chapter 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 chapter, contact Miltenyi Biotec Technical Support as soon as possible.

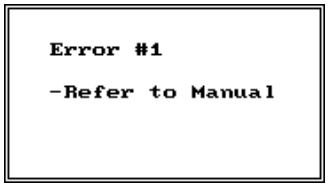
Note: The order of this chapter follows the actual sequence of a separation.

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 the operator do not exactly know the concentration of plasma in the 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 [+66 °F to +77 °F]).

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 Miltenyi Biotec Technical Support.

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

Other than errror messages, malfuctions 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.	Confirm that tubing is correctly inserted. Check whether valves have been cleaned thoroughly. Any valve that has been contaminated by fluid has to be exchanged. Contact Miltenyi Biotec Technical Support.
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 Plus Separation. Confirm 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 priming procedure with sufficient new buffer. Poor performance of the CliniMACS Plus Separation 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 locking forceps during the installation procedure and remove the locking forceps before restarting the priming sequence.

5.3 Automated cell separation

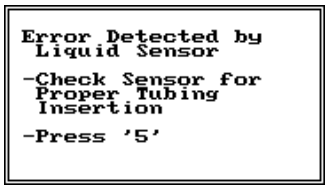
Warning messages

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 Plus Separation, 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 is subdivided accordingly.

Unexpected events – CD34 SELECTION 1/2

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. Confirm that the tubing is not kinked, twisted or taut.

4. Is the Cell Preparation Bag spiked properly? Confirm 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. Contact Miltenyi Biotec Technical Support.

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 Miltenyi Biotec Technical Support. - 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.
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 locking forceps. Adjust the tubing by first depressing the appropriate valve. Remove the locking forceps 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 locking forceps. Depress the valve manually several times to un-stick the stuck valve. Remove the locking forceps and press 'RUN' to continue. The cell separation will be aborted if the 'RUN' key is not pressed within 600 seconds. If the user is unable to un-stick the valve, contact Miltenyi Biotec Technical Support. - Wrong software program used. Check display for name of program currently used. Abort run by pressing the 'STOP' key and immediately contact Miltenyi Biotec Technical Support.
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 Miltenyi Biotec Technical Support. - 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 Miltenyi Biotec Technical Support.

Pump motor stalls during cell separation.

Warning message no. 2

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. The operator then have 600 seconds to correct the position of the pump tubing.

1. Carefully remove the pump tubing from the pump.

2. Confirm 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 separation column to save the cells retained on the column and contact Miltenyi Biotec Technical Support.

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.

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 5–7).

- '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'.

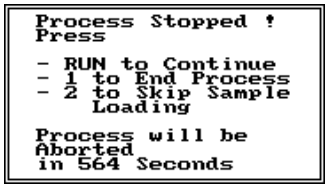
  If, as in this case, 'RUN' is not pressed within 600 seconds, the separation will be aborted and the cell separation will not be completed. Recover as much of the sample as possible from the tubing set by running the EMERGENCY PROGRAM.

- Power failure results in the termination of the CliniMACS Plus 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.

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.

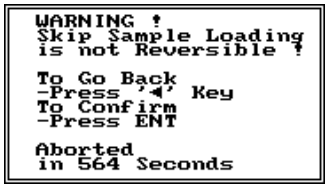
1. Press 'STOP' to interrupt cell loading. Warning message no. 3 will appear.



Warning message no. 3

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

   Warning message no. 4 will appear and will give the operator the opportunity to confirm or amend the decision 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.

EMERGENCY PROGRAM

(to be used with CD34 SELETCTION 1/2 only)

If, for any reason, a run has irreversibly terminated prior to the target cells' being eluted from the separation column, the EMERGENCY PROGRAM can be run to elute the cells from the separation 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

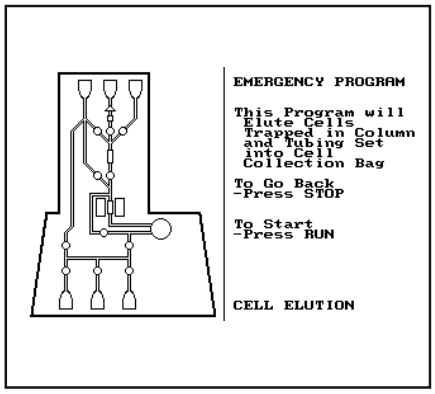
The emergency program will elute approximately 75 mL of fluid. Confirm that a suitable Cell Collection Bag is attached to the tubing set.

1. To confirm that the separation 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 (see Figure 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, contact Miltenyi Biotec Technical Support.

2. Wait until Screen 1.2 appears in the window (see chapter 1).

3. To call up the EMERGENCY PROGRAM, press '4'.

4. Screen 5.1 appears:



Screen 5.1: Emergeny program

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

Target cells do not reach the Cell Collection Bag.	Pump is unable to load the product from the Cell Preparation Bag because the locking forceps next to the Cell Collection Bag is not open during the cell loading sequence. Press 'STOP' and open locking forceps next 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

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 stage.	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.
Pump motor stalls during first elution of labeled cells into Non-Target Cell Bag.	- Locking forceps has not been removed after integrity test. Press 'STOP' and remove locking forceps. 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.

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. Confirm that three large bag hangers are installed for the program DEPLETION 3.1 and refer to installation instructions for appropriate bag heights (see "CliniMACS® Depletion Tubing Set" in chapter 4, STEP 3). - 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.

5.4 Cell separation performance

Unexpected events – CD34 SELECTION 1/2

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 chapter 4, STEP 1, section "Preparation of CliniMACS® PBS/EDTA Buffer"). - 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 [+66 °F to +77 °F]). - Incomplete sample loading due to clogging of separation column, pre-system filter, or pre-column. Check total cell number and depletion efficiency of the remaining cells.
Viability of the target cell 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" in the glossary of terms in chapter 2).

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 was not stored properly. Check storage temperature. Do not use any reagent that has been stored improperly (see package insert of the reagent). • 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 section "Preparation of CliniMACS® PBS/EDTA Buffer", chapter 4, 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 target cell fraction is less than 90% or the color of the supernatant during the washing steps was red" below.
	- Analysis was incorrect. • Sampling error occured. Check cell suspension for clumped or settled cells. Confirm that representative samples have been taken and repeat analysis. • Staining error occured. Check flow cytometry reagents. Repeat staining. • Flow cytometer settings were improper. Check instrument settings.

The purity of target cells is low.

- The leukapheresis product was 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 [+66 °F to +77 °F]).

- The magnetic labeling protocol has not been followed (e.g., incorrect volumes during magnetic labeling). Follow the instructions given for the magnetic labeling (see chapter 4, STEP 1).

  For troubleshooting purposes determine the leukocyte subsets (B cells, T cells, monocytes, granulocytes as well as platelets) contaminating the target cell fraction and contact Miltenyi Biotec Technical Support for advice.

- High numbers of granulocytes contaminated the start product (suboptimal 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 non-target fraction or buffer waste fraction. Inspect tubing placement within the valves to ensure proper functioning. Assess target cell content of the non-target cell fraction and buffer waste. If necessary, pool the target and non-target cell fraction, reduce to suitable volume and repeat the separation with a new tubing set and sufficient new buffer.

- Elution from the separation column was incomplete.

  - Separation program was 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 occured. 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.

The purity of 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. Follow the instructions given for the magnetic labeling (see chapter 4, 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 – 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 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 was exceeded. Refer to capacity limit of the relevant application.
- 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 separation 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 [+66 °F to +77 °F]). The cell concentration should not exceed 0.2×10^9/mL. If necessary, dilute the leukapheresis product with autologous plasma to achieve optimal cell concentration.