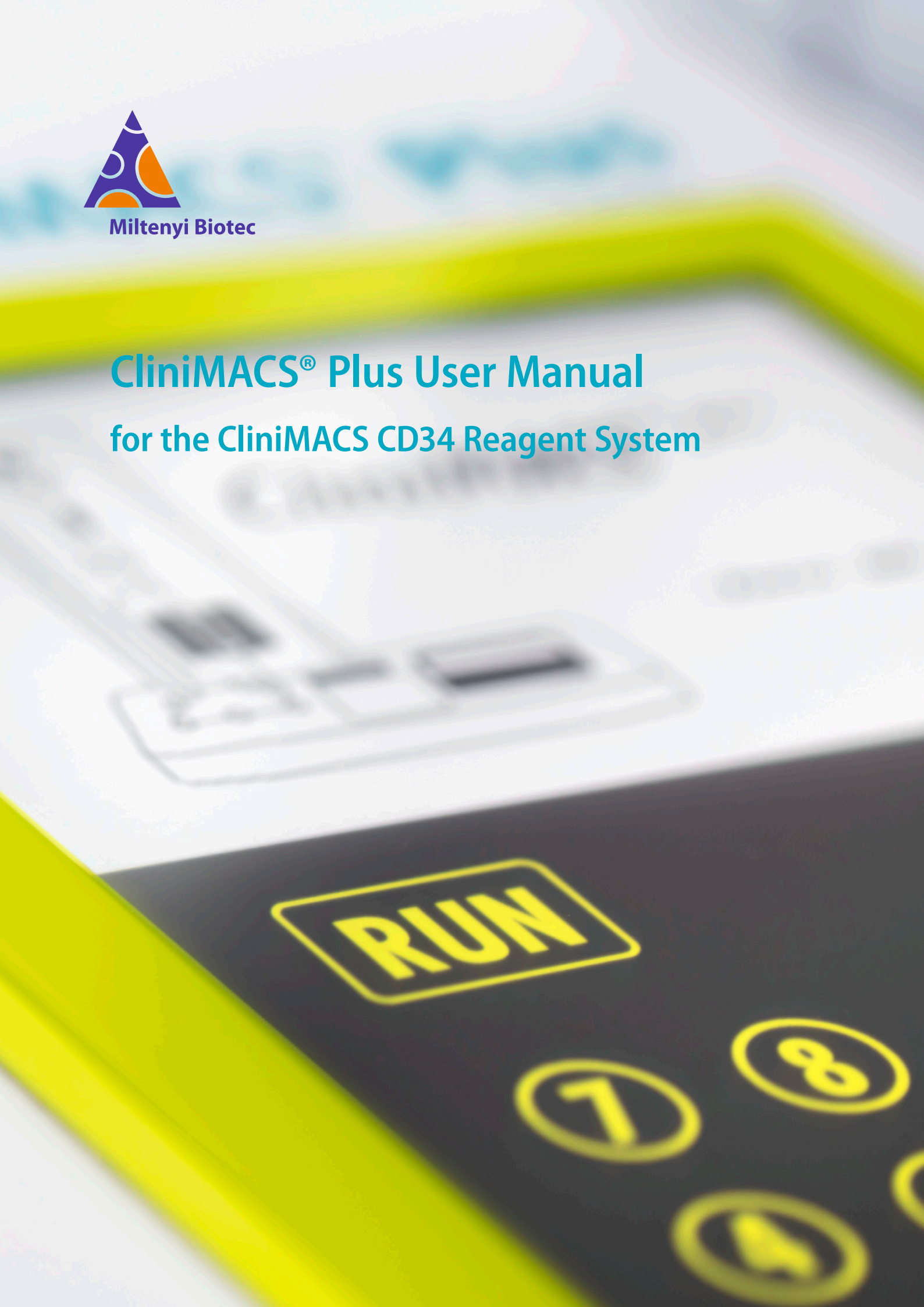
Miltenyi Biotec

CliniMACS® Plus User Manual

for the CliniMACS CD34 Reagent System



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CliniMACS® Plus User Manual

for the CliniMACS CD34 Reagent System

Software version 2.41

Issued: 2023-08
37070/03

HUMANITARIAN DEVICE: 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.

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

INDICATIONS FOR USE: The CliniMACS® CD34 Reagent System is indicated for processing hematopoietic progenitor cells collected by apheresis (HPC, Apheresis) from an allogeneic, HLA-identical, sibling donor to obtain a CD34 positive cell-enriched population for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft versus host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first morphologic complete remission.

CONTRAINDICATIONS: Do not use CD34 positive cells prepared with CliniMACS CD34 Reagent System in patients with known hypersensitivity to murine (mouse) proteins or iron-dextran.

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

Distributed by:
Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602
USA

Miltenyi Biotec Technical Support
+49 2204 8306-3803
technicalsupport@miltenyi.com

www.miltenyibiotec.com

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1 Introduction to the CliniMACS® CD34 Reagent System

1.1 Description

The CliniMACS® System is based on the magnetic cell separation technology (MACS® Technology) developed by Miltenyi Biotec B.V. & Co. KG.

The CliniMACS CD34 Reagent System is a medical device system that consists of the following four components:

- **CliniMACS CD34 Reagent:** a dark colored, non-viscous, colloidal solution containing an antibody conjugate in buffer. The conjugate consists of a murine IgG monoclonal antibody directed against the Class II epitope of the human CD34 antigen, which is chemically conjugated to dextran beads having an iron oxide/hydroxide core. (See the package insert for CliniMACS CD34 Reagent for more information.)

- C**liniMACS Plus Instrument**: a software controlled instrument that processes the HPC, Apheresis.

- **CliniMACS Tubing Set TS (normal scale) and CliniMACS Tubing Set LS (large scale):** single-use, sterile, disposable tubing sets with two proprietary cell separation columns. The CliniMACS Tubing Set TS is for processing HPC, Apheresis preparations containing up to 0.6×10^9 CD34 positive cells out of a total cell number not exceeding 60×10^9 white blood cells (normal fscale). The CliniMACS Tubing Set LS is for larger-scale preparations (up to 1.2×10^9 CD34 positive cells out of a total cell number not exceeding 120×10^9 white blood cells). (See the package insert for CliniMACS Tubing Sets for more information.)

- **CliniMACS PBS/EDTA Buffer (1000 mL):** a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the *in vitro* processing of HPC, Apheresis. (See the package insert for CliniMACS PBS/EDTA Buffer for more information.)

Important

Note that human IgG is not a component of the CliniMACS System. Use only pharmaceutical grade human IgG approved in the country of use. Carefully read the package insert of the human IgG used; in particular the section regarding hypersensitivity reactions and the risk of infection that human IgG as a bloodderived product brings to all patients. All risks arising from these materials must be evaluated by the user.

1.2 Principles of operation

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

The CliniMACS CD34 Reagent System allows the operator to perform an *in vitro* enrichment of human CD34 positive cells from heterogeneous hematological cell populations. The enrichment procedure involves two phases: cell labeling of antigen positive cells (Phase 1) and the automated cell separation process (Phase 2).

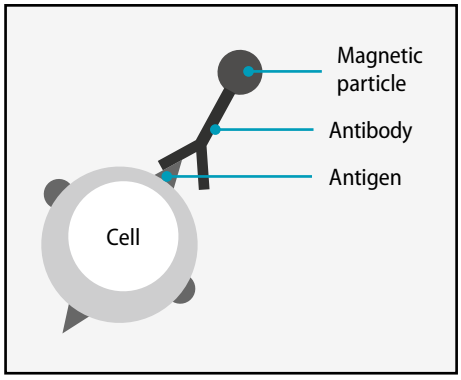


Figure 1.1: Magnetic labeling of cells

The first phase of the procedure is the cell labeling step where cells are directly labeled by antigen-specific antibodies conjugated to super-paramagnetic iron-dextran beads (see Figure 1.1). Prior to labeling with the CliniMACS CD34 Reagent, the HPC, Apheresis is washed with the CliniMACS PBS/EDTA Buffer (1000 mL) for platelet removal. After the platelet wash, the CliniMACS CD34 Reagent is combined with the HPC, Apheresis from the patient's allogeneic, HLA-identical, sibling donor. The HPC, Apheresis and CD34 Reagent are incubated for 30 minutes at room temperature during which the antibody on the CliniMACS CD34 Reagent selectively binds to the surface of cells expressing the CD34 antigen. The mixture is then washed in the CliniMACS PBS/EDTA Buffer (1000 mL) supplemented with HSA (HSA must be provided by the user, see chapter 3). Following centrifugation, the resulting cell pellet is resuspended in the CliniMACS PBS/EDTA Buffer (1000 mL) and the labeled cell suspension is ready for cell separation.

During the second phase of the procedure, the cell suspension, labeled with antibody conjugated to iron-dextran beads, is loaded onto the CliniMACS Plus Instrument prepared with a CliniMACS Tubing Set TS (used for normale scale preparations) or CliniMACS Tubing Set LS (used for large scale preparations). The magnetic cell separation unit consists of a powerful permanent magnet and a separation column with a ferromagnetic matrix. The magnetic field attracts labeled cells to the matrix and retains them. After removing the column from the magnet field, the rapid demagnetization of the column matrix allows the release of retained cells.

The labeled cells (labeled with a CliniMACS CD34 Reagent) are retained in the separation column and the non-labeled cells pass through. The labeled cells (CD34 positive target cells) are collected in the Cell Collection Bag and the non-labeled cells (non-target cells) in the Negative Fraction Bag.

Chapter 3 "Instructions" provides a detailed description regarding the use of the CliniMACS CD34 Reagent System.

For a glossary of terms and symbols, refer to appendix A.1.

1.3 Warnings

1.3.1 CliniMACS® CD34 Reagent System

Do not infuse the CliniMACS® CD34 Reagent or the CliniMACS PBS/EDTA Buffer (1000 mL) into patients directly.

Hypersensitivity reactions

Hypersensitivity reactions including anaphylaxis have been observed during infusion of CD34 positive cells from the CliniMACS CD34 Reagent System. Monitor the patient for hypersensitivity reactions, including anaphylaxis, during infusion of CD34 positive cells from the CliniMACS CD34 Reagent System.

Engraftment failure

Failure to infuse an adequate number of functioning CD34 positive cells can result in engraftment failure. Collect sufficient HPC, Apheresis to yield at least 2.4×10^6 CD34 positive cells per kg of patient body weight after system processing. The clinical trial using the CliniMACS CD34 Reagent System to process HPC, Apheresis did not test allografts with less than 2.4×10^6 CD34 positive cells per kg of recipient body weight. Monitor patients for laboratory evidence of hematopoietic recovery after transplantation. (See the Instructions for Use for the CliniMACS CD34 Reagent System for information regarding Device Performance and Clinical Performance.)

Acute and chronic graft versus host disease (GVHD)

GVHD can occur in patients who receive HPC, Apheresis processed using the CliniMACS CD34 Reagent System. Use pharmacologic prophylaxis if more than 1×10^5 CD3 positive cells per kilogram of recipient body weight are infused.

Delayed immune reconstitution after transplantation

Removal of T cells from the HPC, Apheresis can delay immune reconstitution after transplantation. Patients who receive the CD34 positive cell-enriched population prepared using the CliniMACS CD34 Reagent System are at risk for serious opportunistic viral infections, including post-transplant lymphoproliferative disorder caused by Epstein-Barr virus (EBV) and cytomegalovirus (CMV). Monitor for EBV and CMV in the peripheral blood of patients after transplantation and initiate appropriate treatment promptly.

1.3.2 CliniMACS® Plus Instrument

Risk of serious personal injury! Electronic equipment such as hearing aids, pacemakers, and cerebral/brain shunts may be damaged by the extremely powerful magnet in the CliniMACS® Plus Instrument. Personnel wearing or implanted with such medical devices or equipment should keep a distance of at least 30 cm from the instrument.

1.3.3 CliniMACS® CD34 Reagent

For *in vitro* use only.
Do not infuse into patients.
Not for parenteral application.

1.3.4 CliniMACS® PBS/EDTA Buffer (1000 mL)

For *in vitro* use only.
Do not infuse into patients.
Not for parenteral application.

1.3.5 CliniMACS® Tubing Set TS and CliniMACS Tubing Set LS

Do not connect the tubing set directly to the patient.

1.4 Precautions

1.4.1 CliniMACS® CD34 Reagent System

Safety and probable benefit in children under the age of 17 years have not been established.

Drugs may be incompatible with the CliniMACS® PBS/EDTA Buffer (1000 mL). Do not add drugs to the buffer other than Human Serum Albumin as specified in chapter 3 "Instructions".

Do not use cryopreserved and thawed HPC, Apheresis because cryopreservation promotes cell clumping, which may lead to device performance issues. Process HPC, Apheresis as soon as available, but not longer than 24 hours after collection.

Use only HPC, Apheresis from an allogeneic, HLA-identical sibling donor with the CliniMACS CD34 Reagent System.

Collect HPC, Apheresis according to standard hospital or institutional leukapheresis procedures in standard leukapheresis collection bags. Do not include additional anticoagulants or blood additives, such as heparin, other than those normally used during leukapheresis. Keep the HPC, Apheresis at controlled room temperature +19 °C to +25 °C (+67 °F to +77 °F) if it has to be stored, e.g., overnight, before processing. Do not allow the concentration of leukocytes to exceed 0.2×10^9 cells per mL.

Only trained operators should use the CliniMACS CD34 Reagent System to prepare CD34 positive cells for infusion. Operator training is provided by Miltenyi Biotec authorized personnel.

1.4.2 CliniMACS® Plus Instrument



Figure 1.2: Consult instructions for use

Consult instructions for use (see Figure 1.2). Read and observe all operating instructions carefully to ensure safety of the operator and the equipment.



Figure 1.3: Strong magnetic field

Contains a strong permanent magnet (see Figure 1.3). Measuring and control instruments, computers, and watches and magnetic information carriers (such as credit cards, magnetic tapes and floppy disks) and magnetizable tools and objects may be affected or damaged by the extremely powerful magnet in the CliniMACS® Plus Instrument. Keep these tools and objects at a distance of at least 30 cm from the device. See EMC information provided as appendix A.2.

Install the instrument according to the electromagnetic compatibility (EMC) information (Warning Labeling, Guidance and Manufacturer's Declaration) provided as Appendix 2. Portable and mobile RF communications equipment can affect medical electrical equipment.

Use an uninterruptible power supply or a battery that starts up within 10 milliseconds to supply power to the instrument. Based on technical limitations of the internal power supply voltage, interruptions on power supply input lines for longer than 10 milliseconds may lead to cessation of the separation process (power failure). The separation process cannot be resumed after a power failure.

Use only a grounded connection for the instrument. The instrument is a protection class I device.

Disconnect the power cable cord before cleaning or maintenance of the instrument.

Unplug the cord to disconnect the instrument from the power supply.

Use the originally supplied cord only.

Do not remove the back cover of the instrument to prevent the risk of an electric shock. The instrument may be opened and any spare parts may be exchanged by Miltenyi Biotec authorized personnel only. There are no internal components which can be serviced or calibrated by the operator.

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

Do not leave the instrument unattended during a run. If an error occurs, the cell separation will be interrupted at the current step and the operator will have 10 minutes to correct certain errors. If the instrument has not been restarted after this time period, the run will be aborted.

Do not leave the pump door open at any time during a run. If left open for more than 10 minutes, the run process will be aborted.

Do not open the door of the peristaltic pump when it is moving. Keep away from all moving parts.

Handle all fluid containers 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.

Treat the instrument as a biohazard after running a patient sample and prior to decontamination. Clean the instrument after each run with an aqueous biocidal detergent according to standard hospital or institutional requirements. For additional information regarding cleaning of the instrument refer to section 2.4.

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.

Use only supplies (e.g. CliniMACS Tubing Sets) recommended by the manufacturer.

1.4.3 CliniMACS® CD34 Reagent

Use aseptic working procedures.

Do not use after the use-by date printed on the vial label (see Figure 1.4). Do not use after the use-by date.

Do not use if package is damaged (see Figure 1.5). Use reagent only if vial is undamaged and sealed.

Do not re-use (see Figure 1.6).

The CliniMACS® CD34 Reagent is shipped refrigerated and must be stored at +2 °C to +8 °C (+36 °F to + 46 °F) immediately after receipt (see Figure 1.7). Do not freeze the reagent.



Figure 1.4: Use-by date



Figure 1.5: Do not use if package is damaged



Figure 1.6: For single use only

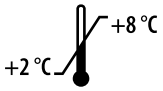


Figure 1.7: Temperature limit, +2 °C to +8 °C (+36 °F to + 46 °F)

1.4.4 CliniMACS® Tubing Set TS or CliniMACS Tubing Set LS

Choose either the CliniMACS® Tubing Set TS or the CliniMACS Tubing Set LS based on the capacity for CD34 and total cell number. Exceeding the capacity for either total cell number or CD34 positive cell number may impact the performance of the device:

- CliniMACS Tubing Set TS: Normal scale capacity for the enrichment of CD34 positive cells using the CliniMACS CD34 Reagent System with one vial of CD34 Reagent and the CliniMACS Tubing Set TS is 0.6×10^9 CD34 positive cells out of a total cell number not exceeding 60×10^9 cells.

- CliniMACS Tubing Set LS: Large scale capacity for the enrichment of up to 1.2×10^9 CD34 positive cells out of a total cell number of 120×10^9 cells (large scale application) requires two vials of the CliniMACS CD34 Reagent and the CliniMACS Tubing Set LS.

Use aseptic working procedures in unpacking, assembly and use of the tubing set.

Perform the integrity test as described in section 3.9.13. Replace tubing set if any leakage is observed during priming or integrity test.

Use universal precautions when handling all blood products and materials that have been in contact with such fluids. Treat used tubing sets as potentially biohazardous materials according to standard hospital or institutional requirements.

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



Figure 1.8: Use-by date

Do not use after the use-by date printed on the product label (see Figure 1.8).



Figure 1.9: Do not use if package is damaged

Do not use if package is damaged (see Figure 1.9). Before opening, inspect the packaging for damage, punctures, or tears. Use tubing set only if package is undamaged and sealed. Do not use if the tubing set is damaged. Do not use if any leakages of the tubing set are observed during priming or separation.



Figure 1.10: For single use only

Do not re-use (see Figure 1.10). The re-use of tubing sets or parts of them leads to the endangerment of the patient due to biological contamination or to inefficient cell separation.

1.4.5 CliniMACS® PBS/EDTA Buffer (1000 mL)

Use universal precautions when handling all blood products and materials that have been in contact with such fluids. Treat used buffer as potentially biohazardous material according to standard hospital or institutional requirements.

Drugs may be incompatible with the buffer. Do not add drugs to the buffer other than Human Serum Albumin (HSA) as specified in this User Manual. See chapter 3 for information regarding the addition of HSA to the CliniMACS® PBS/EDTA Buffer (1000 mL).

Do not use after the use-by date printed on the product label (see Figure 1.8).

Do not use if package is damaged (see Figure 1.9). Use buffer only if the bag is undamaged and sealed.

Do not re-use (see Figure 1.13).



Figure 1.11: Use-by date



Figure 1.12: Do not use if package is damaged



Figure 1.13: For single use only

2 CliniMACS® Plus Instrument (Model CS3)

2.1 Description

The CliniMACS® Plus Instrument is an electromechanical device intended to separate human cells from heterogeneous hematologic cell populations in combination with the CliniMACS CD34 Reagent, CliniMACS Tubing Set TS or CliniMACS Tubing Set LS, and CliniMACS PBS/EDTA Buffer (1000 mL).

The key components of the CliniMACS Plus Instrument include an integrated computer, a magnetic separation unit, a peristaltic pump, a liquid sensor, and pinch valves.

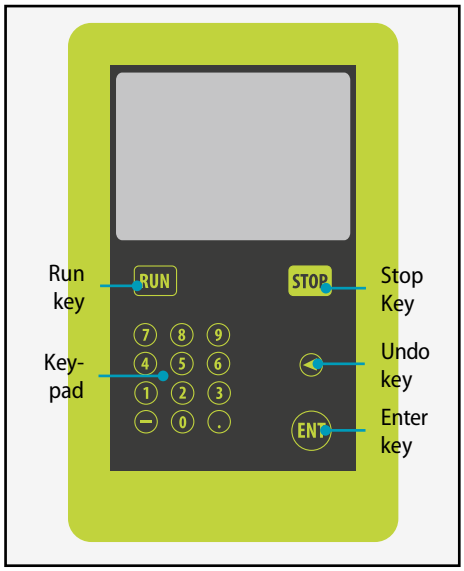


Figure 2.1: Display with touchscreen

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 2.1).

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 of fluid through the tubing set. 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 CD34 SELECTION program to the next phase of the separation process.

Eleven pinch valves ensure controlled flow of buffer and cell suspension throughout the procedure. Figure 2.2 depicts the CliniMACS Plus Instrument.

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

The CliniMACS Plus Instrument software offers the operator the choice between various separation programs. The CD34 SELECTION 1 or CD34 SELECTION 2 program will be used with the CliniMACS CD34 Reagent System. Refer to chapter 3 for additional details regarding the instructions.

Training by Miltenyi Biotec Technical Support or authorized representative is required before using the CliniMACS CD34 Reagent System.

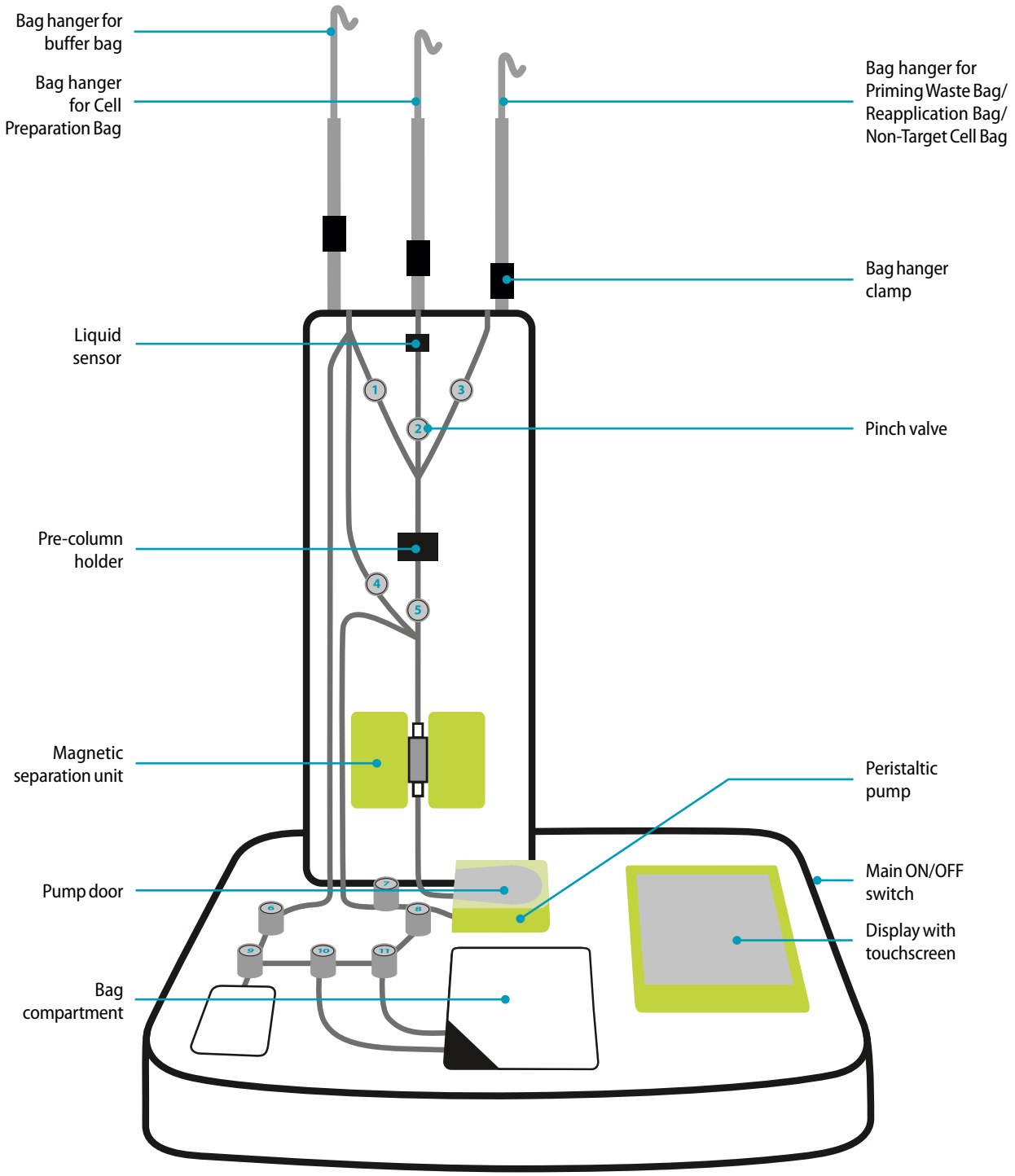


Figure 2.2: The CliniMACS Plus Instrument (Model CS3)

2.2 Specifications

Description

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

Technical data

The technical data of the CliniMACS Plus Instrument are listed in Table 2.1. **WARNING! The CliniMACS Plus 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 2.1: Technical data of the CliniMACS Plus Instrument

Standards

The CliniMACS Plus Instrument, Model CS3, has been tested to and satisfies the requirements for EN 61010-1 for electrical safety and the requirements for EN 60601-1-2 and CISPR 22 for electromagnetic compatibility. Additionally, it has been tested to and satisfies the requirements for UL 3101-1 (File No. E511246) and for CAN/CSA-C22.2 No. 1010.1 (File No. 98SC02331). Therefore, it is listed as laboratory equipment.sealed in the outer packaging provided by the manufacturer.

Instrument power inlet IEC-320-C13, power cords

A USA specific power cord is supplied with the CliniMACS Plus Instrument.

Power connection

The power connection (see Figure 2.3) module is located at the rear of the CliniMACS Plus Instrument. Viewed from behind, the connection consists of three sections. The left section is the recessed male 3-pin connector to which the power cord is attached. The center section is the main power ON/OFF switch. When positioned to the left, the switch is 'OFF' (0). When positioned to the right, the switch is 'ON' (I).

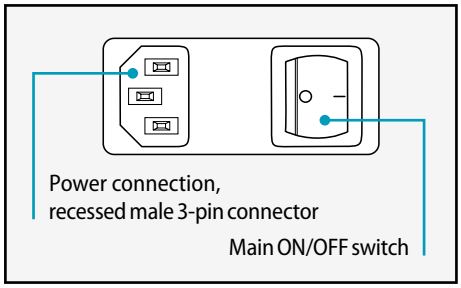


Figure 2.3: Power connection

The right section is the fuse box. The CliniMACS Plus Instrument must be unplugged and switched off before opening the fuse box. To open it, a thin-bladed screwdriver is inserted into the slot and twisted to release the catch. To replace the fuses, remove the fuses from the rear, insert new fuses and slide the module back in until the latch clicks to the closed position. The module will only slide in one direction. Only fuses with UL and European approvals are to be used.

Protection class

The CliniMACS Plus Instrument is a protection class I device (acc. to DIN 61140) and may only be plugged into an outlet with a grounded conductor. The protection category according to DIN EN 60529 is IPX 0.

Note

- Two people are required to safely unpack the CliniMACS Plus Instrument.

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

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.
- Contact the dealer or an experienced radio/TV technician for help.

2.3 Unpacking

The CliniMACS Plus Instrument should be unpacked according to the instructions below.

Unpacking and initial installation of the CliniMACS Plus Instrument must only be performed by an authorized Miltenyi Biotec Service Provider Visually inspect and note any significant damage to the package before unpacking. Damage may require inspection by a representative of the shipping company.

1. Wearing safety glasses, cut the plastic straps using a pair of scissors (see Figure 2.4). It should be noted that the plastic straps are under tension.

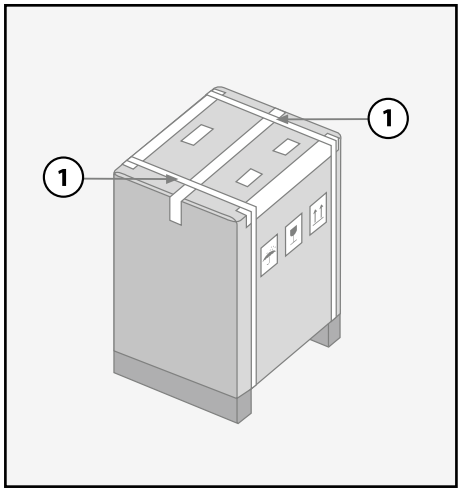


Figure 2.4: Cut plastic straps.

2. Open the top carton by cutting the adhesive tapes (see Figure 2.5).

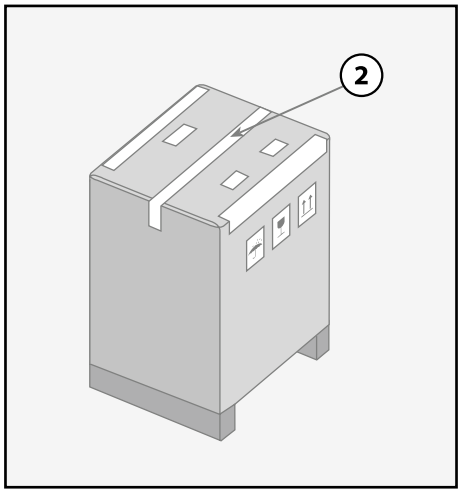


Figure 2.5: Open top carton.

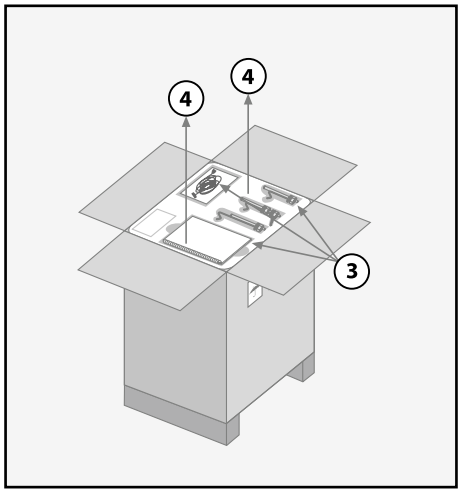


Figure 2.6: Remove the parts and the protective foam.

3. Open the carton and remove the parts (power cord, bag hangers) from the protective foam (see Figure 2.6).

4. Remove the protective foam.

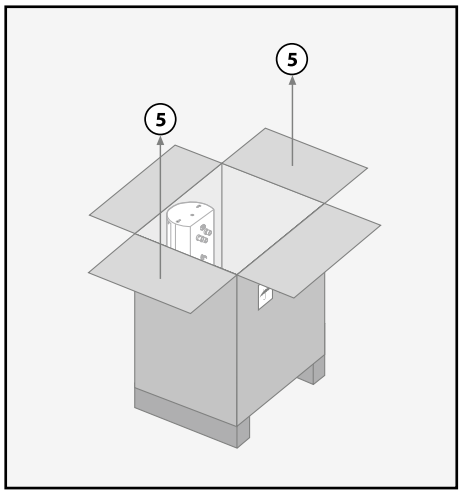


Figure 2.7: Lift the top carton.

5. Lift the top carton vertically off the pallet (see Figure 2.7).

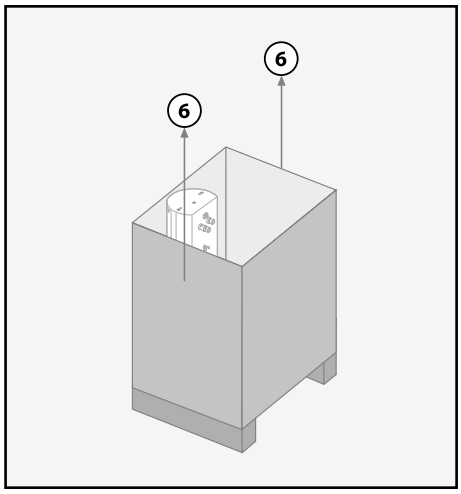


Figure 2.8: Remove the inner carton.

6. Remove the inner carton (see Figure 2.8).

7. Unwrap the large shipping bag (see Figure 2.9). Two people should carefully lift the CliniMACS Plus Instrument onto a flat stable surface which is capable of supporting 100 kg. The instrument should be lifted under each of the four corners at the base of the instrument.

8. Place the instrument at least 10 cm away from the wall to maintain ventilation. Additionally, do not place the instrument next to any vibrating equipment which might cause movement during operation.

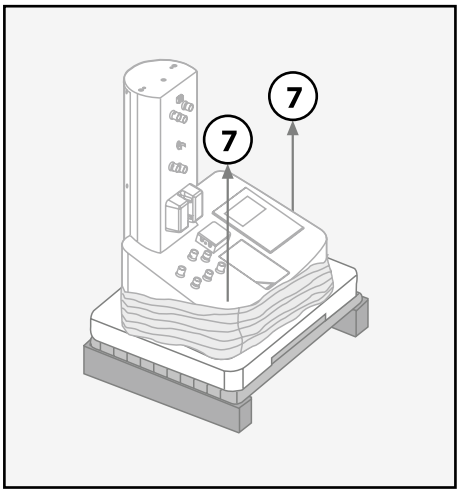


Figure 2.9: Lift the instrument.

9. Attach the bag hangers to the instrument and tighten the rods with clockwise twists until hand tight (see Figure 2.10). The height of the bag hangers may be adjusted by pressing the bag hanger clamps (see Figure 2.10).

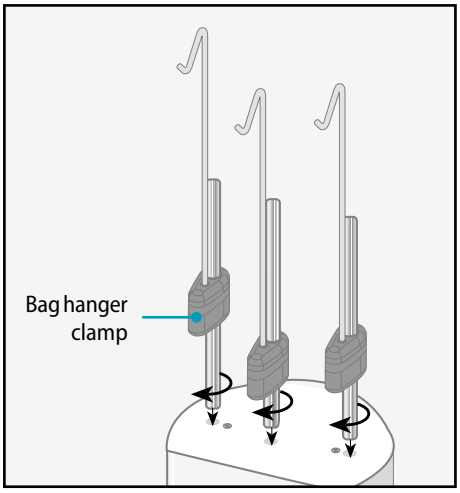


Figure 2.10: Attach bag hangers.

10. A stabilization foot (see Figure 2.11) is included with the instrument. Install the stabilization foot at the back of the instrument.

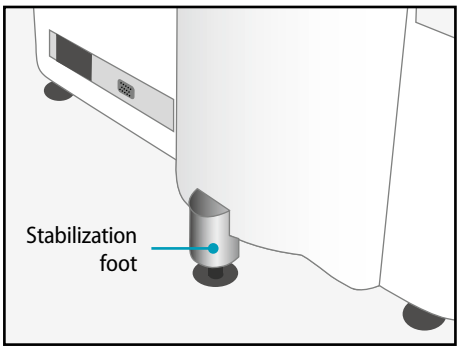


Figure 2.11: Rear of instrument with installed stabilization foot

2.4 Cleaning and maintenance

2.4.1 Cleaning

Caution

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

The surface of the CliniMACS Plus Instrument should be cleaned at regular intervals and after each application with an antiseptic solution (according to your institution's 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, pump head, etc.

2.4.2 Maintenance

The CliniMACS Plus Instrument does not contain any parts that may be serviced by the operator. Routine and preventative maintenance should be conducted by the manufacturer's authorized service personnel at least once per year (see section 2.5). Calibration of the instrument is not required.

2.5 Service and technical support information

2.5.1 CliniMACS® Service and technical support

For any information regarding the CliniMACS® CD34 Reagent System, contact Miltenyi Biotec Technical Support U.S.:

Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602
USA

Miltenyi Biotec Technical Support
+49 2204 8306-3803
technicalsupport@miltenyi.com

www.miltenyibiotec.com

2.5.2 CliniMACS® Plus Instrument Information

Record below the model and serial number located on the back of the CliniMACS® Plus Instrument. Refer to these numbers when calling to request information or service on the instrument.

Approved model no: CS3

Serial no: _____

Software version: 2.41

The software version is displayed during the start-up phase of the instrument.

3 Instructions

Read and observe the warnings and precautions described in chapter 1.

The CliniMACS CD34 Reagent System is for use by trained operators only.

All bag handling should be done in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using a sterile tubing connector may be performed outside the laminar flow hood.

Perform sample preparation and cell separation at room temperature +19 °C to +25 °C (+67 °F to +77 °F). Lower or higher ambient temperature will result in less purity and yield of the separated cells.

3.1 Required materials and equipment

3.1.1 CliniMACS® Materials required

- CliniMACS® Plus Instrument (REF 151-01)

- CliniMACS CD34 Reagent (REF 171-01)

- CliniMACS Tubing Set TS (REF 161-01)
  or
  CliniMACS Tubing Set LS (REF 162-01)

- CliniMACS PBS/EDTA Buffer (1000 mL) (REF 700-25)
  CliniMACS PBS/EDTA Buffer (1000 mL) must be used for the cell preparation and the CliniMACS Plus Separation. For magnetic labeling of the cells, two liters of buffer are required. For the separation, one liter of buffer is required. Before use, supplement the CliniMACS PBS/EDTA Buffer (1000 mL) with HSA to a final concentration of the 0.5% (w/v). HSA is not a component of the CliniMACS CD34 Reagent System and must be provided by the user.

- In addition to the CliniMACS Products, further materials and equipment are required for the CliniMACS Plus CD34 Separation and are supplied by the user.

3.1.2 Additional materials required (supplied by the user)

- Transfer bags, suitable for centrifugation:
  Transfer Bag 150 mL, Transfer Bag 600 mL

- Sampling Site Coupler

- Plasma Transfer Set Coupler/Coupler, Fenwal, 4C2405, or equivalent

- Luer/Spike Interconnector, Charter Medical, 03-220-92, or equivalent

- Pre-system filter, Blood Transfusion Filter, Pall, Ref. No. SQ40S, or equivalent

- Human serum albumin (HSA): Only FDA-licensed HSA should be used.

- Locking forceps or slide clamps: Locking forceps, Qosina: Part No. 16093, or equivalent

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

- Sample tubes

3.1.3 Equipment required (supplied by the user)

- Uninterruptable power supply, rated at a minimum of 180 VA (APC Smart-UPS 1500VA USB & Serial 230 V or equivalent)

- Laminar flow hood

- Orbital rotator (Lab-Line, Model 4635, or equivalent)

- Centrifuge (Sorvall, Model RC3, or equivalent) and buckets for centrifugation with aerosol containment caps

- Plasma extractor (Terumo Equipment, Plasma Separation Stand, Ref. No. 1ME*ACS301, or equivalent)

- Table top balance (Mettler Toledo, Ref. No. 11274-998, or equivalent) with 1 kg capacity; resolution to 0.1 g

- Tubing heat sealer (Baxter, Hematron III, Ref. No. FDR4360, or equivalent)

  Tubing stripper (Baxter, Ref. No. RAR4415, or equivalent)

- Biohazard waste containers

Optional materials and equipment may be used for the CliniMACS Plus CD34 Separation and are supplied by the user.

3.1.4 Optional materials (supplied by the user)

- Human IgG: Only pharmaceutical grade Human IgG should be used.

- 200 µm in-line blood filter, Fenwal, Product Code 4C2160, or equivalent

3.1.5 Optional equipment (supplied by the user)

Sterile tubing connector (Terumo Sterile Connection Device, [1]TSCD® SC-201, or equivalent)

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

3.2 Labeling and preparation of transfer bags

1. Label one 150 mL transfer bag as: **Cell Collection** (Minimally, 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 locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

2. Label one 600 mL transfer bag as: **Cell Preparation** (Minimally, 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 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 as instructed above. Record the weight of the bag.

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

4. Label two 600 mL transfer bag as: **Wash Waste No. 1** and **Wash Waste No. 2**. (Minimally, this should include patient identification, date and time of run, operator identification.)

Important

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.

3.3 Preparation of the CliniMACS® PBS/EDTA Buffer (1000 mL)

Before use, CliniMACS® PBS/EDTA (1000 mL) Buffer must be supplemented with HSA. Supplement three liters of CliniMACS PBS/EDTA Buffer (1000 mL) with HSA to a final concentration of 0.5% (w/v), i.e., add 5 g HSA per liter buffer.

Important

- HSA is not a component of the CliniMACS CD34 Reagent System. HSA is provided by the user and must be used with the CliniMACS CD34 Reagent System. Use only FDA licensed HSA. Carefully read the package insert of the HSA used.

- Store the buffer for cell preparation at +20 °C to +25 °C (+68 °F to +77 °F). Lower or higher ambient temperature will result in less purity and yield of the target cells.

- CliniMACS PBS/EDTA Buffer (1000 mL) is for *in vitro* use only. After the separation, the CliniMACS PBS/EDTA Buffer (1000 mL) contained in the target cell fraction must be exchanged to a medium suitable for application in humans prior to target cell infusion.

3.4 Preparation of the HPC, Apheresis

1. Collect sufficient HPC, Apheresis to yield at least 2.4×10^6 CD34 positive cells per kg of patient body weight after system processing.

2. Collect HPC, Apheresis according to standard hospital or institutional leukapheresis procedures in standard leukapheresis collection bags. Do not include additional anticoagulants or blood additives, such as heparin, other than those normally used during leukapheresis.

3. For transportation or storage, the HPC, Apheresis should be packed in insulated containers and should be kept at controlled room temperature +19 °C to +25 °C (+67 °F to +77 °F) according to standard hospital or institutional blood collection procedures approved for use with the clinical protocol. Do not refrigerate. Do not allow the cell concentration to exceed 0.2×10^9 cells per mL during transportation or storage. If necessary, dilute the HPC, Apheresis with autologous plasma.

4. Begin labeling and separation of cells as soon as possible after the HPC, Apheresis has been collected but not longer than 24 hours after collection.

5. Perform all bag handling in a sterile environment (e.g. laminar flow hood) using aseptic techniques. The connection of tubing using a sterile tubing connector may be performed outside the laminar flow hood.

6. Avoid vigorous mixing of the HPC, Apheresis.

7. Ensure that all required supplies and equipment are available before starting the cell labeling and separation process.

Note

The following sections describe the recommended procedure for the preparation of the HPC, Apheresis using a sterile tubing connector. The operator should be familiar with the operation and use of a sterile tubing connector.

Note

- The normale scale application capacity for the enrichment of CD34 positive cells using the CliniMACS CD34 Reagent System is 0.6×10^9 CD34 positive cells out of a total cell number not exceeding 60×10^9 cells. If either parameter is exceeded, a large scale application must be performed.

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

- If the number of target cells is low in the HPC, Apheresis the mobilization may be insufficient. The user should review the flow analysis for accuracy.

- Poor viability of cells in the HPC, Apheresis may indicate that the product was harvested, stored or transported inappropriately. Refer to chapter 4 "Troubleshooting".

Note

If clumps are suspected or present in the HPC, Apheresis, a 200 µm in-line blood filter may be used to filter the HPC, Apheresis material; connect the Cell Preparation Bag to the original HPC, Apheresis Bag using the 200 µm in-line blood filter.

3.4.1 Analysis

1. Insert a sampling site coupler into the port of the blood collection bag containing the HPC, Apheresis.

2. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler and remove a volume of 0.5 mL of the HPC, Apheresis. Transfer the sample into a sample tube. Label the tube as HPC, APHERESIS and include patient identification information and retain for cell analysis.

3. Determine the following parameters before starting the preparation of the HPC, Apheresis:

   - Total number of leukocytes
   - Percentage of CD34 positive cells
   - Total number of CD34 positive cells
   - Percentage of CD3 positive cells
   - Total number of CD3 positive cells
   - Viability

   Other tests might be required according to individual laboratory practice. Record data.

3.4.2 Transfer into Cell Preparation Bag

1. Record the date and the start time prior to preparing the HPC, Apheresis.

2. Determine the volume of the original HPC, Apheresis by estimating 1 mL of HPC, Apheresis as equivalent to 1 g (1 g $\triangleq$ 1 mL).

3. Hold the HPC, Apheresis bag with both hands and mix the contents thoroughly by using a gentle rotating motion.

4. Connect the Cell Preparation Bag to the original HPC, Apheresis bag using the sterile tubing connector.

5. Open the locking forceps, or the roller clamp on the blood filter, to facilitate the transfer of the HPC, Apheresis material into the Cell Preparation Bag. Clear the tubing of any remaining product using a tubing stripper. Close the locking forceps next to the Cell Preparation Bag, or close the roller clamp.

6. Using the heat sealer, seal the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see Figure 3.1).

7. Tare the balance. Lay the filled Cell Preparation Bag on the balance, let the tubing lie on the table. Record the weight.

8 Determine the actual weight of the HPC, Apheresis by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with HPC, Apheresis. Record the weight.

9. If the weight of the HPC, Apheresis is more than 200 g, but the number of cells is less than 120×10^9 total cells and 1.2×10^9 CD34 positive cells, centrifuge the sample to reduce the volume to 200 g at the most and proceed with "Dilution". Record the reduced volume.

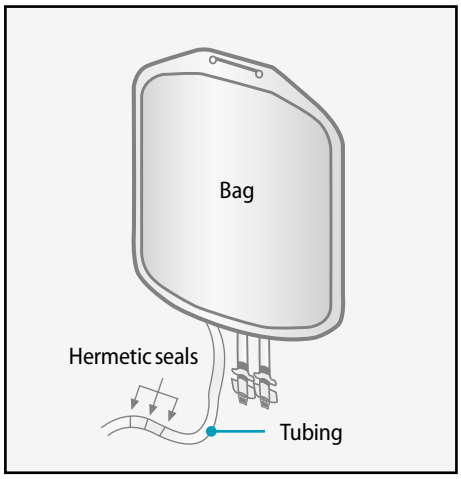


Figure 3.1: Sealing a bag. Use heat sealer to make the seal in the tubing. Sever at center seal.

3.4.3 Dilution

Dilute the HPC, Apheresis with CliniMACS PBS/EDTA Buffer (1000 mL) (supplemented with HSA to a final concentration of 0.5% (w/v)) to remove platelets by centrifugation before magnetic labeling. Centrifuge as instructed below under "Centrifugation" at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C [+67 °F to +77 °F]) following the dilution steps. Calculate the weight of buffer to be added using the following equation and record the weight.

Weight of buffer to be added (g)	=	Weight of HPC, Apheresis product (g)	×	2

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.

2. Connect the buffer bag to the Cell Preparation Bag using the sterile tubing connector.

3. Place the Cell Preparation Bag on the balance and tare the balance. Hang the buffer bag on the bag hanger. Open the locking forceps next to the Cell Preparation Bag.

4. Move the clamp on the plasma transfer set to the open position. Transfer the calculated weight of buffer to the Cell Preparation Bag by visually monitoring the display on the balance.

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

6. Using the heat sealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag. Disconnect the buffer bag.

7. Hold the Cell Preparation Bag with both hands and mix the contents thoroughly by using a gentle rotating motion. Avoid vigorous mixing of the cells.

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

9. Determine the weight of diluted HPC, Apheresis by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

3.4.4 Centrifugation

1. Using the sterile tubing connector, 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 calibrated accurately.

4. Centrifuge the cells at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C [+67 °F to +77 °F]). Record centrifugation conditions.

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

3.4.5 Volume adjustment

1. For magnetic labeling of CD34 positive cells, the optimal weight of the cell sample is:

   a) 95 g (±5 g) for a normale scale preparation, if one vial of CliniMACS CD34 Reagent is sufficient, or,

   b) 190 g (±5 g) for a large scale preparation if two vials of reagent are needed (see Table 3.1).

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

   a) Weight of supernatant to be removed (g) = Weight of diluted HPC, Apheresis (g) – 95 g

   b) Weight of supernatant to be removed (g) = Weight of diluted HPC, Apheresis (g) – 190 g

   Record the weight of supernatant 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. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Use the plasma extractor to carefully press out excess supernatant. Continue until the calculated weight of supernatant has been transferred into the Plasma Waste Bag so that a) 95 g or b) 190 g remain in the Cell Preparation Bag.

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

5. Seal off the tubing to disconnect the Plasma Waste Bag using the heat sealer. Leave at least 15 cm of tubing on the Cell Preparation Bag for further connections.

6. Weigh the Cell Preparation Bag and record the weight.

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

Table 3.1: Optimal labeling weight for the enrichment of CD34 positive cells

Important

- Ideally, magnetic labeling is performed on diluted HPC, Apheresis. Adjust the product to a final dilution of approximately 1:3. If the product received is diluted more than 1:3, or if the concentration of plasma in the sample is unknown, add immunoglobulin (IgG) to the product prior to the addition of the CliniMACS CD34 Reagent. The recommended concentration of IgG in the labeling mixture is 1.5 mg/mL. It is important to include the appropriate amount of IgG in the sample during the labeling in order to minimize non-specific binding of the CliniMACS CD34 Reagent. If a final concentration of about 30% autologous plasma in the sample during magnetic labeling cannot be guaranteed, add IgG to the sample. The volume of IgG added should be included in the final labeling weight; do not exceed 95 g or 190 g.

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

Important

If the CD34 positive cell recovery is low following completion of the separation process, the content of the Plasma Waste Bag may be analyzed to determine whether cells were lost during the removal of the supernatant.

7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid vigorous mixing of the cells. Ensure that all cells are resuspended. Determine the weight of the HPC, Apheresis product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Note the calculated weight.

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

3.5 Labeling of the cells

3.5.1 Determination of the scale of the CliniMACS® Plus CD34 Separation

Use the data (total number of leukocytes and total number of CD34 positive cells) from „Analysis“ to determine the scale of enrichment to be performed. The normale scale capacity for the enrichment of CD34 positive cells using the CliniMACS® CD34 Reagent System with one vial of CliniMACS CD34 Reagent and the CliniMACS Tubing Set TS is 0.6×10^9 CD34 positive cells out of a total cell number not exceeding 60×10^9 cells. Large scale capacity for the enrichment of up to 1.2×10^9 CD34 positive cells out of a total cell number of 120×10^9 cells (large scale application) requires two vials of the CliniMACS CD34 Reagent and the CliniMACS Tubing Set LS.

3.5.2 Preparation of the CliniMACS® CD34 Reagent

The CliniMACS® CD34 Reagent should be used cold, directly from the refrigerator. DO NOT warm up before use. The use-by date and lot number of the reagent are printed on the vial. DO NOT use the reagent after the use-by date.

3.5.3 Incubation with the CliniMACS® CD34 Reagent

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

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from one vial CliniMACS CD34 Reagent (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one vial for a normale scale application, or respectively, a 20 mL syringe is sufficient to remove the contents of two vials of reagent for a large scale application. Use a syringe with a 20 gauge needle.

3. Inject the entire volume of reagent into the Cell Preparation Bag using the injection port on the sampling site coupler. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.

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

5. Partially inflate the Cell Preparation Bag to ensure that cells and reagent are thoroughly mixed. Use an appropriate sterile syringe and needle (i.e. a 50 mL syringe with 20 gauge needle) to inject 100 mL of sterile air from the laminar flow hood into the injection port of the sampling site coupler.

6. Place the Cell Preparation Bag flat on the orbital rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 30 minutes at controlled room temperature (+19 °C to +25 °C [+67 °F to +77 °F]). Record the incubation stop time.

3.5.4 Removal of excess reagent

Wash no. 1

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

2. Connect the buffer bag to the Cell Preparation Bag using the sterile tubing connector.

3. Place the Cell Preparation Bag on the balance. Hang the buffer bag on the bag hanger. Tare the balance.

4. Open the locking forceps next to the Cell Preparation Bag. Then open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e. add 400 g to 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag.

5. Close the locking forceps next to the Cell Preparation Bag. Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

6. Hold the Cell Preparation Bag with both hands and mix the contents (HPC, Apheresis product and buffer) thoroughly by using a gentle rotating motion.

Important

- For the normale scale preparation (one vial of CliniMACS CD34 Reagent), remove at least 500 g of supernatant weight.
- For the large scale preparation (two vials of CliniMACS CD34 Reagent), remove at least 450 g of supernatant weight.
- If the volume of supernatant removed is less than the weight listed above a total of three washing steps (instead of only two) is recommended. Otherwise the removal of unbound reagent may be insufficient. Unbound reagent may bind to the separation column and this may decrease the selection efficiency.
- If the CD34 positive cell recovery is low following completion of the separation process, the content of the Wash Waste Bag No. 1 may be analyzed to determine whether cells were lost during the removal of the supernatant.

7. Connect the empty Wash Waste Bag No. 1 to the Cell Preparation Bag using the sterile tubing connector.
8. Fold any loose parts of the bags or tubing downwards. Transfer Cell Preparation Bag and Wash Waste Bag No. 1 securely to the centrifuge bucket.
9. Balance the loaded bucket with a suitable weighted bucket. It is essential that the centrifuge is calibrated accurately.
10. Centrifuge at 200 × g for 15 min (without brake) at room temperature (+19 °C to +25 °C [+67 °F to +77 °F]). Note the centrifugation conditions.
11. Remove the bags from the centrifuge without disturbing the cell pellet.
12. Hang the Cell Preparation Bag on the plasma extractor.
13. Place the Wash Waste Bag No. 1 on the balance. Tare the balance.
14. Open the locking forceps next to the Cell Preparation Bag. Remove as much excess supernatant as possible from the Cell Preparation Bag using the plasma extractor. Be careful not to remove cells. Note the weight of supernatant removed.
15. Close the locking forceps and heat seal off the tubing to disconnect the Wash Waste Bag No. 1 leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.
16. Keep the Wash Waste Bag no.1 until the separation and final analysis of all cells has been accomplished.
17. Resuspend the cell pellet in the Cell Preparation Bag. Avoid vigorous mixing of the cells. Ensure that all cells are resuspended.

Wash no. 2

1. Connect the plasma transfer set, inserted into a buffer bag containing at least 500 mL of buffer, to the Cell Preparation Bag using the sterile tubing connector. Confirm the clamp on the plasma transfer set is closed.

2. Place the Cell Preparation Bag on the balance. Hang the buffer bag on the bag hanger. Tare the balance.

3. Open the locking forceps next to the Cell Preparation Bag. Next open the clamp on the plasma transfer set and completely fill the Cell Preparation Bag with buffer (i.e., add approximately 500 g of buffer). To stop the liquid flow, close the clamp on the plasma transfer set. Record the weight of buffer transferred into the Cell Preparation Bag.

4. Close the locking forceps next to the Cell Preparation Bag. Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

5. Hold the Cell Preparation Bag with both hands and mix the contents (HPC, Apheresis product and buffer) thoroughly by using a gentle rotating motion.

6. Connect the empty Wash Waste Bag No. 2 to the Cell Preparation Bag using the sterile tubing connector.

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

8. Centrifuge at 200×g for 15 min (without brake) at room temperature (+19 °C to +25 °C [+67 °F to +77 °F]). Record the centrifugation conditions.

9. Remove the bags from the centrifuge without disturbing the cell pellet. Carefully hang the Cell Preparation Bag on the plasma extractor.

10. Place the Wash Waste Bag No. 2 on the balance. Tare the balance.

11. Open the locking forceps next to the Cell Preparation Bag. Remove as much excess supernatant as possible from the Cell Preparation Bag using the plasma extractor. Record the weight of supernatant removed.

Important

If the CD34 positive cell recovery is low following completion of the separation process, the content of the Wash Waste Bag No. 2 may be analyzed to determine whether cells were lost during the removal of the supernatant.

12. Close the locking forceps and heat seal off the tubing to disconnect the Wash Waste Bag No. 2.

13. Keep the Wash Waste Bag No. 2 until the separation and final analysis of all cells has been accomplished.

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

15. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the HPC, Apheresis after the washes by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

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

17. Connect the buffer bag to the Cell Preparation Bag using the sterile tubing connector.

18. Place the Cell Preparation Bag on the balance and tare the balance. Hang the buffer bag on the bag hanger. Open the locking forceps next to the Cell Preparation Bag.

19. Move the clamp on the plasma transfer set to the open position. Transfer the calculated weight of buffer to the Cell Preparation Bag by visually monitoring the scale on the balance.

20. Close the clamp on the plasma transfer set to stop the liquid flow when the appropriate weight of buffer has been transferred. Close the locking forceps next to the Cell Preparation Bag.

21. Make three hermetic seals between both clamps using the heat sealer. Disconnect the buffer bag.

22. Insert a sampling site coupler into the port of the bag containing the HPC, Apheresis.

23. 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. This sample will be used to calculate the CD34 positive cell recovery based on the product actually loaded on the instrument. Determine the following parameters before starting the separation process:

    - Total number of leukocytes
    - Percentage of CD34 positive cells
    - Total number of CD34 positive cells
    - Percentage of CD3 positive cells
    - Total number of CD3 positive cells
    - Viability

    Other tests might be required according to individual laboratory practice. Record data.

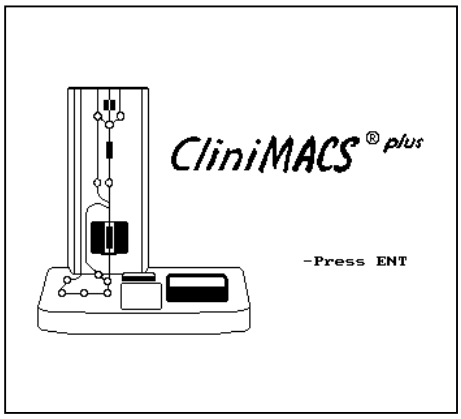
24. Weigh the Cell Preparation Bag and record the weight. Determine the weight of the HPC, Apheresis (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.

Continue to STEP 2: Start of the instrument and choice of separation program.

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

3.6 Operation of the CliniMACS® Plus Instrument

3.6.1 Switching on the CliniMACS® Plus Instrument



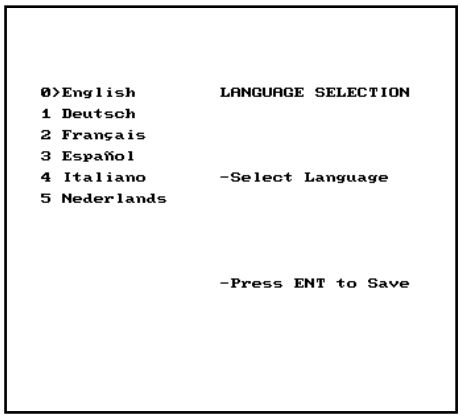
Screen no. 3.1: Main screen

Switch on the CliniMACS® Plus Instrument by using the ON/OFF switch located on the back panel of the instrument. Record the date and time detailing when the instrument run has been started. The instrument performs self-check procedures.

The program will automatically load and a screen depicting the CliniMACS Plus Instrument appears (see Screen no. 3.1).

3.6.2 Program menu, language selection and service menu

From Screen no. 3.1 the operator may proceed to the program menu by pressing 'ENT', or access menus that facilitate language selection or a service menu.



Screen no. 3.2: Language selection

Language selection

To change the language selection, press 2

The window will display Screen no. 3.2 as shown.

To select a language, press the corresponding number. To save the language,
press ENT

Service menu

To enter the service menu, press 5

The window will display Screen no. 3.3 as shown.

From the Service menu the operator may change the date and time, call up the process codes of the last 15 runs, enter Access to Program Menu (as directed by Miltenyi Biotec Technical Support) or perform an instrument check.

Date and time setting

To set the date and time, press [0]

Follow the instructions shown on Screen no. 3.4. The date or time may be changed when the respective field is highlighted by the black bar.

To move the black bar between the date and time input, press 'ENT'. Enter the current date (order: day/month/year) and time (order: hours/minutes/seconds). A wrong input may be amended by pressing the "UNDO" key.

To save the data and leave, press [RUN]

To leave the program without saving the changes, press 'STOP'. After pressing 'RUN', the program will automatically return to the service menu.

Process code review

This menu permits the operator to view the process codes of the last 15 operations. A process code is saved when the installation of a tubing set has begun, or when the emergency program has been used (see chapter 4 "Troubleshooting"). Saving a process code is independent of whether a separation sequence was completed or interrupted.

To review the process codes, press [1]

The process codes for the last 15 operations are listed on the screen chronologically as depicted on Screen no. 3.5. The most recent operation is listed first.

To return to the service menu, press [ENT]

Access to programs

This menu should not be entered by the user unless instructed to do so by Miltenyi Biotec Technical Support. If the program is entered by mistake, press 'STOP' to leave the program and return to the service menu.

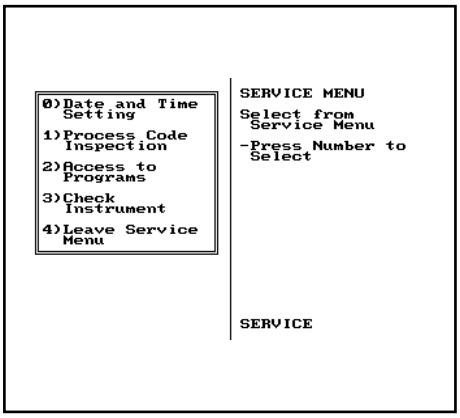
Check instrument

This menu allows the operator to initiate an automated instrument check sequence.

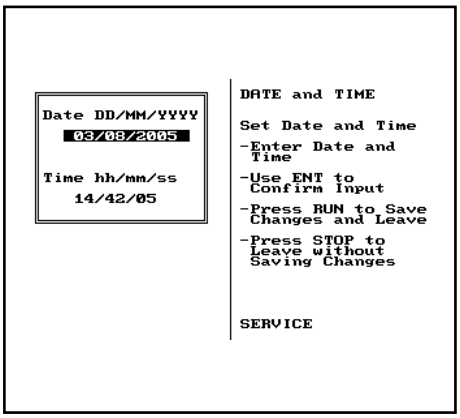
To begin the instrument check, press [3]

To leave the service menu, press [4]

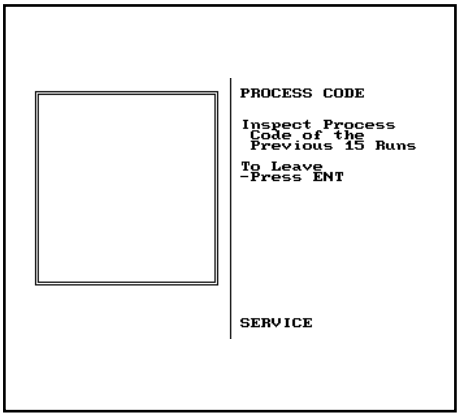
The program will return to Screen no. 3.1. To proceed to the Program Menu and choose a separation program, press [ENT]



Screen no. 3.3: Service menu



Screen no. 3.4: Sercive menu (Date and time)

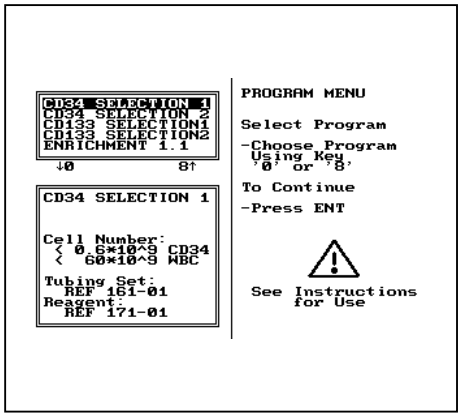


Screen no. 3.5: Service menu (Process code)

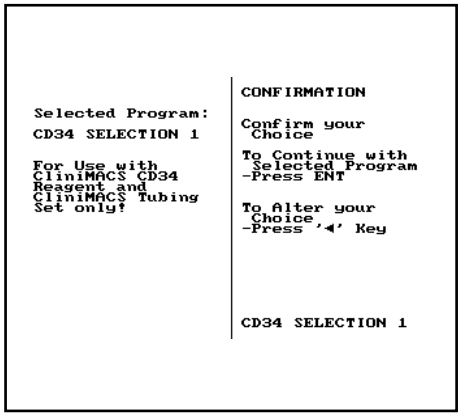
Note

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.

3.7 Choice of separation program: CD34 SELECTION 1 or CD34 SELECTION 2



Screen no. 3.6: Choice of separation program



Screen no. 3.7: Confirmation

The window will display Screen no. 3.6 as shown.

Choose either the CD34 SELECTION 1 (normal scale applications) or CD34 SELECTION 2 (large scale applications), as appropriate.

To choose the appropriate CD34 SELECTION 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

3.7.1 Confirmation of the program choice

The window will display Screen no. 3.7 as shown. Confirm the correct program has been chosen.

If not, press the "UNDO" key on the instrument keypad to return to the previous step in order to amend the choice.

To confirm and proceed, press ENT

3.7.2 Material check

The window will show Screen no. 3.8 as shown.

CD34 SELECTION 1 and CD34 SELECTION 2 separation programs 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 tubing set reference number (REF) in the query box. The instrument will check whether the materials can be used in combination with the chosen separation program.

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

   To confirm and proceed, press ENT

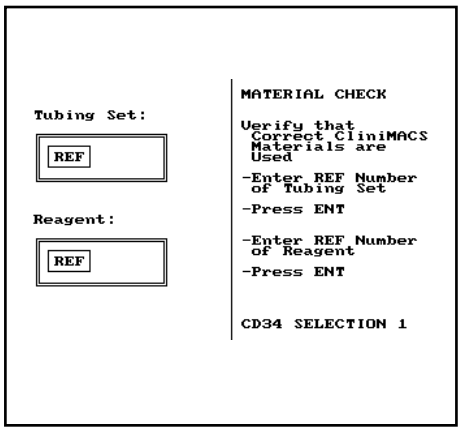
2. Enter reference number of the reagent that has been used for cell labeling.

   To confirm and proceed, press ENT

If the reference number of a tubing set or a reagent not specified for the chosen separation program has been entered, a message appears. Press 'ENT' to confirm and enter the correct reference number again. If the reference number entered is still incorrect, the message will appear a second time. After pressing 'ENT' the program will return to the program menu (see Screen no. 3.6).

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

Proceed to STEP 3: Installation of the CliniMACS Tubing Sets.



Screen no. 3.8: Material check

Note

- CD34 SELECTION 1 must only be used in combination with the CliniMACS Tubing Set TS (REF 161-01), while CD34 SELECTION 2 must only be used in combination with the CliniMACS Tubing Set LS (REF 162-01). Check that the tubing set chosen for installation matches the separation process before proceeding.

- Press the "UNDO" key located on the instrument keypad to correct a mistake during data input.

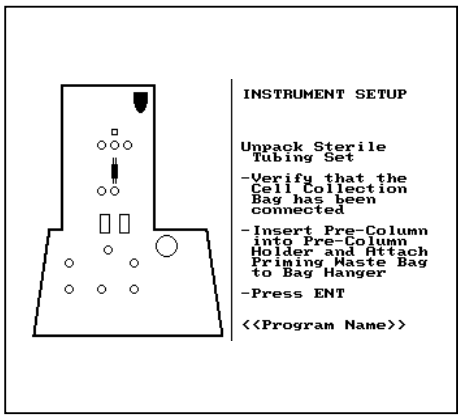
3.8 Installation of the CliniMACS® Tubing Set TS and CliniMACS Tubing Set LS

The installation of the CliniMACS® Tubing Set TS and CliniMACS Tubing Set LS, may be performed using one of two sets of instructions. Section 3.9 describes the installation of the tubing set in a clean room environment (controlled laboratory environment) or section 3.10 describes the use of alternative installation instructions for use in a laminar air flow hood. Two sets of instructions are provided so the operator may select the appropriate set of installation instructions based on the location of the CliniMACS Plus Instrument and the design of the individual processing facility.

The CliniMACS CD34 Reagent System itself is a functionally closed system which does not necessarily need to be operated in a clean room.

Aseptic technique must be followed when installing the tubing set regardless of the installation method chosen.

3.8.1 Preparation for tubing set installation



Screen no. 3.9: Unpack tubing set

The window will display Screen no. 3.9 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 TS (REF 161-01) and the CliniMACS Tubing Set LS (REF 162-01) are each provided in sealed, sterilized packages. Each tubing set contains pre-assembled tubing and columns for one cell separation (see Figure 3.2). When the packaging is intact, a sterile fluid path is provided.

Note that both the CliniMACS Tubing Set TS and the CliniMACS Tubing Set LS may be installed using the following instructions.

1. Record the lot number and use-by date of the tubing set on the worksheet. Unpack the sterile tubing set under sterile conditions (e.g. laminar flow hood).

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

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, located on the instrument keypad, can be pushed to return to the previous step.

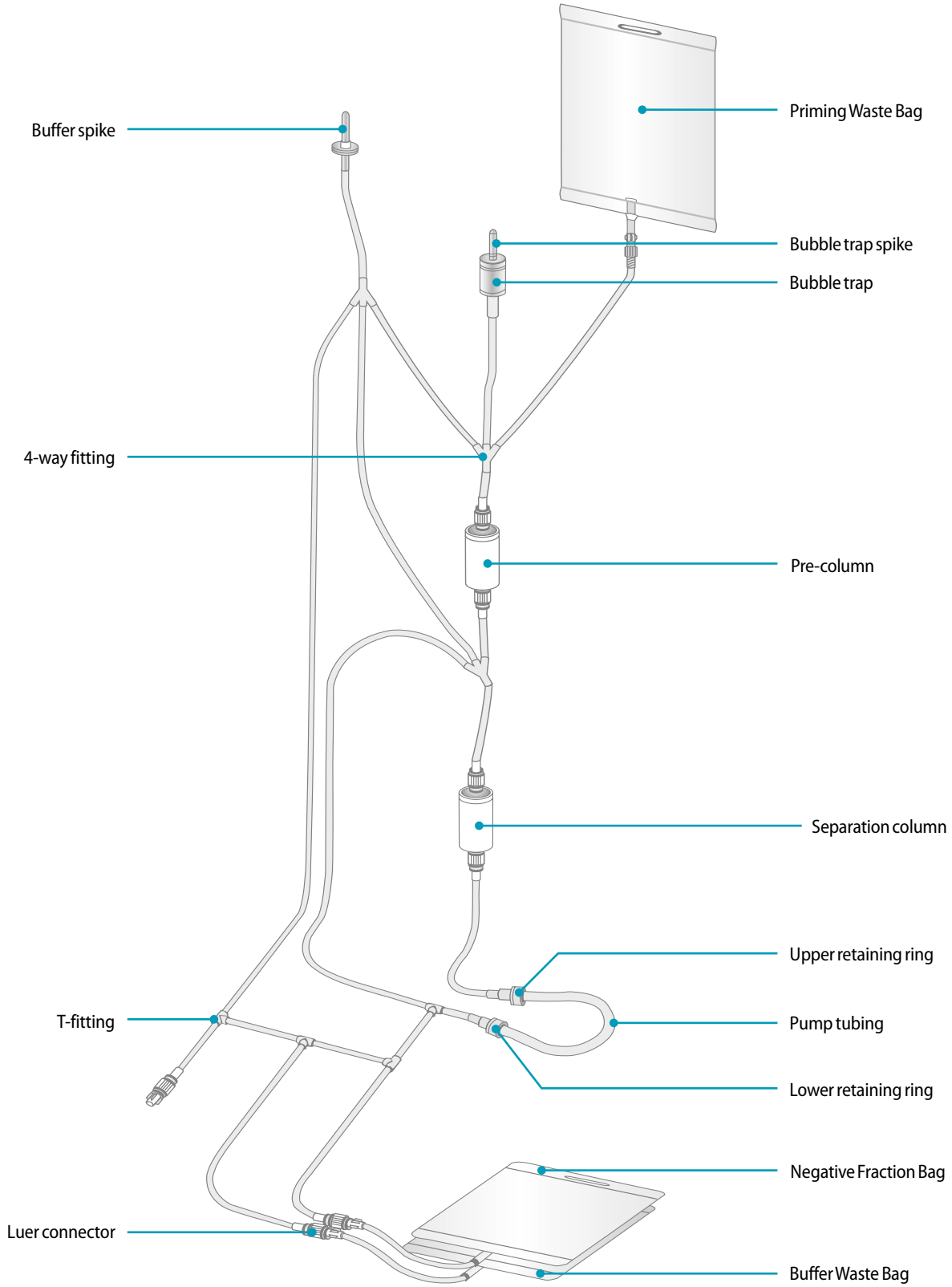


Figure 3.2: General construction of a CliniMACS Tubing Set (e.g. CliniMACS Tubing Set TS, REF 161-01, note the construction of the CliniMACS Tubing Set LS, REF 162-01, is identical)

3.8.2 Attach Cell Collection Bag

1. Record the weight of the empty Cell Collection Bag. Remove caps and attach the sterile Cell Collection Bag to the luer connector on the tubing set in an aseptic environment before loading the tubing set onto the CliniMACS Plus Instrument.

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

3. Proceed with the installation of the tubing set using either the clean room installation instructions described in section 3.9 or the alternative installation instructions for non-clean room settings described in section 3.10.

3.9 Clean room installation instructions for the CliniMACS® Tubing Sets

3.9.1 Attach Priming Waste Bag and insert Pre-column

The window will display the INSTRUMENT SETUP Screen (see Screen no. 3.9).

1. Attach the Priming Waste Bag to the right hand bag hanger on the instrument as shown (see Figure 3.3).

2. Place the pre-column into the holder as instructed on the INSTRUMENT SETUP screen (see Screen no. 3.9).

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the Priming Waste Bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow of fluid, and that it is low enough to avoid the tubing or connections being stretched.

To proceed, press ENT

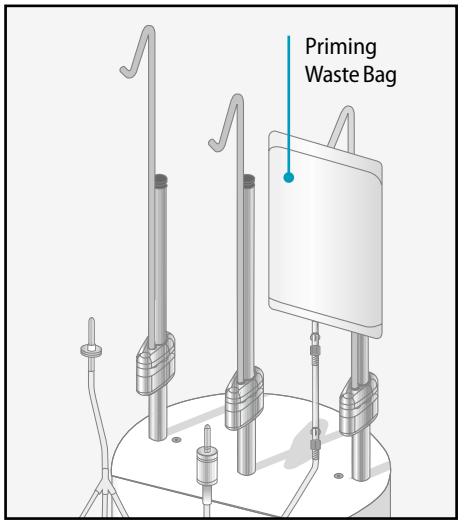


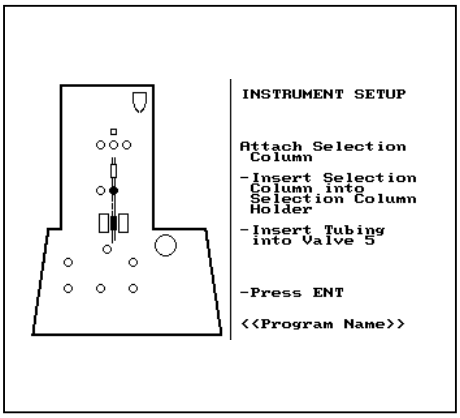
Figure 3.3: Attach Priming Waste Bag to bag hanger

Note

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

3.9.2 Insert separation column and load valve no. 5

The window will display Screen no. 3.10 as shown.



Screen no. 3.10: Insert separation column and load valve no. 5

1. Insert the separation column into the separation column holder as follows to avoid possible pinch injury: Hold the top and bottom of the column between thumb and index finger, then carefully insert the separation column into the separation column holder (see Figure 3.4).

2. Load the tubing into valve no. 5.

Note

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

Tubing must only be inserted into open valves. The tubing will not fit correctly if inserted into a closed valve. Press buttons on valves to open.

If the tubing requires adjustment after a valve has been closed, press the valve button to open the valve before adjusting the tubing (see Figure 3.5).

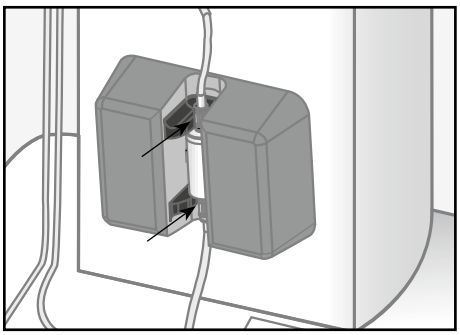


Figure 3.4: Separation column in separation column holder

If any of the valves fail to open/close properly, confirm that the tubing has been placed in the valve correctly and that the valve is clean and has not been contaminated by fluid. Refer to chapter 4 "Troubleshooting" for additional information.

To proceed, press ENT

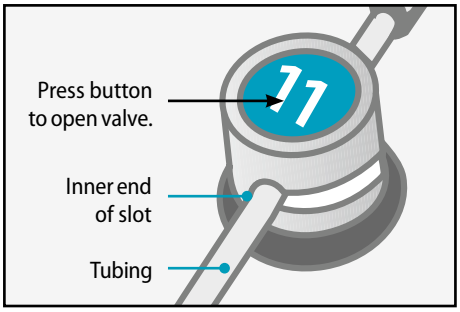
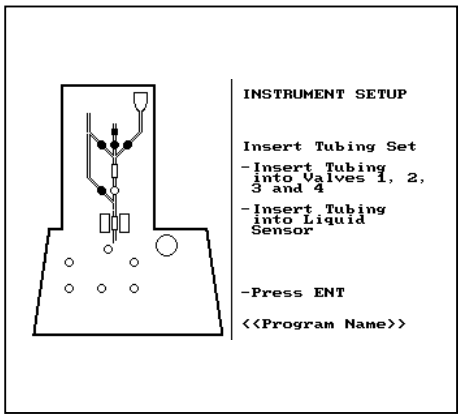


Figure 3.5: Correctly inserted tubing



Screen no. 3.11: Load valves nos. 1, 2, 3, 4, and liquid sensor

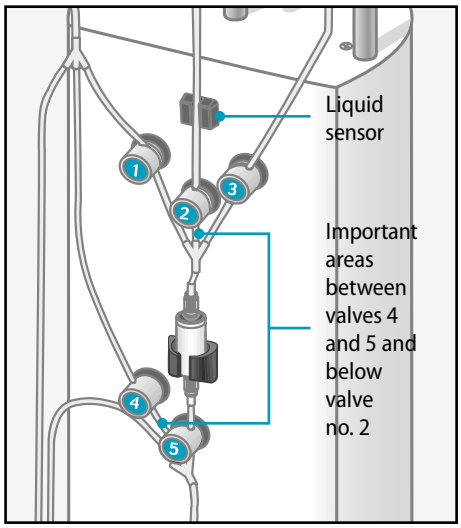


Figure 3.6: Tubing in valves

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

The window will display Screen no. 3.11 as shown.

1. Load the tubing into valve no. 4. Confirm that the tubing is placed securely in the valve opening (see Figure 3.6). Pay particular attention to the area between valves nos. 4 and 5 (see Figure 3.6).

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 3.6) and avoid bends or kinks in the tubing.

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 3.6). Confirm that the tubing is placed correctly into the sensor fitting.

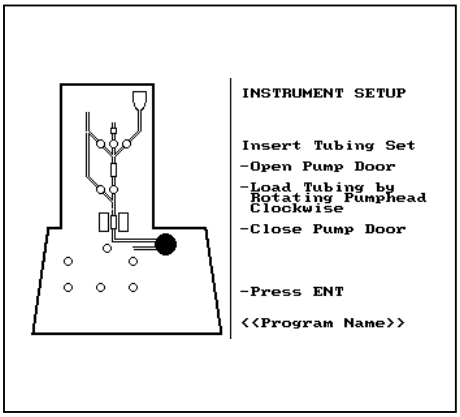
Note

To ensure proper operation, both the liquid sensor and the tubing being inserted must be dry. Inspect both and if any liquid is present, dry the area with a soft, lint-free cloth.

To proceed, press ENT

3.9.4 Load pump tubing

The window will display Screen no. 3.12 as shown.



Screen no. 3.12: 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 (see Figure 3.7) on the pump housing.
3. Rotate the pump roller clockwise (see Figure 3.7) until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins. If adjustment of the tubing inside the pump is necessary, the tubing can be unloaded by lifting the lower end and turning the pump roller anti-clockwise.
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove (see Figure 3.7) on the pump housing.
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely.
6. Close the pump door.

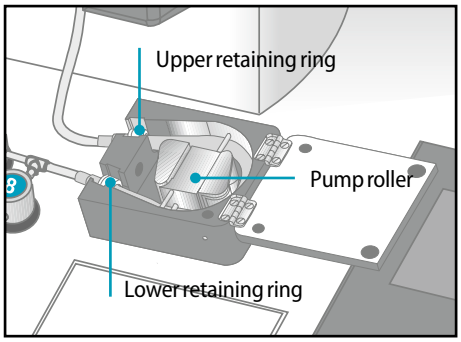


Figure 3.7: Load pump tubing

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 10 minutes the instrument will abort the run in progress. The run may not be resumed.

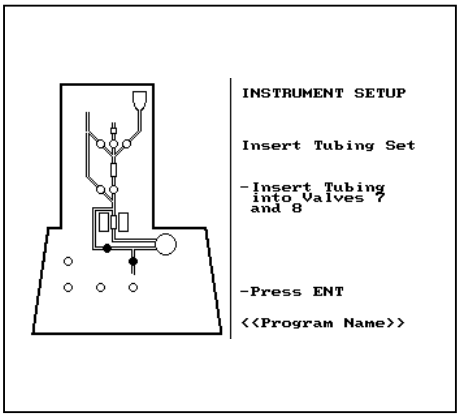
To proceed, press ENT

3.9.5 Load valves nos. 7 and 8

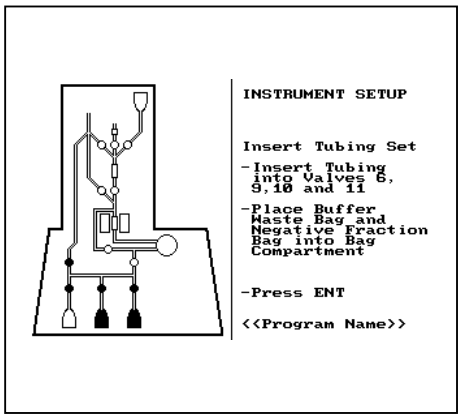
The window will display Screen no. 3.13.

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

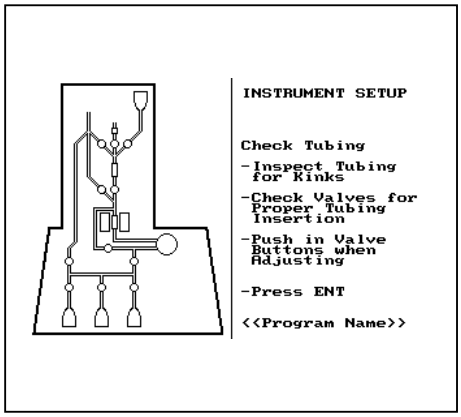
To proceed, press ENT



Screen no. 3.13: Load valves nos. 7 and 8



Screen no. 3.14: Load valves nos. 6, 9, 10, and 11



Screen no. 3.15: Recheck all tubing and attachments

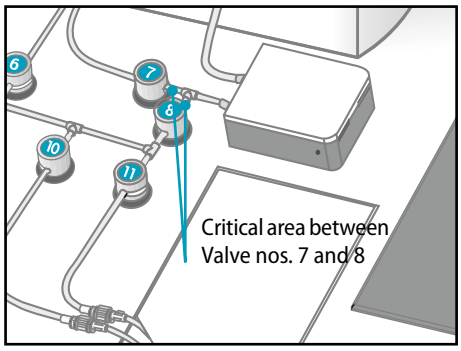


Figure 3.8: Tubing in valves

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

The window will display Screen no. 3.14.

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. Confirm that the tubing is not compressed under the bag compartment lid.

To proceed, press ENT

3.9.7 Recheck all tubing and attachments

The window will display Screen no. 3.15.

Before beginning the run, verify that all tubing and attachments are correctly placed.

Note

- Check all valves for proper tubing insertion. Confirm 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 3.8), and between valves nos. 4 and 5 (see Figure 3.6).

- If the tubing must be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve. If a section of tubing has been adjusted, it is absolutely necessary to press the corresponding valves firmly two times to properly seat the tubing.

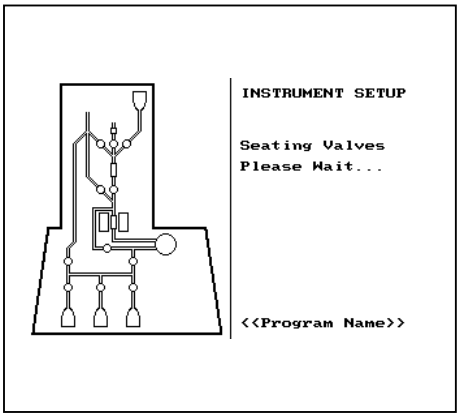
To proceed, press ENT

3.9.8 Seating of valves

The window will display Screen no. 3.16.

In order to ensure the proper fit of tubing in the valves, the CliniMACS Plus Instrument will operate all of the valves in sequence, twice. Watch and listen to confirm that all valves are working properly. Refer to chapter 4 "Troubleshooting" if any of the valves do not operate correctly. This step can be repeated by using the "UNDO" key followed by the 'ENT' key. Both keys are located on the instrument keypad.

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



Screen no. 3.16: Seating of valves

3.9.9 Attach buffer bag

The window will display Screen no. 3.17.

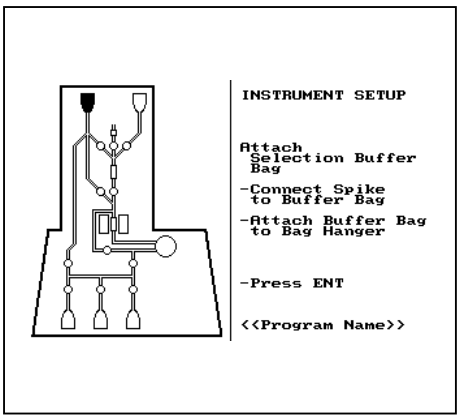
The buffer required for CliniMACS Plus Separation is the CliniMACS PBS/EDTA Buffer (1000 mL) supplemented with HSA to a final concentration of 0.5% (w/v). The HSA is not a component of the CliniMACS CD34 Reagent System and must be supplied by the user.

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

2. Attach the buffer bag to the buffer bag hook on the bag hanger (see Figure 3.9).

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow of fluids, and that it is low enough to avoid the tubing or connections being stretched (see Figure 3.9).

To proceed, press ENT



Screen no. 3.17: Attach buffer bag

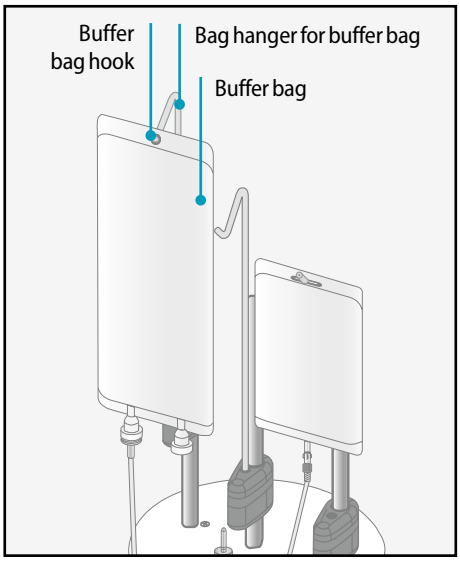
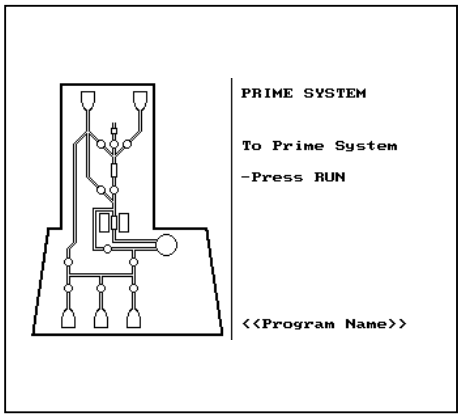
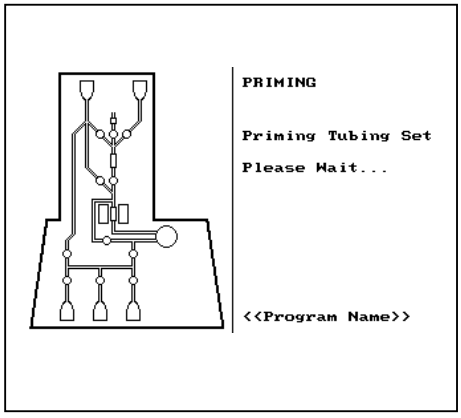


Figure 3.9: Attach buffer bag



Screen no. 3.18: Start priming



Screen no. 3.19: Priming in process

3.9.10 Start priming

The window will display Screen no. 3.18.

To start priming, press RUN

The window will display Screen no. 3.19. 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 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.

3.9.11 Check during the priming

1. 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.

2. If leaks or malfunctions are observed, stop run by pressing 'STOP'. The operator has 10 minutes to resolve the problem. Restart the process by pressing 'RUN'.

   After 10 minutes, the separation will be aborted. However, if the operator requires additional time to assess the problem, 'STOP' may be pressed multiple times to acquire additional time; each time 'STOP' is pressed the 10 minute timer resets.

Note

- **If at any time the operator allows the entire 10 minutes (shown on the screen) to elapse without pressing 'STOP' again to acquire additional time, the separation sequence will be aborted and the procedure cannot be resumed.**

- If the operator successfully resolves the problem and the tubing set is not defective (i.e. no leaks or folds that restrict the flow of fluid) continue with the procedure.

- If the operator cannot resolve the problem or if the tubing set is defective, the operator should shut down the instrument and remove the tubing set. Following the removal of the tubing set, the operator should obtain a new tubing set and return to section 3.6 and follow the instructions to begin a new procedure.

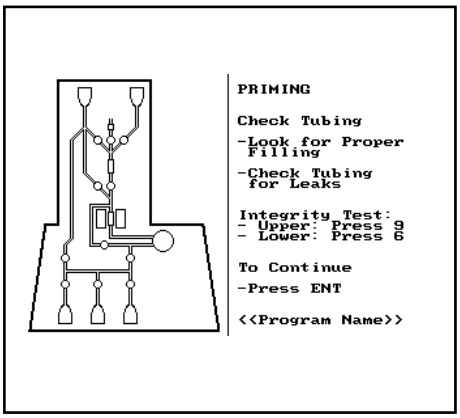
- It is not possible to return to the instrument set-up procedure once priming has begun.

3.9.12 Final check of all tubing and attachments

The window will display Screen no. 3.20.

Check the following before beginning the run:

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



Screen no. 3.20: Final check of tubing

Note

- If leaking is observed during priming, the tubing set is defective and must be replaced. Remove the defective tubing set and replace it with a new tubing set and buffer bag.

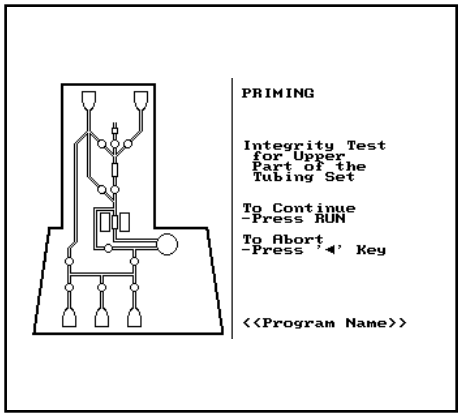
- Excessive air in the tubing set following priming indicates that the buffer bag was not properly spiked. Remove the tubing set, replace it with a new tubing set and buffer bag.

- Only a small amount of buffer should be present in the Priming Waste Bag and Buffer Waste Bag following completion of priming. Excess buffer in the Priming Waste Bag or the Buffer Waste Bag, or buffer in other bags (i.e. Negative Fraction Bag or Cell Collection Bag) indicates that the tubing set was not mounted properly. Remove the tubing set, replace it with a new tubing set and buffer bag.

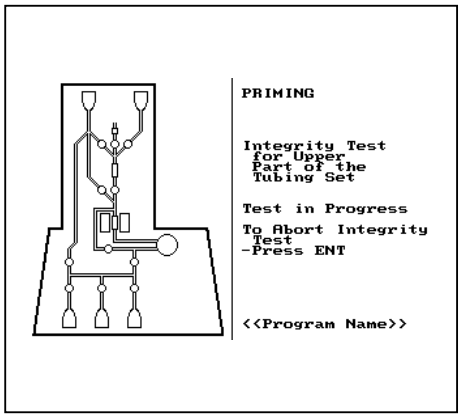
3.9.13 Integrity test

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



Screen no. 3.21: Start integrity test for upper part



Screen no. 3.22: Integrity test for upper part in process

1. When the operator performs "Final check of all tubing and attachments" the window displays Screen no. 3.20.

2. **Do not** press **'ENT'** after performing the "Final check of all tubing and attachments".

3. To enter the integrity test for the upper part of the tubing set, press 9

4. The window will display Screen no. 3.21.

5. To start the test sequence, press RUN

   To return to the display Screen no. 3.20, press ◄

   Once the 'RUN' button has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set. The window will display Screen no. 3.22.

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

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

6. After 2 minutes the pressure is automatically released, and the window displays Screen no. 3.20.

   Use a tissue to determine if any leaks have occurred during the test sequence by dabbing the tissue around the connections of the tubing set. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Keep the defective tubing set and contact Miltenyi Biotec Technical Support for further instructions.

Note

- As previously described in section 3.8, the installation of the tubing set, may be performed using one of two sets of instructions. Section 3.9 describes the installation of the tubing set in a clean room environment or section 3.10 describes the use of alternative installation instructions (i.e. laminar air flow hood).

- If the alternative installation instructions were used for the installation of the tubing set, the Cell Preparation Bag was connected to the tubing set prior to priming or initiation of the Integrity test for the upper part of the tubing set.

- If a leak was observed the operator should first use the heat sealer to seal the tubing below the Cell Preparation Bag and pre-system filter and disconnect them from the tubing set. The magnetically labeled and washed cells should be transferred a new transfer bag (Cell Preparation Bag) and retained. The operator must then remove the defective tubing set, install a new tubing set and start a new procedure.

7. Continue with the integrity test of the lower part of the tubing set if no leaks are observed.

Integrity test for the lower part of the tubing set

1. The window will display Screen no. 3.20.

2. Enter the integrity test for the lower part of the tubing set, press [6]

   Do not press **'ENT'**.

3. The window will display Screen no. 3.23.

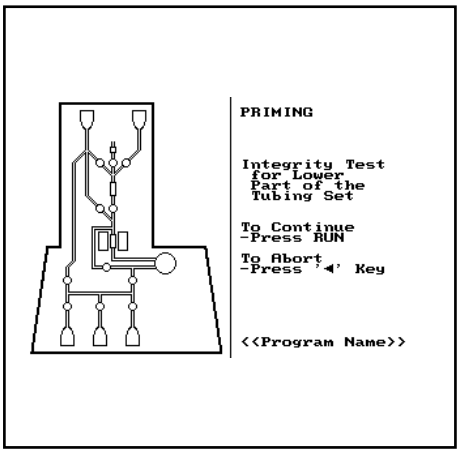
4. To start the test sequence, press [RUN]

   To return to Screen no. 3.20, press [◀]

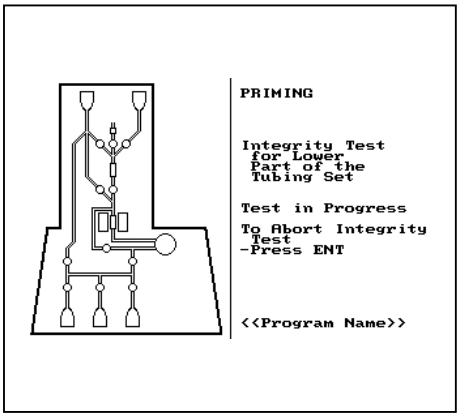
5. Once the 'RUN' button has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set. The window will display Screen no. 3.24.

   Overpressure will be created and held for 30 seconds. During this time the operator should watch for leaks around 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'.



Screen no. 3.23: Start integrity test for lower part



Screen no. 3.24: Integrity test for lower part in progress

6. After 30 seconds the pressure is automatically released, and the window displays Screen no. 3.20.

   Use a tissue to determine if any leaks have occurred during the test sequence by dabbing the tissue around the connections of the tubing set. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Keep the defective tubing set and contact Miltenyi Biotec Technical Support for further instructions.

   Note

   - As previously described in section 3.8, the installation of the tubing sets, may be performed using one of two sets of instructions. Section 3.9 describes the installation of the tubing set in a clean room environment or section 3.10 describes the use of alternative installation instructions (i.e. laminar air flow hood).

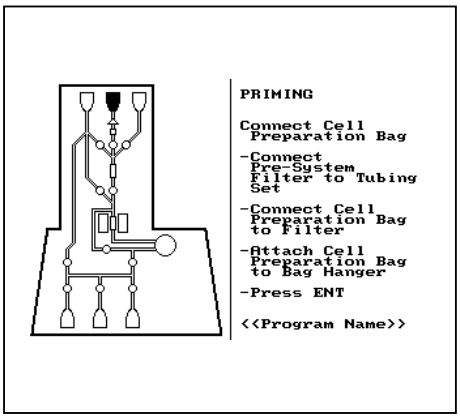
   - If the alternative installation instructions were used for the installation of the tubing set, the Cell Preparation Bag was connected to the tubing set prior to priming or initiation of the Integrity test for the upper part of the tubing set.

   - If a leak was observed the operator should first use the heat sealer to seal the tubing below the Cell Preparation Bag and pre-system filter and disconnect them from the tubing set. The magnetically labeled and washed cells should be transferred a new transfer bag (Cell Preparation Bag) and retained. The operator must then remove the defective tubing set, install a new tubing set and start a new procedure.

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

3.9.14 Connect the Cell Preparation Bag

The window will display Screen no. 3.25.



Screen no. 3.25: Connect Cell Preparation Bag

Attach the Cell Preparation Bag after the priming phase has been completed and no leaks or malfunctions are observed (see Figure 3.10 [fully assembled CliniMACS Plus Instrument]). Use aseptic techniques for all steps.

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

Note

If the alternative installation instructions were used for the installation of the tubing set, the Cell Preparation Bag was connected to the tubing set prior to prime or initiation of the upper or lower Integrity test proceed to Step 7 below.

1. Remove the cap from the bubble trap spike of the bubble trap (see Figure 3.2).

2. Remove the cap from the lower opening of the pre-system filter (see Figure 3.10). 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 3.10).

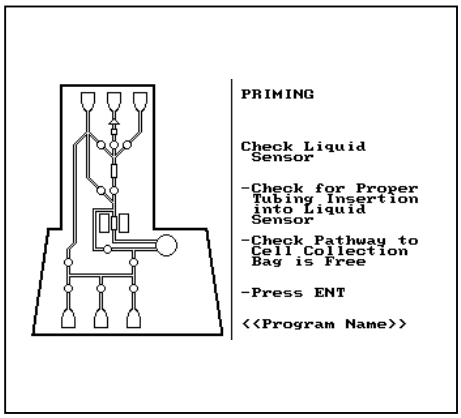
4. Spike the Cell Preparation Bag with the pre-system filter (see Figure 3.10) ensuring that the septum is punctured, allowing the free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.

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 (see Figure 3.10).

7. Adjust the bag hanger for the Cell Preparation Bag (see Figure 3.10) to hold the Cell Preparation Bag in an upright position.

To proceed, press ENT



Screen no. 3.26: Final check of the liquid sensor

3.9.15 Final check of the liquid sensor

The window will display Screen no. 3.26.

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 unrestricted flow to the Cell Collection Bag is possible.

To proceed, press ENT

Proceed to STEP 4: CliniMACS Plus Separation.

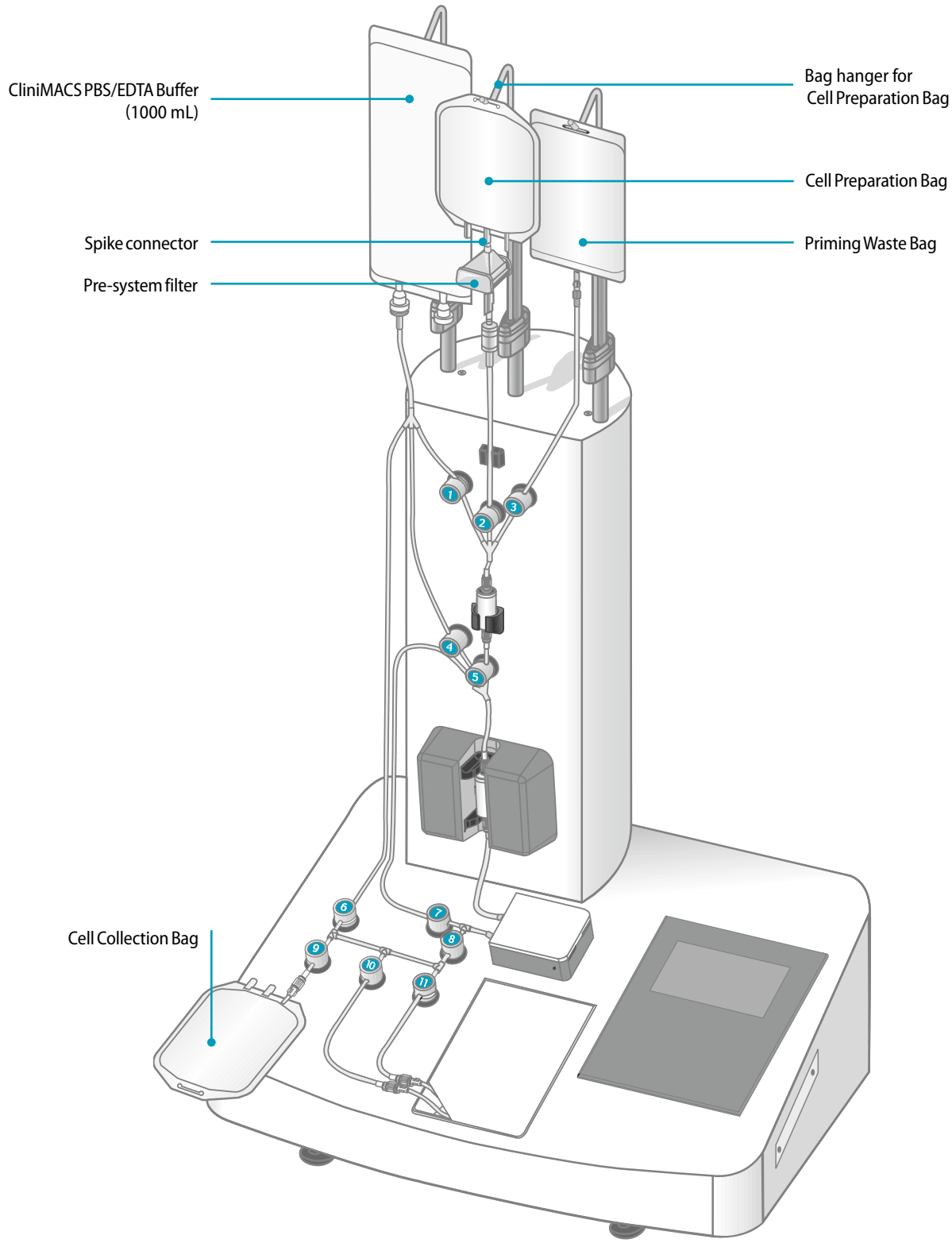


Figure 3.10: CliniMACS Plus Instrument with CliniMACS Tubing Set TS (note that configurations are identical for the TS and LS), CliniMACS PBS/EDTA Buffer (1000 mL), Cell Preparation Bag, and Cell Collection Bag

3.10 Alternative installation instructions for the CliniMACS® Tubing Sets for non-clean room settings

The previous instructions described in section 3.9 and the screens displayed by the CliniMACS® Plus Instrument describe the installation of the CliniMACS Tubing Sets in a clean room. The CliniMACS CD34 Reagent System itself is a functionally closed system which does not necessarily need to be operated in a clean room. However, if operated outside a clean room, the alternative installation instructions for the tubing set must be used since the sterility of the cell separation process may be compromised during the attachment of the Cell Collection Bag, the buffer bag, 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) using aseptic technique.

When operating instrument outside a clean room, follow the instructions below.

3.10.1 Preparation for tubing set installation

The window will display Screen no. 3.9 as previously described.

1. Unpack the tubing set, the CliniMACS PBS/EDTA Buffer (1000 mL), the pre-system filter and the Cell Preparation Bag under sterile conditions (e.g. laminar flow hood) using aseptic technique when outside of the clean room environment.

2. **Attachment of the Cell Collection Bag**
   Follow the instructions as previously described in section 3.8.2.

3. **Attachment of the CliniMACS PBS/EDTA Buffer (1000 mL)**
   Clamp the tubing just below the buffer spike with forceps in order to prevent the buffer from flowing into the tubing set during its installation (see Figure 3.11, clamp 1). Using aseptic technique 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.

4. **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 forceps (see Figure 3.11, clamp 2). This prevents the prepared cell suspension in the Cell Preparation Bag from entering the pre-system filter.

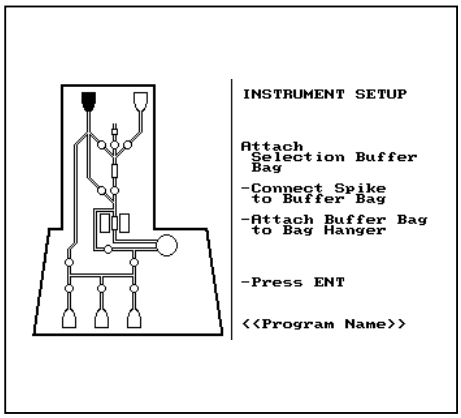
5. **Attachment of Cell Preparation Bag**
   Connect the Cell Preparation Bag containing the magnetically labeled and washed cells to the tubing set. Spike the Cell Preparation Bag with the pre-system filter ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to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

6. **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 of the instrument.

To proceed, press ENT

Follow the instructions as described in the clean room procedure for installation of the CliniMACS Tubing Sets:

- **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**



Screen no. 3.27: Attach buffer bag

3.10.2 Attach buffer bag

The window will display Screen no. 3.27.

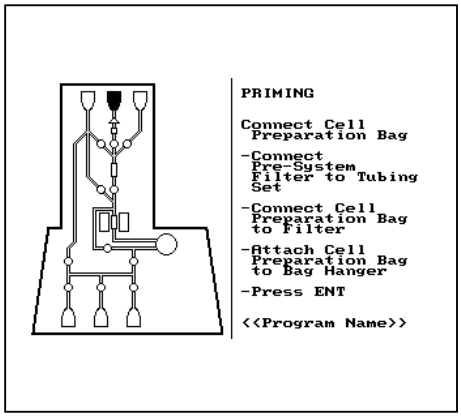
1. When using the alternative installation instructions for installation of the tubing set for non-clean room settings, the buffer bag was previously attached during „Preparation for tubing set installation". Therefore, the height of the buffer bag hanger may require adjustment. Raise or lower the hanger to accommodate the size of the buffer bag, ensuring that the height allotted is high enough to prevent the tubing from severe bending that could restrict the flow of buffer, and low enough to avoid stretching the tubing or connections.

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

To proceed, press ENT

Follow the instructions:

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



Screen no. 3.28: Connect Cell Preparation Bag

3.10.3 Connect Cell Preparation Bag and Pre-system filter

The window will display Screen no. 3.28.

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

2. Remove the clamp below the bubble trap.

To proceed, press ENT

Follow the instructions:

- **Final check of the liquid sensor**

Proceed to the CliniMACS Plus Separation.

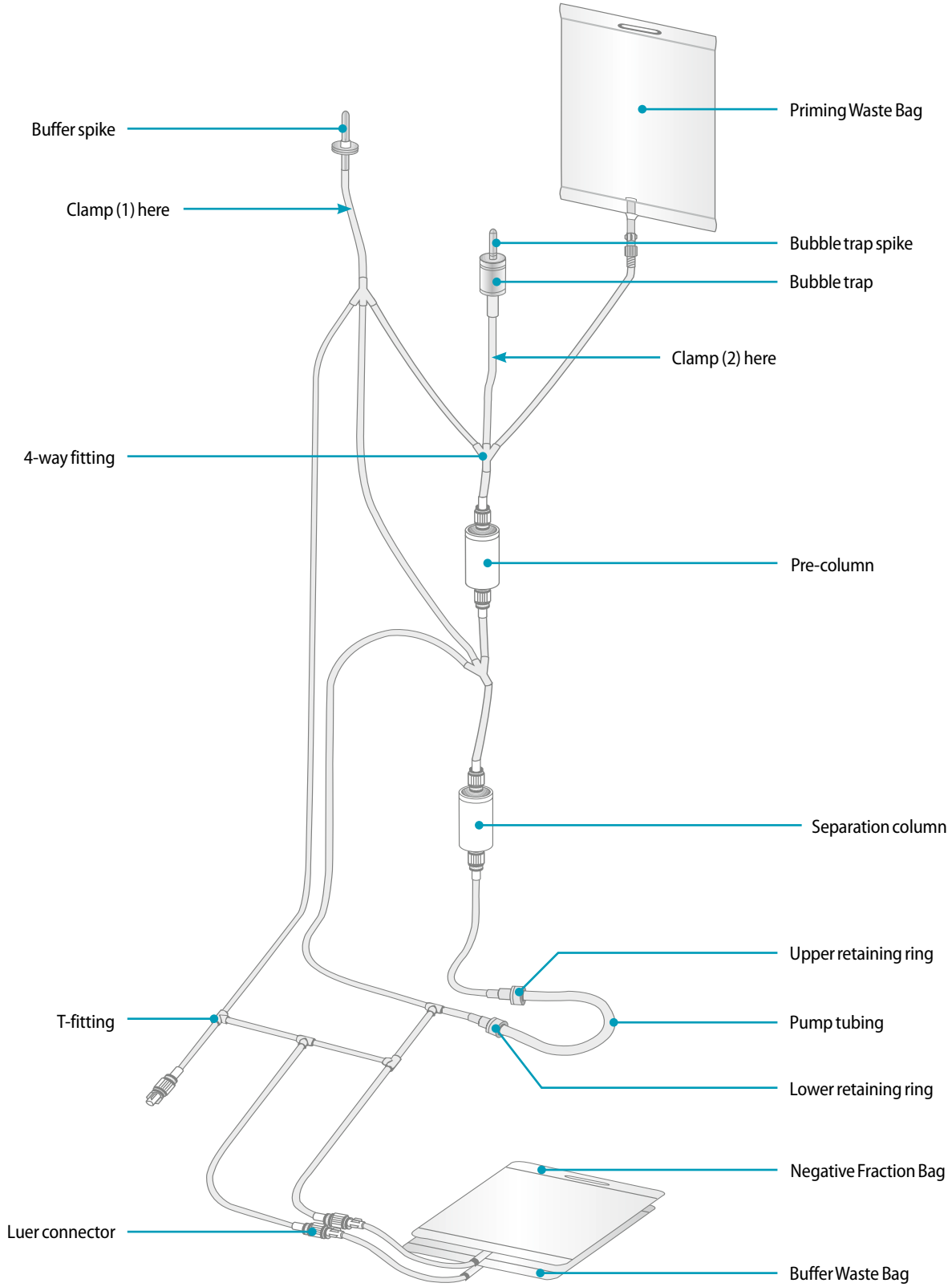
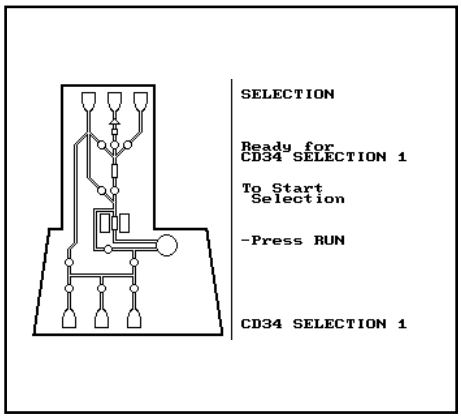


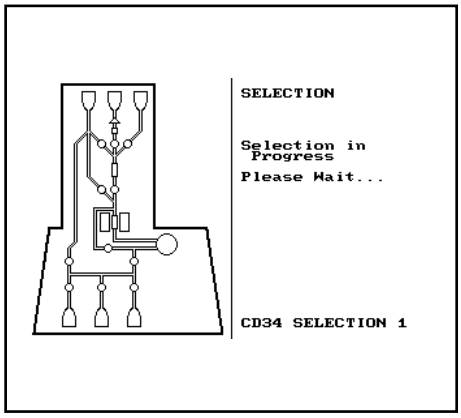
Figure 3.11: General construction of a CliniMACS Tubing Set (e.g. CliniMACS Tubing Set TS, REF 161-01, note the construction of the CliniMACS Tubing Set LS, REF 162-01, is identical)



Screen no. 3.29: Start separation

Important

At the beginning of the separation sequence, buffer is pumped upwards towards the Cell Preparation Bag to fill the Pre-system filter. Tap the side of the pre-system filter several times to remove any bubbles which might be trapped in the filter.



Screen no. 3.30: Separation in process

Note

At each phase of the operation, the status of the separation process is shown on the screen.

3.11 CliniMACS® Plus Separation

Once the final check of the liquid sensor has been completed, the CliniMACS® Plus Instrument is ready to begin the separation process, the window will display Screen no. 3.29 as shown.

To proceed, press RUN

Once 'RUN' has been pressed, the instrument will automatically perform the CD34 SELECTION program chosen.

At each phase of the operation, a screen similar to Screen no. 3.30 is displayed to show the status of the separation.

On the screen, 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 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.

3.11.1 Separation process

The general steps of the separation process are the same for both CD34 SELECTION programs. CD34 SELECTION 1 is for use with normal scale applications and CD34 SELECTION 2 is for use with large scale applications. Figure 3.12 depicts the CD34 enrichment strategy.

Loading cells

The separation process starts with the filling of the pre-system filter to complete the priming of the system. Separation of the labeled cells then begins. The pump draws the contents of the Cell Preparation Bag into the tubing set. The magnetically labeled CD34 positive cells are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and collected in the Negative Fraction Bag. When the Cell Preparation Bag is empty (detected automatically by the liquid sensor) the pre-system filter is rinsed twice with buffer.

Column wash I

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 process is shown on the screen.

Release of cells I

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

Reloading of cells I

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

Column wash II

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

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

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

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

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

Final elution of the cells

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

CD34 Separation strategy using the CliniMACS Tubing Set TS or CliniMACS Tubing Set LS

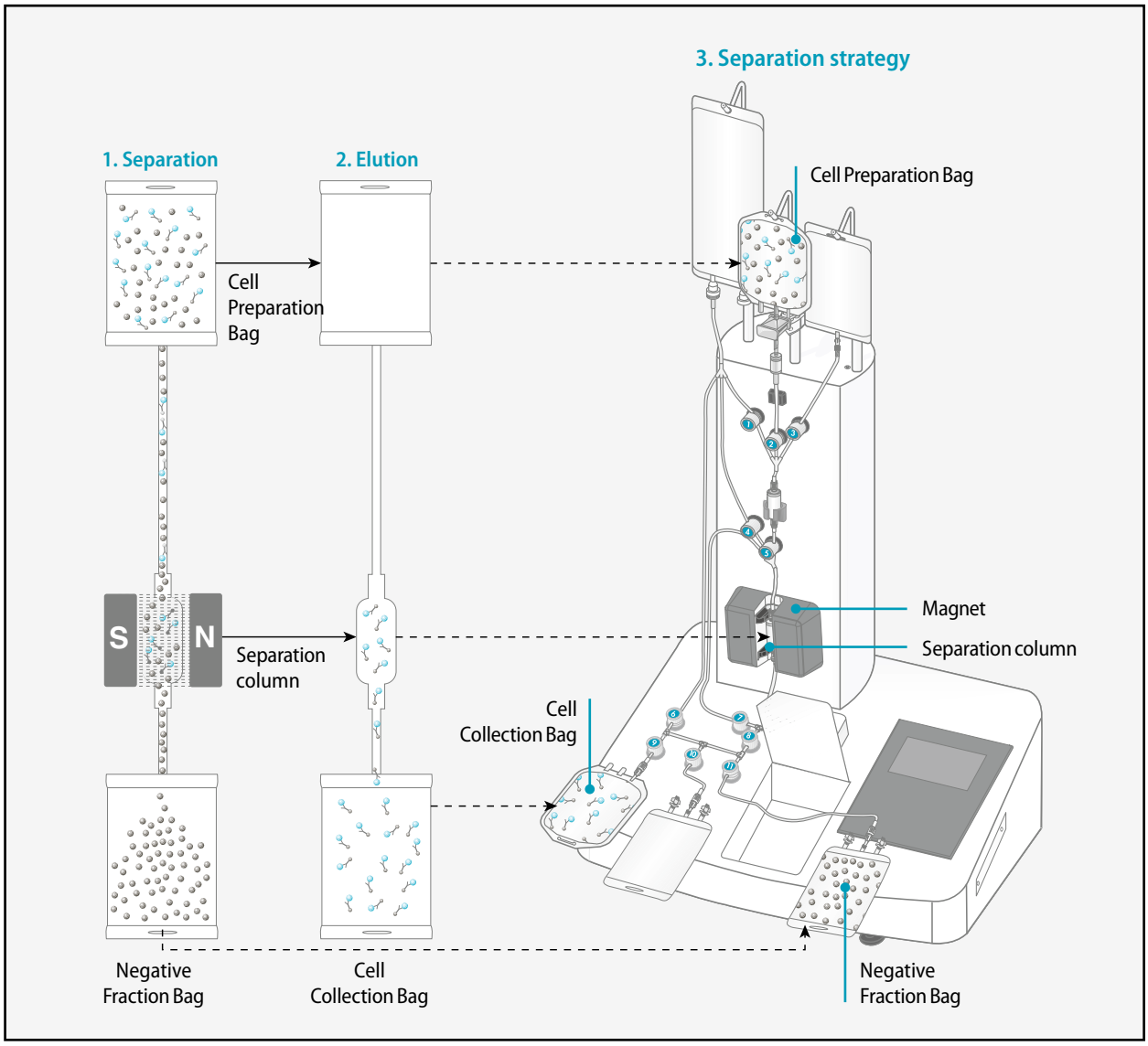


Figure 3.12: CD34 Separation strategy

1. The magnet is in the “ON”-position. The labeled CD34 positive 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 labeled cells (CD34 positive cells) are released from the separation column and collected in the Cell Collection Bag.

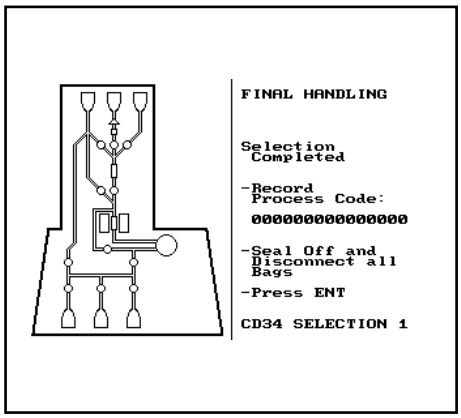
3. The CD34 SELECTION 1/2 separation programs retain the labeled CD34 positive cells in the separation column, the non-labeled cells (non-target cells) flow through the column and are collected in the Negative Fraction Bag. When the magnet is moved into the “OFF”-position, the labeled cells (CD34 positive cells) are released from the column and collected in the Cell Collection Bag.

Important

- The CD34 positive cell fraction is always collected in the Cell Collection Bag.

- The CD34 positive cell fraction collected after completion of the separation process should not be re-processed. The CliniMACS CD34 Reagent System is not designed to reprocess HPC/Apheresis.

- If for any reason, a run has irreversibly terminated prior to the CD34 positive cell fraction 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 CD34 SELECTION 1 or CD34 SELECTION 2 together with either a CliniMACS Tubing Set TS (REF 161-01) or a CliniMACS Tubing Set LS (REF 162-01).



Screen no. 3.31: Disconnect bags and record process code

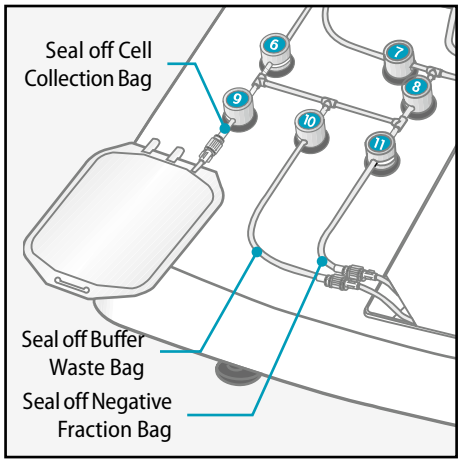
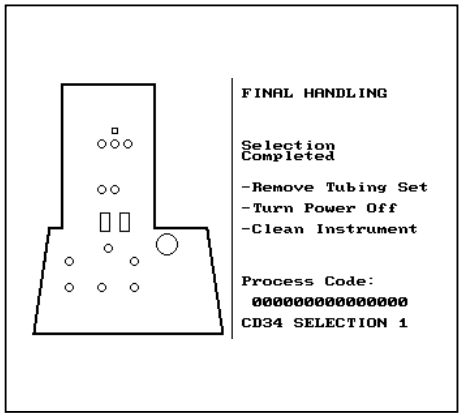


Figure 3.13: Disconnect bags

Important

Prior to infusion of the CD34 positive cells to the patient, the CliniMACS PBS/EDTA Buffer (1000 mL) contained in the target fraction must be exchanged to a medium suitable for applications in humans.



Screen no. 3.32: Unload tubing set and shutdown

3.11.2 Disconnection of bags and recording of process code

When the run has been completed, the CliniMACS Plus Instrument will display Screen no. 3.31 as shown.

1. Record the process code displayed on the screen.

2. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 3.13). Make three hermetic seals in the tubing directly below valve no. 9 using the heat sealer. Sever the middle seal carefully to disconnect the Cell Collection Bag from the tubing set.

3. Weigh the filled Cell Collection Bag and 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 CD34 positive cells thoroughly by rotating the bag. Remove an aliquot of 0.5 mL and retain for analysis.

5. Heat seal the tubing above, the luer lock of the Negative Fraction Bag (see Figure 3.13). 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 manner (see Figure 3.13).

7. Remove the Negative Fraction Bag and Buffer Waste Bag. Keep both bags until analysis of the CD34 positive cells has been completed. The CD34 positive cells can now be prepared for infusion in accordance with clinical protocols.

To proceed, press ENT

3.11.3 Unload tubing set and shutdown

The window will display Screen no. 3.32.

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 provided in section 2.4. Follow the standard procedures for the treatment of infectious material.

3.11.4 Analysis of the cells

Establish the suitability of the target CD34 positive cells before infusion. Examine the following parameters:

This must include the following parameters:

- Total number of leukocytes
- Viability
- Total number of CD34 positive cells
- Total number and CD3 positive cells
- Purity and recovery of CD34 positive cells
- CD3 log depletion

Assessment of the non-target fraction for the total number and viability of leukocytes is recommended to assess the performance of the device and quality of the device output (CD34 positive cells).

Other tests may be included based on individual laboratory practice.

Record the data analysis.

Note

- If the yield of CD34 positive cells is low:

  - The CD34 positive cell content may have been over-estimated in the starting HPC, Apheresis.
  - CD34 positive cells may have been poorly labeled with the CliniMACS CD34 Reagent.
  - Cells may have been lost during the preparation steps.
  - Flow analysis may be incorrect.

  Refer to chapter 4 “Troubleshooting“ for additional information regarding a low yield of CD34 positive cells.

- Other factors may also result in a low purity of CD34 positive cells. These factors include the following:

  - incorrect storage of the HPC, Apheresis
  - failure to follow the labeling procedure correctly
  - granulocyte contamination in the starting product
  - valve malfunctions
  - incomplete elution from the separation column
  - a pump failure,
  - blockage of the tubing to the Cell Collection Bag

  Refer to chapter 4 “Troubleshooting“ for additional information regarding a low yield of CD34 positive cells. See the Instructions for Use for the CliniMACS CD34 Reagent System for information regarding device performance.

4 Troubleshooting

This troubleshooting chapter is intended as a reference to provide information regarding possible unexpected events that might occur during the use of the CliniMACS CD34 Reagent System and to suggest appropriate corrective action.

For information not covered in this manual or further technical assistance, contact Miltenyi Biotec Technical Support at:

Miltenyi Biotec Technical Support
+49 2204 8306-3803
technicalsupport@miltenyi.com

For complaint registration regarding any of the components included in the CliniMACS CD34 Reagent System, contact Miltenyi Biotec Technical Support at:

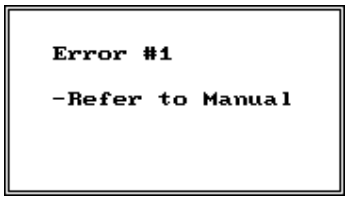
Miltenyi Biotec Technical Support
+49 2204 8306-3803
technicalsupport@miltenyi.com

4.1 Preparation of the HPC, Apheresis

The HPC, Apheresis received is diluted.	Ideally, magnetic labeling is performed in diluted HPC, Apheresis. Adjust the HPC, Apheresis to a final dilution of approximately 1:3. If the product received is diluted more than 1:3 or, if the concentration of plasma in the sample is unknown, add immunoglobulin (IgG) to the sample prior to the addition of the CliniMACS CD34 Reagent (recommended concentration of IgG in the labeling volume: 1.5 mg/mL). It is important to include an appropriate amount of immunoglobulin in the sample during the labeling in order to minimize non-specific binding of the CliniMACS CD34 Reagent.
The number of target cells is low in the HPC, Apheresis.	The mobilization of stem cells was insufficient. Check analysis of the HPC, Apheresis.
Poor viability of cells in the HPC, Apheresis.	The HPC, Apheresis may have been harvested, stored or transported inappropriately. To ensure better sample quality, the preparation and separation of the HPC, Apheresis should be performed immediately after leukapheresis. Keep the HPC, Apheresis at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the HPC, Apheresis with autologous plasma. The HPC, Apheresis should not be older than 24 hours when starting the labeling and separation process. If the HPC, Apheresis has to be stored, e.g., overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+67 °F to +77 °F]).

4.2 CliniMACS® Plus Instrument and CliniMACS Tubing Sets

Error messages



Error message no. 1

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.

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

3. Other than error messages, malfunctions that can be corrected by the operator are marked "Warning messages". These are described under 4.3 “Automated cell separation“ below.

Loading and priming of the CliniMACS® Tubing Sets

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, refer to below to the topic "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 must 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, install a new tubing set, and restart priming with sufficient new buffer.
Excessive air occurs in tubing set after priming.	Buffer bag is not properly spiked (punctured). Use a new tubing set and sufficient new buffer and restart the run. 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 present 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 separation performance may result if the tubing set is not inserted properly.
Pump motor stalls during priming.	Pump tubing has not been inserted correctly. Press 'STOP' to interrupt the priming and turn the power "OFF" and then "ON" again. Clamp the buffer line with a hemostat during the installation procedure and remove the clamp before restarting the priming sequence.

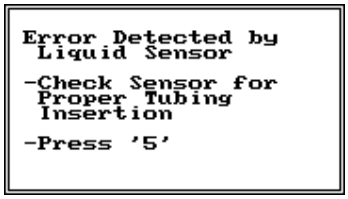
4.3 Automated cell separation

Warning messages

Unlike error messages described above, 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 run, follow the instructions on the screen to proceed with the cell separation. Generally speaking, warning messages appear when the 'STOP' key is pressed, when the pump door is opened, when the pump stalls or when the liquid sensor detects an error.

Unexpected events CD34 SELECTION 1/2

Error detected by the 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, inspect the tubing for kinks blocking the buffer flow upwards into the pre-system filter and Cell Preparation Bag. Adjust the position of the tubing set. If necessary, raise or lower the bag hangers using the bag hanger clamps. Adjust the position of the tubing in the valves. To alter the position of the tubing, open the valve by manually pressing the button. Make sure that the tubing is not kinked, twisted or taut.

3. Is the Cell Preparation Bag spiked properly? Confirm the pre-system filter spike has penetrated the septum of the Cell Preparation Bag port.

4. If the tubing set has not been completely filled with buffer, has the buffer spike of the tubing set penetrated the buffer bag? For corrective action refer to section 4.2, "Excessive air occurs in the tubing set after priming".

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 defective. 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.

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

1. Tubing set has not been properly inserted. If the run is ongoing, press the 'STOP' and clamp the line with a hemostat. Adjust the tubing by first depressing the appropriate valve. Remove the clamp and press 'RUN' to resume separation. The cell separation will be aborted if 'RUN' is not pressed within 10 minutes.

2. Valve is not functioning properly. Press 'STOP'. Clamp the line with a hemostat. Depress the valve manually several times to un-stick the stuck valve. Remove the clamp and press 'RUN' to continue. The cell separation will be aborted if the 'RUN' key is not pressed within 10 minutes. If the valve cannot be un-stuck, contact Miltenyi Biotec Technical Support.

3. 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.

1. 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 for assistance.

2. A magnet drive failure has occurred. An error message will be displayed (see section 4.2, "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

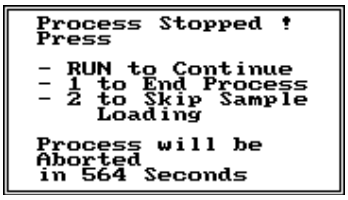
Pump tubing has not been inserted correctly, therefore, the pump might be unable to rotate. In this case warning message no. 2 will appear on the screen window. The operator then has 10 minutes to correct the position of the pump tubing.

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.

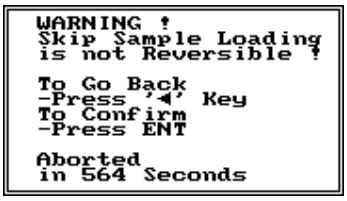
3. If the pump tubing has not been damaged, it can be reinserted into the pump housing.

4. Press 'RUN' to restart the separation process within 10 minutes 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 is wet. Press'STOP' to interrupt the separation process. 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 10 minutes or the cell separation will be terminated. If it is not possible to activate the liquid sensor following these steps, press 'STOP', then '2' and confirm with 'ENT' to skip sample loading and to continue the separation process.
Loading stopped before complete sample has been loaded onto the columns.	1. 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 was created which led to the generation of air bubbles activating the liquid sensor. Therefore, the separation process proceeded 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. 2. 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 process has finished, filter the remaining sample in the Cell Preparation Bag 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. Do not reprocess the cells that completed the separation process.
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 process 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 an opportunity to confirm or amend the decision made because skipping of sample loading is not reversible.



Warning message no. 4

3. Press 'ENT' and the separation program will stop the sample loading and continue with column washes. Clamp the tubing below the pre-system filter to prevent cells from leaking out of the Cell Preparation Bag into the tubing set. After the separation process 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 using a new tubing set and sufficient new buffer.

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.

- '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, 'RUN' is not pressed within 600 seconds, the separation will be aborted and the cell separation will not be completed. Recover as much of the sample as possible from the tubing set by running the emergency program.

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

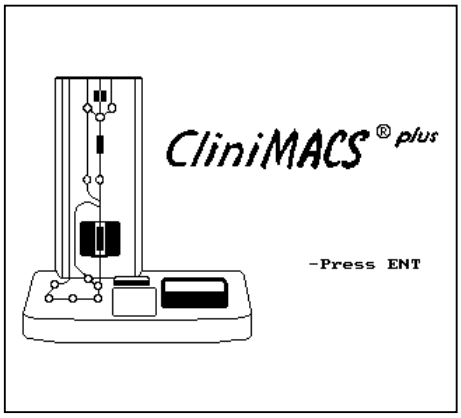
4.4 Emergency program

If for any reason a run has irreversibly terminated prior to the CD34 positive 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 CD34 SELECTION 1 (normal scale applications) or CD34 SELECTION 2 (large scale applications) together with either a CliniMACS Tubing Set TS (REF 161-01) or a CliniMACS Tubing Set LS (REF 162-01), respectively.

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. 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.

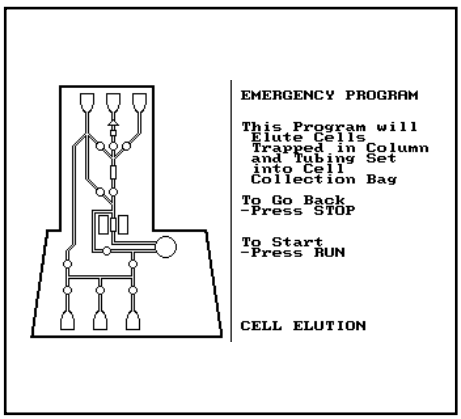
2. Wait until the following screen appears:



Screen no. 4.1: Main screen

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

4. The following screen appears:



Screen no. 4.2: 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. Pool any cells remaining in the Cell Preparation Bag with the cells eluted by the emergency program. Start a new separation process using a new tubing set and sufficient new buffer.

To leave the emergency program without starting the elution of the trapped cells, press 'STOP'.

4.5 Cell separation performance – Unexpected events

The yield of target cells is low.

1. Target cell content was over-estimated in the HPC, Apheresis. During analysis, target cells were incorrectly counted or an error occurred during counting of leukocytes. Repeat the analysis of HPC, Apheresis for starting target cell content.

2. Target cells were poorly labeled with the reagent:
   - CD34 Reagent was expired. Check use-by date. Do not use any CD34 Reagent after the use-by date.
   - CD34 Reagent was not stored properly. Check storage temperature. Do not use any CD34 reagent that has been stored improperly (see reagent package insert).
   - Recommended cell labeling procedure was not followed. Refer to sample preparation and labeling procedures in chapter 3.

3. 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 HPC, Apheresis and the labeled HPC, Apheresis. 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), (refer to section 3.3 "Preparation of the CliniMACS® PBS/EDTA Buffer (1000 mL)").
   - Centrifuge settings were suboptimal. Check centrifugation settings.
   - Centrifuge imbalance or use of brake or asymmetrical loading of centrifuge.

4. Cell viability decreased during preparation. See section "Viability of the positive fraction is less than 90% or the color of the supernatant during the washing steps was red" below.

The yield of target cells is low (continued).	5. Non-target cells were retained. - Mobilization of CD34 positive target cells was poor. Very low number of CD34 positive target cells was present in the HPC, Apheresis. Therefore, a low number of contaminating non-target cells (e.g. granylocytes, monocytes, platelets) may lead to decreased purity. - Insufficient plasma or IgG were present during magnetic labeling. Follow the instructions given for the magnetic labeling. If a final concentration of about 30% autologous plasma in the sample during magnetic labeling cannot be guaranteed, add IgG 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.
The purity of target cells is low.	1. The HPC, Apheresis was stored inappropriately. Preparation and selection of the HPC, Apheresis should be performed immediately after leukapheresis. Keep the HPC, Apheresis at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the HPC, Apheresis with autologous plasma. The HPC, Apheresis should not be older than 24 hours when starting the labeling and selection procedure. If the HPC, Apheresis has to be stored, e.g., overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+67 °F to +77 °F]). 2. The magnetic labeling protocol has not been followed (e.g., incorrect volumes during magnetic labeling). Follow the instructions given for the magnetic labeling. For troubleshooting purposes determine the leukocyte subsets (B cells, T cells, monocytes, granulocytes as well as platelets) contaminating the target fraction and contact Miltenyi Biotec Technical Support. 3. High numbers of granulocytes contaminated the starting HPC, Apheresis (suboptimal apheresis setting). Dying granulocytes will then bind the CliniMACS CD34 Reagent non-specifically which may lead to decreased purity of the CD34 positive target cells. 4. 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.“ (section 4.3). - Pump failure or valve failure occurred. Recover cells from the tubing set following the emergency program as previously described. 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 was not properly inserted. Check all valves for proper tubing insertion.

The purity of target cells is low (continued).	5. Non-target cells were retained. - Mobilization of CD34 positive target cells was poor. Very low number of CD34 positive target cells was present in the HPC, Apheresis. Therefore, a low number of contaminating non-target cells (e.g., granylocytes, monocytes, platelets) may lead to decreased purity. - Insufficient plasma or IgG were present during magnetic labeling. Follow the instructions given for the magnetic labeling. If a final concentration of about 30% autologous plasma in the sample during magnetic labeling cannot be guaranteed, add IgG 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.
The purity of target cells is low (continued).	5. Non-target cells were retained. - Mobilization of CD34 positive target cells was poor. Very low number of CD34 positive target cells was present in the HPC, Apheresis. Therefore, a low number of contaminating non-target cells (e.g., granylocytes, monocytes, platelets) may lead to decreased purity. - Insufficient plasma or IgG were present during magnetic labeling. Follow the instructions given for the magnetic labeling. If a final concentration of about 30% autologous plasma in the sample during magnetic labeling cannot be guaranteed, add IgG 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.
Viability of the positive fraction is less than 90% or the color of the supernatant during the washing steps was red.	Cell lysis occurred due to incorrect osmolarity of the buffer. Check buffer and use recommended buffer.
Non-specific retention of dead cells from HPC, Apheresis or high non-specific cell losses throughout the procedure.	1. Buffer does not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5 % (w/v), (see section 3.3 “Preparation of the CliniMACS® PBS/EDTA Buffer (1000 mL)“). 2. The HPC, Apheresis may have been stored inappropriately. Preparation and separation of the HPC, Apheresis should be performed immediately after leukapheresis. Keep the HPC, Apheresis at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis with autologous plasma. The HPC, Apheresis should not be older than 24 hours when starting the labeling and separation process. If the HPC, Apheresis has to be stored, e.g., overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+67 °F to +77 °F]). 3. 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.

A.1 Glossary

A.1.1 Glossary of symbols

Safety symbols



General warning sign



Warning: Magnetic field

Symbols used for labeling products

MD

Medical Device

CE 0123

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

UK CA

UK Conformity Assessed mark

c UL us LISTED LABORATORY EQUIPMENT

UL listing mark, listed as laboratory equipment



Consult instructions for use



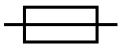
Caution



Manufacturer



Date of manufacture



Fuse



Non-ionizing radiation



“OFF” (power)

“ON” (power)



Separate collection for waste of electrical and electronic equipment



Keep dry



Fragile, handle with care



This way up



Do not re use



Do not use if package is damaged



Use-by date



Temperature limit



Non-pyrogenic fluid path



The packaging is PVC free



Non-pyrogenic



Batch code



Part number



Catalogue number (REF)



Serial number



Sterilized using aseptic processing techniques



Sterilized using steam or dry heat



Sterilized using ethylene oxide



Phone



Fax



E-mail



Website

A.1.2 Glossary of terms

Apheresis	The method of collecting blood in which whole blood is withdrawn, a desired component selected and retained, and the remainder of the blood returned to the donor
Bag compartment	Compartment of the CliniMACS Plus Instrument where 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, and CliniMACS PBS/EDTA Buffer (1000 mL)
Buffer Waste Bag	Waste bag to collect buffer during cell separation using the CliniMACS Plus Instrument
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 (CD34 positive cells) are accumulated after separation. This bag is provided by the user.
Cellular starting Product	Cell-containing apheresis product used as starting material for the CliniMACS Plus Separation, e.g., HPC, Apheresis
Clean room	A room in which the concentration of airborne particles is controlled, and which is constructed and used in a manner to minimize the introduction, generation, and retention of particles inside the room and in which other relevant parameters, e.g., temperature, humidity, and pressure, are controlled as necessary (ISO 14664-1).
CliniMACS CD34 Reagent	Reagent for magnetic labeling of cells expressing the CD34 antigen
CliniMACS PBS/EDTA Buffer (1000 mL)	Buffer used for cell preparation and cell separation with the CliniMACS CD34 Reagent System: PBS (phosphate buffered saline), supplemented with 1 mM EDTA, pH 7.2. Before use, CliniMACS PBS/EDTA Buffer (1000 mL) must be supplemented with pharmaceutical grade HSA to a final concentration of 0.5% (weight/volume, i.e. 5 g HSA per liter buffer). HSA is not a component of the CliniMACS CD34 Reagent System and is supplied by the user.
CliniMACS Tubing Set TS or CliniMACS Tubing Set LS	Set of tubes, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place
CliniMACS Plus Instrument	Magnetic cell separation instrument based on the MACS Technology
EDTA	Ethylene-diamine-tetra-acetic acid

Heat Sealer	Heating device used to sterile seal PVC tubing. Additional equipment provided by the user
Hematopoietic progenitor cells	Progenitor cells of lymphoid, myeloid, and erythroid lineages
HPC, Apheresis	Hematopoietic Progenitor Cells, Apheresis
Labeling	Reaction of cells with magnetic labeling reagent, e.g., CliniMACS CD34 Reagent to CD34 positive cells
Leukapheresis	Apheresis collecting leukocytes. HPC, Apheresis.
Liquid sensor	Component of the CliniMACS Plus Instrument that detects liquid in the tubing
Luer connector	Screw coupling, part of the tubing set
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 TS and CliniMACS Tubing Set LS containing the non-target cell fraction (CD34 negative cells)
Orbital rotator	Device used to mix HPC, Apheresis during the reaction with CliniMACS CD34 Reagent. Additional equipment provided by the user.
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. Additional equipment provided by the user.
Pre-column holder	Support mounted on the CliniMACS Plus Instrument that holds the pre-column in place
Pre-column	First column in the CliniMACS Tu[b]ing Set TS and the CliniMACS Tubing Set LS, serves as filter to trap cells having non-specific interactions with column matrix
Pre-system filer	40 µm filter device between Cell Preparation Bag and pre-column used to trap clumps and cell debris. Additional material provided by the user.
Priming Waste Bag	Buffer used to flush the tubing set during the priming step is collected in this bag
Priming	Step prior to cell separation in which buffer is flushed through the tubing set
Pump safety switch	Sensor that prevents pump operation when the pump door is open
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 the CliniMACS CD34 Reagent to the Cell Preparation Bag. Additional material provided by the user.
Separation column holder	Molded guides in the magnet housing that hold the separation column in place
Separation column	Column where the magnetically labeled cells are separated when exposed to the magnetic field
Separation program	Software program designed for the enrichment of magnetically labeled cell subsets (CD34 positive cells) from a mixed cell population. The operator can choose from a menu of separation programs depending on the intended procedure (CD34 SELECTION 1 for normale scale applications or CD34 SELECTION 2 for large scale applications).
Sterile tubing connector	A device used to connect tubing aseptically outside a laminar flow hood. Additional equipment provided by the user.
T-fitting	T-shaped fitting on a tubing set where three tubing meet
Transfer bag	Bag with a tubing and a spike at the end. Additional material provided by the user.
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
×g	Multiples of the earth's gravitational acceleration

A.2 Guidance and manufacturer`s declaration

Warning Labeling, Guidance and Manufacturer's Declaration in Accompanying Documents of Medical Elecrical Equipment According to IEC 60601-1-2:2014 +AMD1:2020.

A.2.1 Instructions for use

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

A.2.2 Technical description

EMC compliance with IEC 60601-1-2:2014 +AMD1:2020 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
RF Emissions CISPR 32	Class A
Harmonic emissions IEC 61000-3-2	Class A
Voltage fluctuations/Flicker emissions IEC 61000-3-3	

Table A.1: Guidance and manufacturer's declaration – Electromagnetic emissions

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

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

Guidance and manufacturer's declaration – Electromagnetic immunity		
The CliniMACS Plus Instrument is intended for 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 61000-4-6	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 A.3 below: Specifications for immunity to RF wireless communication equipment	See table A.3. below: Specifications for immunity to RF wireless communication equipment
Proximity magnetic fields in the frequency range from 9 kHz to 13.56 MHz IEC 61000-4-39	See table A.4 below: Specification for immunity to proximity magnetic fields in the frequency range from 9 kHz to 13.56 MHz	See table A.4 below: Specification for immunity to proximity magnetic fields in the frequency range from 9 kHz to 13.56 MHz
NOTE U_T is the a.c. mains voltage prior to application of the test level.		

Table A.2: Guidance and manufacturer's declaration – Electromagnetic immunity

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

Table A.3: Guidance and manufacturer's declaration – Electromagnetic immunity to RF wireless communication equipment

Guidance and manufacturer's declaration - Electromagnetic immunity to proximity magnetic fields in the frequency range 9 kHz to 13.56 MHz			
Test Frequency (MHz)	**Modulation**	**Immunity test Level (V/m)**	**Compliance Level (V/m)**
134.2 kHz	Pulse modulation 2.1 kHz	65	65
13.56 MHz	Pulse modulation 50 kHz	7.5	7.5

Table A.4: Guidance and manufacturer's declaration – Electromagnetic immunity to proximity magnetic fields in the frequency range 9 kHz to 13.56 MHz (model CS3)



Miltenyi Biotec

Germany/Austria
Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68
51429 Bergisch Gladbach
Germany
+49 2204 8306-0
+49 2204 85197
macsde@miltenyi.com

USA/Canada
Miltenyi Biotec Inc.
2303 Lindbergh Street
Auburn, CA 95602
USA
800 FOR MACS
+1 866 811 4466
+1 877 591 1060
macsus@miltenyi.com

Australia
Miltenyi Biotec
Australia Pty. Ltd.
Unit 11, 2 Eden Park Drive
Macquarie Park NSW 2113
Australia
+61 2 8877 7400
+61 2 9889 5044
macsau@miltenyi.com

Benelux
Miltenyi Biotec B.V.
Dellaertweg 9C
2316 WZ Leiden
The Netherlands
macsnl@miltenyi.com
Customer service The Netherlands
0800 4020120
0800 4020100
Customer service Belgium
0800 94016
0800 99626
Customer service Luxembourg
800 24971
800 24984

China
Miltenyi Biotec Technology &
Trading (Shanghai) Co., Ltd.
Room A401, 4/F
No. 1077, Zhangheng Road
Pudong New Area
201203 Shanghai
P.R. China
+86 21 6235 1005-0
+86 21 6235 0953
macscn@miltenyi.com.cn

France
Miltenyi Biotec SAS
10 rue Mercoeur
75011 Paris
France
+33 1 56 98 16 16
macsfr@miltenyi.com

Hong Kong
Miltenyi Biotec Hong Kong Ltd.
Unit 301, Lakeside 1
No. 8 Science Park West Avenue
Hong Kong Science Park
Pak Shek Kok, New Territories
Hong Kong
+852 3751 6698
+852 3619 5772
macshk@miltenyi.com.hk

Italy
Miltenyi Biotec S.r.l.
Via Paolo Nanni Costa, 30
40133 Bologna
Italy
+39 051 6 460 411
+39 051 6 460 499
macsit@miltenyi.com

Japan
Miltenyi Biotec K.K.
NEX-Eitai Building 5F
16-10 Fuyuki, Koto-ku
Tokyo 135-0041
Japan
+81 3 5646 8910
+81 3 5646 8911
macsjp@miltenyi.com

Nordics and Baltics
Miltenyi Biotec Norden AB
Medicon Village
Scheeletorget 1
223 81 Lund
Sweden
macsse@miltenyi.com
Customer service Sweden
0200 111 800
+46 280 72 99
Customer service Denmark
80 20 30 10
+46 46 280 72 99
Customer service
Norway, Finland, Iceland,
and Baltic countries
+46 46 280 72 80
+46 46 280 72 99

Singapore
Miltenyi Biotec Asia Pacific Pte Ltd.
438B Alexandra Road, Block B
Alexandra Technopark
#06-01
Singapore 119968
+65 6238 8183
+65 6238 0302
macssg@miltenyi.com

South Korea
Miltenyi Biotec Korea Co., Ltd.
Donggeuk 7F
562 Nonhyeon-ro
Gangnam-gu
Seoul 06136
South Korea
+82 2 555 1988
+82 2 555 8890
macskr@miltenyi.com

Spain
Miltenyi Biotec S.L.
C/Luis Buñuel 2
Ciudad de la Imagen
28223 Pozuelo de Alarcón (Madrid)
Spain
+34 91 512 12 90
+34 91 512 12 91
macses@miltenyi.com

Switzerland
Miltenyi Biotec Swiss AG
Gibelinstrasse 27
4500 Solothurn
Switzerland
+41 32 623 08 47
+49 2204 85197
macsch@miltenyi.com

United Kingdom
Miltenyi Biotec Ltd.
Almac House, Church Lane
Bisley, Surrey GU24 9DR
United Kingdom
+44 1483 799 800
+44 1483 799 811
macsuk@miltenyi.com

www.miltenyibiotec.com

37070-03 | Issued: 2023-08

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