Miltenyi Biotec

CliniMACS® Plus Applications

User manual (GMP)



The CliniMACS System components, including Reagents, Tubing Sets, Instruments, and PBS/EDTA Buffer, are designed, manufactured and tested under a quality system certified to ISO 13485. In the EU, the CliniMACS System components are available as CE-marked medical devices for their respective intended use, unless otherwise stated. In the US, the CliniMACS CD34 Reagent System, including the CliniMACS Plus Instrument, CliniMACS CD34 Reagent, CliniMACS Tubing Set TS and LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE) or FDA approval. MACS GMP Products and CR/GMP products are for *ex vivo* cell processing only and are not intended for human *in vivo* applications. For the manufacture and use of cellular products, national and international legislation and regulations must be followed.

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CliniMACS Plus Applications
User manual (GMP)

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1

About this user manual

This chapter provides general information about the importance of reading this user manual before using the instrument. It also covers the associated document series and the presentational conventions used in this manual.

This user manual provides instructions, warnings, precautions, and other important information for the use of the CliniMACS Plus Applications.

For further details on the instrument hardware, refer to the CliniMACS Plus Instrument user manual. For further details on the software running on the instrument, refer to the CliniMACS Plus Software user manual.

1.1 Read the manual first

⚠ WARNING

Risk of electrical shock, physical injuries, instrument damage, or malfunction.

Disregard of any instructions, warnings, or cautions at any time can endanger users and others and may compromise the electrical, electronical, electromagnetic, and mechanical safety. Disregard also excludes the manufacturer from any liability.

- The operation of the instrument must be performed by trained users only.
- Pay special attention to all warnings shown on the instrument or provided with consumables and accessories.
- Adhere to all safety information, warnings, precautions, and instructions at all times while the instrument is in operation.
- Retain the user manuals for future reference. They should be kept accessible and readily available together with all other safety and operating documentation during the entire life cycle of the instrument.

1.2 Associated documents

This user manual is part of a series and should only be used in conjunction with the other user manuals of the series.

- CliniMACS Plus Instrument user manual
- CliniMACS Plus Software user manual
- MACS® TubeSealer user manual

1.3 Presentational conventions

The following text styles are used to indicate specific information types in this user manual that are not safety-related. For safety-related information types, see section 'Hazard levels' and section 'Structure of warning messages'.

1.3.1 Text styles

• Bullet point list item with text	Indicates a list-item of an unordered list
1. Numbered list item with text	Indicates a list-item of a step-by-step instruction
Bold, colored text	Indicates a software element (for example a button or tab of the graphical user interface)
See section 'Text styles'	Indicates a reference to the section with the heading 'Text styles'
See Figure 4.2/Table 4.2	Indicates a reference to the second figure/table in chapter four

IMPORTANT:
Advises the user of important practices or information not related to personal injury nor property damage

1.3.2 Screens and figures

The screens and the figures in this manual are only representative screens and may slightly differ from those shown on the instrument.

2

Safety notes

This chapter explains the potential risks associated with the operation of the CliniMACS Plus System. At all times, local working area safety instructions, policies, standards regarding good manufacturing practice, health, safety, and prevention of accidents must be adhered to. For instructions for use, e.g., warnings and precautions, concerning the handling of biohazardous materials and starting cell product, as well as the CliniMACS Plus System components, refer to the relevant sections in the instructions for use of the respective component. Detailed instructions for use regarding the CliniMACS Plus Instrument are described in the CliniMACS Plus Instrument user manual.

2.1 Hazards

2.1.1 Hazard levels

The color and signal word of a warning message depend on the hazard level. The hazard level classifies the hazard as follows:

⚠ WARNING

indicates a hazardous situation that, if not avoided, could result in death or serious injury.

⚠ CAUTION

indicates a hazardous situation that, if not avoided, could result in minor or moderate injury.

NOTICE

indicates a hazardous situation that, if not avoided, could result in property damage. This does not indicate a risk of personal injury.

2.1.2 Structure of warning messages

Warning messages warn of hazardous situations. Warning messages specify the hazard level, the type and source of the hazard, the possible consequences, and the measures to avoid the hazard.

The following examples show the structure of warning messages at the beginning of a chapter or section where a hazard can occur:

⚠ WARNING

Type and source of hazard.

Consequences of noncompliance.

- Measures to avoid the hazard.

⚠ CAUTION

Type and source of hazard.

Consequences of noncompliance.

- Measures to avoid the hazard.

NOTICE

Type and source of hazard.

Consequences of noncompliance.

- Measures to avoid the hazard.

The following examples show the structure of embedded warning messages before an instructional step:

1. **WARNING! Type and source of hazard. Consequences of noncompliance. Measures to avoid the hazard.** Instructional step.

1. **CAUTION! Type and source of hazard. Consequences of noncompliance. Measures to avoid the hazard.** Instructional step.

1. **NOTICE Type and source of hazard. Consequences of noncompliance. Measures to avoid the hazard.** Instructional step.

2.2 Risks related to the process and cell product

⚠ WARNING

Risk of process failure or damage to the instrument.

Risk of process failure or damage to the instrument if procedures are performed by untrained operators.

- All processing procedures must be performed by trained operators only. Operator training will be provided by a qualified Miltenyi Biotec representative.

⚠ CAUTION

Risk of reduced process performance.

Temperatures lower or higher than room temperature may result in less viability, purity and yield of the target cells.

- Perform sample preparation and cell separation at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

⚠ CAUTION

Equipment safety may be compromised.

Equipment safety may be compromised if the instrument is not used according to the manufacturer's instructions.

- Fully read and understand the safety information, warnings, precautions, and instructions for proper operation of the CliniMACS Plus System provided in the CliniMACS Plus instructions for use and in any safety-related recommendations issued by Miltenyi Biotec.

- For the manufacture and use of the target cell product, national and international legislation and regulations must be followed. Miltenyi Biotec as the provider of the CliniMACS Plus System does not provide any recommendations regarding the use of the manufactured cells for therapeutic purposes and does not make any claim regarding a clinical benefit.
- All materials that have come into contact with blood and blood products, must be treated as infectious material. Regulations for the handling of infectious material must be observed.
- All tubing, fittings, valves, the pre-column, and the separation column should be checked thoroughly for leaks during the priming step.
- All bags, including those used in sample preparation, should be preserved until final analysis of the collected cells has been completed and successful separation of the target cells has been confirmed.

2.3 Handling of biohazardous material

⚠ WARNING

Biological hazard.

Depending on the biological material used, contact may lead to severe personal injury or death.

- Observe the following safety instructions/measures.

- To avoid contamination of the starting cell product, all preparation steps should be performed under sterile conditions using aseptic techniques (for example, in the laminar flow hood).
- The user performing the cell separation must be trained in the proper use of the equipment and in the handling of blood products and bone marrow aspirate.
- The user performing the cell separation should wear appropriate clothing (for example, lab coat, gloves, and eyeglasses or goggles) when working with patient samples and handling potentially biohazardous material.
- All blood products must be treated as a potential biohazard. Leukapheresis product, blood product, bone marrow aspirate, collected cells, used buffer, used tubing set, and other materials that have been in contact with these fluids must be treated as biohazardous materials according to standard hospital or institutional requirements.
- The CliniMACS Plus Instrument should be considered a potential biohazard after each separation run and cleaned according to standard hospital or institutional requirements. Refer to the CliniMACS Plus Instrument user manual for detailed information on cleaning and disinfection of the instrument.
- Disposable materials must be treated as biohazardous materials according to standard hospital or institutional requirements.

2.4 Starting cell product

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

- The starting cell product (for example, leukapheresis product) should be collected according to standard hospital or institutional procedures in standard collection bags. Bone marrow aspirate should be collected in heparin-coated containers (e.g. 5 mL syringes).
- The container containing the starting cell product should be labeled with patient identification, time, date, and place of collection according to procedures specified for use with the clinical protocol.
- For transportation, the starting cell product should be packed in insulated containers and should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]) according to standard hospital or institutional blood collection procedures approved for use with the clinical protocol. Do not refrigerate. The cell concentration should not exceed 0.2×10^9 cells per mL during transportation. For transportation of bone marrow aspirate, the product should be packed in insulated containers and should be kept at controlled temperature (+4 °C [+39 °F]).
- Avoid intensive mixing of the cellular starting material.

- If the starting cell product has to be stored, for example, overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). During storage, the concentration of leukocytes should never exceed 0.2×10^9 cells per mL.
- Cells should be stored in autologous plasma. If the cell concentration is higher than 0.2×10^9 cells per mL, dilute the starting cell product with autologous plasma.

3

Principles of CliniMACS magnetic cell separation

3.1 Labeling of cells with CliniMACS reagents

MACS magnetic cell separation is a well proven powerful tool for the separation of many cell types, in research laboratories as well as in clinical applications. Cell mixtures are separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, for example, human CD14-positive cells from heterogeneous hematologic cell populations.

Using the CliniMACS Plus system, cells can be magnetically labeled directly in a one-way labeling procedure by antigen-specific antibodies conjugated to superparamagnetic iron-dextran beads (see Figure 3.1).

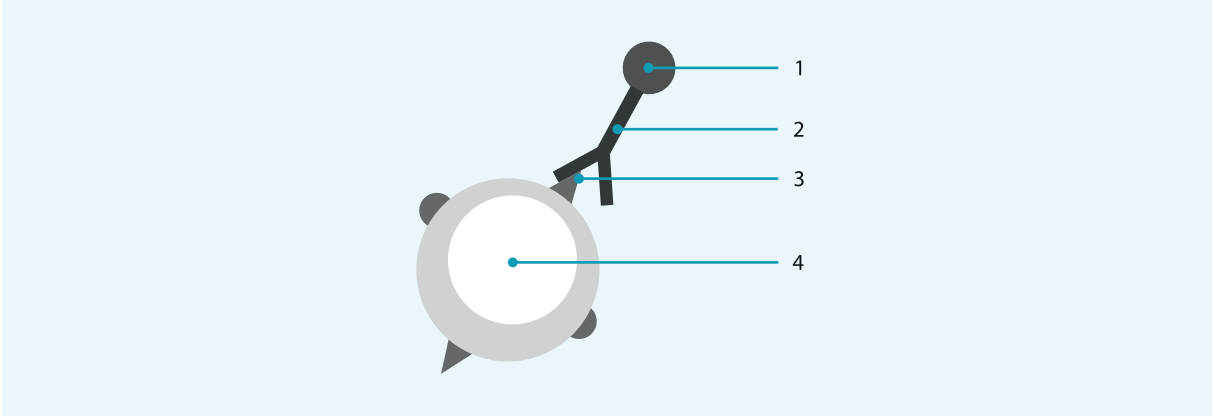


Figure 3.1: Magnetic labeling of cells

1 Magnetic particle
2 Antibody
3 Antigen
4 Cell

3.2 High-gradient magnetic cell separation

The magnetically labeled cell suspension is loaded onto the CliniMACS Plus Instrument prepared with a tubing set. The magnetic separation unit of the instrument contains a strong permanent magnet which enables the rapid generation of a high gradient magnetic field within the ferromagnetic matrix in the separation column.

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

them. After removing the column from the magnet, the rapid demagnetization of the column matrix allows the release of retained cells.

3.3 Enrichment of magnetically labeled cells

The CliniMACS Plus System provides the user with a variety of separation programs (CD133 Selection 1/2, Enrichment 1.1, and Enrichment 3.2).

When choosing an enrichment strategy, the magnetically labeled cells (labeled with a CliniMACS Reagent) are retained in the separation column and the unlabeled cells pass through. The labeled cells (target cells) are collected in the Cell Collection Bag and the unlabeled cells (non-target cells) in the Negative Fraction Bag (see Figure 3.2).

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

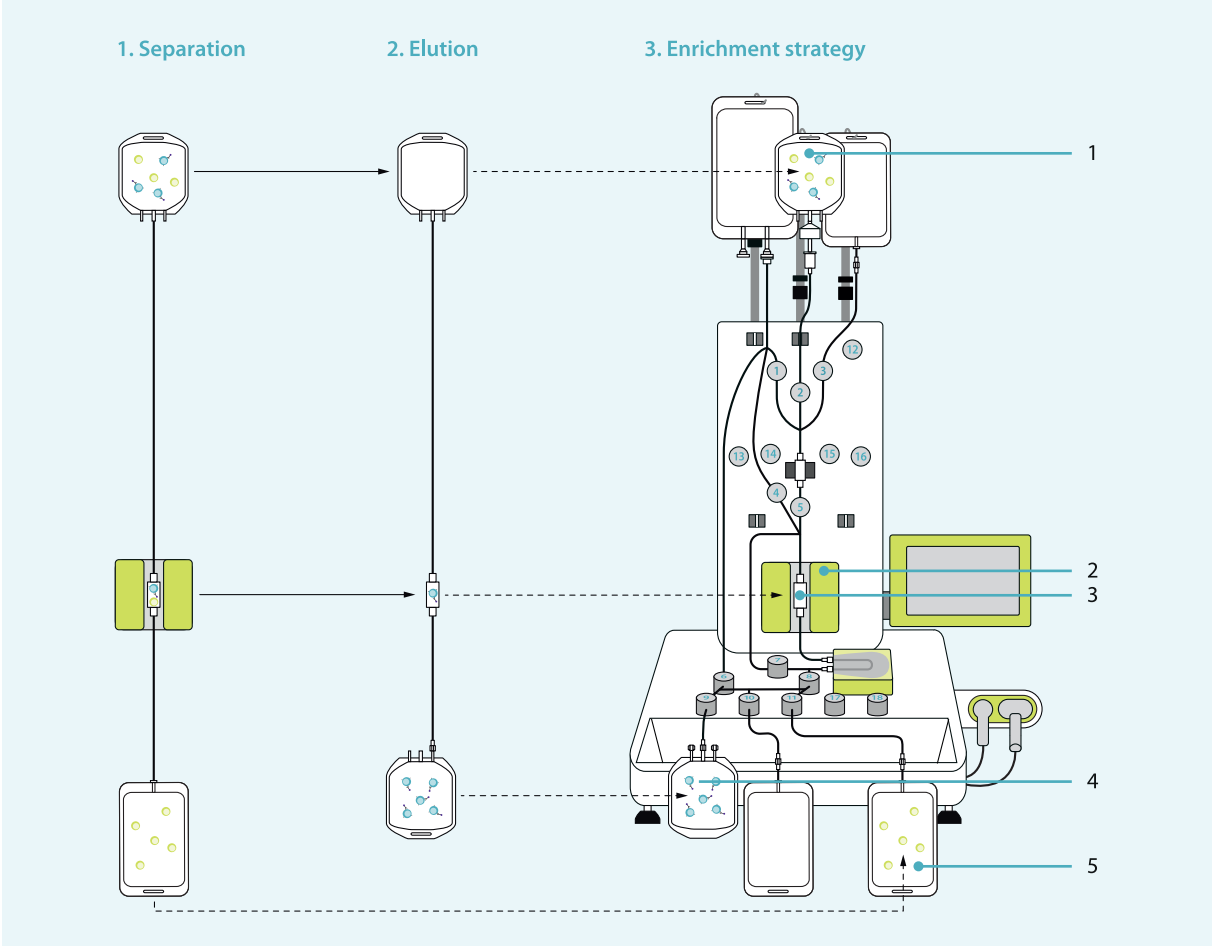


Figure 3.2: Strategy of enrichment programs using the CliniMACS Tubing Set or CliniMACS Tubing Set LS

1 Cell Preparation Bag
2 Magnet
3 Separation column
4 Cell Collection Bag
5 Negative Fraction Bag

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

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

IMPORTANT:
The target cells are always collected in the Cell Collection Bag.

4

About the CliniMACS Plus System

The CliniMACS Plus System offers a set of tools making high-quality standard cell separations available for therapeutic applications.

4.1 Intended purpose

The CliniMACS Reagents in combination with the CliniMACS Plus System are intended to separate human cells.

The CliniMACS Plus System can be used as preparative step as part of the manufacturing process of a medicinal product, e.g. an Advanced Therapy Medicinal Product (ATMP).

IMPORTANT:
For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Plus System components, refer to the instructions for use provided for the respective component.

4.2 Regulatory information

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

For the manufacture and use of the target cell product, national and international legislation and regulations must be followed. Miltenyi Biotec as the provider of the CliniMACS Plus System does not provide any recommendations regarding the use of the manufactured cells for therapeutic purposes and does not make any claim regarding a clinical benefit.

4.3 CliniMACS Plus Instrument and additional components

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

The CliniMACS Plus Instrument is delivered with additional accessories and components. For further details, refer to the CliniMACS Plus Instrument user manual.

MACS® TubeSealer

The MACS® TubeSealer is used for sealing PVC and EVA tubing of the tubing sets installed on the instrument. A detailed description of the MACS® TubeSealer and relevant safety information are provided in the MACS® TubeSealer user manual.

Barcode reader

The barcode reader is intended to scan barcodes printed on CliniMACS materials required for process execution. The scanned data serve as input of part and lot numbers of the CliniMACS materials required for an application. These numbers and any other process-related parameters may also be entered manually.

External keyboard

The external keyboard is intended to enable administrators to set up user accounts.

IMPORTANT:
DO NOT use the external keyboard when running any of the installed applications on the instrument. The external keyboard must be used for the "User Management" only! The external keyboard must be unplugged from the instrument before starting any application and tool.

Refer to the CliniMACS Plus Software user manual for detailed information on User Management.

4.4 CliniMACS Reagents

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

4.5 CliniMACS Tubing Sets

The CliniMACS tubing sets are sterile, single-use disposables designed to be used in combination with the CliniMACS Plus Instrument for the *in vitro* enrichment or depletion of human cells from heterogeneous hematologic cell populations.

4.6 CliniMACS PBS/EDTA Buffer

The CliniMACS PBS/EDTA Buffer is a wash and transport fluid to enable the *in vitro* separation of human cells.

IMPORTANT:
The CliniMACS PBS/EDTA Buffer is intended for *in vitro* use only. It is not intended for intravenous infusion or for parenteral applications. It must be exchanged to an infusion solution approved in the country of the user by appropriate means, for example, through centrifugation or buffer exchange.

4.7 CliniMACS Accessories

- **Plasma transfer set:**
  transfer set with two spikes for plasma or fluids, Transfer Set Coupler/Coupler (REF 200-073-902), or equivalent
- **Luer/Spike Interconnector:**
  transfer set with spike and syringe adapter (female luer) for plasma or fluids, Luer/Spike Interconnector (REF 200-073-903), or equivalent

4.8 Additional materials required

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

- **Cell culture bags, gas-permeable:**
  MACS®GMP Cell Differentiation Bag – 100 mL (REF 170-076-400), or equivalent
- **Transfer bags, suitable for centrifugation:**
  Transfer bag 150 mL, Terumo, or equivalent
  Transfer bag 600 mL, Terumo, or equivalent
  Transfer bag 1,000 mL, Terumo, or equivalent
- **Sampling site coupler:**
  Sampling site coupler, Terumo, or equivalent
- **Pre-system filter:**
  Blood Transfusion Filter, GVS, or equivalent
- **200 µm in-line blood filter:**
  Fenwal, Product Code 4C2160 or equivalent
- **Syringes and needles:**
  Appropriate syringes (5 mL, 10 mL, 20 mL, 50 mL) and hypodermic 20-gauge needles
- **Locking forceps**
- **Sample tubes for quality control**
- **Human serum albumin (HSA):**
  Use HSA of pharmaceutical grade quality only, which is available as approved product in the country of the user.
- **Clinical grade immunoglobulin G:**
  Use IgG of pharmaceutical grade quality only, which is available as approved drug in the country of the user, for example, for Germany: Gamunex® 10%, or equivalent.
- **Cell culture medium:**
  For example, RPMI 1640, or equivalent

4.9 Equipment required

- **Uninterruptible power supply (UPS):**
  Recommended UPS: APC Smart-UPS 1500VA USB & Serial 230 V, manufactured by APC (American Power Conversion), or equivalent
- **Laminar flow hood**
- **Sterile tubing connector:**
  Terumo Sterile Connection Device (TSCD®), or equivalent
- **Orbital rotator:**
  Platform shakers, Heidolph Instruments, or equivalent
- **Centrifuge:**
  Centrifuge, Sorvall, or equivalent, and buckets for centrifugation with aerosol containment caps
- **Plasma extractor:**
  Plasma Separation Stand, Terumo Equipment, or equivalent
- **Table top balance:**
  Laboratory Balance, Mettler Toledo, or equivalent, with 1 kg capacity; resolution to 0.1 g
- **Tubing stripper:**
  Tube Stripper, Baxter, or equivalent
- **Biohazard waste containers**

5

Overview of applications

5.1 Four Steps to your target cells

In the following chapter an overview of the CliniMACS Plus separation is presented. The CliniMACS Plus separation is carried out in four Steps.

Recording of application-related process data may be required. For supporting worksheets, contact Miltenyi Biotec Technical Support.

Step 1: Cell preparation and magnetic labeling

Step 1 describes the preparation of the cell product and the magnetic labeling of the specific cells expressing the respective antigen. Follow the instructions in the applicable section. Continue with Step 2.

Step 2: Choice of separation program

Step 2 describes the selection of a separation program on the CliniMACS Plus Instrument. Depending on cell type and cell number to be separated, the appropriate program must be selected. To determine which program is applicable, refer to the overview table on the next page. Read the respective section for the selected separation program. Follow the instructions given in the applicable section. Continue with Step 3.

Step 3: Installation of CliniMACS tubing sets

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

Step 4: CliniMACS Plus separation

Step 4 describes the automated CliniMACS Plus separation. The CliniMACS Plus Instrument performs the automated cell separation procedure (chosen in Step 2). Follow the instructions in the applicable section.

5.2 Overview table

The following overview table summarizes information on typical applications of the CliniMACS Reagents. When the separation of a different number of target cells is to be performed, refer to the instructions in the application-specific overview to find information on the maximum capacity of the respective application. Follow the instructions in the application-specific sections and detailed explanations of Steps 1–4.

Application capacity	Reagent vial(s)	Tubing set	Separation program	Application-specific section	Detailed explanation of Steps 1–4 sections			
					Step 1	Step 2	Step 3	Step 4
CD133 – Enrichment (leukapheresis product, normal-scale application)								
≤0.6×10^9 CD133 positve cells from ≤60×10^9 total cells	1× CD133 Reagent	1× CliniMACS Tubing Set	CD133 Selection 1	6.1	7.1	7.2.3	7.3.1	7.4.1
CD133 – Enrichment (leukapheresis product, large-scale application)								
>0.6–1.2×10^9 CD133 positve cells from >60–120×10^9 total cells	2× CD133 Reagent	1× CliniMACS Tubing Set LS	CD133 Selection 2	6.1	7.1	7.2.3	7.3.1	7.4.1
CD133 – Enrichment (bone marrow)								
≤50 mL aspirate and ≤120×10^9 total cells	1–2× CD133 Reagent	1× CliniMACS Tubing Set LS	CD133 Selection 2	6.2	7.1	7.2.3	7.3.1	7.4.1
CD14 – Enrichment								
≤4×10^9 CD14 - positive cells from ≤20×10^9 total cells	1–2× CD14 Reagent	1× CliniMACS Tubing Set	Enrichment 1.1	6.3	7.1	7.2.4	7.3.1	7.4.2
CD271 – Enrichment								
≤100 mL aspirate and ≤1×10^7 CD271-positive cells	1–2× CD271 Reagent	1× CliniMACS Tubing Set	Enrichment 3.2	6.4	7.1	7.2.5	7.3.1	7.4.3
CD62L – Enrichment								
≤4×10^9 CD62L positve cells from ≤10×10^9 total cells	1× CD62L Reagent	1× CliniMACS Tubing Set LS	Enrichment 1.1	6.5	7.1	7.2.4	7.3.1	7.4.2

Table 5.1: Overview of available applications with corresponding tubing sets and separation programs

6

Application-specific overview of Steps 1-4

6.1 Enrichment of CD133-positive cells (leukapheresis product)

For bone marrow aspirate as starting material, see section 'Enrichment of CD133-positive cells (bone marrow)'.

6.1.1 Step 1: Cell preparation and magnetic labeling

First actions required

Refer to section 'First actions required' (Detailed explanation of Step 1–4) for a detailed description of the first actions required.

Application capacity

- **Normal-scale application:** up to 0.6×10^9 CD133-positive cells out of up to 60×10^9 total cells (WBC)
- **Large-scale application:** greater than 0.6×10^9 and up to 1.2×10^9 CD133-positive cells out of up to 120×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells.

If the application specifications are exceeded, there is the risk of insufficient specific labeling.

- Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Quantity	Separation program
CliniMACS Plus Instrument	1	–
CliniMACS CD133 Reagent	• 1 vial (normal-scale) • 2 vials (large-scale)	–
CliniMACS Tubing Set	1 tubing set	CD133 Selection 1
CliniMACS Tubing Set LS	1 tubing set	CD133 Selection 2
CliniMACS PBS/EDTA Buffer	3×1,000 mL bags[1]	–
[1] Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).		

Table 6.1: CliniMACS Materials required for enrichment of CD133-positive cells (leukapheresis product)

Additional materials required

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

Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 'First actions required' (Detailed explanation of Step 1–4).

Magnetic labeling of cells

Refer to section 'Magnetic labeling of cells' (Detailed explanation of Step 1–4) for a detailed description of the following steps.

- **Analysis of starting cell product**
- **Transfer of starting cell product into Cell Preparation Bag**
  If the weight of the starting cell product is more than 200 g, but the number of cells is less than 120×10^9 total cells and 1.2×10^9 CD133-positive cells, centrifuge the sample to reduce the volume to 200 g at the most and then continue with the following steps.
- **Dilution of starting cell product**
  Add buffer using the following equation:
  Weight of buffer to be added = Weight of starting cell product × 2
  If the volume has been reduced:
  Weight of reduced cell product × 2
- **Centrifugation**
  Centrifuge at 200×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).
- **Volume adjustment: labeling volume 95 g (±5 g) (normal-scale application) or 190 g (±5 g) (large-scale application)**
  1. Remove supernatant to adjust the cell product using the following equation:
     Weight of supernatant to be removed = Weight of diluted cell product – Labeling volume
  2. Resuspend the cell pellet.

- **Incubation with the CliniMACS CD133 Reagent**
  1. Add 1 or 2 vials of CliniMACS CD133 Reagent (see Table 6.1). Mix contents gently.
  2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.
- **Removal of excess reagent**
  1. Fill Cell Preparation Bag with buffer.
  2. Centrifuge (200×g, without brake, 15 minutes, RT).
  3. Remove supernatant as much as possible. Recommendation: Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g, a total of three washing steps (instead of only two) is recommended. Otherwise, the removal of unbound reagent may be insufficient.
  4. Resuspend the cell pellet.
  5. Fill Cell Preparation Bag with buffer.
  6. Centrifuge (200×g, without brake, 15 minutes, RT).
  7. Remove supernatant as much as possible.
  8. Resuspend the cell pellet.
  9. Adjust Cell Preparation Bag volume to 150 g (normal-scale application) or 275 g (large-scale application).

  Optional: Take a sample after removal of excess reagent for later analysis.

6.1.2 Step 2: Choice of separation program

- **Basic operation**
- **Choosing separation program CD133 Selection 1 or CD133 Selection 2**
  (CliniMACS Tubing Set: CD133 Selection 1)
  (CliniMACS Tubing Set LS: CD133 Selection 2)
- **Entering tubing set parameters**
- **Entering CliniMACS CD133 Reagent parameters**

See section 'CD133 Selection 1/ CD133 Selection 2' in the detailed explanation.

6.1.3 Step 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' in the detailed explanation.

6.1.4 Step 4: CliniMACS Plus separation

- **Separation procedure**
- **Disconnecting bags and removing tubing set**
- **Shutting down and cleaning the instrument**
- **Analysis of cells**

See section 'CD133 Selection 1/2' in the detailed explanation.

6.2 Enrichment of CD133-positive cells (bone marrow)

For peripheral blood cells as starting material, see section 'Enrichment of CD133-positive cells (leukapheresis product)'.

6.2.1 Step 1: Cell preparation and magnetic labeling

First actions required

Refer to section 'First actions required' (Detailed explanation of Step 1–4) for a detailed description of the first actions required.

Application capacity

Up to 50 mL aspirate and up to 120×10^9 total cells

⚠ CAUTION

Risk of insufficient separation performance of labeled cells.

If the application specifications are exceeded, there is the risk of insufficient specific labeling.

- Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Quantity
CliniMACS Plus Instrument	1
CliniMACS CD133 Reagent	1 or 2 vials
CliniMACS Tubing Set LS	1 tubing set
CliniMACS PBS/EDTA Buffer	2×1,000 mL bags[1]

[1] Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.2: CliniMACS Materials required for enrichment of CD133-positive cells (bone marrow)

Additional materials required

- **Cell Preparation Bag:**
  One 400 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bags:**
  Three 400 mL transfer bags, suitable for centrifugation
- **Bone Marrow Aspirate Bag**:
  One 400 mL transfer bag in combination with a Luer/Spike Interconnector
- **Cell Collection Bag:**
  One 150 mL transfer bag in combination with a Luer/Spike Interconnector in to connect the Cell Collection Bag to the tubing set
- **One pre-system filter**
- **One 200 µm in-line blood filter**
- **Locking forceps**
- **Appropriate syringes (different sizes) and hypodermic 20 gauge needles**
- **Human serum albumin (HSA)** to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- **Sample tubes for quality control**

Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 'First actions required' (Detailed explanation of Step 1–4).

Magnetic labeling of cells

The following sections describe the recommended procedure for the preparation of the bone marrow aspirate using a sterile tubing connector:

- The user must be familiar with the operation and use of sterile tubing connectors.
- Before starting the cell labeling and separation procedure ensure that all needed supplies and equipment are available

Analysis of bone marrow aspirate

Before
starting the preparation of the bone marrow aspirate the following parameters should be determined:

- Total number of leukocytes
- Viability

Filtration of bone marrow aspirate and transfer into Cell Preparation Bag

1. Combine all bone marrow aspirates by transferring each aspirate into the Bone Marrow Aspirate bag through the luer lock interconnector or by using the sterile tubing connector.
2. Holding the Bone Marrow Aspirate bag with both hands, mix the contents thoroughly by using a gentle rotating motion.
3. Using the sterile tubing connector, connect the filled Bone Marrow Aspirate bag to one site of the in-line blood filter, and the Cell Preparation Bag to the other site of the filter. Secure the tubing with clamps to close the fluid pathway.
4. Open the fluid pathway and hold the Bone Marrow Aspirate bag up to let the bone marrow aspirate run through the in-line blood filter into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the clamp on the Cell Preparation Bag.
5. Using the heat sealer, seal off the tubing and separate, leaving at least 15 cm of tubing on the Cell Preparation for further connections (see Figure 6.1). Retain the original Bone Marrow Aspirate bag until cell separation and final cell analysis have been completed.
6. Determine the volume of the filtered bone marrow aspirate by estimating 1 mL of cellular product as equivalent to 1 g (1 g ≙ 1 mL).
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 weight of the bone marrow aspirate by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with bone marrow aspirate. Record the weight.
   Weight of bone marrow aspirate (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)

9. 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 bone marrow aspirate. Transfer the sample into a sample tube. Label the tube as BONE MARROW ASPIRATE (this should include patient identification) and retain for cell analysis. Analysis of the WBC content, CD133 enumeration, and viability testing are recommended.

Dilution of bone marrow aspirate

⚠ WARNING

Risk of contamination.

If the bags are not suitable for cell preparation or not placed correctly into the centrifuge bucket, there is the risk of contamination due to bag damage.

- Use suitable bags, fold any loose parts of the bags or tubing downwards, taking care not to impair bag integrity and place the bags securely in the centrifuge bucket.

⚠ CAUTION

Risk of lower cell separation performance.

If the bags are not placed correctly into the centrifuge bucket, there is the risk of cell loss during supernatant removal.

- Fold any loose parts of the bags or tubing downwards and place the bags securely in the centrifuge bucket.

The bone marrow aspirate must be diluted with CliniMACS PBS/ EDTA Buffer (supplemented with HSA to a final concentration of 0.5% [w/v]) before magnetic labeling. Dilute the bone marrow aspirate with buffer. Calculate the amount of buffer to be added using the following equation.

Weight of buffer to be added (g) = Weight of bone marrow aspirate (g) × 4

1. Take a plasma transfer set and ensure the clamp is in the closed position. Insert the spike of the plasma transfer set into a port of the buffer bag.
2. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.
3. Place the Cell Preparation Bag on the balance and tare the balance. Open the locking forceps next to the Cell Preparation Bag.
4. Move the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated amount of buffer to the Cell Preparation Bag.
5. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the actual amount of buffer added.
6. Using the MACS TubeSealer, make three hermetic seals between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.

7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.
8. Weigh the Cell Preparation Bag and record the weight.
9. Determine the weight of diluted bone marrow aspirate by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the weight.
   Weight of diluted bone marrow aspirate (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)

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 balanced accurately.
4. Centrifuge the cells at 300×g for 15 min (without brake) at room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Record centrifugation conditions.
5. Remove the bag from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor.

Volume adjustment

1. For magnetic labeling of CD133-positive cells, the optimal weight of the cell sample is 95 g (± 5 g) using one vial of CliniMACS CD133 Reagent. Calculate the amount of supernatant to be removed to adjust the sample to 95 g using the equation below:
   Weight of supernatant to be removed (g) = Weight of diluted bone marrow aspirate (g) – 95 g
   Record the amount of supernatant to be removed.
2. Place the empty Plasma Waste Bag on the balance and tare the balance.
3. Open the locking forceps next to the Cell Preparation Bag. Use the balance display to monitor the weight of the Plasma Waste Bag while removing the supernatant. Using the plasma extractor, carefully press out excess supernatant until the calculated "weight of supernatant to be removed" has been transferred into the Plasma Waste Bag so that 95 g remain in the Cell Preparation Bag. If necessary, stop the supernatant removal before cells get transferred into the Plasma Waste Bag.

   IMPORTANT:
   - Using the plasma extractor, maintain constant control of the extractor release handle and ensure that the locking forceps next to the Cell Preparation Bag is open before beginning the transfer. Release the extractor handle slowly.
   - During removal of supernatant be careful not to lose cells.
4. When the appropriate amount of supernatant has been transferred, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed.
5. Using the MACS TubeSealer, seal off the tubing to disconnect the Plasma Waste Bag leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.

6. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
7. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
8. Determine the weight of the bone marrow aspirate after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.

   Weight of bone marrow aspirate after volume adjustment (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)

9. **If the weight of the bone marrow aspirate exceeds 95 g (±5 g), the product must be adjusted to 190 g (±5 g) and two vials of CliniMACS CD133 Reagent are required. If not, please proceed to number 19 of the protocol.**
10. Calculate the amount of buffer to be added using the following equation and record it.

    Weight of buffer to be added (g) = 190 g – Weight of diluted bone marrow aspirate after supernatant removal (g)

11. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on the plasma extractor or, alternatively, hang the buffer bag on a bag hanger.
12. Place the Cell Preparation Bag on the balance and tare the balance.
13. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the buffer bag plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.
14. When the appropriate amount of buffer has been transferred, close the clamp on the plasma transfer set to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the actual amount of buffer added.
15. Using the MACS TubeSealer, 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.
16. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
17. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
18. Determine the weight of the bone marrow aspirate after the second volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag. Record the calculated weight.
19. Keep the Plasma Waste Bag until the separation and final analysis of all cells has been completed.

Incubation with clinical grade immunoglobulin

1. Disinfect the septum of the sampling site coupler of the Cell Preparation Bag.
2. Use an appropriate sterile syringe and needle to add 1.5 mg human IgG per mL cellular product (e.g. 1.6 mL of Gamunex 10% for 95 mL cellular product). Mix the contents thoroughly.
3. After adding the human IgG solution, the total volume should not exceeded 100 mL for a 95 mL cellular product, or 195 for a 190 mL cellular product.

4. Incubate for 5 minutes at controlled room temperature by using a gentle rotating motion (e.g., 25 rpm on orbital rotator).

Incubation with the CliniMACS CD133 Reagent

The CliniMACS CD133 Reagent vials are ready to use and sufficient for the application as described below. The reagents are not for parenteral administration.

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

1. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from the reagent vial (7.5 mL). A 10 mL syringe is sufficient to remove the contents of one reagent vial or a 20 mL syringe is sufficient to remove the contents of two reagent vials, respectively. The syringe should be equipped with a 20 gauge needle.
2. **If the labeling volume of the bone marrow aspirate was adjusted to 190 g (±5 g), a second CliniMACS CD133 Reagent vial must be added.**
3. Using the injection port on the sampling site coupler, inject the entire volume of reagent into the Cell Preparation Bag. Take care not to puncture the Cell Preparation Bag. Immediately begin the 30 minute incubation.
4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Record the incubation start time.
5. Place the Cell Preparation Bag flat on the orbital rotator at approximately 25 rpm and ensure that the bag is not creased or bent. Incubate the bag for a total of 15 minutes at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]). Record the incubation stop time.

6.2.2 Step 2: Choice of separation program

- **Basic operation**
- **Choosing separation program CD133 Selection 1 or CD133 Selection 2**
- **Entering tubing set parameters**
- **Entering CliniMACS CD133 Reagent parameters**

See section 'CD133 Selection 1/ CD133 Selection 2' in the detailed explanation.

6.2.3 Step 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' in the detailed explanation.

6.2.4 Step 4: CliniMACS Plus separation

- **Separation procedure**
- **Disconnecting bags and removing tubing set**
- **Shutting down and cleaning the instrument**
- **Analysis of cells**

See section 'CD133 Selection 1/2' in the detailed explanation.

6.3 Enrichment of CD14-positive cells

6.3.1 Step 1: Cell preparation and magnetic labeling

First actions required

Refer to section 'First actions required' (Detailed explanation of Step 1–4) for a detailed description of the first actions required.

Application capacity

Up to 4×10^9 CD14-positive cells out of up to 20×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells.

If the application specifications are exceeded, there is the risk of insufficient specific labeling.

- Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Quantity
CliniMACS Plus Instrument	1
CliniMACS CD14 Reagent	1 vial
CliniMACS Tubing Set	1 tubing set
CliniMACS PBS/EDTA Buffer	3×1,000 mL bags[1]

[1] See Table 6.4 and Table 6.5. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.3: CliniMACS Materials required for enrichment of CD14-positive cells

Additional materials required

- **Cell Preparation Bag**:
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag**:
  One 150 mL or one 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set (see Table 6.5)

- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary:**
  Two 1,000 mL transfer bags in combination with two Luer/Spike Interconnector to connect the bags to the tubing set (see Table 6.5)
- **If more than one liter of buffer is required for the separation:**
  One plasma transfer set to connect the buffer bags (see Table 6.5)
- **One pre-system filter**
- **Locking forceps**
- **Appropriate syringes (different sizes) and hypodermic 20-gauge needles**

Overview of materials required

Cell preparation	
Total cells (WBC)	$\leq 20\times10^9$
Labeled cells	$\leq 4\times10^9$
Number of reagent vials	1–2
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1

Table 6.4: Material required for cell preparation of CD14-positive cells

Separation		
Labeled cells	$\leq 2\times10^9$	$>2–4\times10^9$
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	150 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL
Plasma transfer set	–	1

Table 6.5: Material required for enrichment of CD14-positive cells

Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 'First actions required' (Detailed explanation of Step 1–4).

Magnetic labeling of cells

Refer to section 'Magnetic labeling of cells' (Detailed explanation of Step 1–4) for a detailed description of the following steps.

- **Analysis of starting cell product**
- **Transfer of starting cell product into Cell Preparation Bag**
- **Dilution of starting cell product**
  Add buffer using the following equation:
  Weight of buffer to be added = 600 g – Weight of starting cell product

- **Centrifugation**
  Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).
- **Volume adjustment: labeling volume 50 g (±5 g)**
  1. Remove supernatant to adjust the sample using the following equation:
     Weight of supernatant to be removed = Weight of diluted starting cell product – Labeling volume
  2. Resuspend the cell pellet.
- **Incubation with the CliniMACS CD14 Reagent**
  1. Add 1 vial of CliniMACS CD14 Reagent. Mix contents gently.
  2. Incubate on orbital rotator (25 rpm) for 15 minutes at RT.
- **Removal of excess reagent**
  1. Fill Cell Preparation Bag with buffer.
  2. Centrifuge (300×g, without brake, 15 minutes, RT).
  3. Remove supernatant as much as possible. Recommendation: Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g, a total of two washing steps (instead of only one) is recommended. Otherwise, the removal of unbound reagent may be insufficient.
  4. Resuspend the cell pellet.
  5. Adjust Cell Preparation Bag volume to 100 g.

  Optional: Take a sample after removal of excess reagent for later analysis.

6.3.2 Step 2: Choice of separation program

- **Basic operation**
- **Separation planner tool**
- **Choosing separation program Enrichment 1.1**
- **Entering tubing set parameters**
- **Entering starting cell product parameters**

See section 'Enrichment 1.1' in the detailed explanation.

6.3.3 Step 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' in the detailed explanation.

6.3.4 Step 4: CliniMACS Plus separation

- **Separation procedure**
- **Disconnecting bags and removing tubing set**
- **Shutting down and cleaning the instrument**
- **Analysis of cells**

See section 'Enrichment 1.1' in the detailed explanation.

6.4 Enrichment of CD271-positive cells

6.4.1 Step 1: Cell preparation and magnetic labeling

First actions required

Refer to section 'First actions required' (Detailed explanation of Step 1–4) for a detailed description of the first actions required.

Application capacity

Up to 1×10^7 CD271-positive cells out of up to 100 mL of bone marrow aspirate

⚠ CAUTION

Risk of insufficient separation performance of labeled cells.

If the application specifications are exceeded, there is the risk of insufficient specific labeling.

- Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Quantity
CliniMACS Plus Instrument	1
CliniMACS CD271 Reagent	1 or 2 vials
CliniMACS Tubing Set	1 tubing set
CliniMACS PBS/EDTA Buffer	2×1,000 mL bags[1]

[1] Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.6: CliniMACS Materials required for enrichment of CD133-positive cells (bone marrow)

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag, suitable for centrifugation, as well as one sampling site coupler and one plasma transfer set, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation
- **Bone Marrow Aspirate Bag**:
  One 600 mL transfer bag in combination with a Luer/Spike Interconnector
- **Cell Collection Bag:**
  One 150 mL transfer bag in combination with a Luer/Spike Interconnector in to connect the Cell Collection Bag to the tubing set
- **One pre-system filter**
- **One 200 µm in-line blood filter**
- **Locking forceps**
- **Appropriate syringes (different sizes) and hypodermic 20 gauge needles**

- **Human serum albumin (HSA)** to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- **Sample tubes for quality control**

Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 'First actions required' (Detailed explanation of Step 1–4).

Magnetic labeling of cells

Refer to section 'Magnetic labeling of cells' (Detailed explanation of Step 1–4) for a detailed description of the following steps.

- **Analysis of starting cell product (bone marrow aspirate)**
- **Transfer of starting cell product into Cell Preparation Bag**
- **Dilution of starting cell product**
  Add buffer using the following equation:
  Weight of buffer to be added = Weight of starting cell product × 4
- **Centrifugation**
  Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).
- **Volume adjustment: labeling volume 95 g (±5 g)**
  1. Remove supernatant to adjust the sample using the following equation: Weight of supernatant to be removed = Weight of diluted cell product – 95 g
  2. Remove the supernatant taking care not to resuspend the cell pellet. Stop the removal of supernatant before losing cells.
  3. If the recommended labeling volume exceeds 95 g, adjusts the labeling volume to 190 g using the following equation:
     Weight of buffer to be added = 190 g – Weight of diluted cell product after supernatant removal
  4. Resuspend the cells.
- **Incubation with the CliniMACS CD271 Reagent**
  1. Add 1 vial of CliniMACS CD271 Reagent if the labeling volume was adjusted to 95 g or 2 vials if the labeling volume is 190 g. Mix contents gently.
  2. Incubate on orbital rotator (25 rpm) for 30 minutes at RT.
- **Removal of excess reagent**
  1. Fill Cell Preparation Bag with buffer.
  2. Centrifuge (200×g, without brake, 15 minutes, RT).
  3. Remove supernatant as much as possible. Recommendation: Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g, a total of two washing steps (instead of only one) is recommended. Otherwise, the removal of unbound reagent may be insufficient.
  4. Resuspend the cell pellet carefully after removal of supernatant.
  5. Adjust Cell Preparation Bag volume. (The volume may range between 100 g and 275 g but must not exceed 275 g).

6.4.2 Step 2: Choice of separation program

- **Basic operation**
- **Choosing of separation program Enrichment 3.2**
- **Entering tubing set parameters**

See section 'Enrichment 3.2' in the detailed explanation.

6.4.3 Step 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' in the detailed explanation.

6.4.4 Step 4: CliniMACS Plus separation

- **Separation procedure**
- **Disconnecting bags and removing tubing set**
- **Shutting down and cleaning the instrument**
- **Analysis of cells**

See section 'Enrichment 3.2' in the detailed explanation.

6.5 Enrichment of CD62L-positive cells

6.5.1 Step 1: Cell preparation and magnetic labeling

First actions required

Refer to section 'First actions required' (Detailed explanation of Step 1–4) for a detailed description of the first actions required.

Application capacity

Up to 4×10^9 CD62L-positive cells out of up to 10×10^9 total cells (WBC)

⚠ CAUTION

Risk of insufficient separation performance of labeled cells.

If the application specifications are exceeded, there is the risk of insufficient specific labeling.

- Use the required amount of cells and reagent as described for the respective application.

CliniMACS Materials required

Material	Quantity
CliniMACS Plus Instrument	1
CliniMACS CD62L Reagent	1 vial
CliniMACS Tubing Set LS	1 tubing set
CliniMACS PBS/EDTA Buffer	4×1,000 mL bags[1]

[1] See Table 6.8 and Table 6.9. Before use, supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v).

Table 6.7: CliniMACS Materials required for enrichment of CD62L-positive cells

Additional materials required

- **Cell Preparation Bag:**
  One 600 mL transfer bag suitable for centrifugation as well as one sampling site coupler and two plasma transfer sets, for use during the cell preparation procedure
- **Plasma Waste Bag and Wash Waste Bag:**
  Two 600 mL transfer bags, suitable for centrifugation
- **Cell Collection Bag:**
  One 600 mL transfer bag in combination with a Luer/Spike Interconnector to connect the Cell Collection Bag to the tubing set (see Table 6.9)
- **If the replacement of the Negative Fraction Bag and the Buffer Waste Bag is necessary:**
  Two 1,000 mL transfer bags in combination with two Luer/Spike Interconnectors to connect the bags to the tubing set (see Table 6.9)
- **If more than one liter of buffer is required for the separation:**
  One to two plasma transfer set to connect the buffer bags (see Table 6.9)
- **One pre-system filter**
- **Locking forceps**
- **Syringes (different sizes) and hypodermic 20-gauge needles**
- **Human serum albumin (HSA)** to be added to the CliniMACS PBS/EDTA Buffer to a final concentration of 0.5% (w/v)
- **Sample tubes for quality control**

Overview of materials required

Cell preparation	
Total cells (WBC)	10×10^9
Labeled cells	$\leq4\times10^9$
Number of reagent vials	1
CliniMACS PBS/EDTA Buffer	1,000 mL
Plasma transfer set	1

Table 6.8: Materials required for the cell preparation of CD62L-positive cells

Cell preparation		
Labeled cells	≤2×10^9	>2–4×10^9
Negative Fraction Bag	–	Replace original bags of the tubing set with 1,000 mL transfer bags
Buffer Waste Bag	–	
Priming Waste Bag	–	–
Cell Collection Bag	600 mL	600 mL
Luer/Spike Interconnector	1	3
CliniMACS PBS/EDTA Buffer	1,000 mL	2,000 mL–3000 mL
Plasma transfer set	–	1–2

Table 6.9: Materials required for the enrichment of CD62L-positive cells

Preparation of bags

Determine the weight of the empty Cell Collection Bag and Cell Preparation Bag as described in section 'First actions required' (Detailed explanation of Step 1–4).

Magnetic labeling of cells

Refer to section 'Magnetic labeling of cells' (Detailed explanation of Step 1–4) for a detailed description of the following steps.

- **Analysis of starting cell product**
- **Transfer of starting cell product into Cell Preparation Bag**
- **Dilution of depleted starting cell product**
  Add buffer using the following equation:
  Weight of buffer to be added = 600 g – Weight of cellular starting product
- **Centrifugation**
  Centrifuge at 300×g, without brake, 15 minutes, room temperature (RT: +19 °C to +25 °C [+66 °F to +77 °F]).
- **Volume adjustment: labeling volume 95 g (±5 g)**
  1. Remove supernatant completely taking care not to resuspend the cell pellet.
  2. Resuspend the cell pellet carefully after removal of supernatant.
  3. Adjust the weight of the Cell Preparation Bag (CPB) by adding buffer using the following equation:
     Target weight of filled CPB = 95 g + Weight of empty CPB
  4. Resuspend the cell pellet.
- **Incubation with the CliniMACS CD62L Reagent**
  1. Add 1 vial of CliniMACS CD62L Reagent. Mix contents gently.
  2. Incubate on orbital rotator (25 rpm) for 15 minutes at RT.
- **Removal of excess reagent**
  1. Fill Cell Preparation Bag with buffer.
  2. Centrifuge (300×g, without brake, 15 minutes, RT).
  3. Remove supernatant as much as possible. Recommendation: Remove at least 500 g of supernatant. If the supernatant removed is less than 500 g, a total of two washing steps (instead of only one) is recommended. Otherwise, the removal of unbound reagent may be insufficient.
  4. Resuspend the cell pellet carefully after removal of supernatant.
  5. Adjust Cell Preparation Bag volume to 100 g.

6.5.2 Step 2: Choice of separation program

- **Basic operation**
- **Separation planner tool**
- **Choosing separation program Enrichment 1.1**
- **Entering tubing set parameters**
- **Entering starting cell product parameters**

See section 'Enrichment 1.1' in the detailed explanation.

6.5.3 Step 3: Installation of CliniMACS Tubing Sets

- **CliniMACS Tubing Set and CliniMACS Tubing Set LS**

See section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' in the detailed explanation.

6.5.4 Step 4: CliniMACS Plus separation

- **Separation procedure**
- **Disconnecting bags and removing tubing set**
- **Shutting down and cleaning the instrument**
- **Analysis of cells**

See section 'Enrichment 1.1' in the detailed explanation.

7

Detailed explanation of Step 1–4

7.1 Step 1: Cell preparation and magnetic labeling

This chapter gives the detailed explanations of the procedures that are needed for cell separation using the CliniMACS Plus System.

7.1.1 First actions required

For the materials required for a particular application, refer to the application-specific section (see section 'Overview table').

Preparation of the CliniMACS PBS/EDTA Buffer (process buffer)

Supplement the CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v). The supplemented buffer is henceforth referred to as "process buffer".

Preparation of bags

Refer to the application-specific section for the bags needed:

- **Cell Preparation Bag:**

  Insert a sampling site coupler (or equivalent) into the outside port of the Cell Preparation Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Preparation Bag with the locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

  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.

- **Cell Collection Bag:**

  - CliniMACS Tubing Set/CliniMACS Tubing Set LS: Insert a Luer/Spike Interconnector (or equivalent) into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with the locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

  - CliniMACS Depletion Tubing Set: One Cell Collection Bag is already attached to the tubing set. Insert a Luer/Spike Interconnector (or equivalent) into the outside port of the Cell Collection Bag. Place a locking forceps on the tubing close to the bag and close the locking forceps. Weigh the empty Cell Collection Bag with locking forceps positioned close to the bag and the tubing hanging on the table next to the balance. Record the weight.

7.1.2 Magnetic labeling of cells

The following sections describe the recommended in-bag procedure for the preparation of the cell product using the sterile tubing connector.

- The user must be familiar with the operation and use of the sterile tubing connector.
- Before starting the cell labeling and separation procedure, ensure that all needed supplies and equipment are available.

IMPORTANT:

- All bag handling should be done in a sterile environment (for example, laminar flow hood) using aseptic techniques. The connection of tubing using the sterile tubing connector may be performed outside the laminar flow hood.
- Perform sample preparation and cell separation at the temperature conditions defined in the application-specific section. Lower or higher ambient temperature will result in less purity and yield of the target cells.

In short, the magnetic labeling of the cell product is performed by the following steps:

1. Analysis of starting cell product
2. Transfer of starting cell product into Cell Preparation Bag
3. Dilution of the starting cell product
4. Centrifugation
5. Volume adjustment
6. Magnetic labeling of the cells (incubation with CliniMACS Reagent)
7. Removal of excess reagent
8. Volume adjustment for cell separation

Analysis of starting cell product

Before starting the preparation of the starting cell product, determine the following parameters:

- Total number of leukocytes
- Percentage of cells to be labeled
- Total number of leukocytes to be labeled
- Viability

Ensure working within the application specification.

Recheck the parameters after the labeling procedure. This is necessary for the staged-loading programs (Enrichment 1.1/Depletion 2.1/Depletion 3.1), as the following three sample parameters have to be entered in the software during the setup of the CliniMACS Plus Instrument prior to loading the tubing set onto the instrument:

- Concentration of leukocytes per mL
- Percentage of labeled cells
- Final volume of the cell product (cell product loading volume)

Other tests might be required depending on the intended use of the cells (for example, number of T cells, phenotype). Record all data.

Transfer of starting cell product into Cell Preparation Bag

1. Record the date and the start time before beginning to prepare the starting cell product.
2. Determine the volume of the starting cell product by assuming 1 mL of starting cell product as equivalent to 1 g (1 g ≙ 1 mL).
3. Holding the starting cell product bag with both hands, mix the contents thoroughly by using a gentle rotating motion.
4. Using the sterile tubing connector, connect the Cell Preparation Bag to the original starting cell product bag.
5. Open the locking forceps to transfer the starting cell product into the Cell Preparation Bag. Use a tubing stripper to clear the tubing from any remaining blood. Close the locking forceps on the tubing as close to the bag as possible.

6. Seal off the tubing and separate bags, leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections (see Figure 7.1. Use the MACS TubeSealer to produce three adjacent seals in the tubing, with a distance of at least 1 cm between each seal. Make sure the seals are thoroughly established. Sever at the center seal. Keep the original leukapheresis/cellular starting bag until the separation and final analysis of all cells have been accomplished.

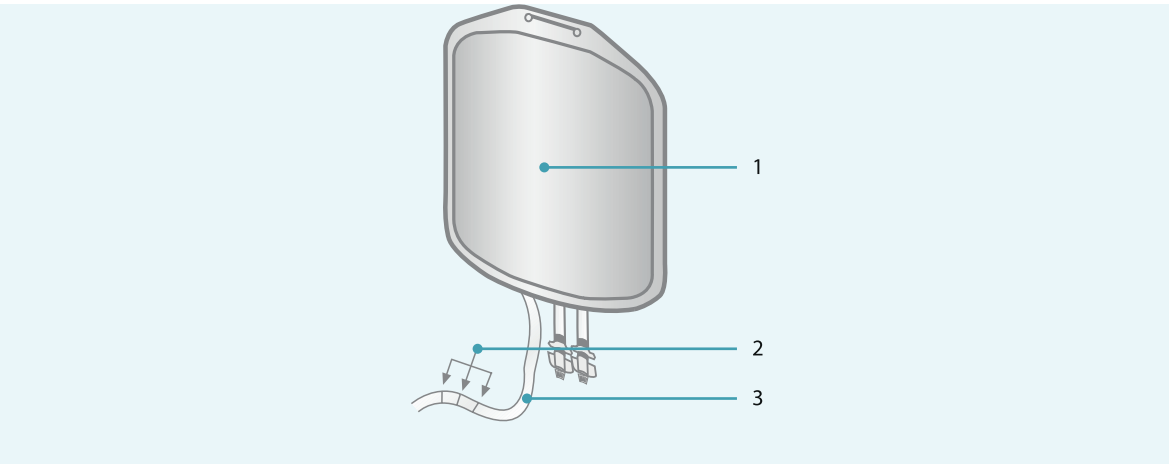


Figure 7.1: Sealing a bag

1 Bag
2 Hermetic seals
3 Tubing

7. To take a sample, disinfect the septum of the sampling site coupler. Mix the contents of the bag. 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. Transfer the sample into a sample tube. Label the tube as LEUKAPHERESIS/starting cell product (should include patient identification) and retain for cell analysis.

IMPORTANT:
Ensure that the starting cell product is mixed thoroughly before taking the sample. In order to get a representative sample, use a syringe with a capacity of at least 5 mL.

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

9. Determine the weight of the starting cell product by subtracting the weight of the empty Cell Preparation Bag from the weight of the Cell Preparation Bag filled with starting cell product. Record the calculated weight.
Weight of starting cell product (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)

Dilution of starting cell product

Refer to the application-specific section for the dilution ratio needed.
The starting cell product must be diluted with CliniMACS PBS/EDTA Buffer (supplemented with HSA to a final concentration of 0.5% [w/v]) before magnetic labeling. Calculate the weight of buffer to be added using either of the following equations (1 or 2) depending on the application and record it.

1. Weight of buffer to be added (g) = 600 – Weight of starting cell product (g)
2. Weight of buffer to be added (g) = Weight of starting cell product (g) × 2

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

2. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Make sure to position the buffer bag higher than the Cell Preparation Bag.
3. Place the Cell Preparation Bag on the balance and tare the balance.
4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.
5. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps on the tubing as near to the bag as possible.
6. Use the MACS TubeSealer to seal off the tubing between the clamp and the locking forceps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.
7. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion. Avoid intensive mixing of the cells.
8. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
9. Determine the weight of the diluted starting cell product by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.
   Weight of diluted starting cell product (g) = Weight of filled Cell Preparation Bag (g)
   – Weight of empty Cell Preparation Bag (g)

Centrifugation

⚠ WARNING

Risk of contamination.

If the bags are not suitable for cell preparation or not placed correctly into the centrifuge bucket, there is the risk of contamination due to bag damage.

- Use suitable bags, fold any loose parts of the bags or tubing downwards, taking care not to impair bag integrity and place the bags securely in the centrifuge bucket.

⚠ CAUTION

Risk of lower cell separation performance.

If the bags are not placed correctly into the centrifuge bucket, there is the risk of cell loss during supernatant removal.

- Fold any loose parts of the bags or tubing downwards and place the bags securely in the centrifuge bucket.

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 suitably weighed bucket. It is essential that the centrifuge is balanced accurately.

4. Centrifuge the cells at conditions defined in the application-specific section. Record the centrifugation conditions.
5. Remove the bags from the centrifuge, taking care not to disturb the cell pellet. Load the Cell Preparation Bag onto the plasma extractor and the connected Waste Bag on the table top balance.

Volume adjustment

For magnetic labeling of the cells, the optimal weight of the cell product varies depending on the application. Refer to the application-specific section for the labeling volume.

Depending on the application, it may be necessary either to only remove some amount of the supernatant to reach the labeling volume (tailored supernatant removal) or remove the supernatant completely and add buffer to reach the labeling volume (complete supernatant removal). Refer to the application-specific section for the applicable option and continue accordingly.

IMPORTANT:

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

Tailored supernatant removal

Calculate the weight of supernatant to be removed to adjust the sample to the labeling volume using the following equation: Weight of supernatant to be removed (g) = Weight of cell product after the wash (g) – Labeling volume (g)
Record the weight of supernatant to be removed.

1. Place the empty Plasma Waste Bag or Wash Waste Bag No. 1 on the balance and tare the balance.
2. Open the locking forceps next to the Cell Preparation Bag. By visually monitoring the scale on the balance, remove the supernatant using the plasma extractor. Carefully press out excess supernatant until the calculated "weight of supernatant to be removed" is reached.
3. When the appropriate weight of supernatant is removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow. Record the weight of the supernatant removed.
4. Using the MACS TubeSealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Plasma Waste Bag or Wash Waste Bag No. 1.
5. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
6. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
7. Determine the weight of the cell product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.
   Weight of cell product after volume adjustment (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)
8. Keep the Plasma Waste Bag or Wash Waste Bag until the final separation and analysis of all cells have been accomplished.

Complete supernatant removal

1. Open the locking forceps next to the Cell Preparation Bag.
2. Remove the supernatant completely using the plasma extractor, taking care not to resuspend the cell pellet during removal of supernatant.
3. When the supernatant has been removed, close the locking forceps next to the Cell Preparation Bag to stop the liquid flow.
4. Using the MACS TubeSealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections.
5. Disconnect the Plasma Waste Bag.
6. Resuspend the pellet carefully.
7. Adjust the weight of the Cell Preparation Bag by adding buffer. Calculate the target weight of the Cell Preparation Bag (CPB) filled with diluted cell product using the following equation:
   Target weight of CPB filled with washed cell product (g) = Labeling volume (g) + Weight of empty CPB (g)
8. Insert the spike of a plasma transfer set to a port of a buffer bag. Confirm the clamp on the plasma transfer set is in the closed position.
9. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.
10. Tare the balance. Place the Cell Preparation Bag with the cell pellet on the balance.
11. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag with buffer until the calculated "Target weight of Cell Preparation Bag filled with washed cell product" is reached. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.
12. Using the MACS TubeSealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.
13. Resuspend the cells in the Cell Preparation Bag carefully. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
14. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
15. Determine the weight of the cell product after volume adjustment by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.
    Weight of cell product after volume adjustment (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)
16. Keep the Plasma Waste Bag until the separation and final analysis of all cells have been accomplished.

Magnetic labeling of the cells: incubation with the CliniMACS Reagent

The CliniMACS Reagent vials are ready to use and sufficient for the application as described below. The reagents are not for parenteral administration.
Store the reagents at +2 °C to +8 °C (+36 °F to +46 °F). DO NOT freeze. The reagents must be used cold directly from the refrigerator. DO NOT warm up before use. The lot number and use-by date of the reagents are printed on the vial label. DO NOT use the reagents after the use-by date.

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

2. Disinfect the septum of the sampling site coupler. Use an appropriate sterile syringe and needle to remove the entire volume from a) one vial or b) two vials (7.5 mL each). A 10 mL syringe is sufficient to remove the contents of one vial, or respectively, a 20 mL syringe is sufficient to remove the contents of two reagent vials. The syringe should be equipped with a 20-gauge needle.
3. Using the injection port of the sampling site coupler, inject the entire volume of the reagent into the Cell Preparation Bag (optionally, in depletion applications transfer the content of the Cell Preparation Bag into a new transfer bag to ensure high depletion efficiency). The incubation time starts at this point.
4. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly by using a gentle rotating motion.
5. Place the Cell Preparation Bag flat on the orbital rotator, set to a speed of approximately 25 rpm, and ensure that the bag is not creased or bent. Incubate the bag in the conditions and duration defined in the application-specific section.

Removal of excess reagent

To remove the unbound reagent after incubation is completed, washing the cells is required. Refer to the application-specific section for the required number of washes.

1. Insert the spike of a plasma transfer set to a port of a buffer bag containing at least 500 mL of buffer. Confirm the clamp on the plasma transfer set is in the closed position.
2. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.
3. Place the Cell Preparation Bag on the balance and tare the balance.
4. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. Fill the Cell Preparation Bag with buffer to 600 mL. Slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag. Record the weight of the filled Cell Preparation Bag.
5. Seal off the tubing between both clamps leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the buffer bag.
6. Holding the Cell Preparation Bag with both hands, mix the contents thoroughly with a gentle rotating motion.
7. Using the sterile tubing connector, connect the empty Wash Waste Bag to the Cell Preparation Bag.
8. Fold any loose parts of the bags or tubing downwards. Transfer the Cell Preparation Bag and Wash Waste Bag securely to the centrifuge bucket.
9. Centrifuge the cells in the conditions and duration defined in the application-specific section.
10. Taking care not to disturb the cell pellet, remove the bags from the centrifuge.
11. Carefully hang the Cell Preparation Bag on the plasma extractor.
12. Place the Wash Waste Bag on the balance and tare the balance.
13. Open the locking forceps next to the Cell Preparation Bag, and open the sterile welds between the bags, if not yet done. Using the plasma extractor, remove as much supernatant as possible from the Cell Preparation Bag taking care not to remove cells. Close the locking forceps next to the Cell Preparation Bag to stop the liquid flow.

14. Using the MACS TubeSealer, seal off the tubing leaving at least 15 cm of tubing on the Cell Preparation Bag for further connections. Disconnect the Wash Waste Bag.
15. Keep the Wash Waste Bag until the separation and final analysis of all cells have been accomplished.
16. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
17. If more washes are required (for example, for enrichment of CD34-positive cells), repeat steps 1–16.
18. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
19. Determine the weight of the cell product after the wash by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.
    Weight of cell product after the wash (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)
20. Adjust sample loading volume: Calculate the weight of buffer necessary to adjust the weight of the cell suspension to the appropriate sample loading volume according to the application using the following equation. For loading the labeled and washed cells on the tubing set, a maximum cell concentration of 0.4×10^9 cells per mL is recommended.
    Weight of buffer to be added (g) = Sample loading volume (according to application) – Weight of cell product after the wash (g)
21. Using the sterile tubing connector, connect the buffer bag to the Cell Preparation Bag. Hang the buffer bag on a bag hanger.
22. Place the Cell Preparation Bag on the balance and tare the balance.
23. Open the locking forceps next to the Cell Preparation Bag. Slide the clamp on the plasma transfer set to the open position. By visually monitoring the scale on the balance, transfer the calculated weight of buffer to the Cell Preparation Bag.
24. When the appropriate weight of buffer has been transferred, slide the clamp on the plasma transfer set to the closed position to stop the liquid flow. Close the locking forceps next to the Cell Preparation Bag.
25. Using the MACS TubeSealer, seal off the tubing between both clamps. Disconnect the buffer bag.
26. Resuspend the cell pellet in the Cell Preparation Bag. Avoid intensive mixing of the cells. Ensure that all cells are resuspended.
27. Disinfect the septum of the sampling site coupler. Insert a needle with an appropriate syringe into the sampling site coupler of the Cell Preparation Bag and remove a volume of 0.5 mL of the labeled product. Transfer the sample into a sample tube. Label the tube as ORIGINAL (should include patient identification) and retain for analysis.
28. Tare the balance and weigh the filled Cell Preparation Bag. Record the weight.
29. Determine the weight of the cell product after the addition of buffer (sample loading volume) by subtracting the weight of the empty Cell Preparation Bag from the weight of the filled Cell Preparation Bag. Record the calculated weight.
    Weight of cell product after addition of buffer (g) = Weight of filled Cell Preparation Bag (g) – Weight of empty Cell Preparation Bag (g)

Proceed to Step 2.

DO NOT connect the Cell Preparation Bag to the tubing set until instructed by the screen.

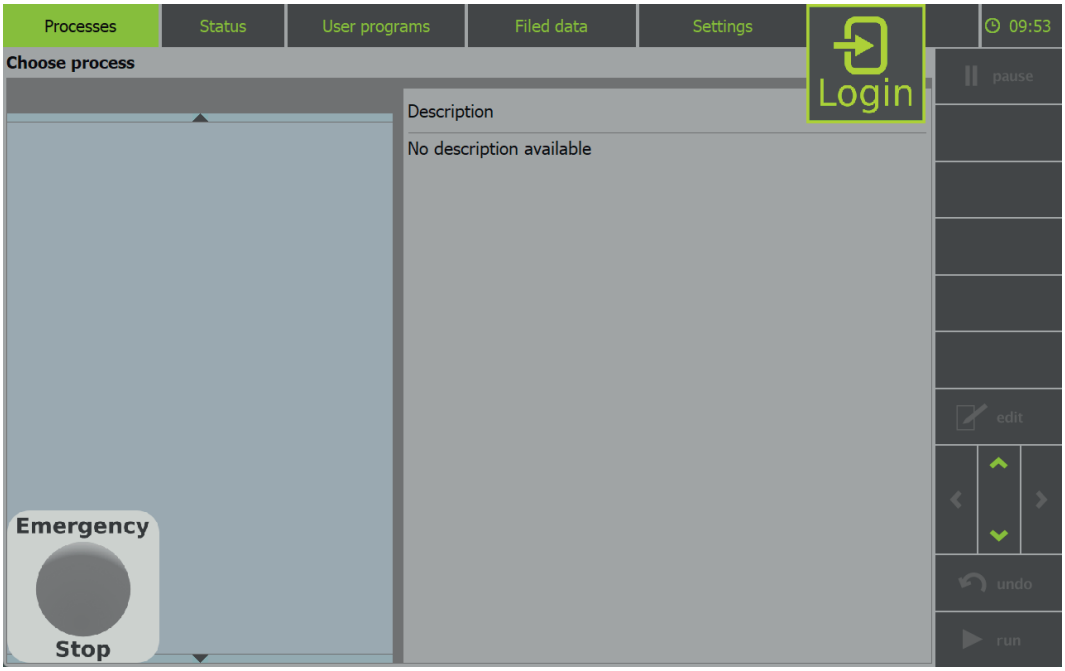
7.2 Step 2: Choice of separation program

7.2.1 Basic operation

This section describes the basic instrument operations required before and after the execution of a separation
program. For information on additional instrument operations and software functions, refer to the CliniMACS Plus Software user manual.

Switching on the instrument

1. Plug the power cable of the instrument into a grounded electrical outlet.
2. Switch on the CliniMACS Plus with the power switch located on the rear side of the instrument.
   The start screen appears and shows the current software version. During the initialization, the instrument performs self-checking procedures. After the initialization process, a locked screen appears and only the Login button is active.



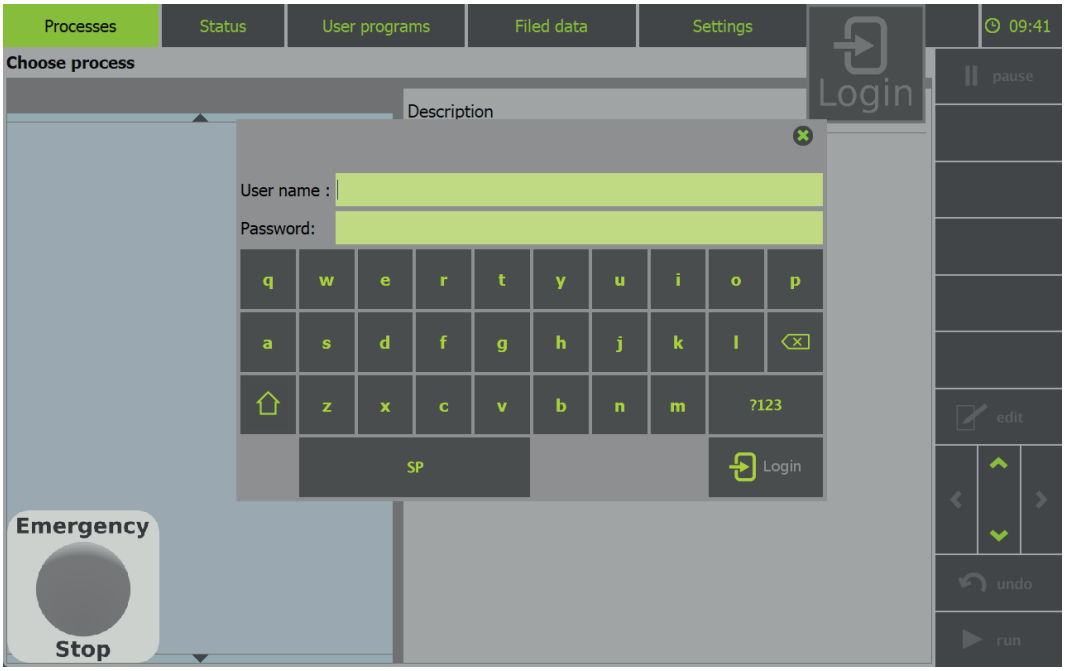
   If the instrument does not start up, switch off the instrument by using the power switch and disconnect it from the power supply. Check the power port. Then switch on the instrument again. If the instrument does not start correctly or the window shows an error message, note the error message number, switch off the instrument, and contact Miltenyi Biotec Technical Support.

Login

IMPORTANT:

- The username and password should be set up before login (refer to the CliniMACS Plus Software user manual for details).
- Maxing out the login attempts locks the user completely. There is no option to reset the password, also not by Miltenyi Biotec Technical Support.
- In case of login failure, refer to the CliniMACS Plus Software user manual.

1. To open the login window, tap **Login** in the upper right corner of the locked screen.
   A pop-up window appears, allowing the user to enter the user credentials:



2. Enter username and password and tap **Login** within the pop-up window.

Logout

IMPORTANT:
After 20 minutes (default) without interaction with the software, the user is logged out automatically.

1. Tap (**quick access menu**) and select **Logout** from the list.
2. To confirm the logout, tap **ok** in the pop-up window.

Shutting down the instrument

1. To shut down the instrument, choose one of the following options:
   - Tap (**quick access menu**) and select **Shutdown** from the list or
   - navigate to **Settings** > **Tools** > **Shutdown** and tap **run**.
2. In the pop-up window, confirm with **shutdown**.
3. Wait for the shutdown process to finish and switch off the instrument using the power switch located at the rear side of the instrument.

Navigating to the processes menu

To view the standard processes, navigate to the Processes menu (see Figure 7.2).

Tap Processes in the menu bar.
The left-hand side of the screen shows a list of the available processes. The right-hand side of the screen shows a description of the selected process.

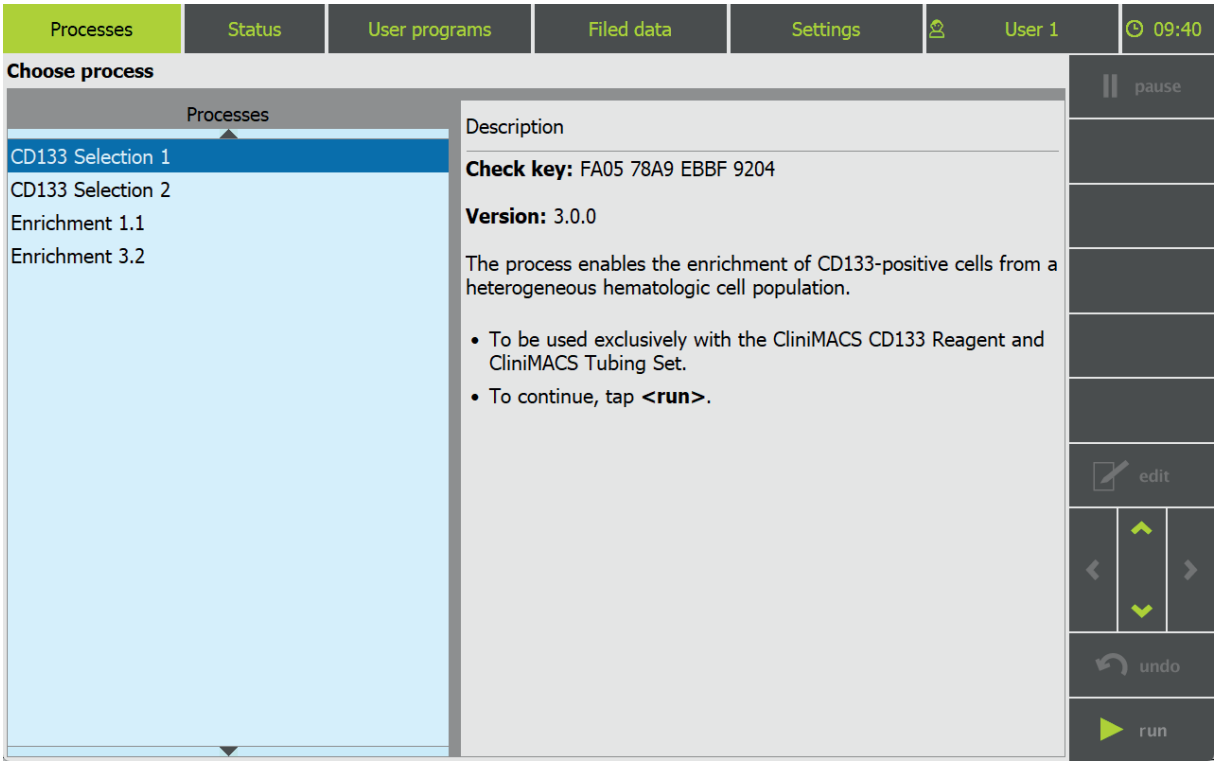


Figure 7.2: Processes menu

Parameter input

Multiple parameters must be entered for the process either with the barcode reader connected to the CliniMACS Plus or the keyboard shown on the screen. Optional parameters are indicated as such and can be skipped. Tap ok to confirm each entry. All entered parameters are saved in the process-related protocol.

If unexpected events occur during barcode scanning, refer to section 'Barcode scanning' in the Troubleshooting chapter for information on corrective actions.

IMPORTANT:

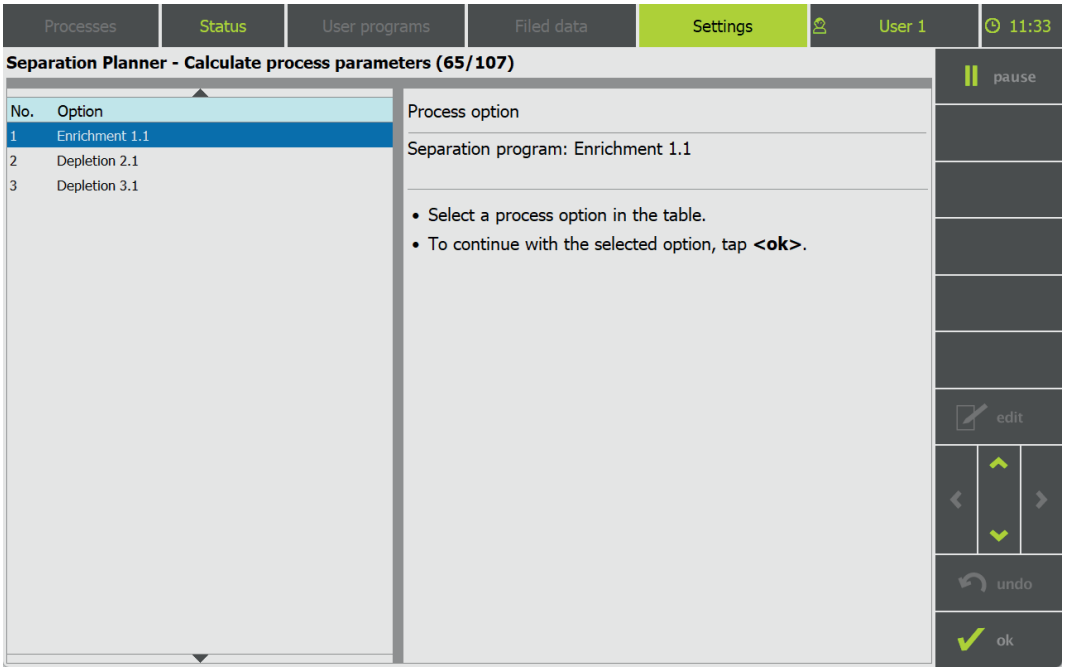
- The user interface shows screens with instructions and images to guide the user through the entire procedure. Use the touchscreen functionality and buttons on the menu bar and tool bar to operate the CliniMACS Plus Instrument. For details, refer to the CliniMACS Plus Software user manual.
- DO NOT use the external keyboard when running any of the installed applications on the instrument. The external keyboard must be used for the "User Management" only! The external keyboard must be unplugged from the instrument before starting any application and tool.

7.2.2 Separation planner tool

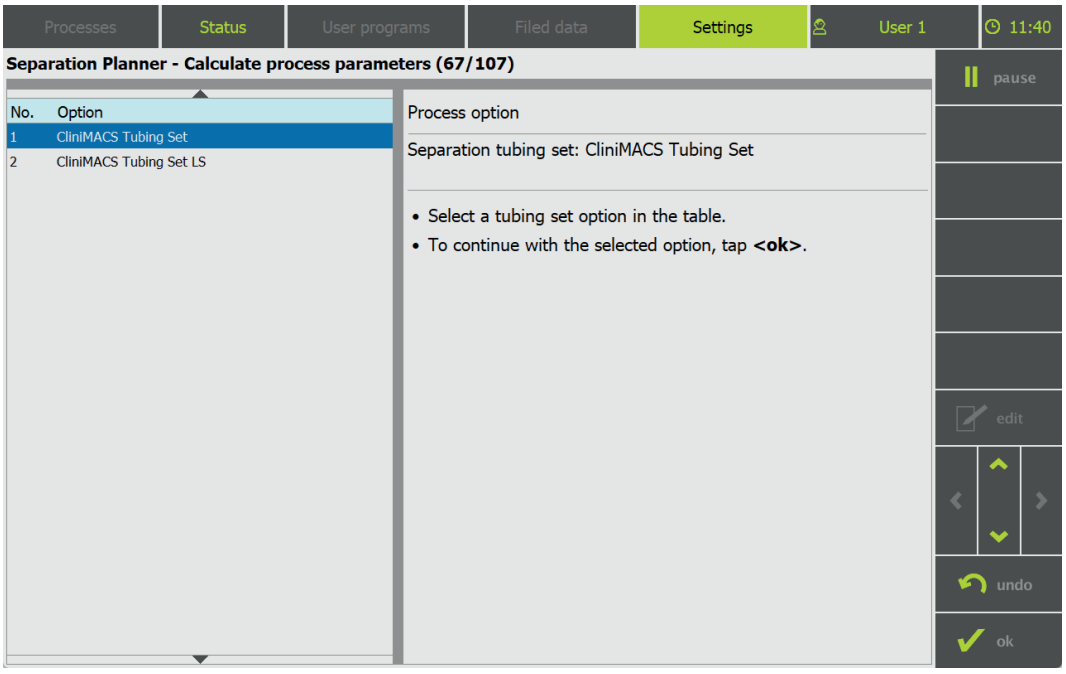
The Separation planner tool allows the user to calculate and estimate process parameters for Enrichment 1.1, Depletion 2.1, and Depletion 3.1 based on entered starting cell product parameters.

IMPORTANT:
Before starting a separation program, use the Separation Planner tool to support the planning and preparation of the separation run.

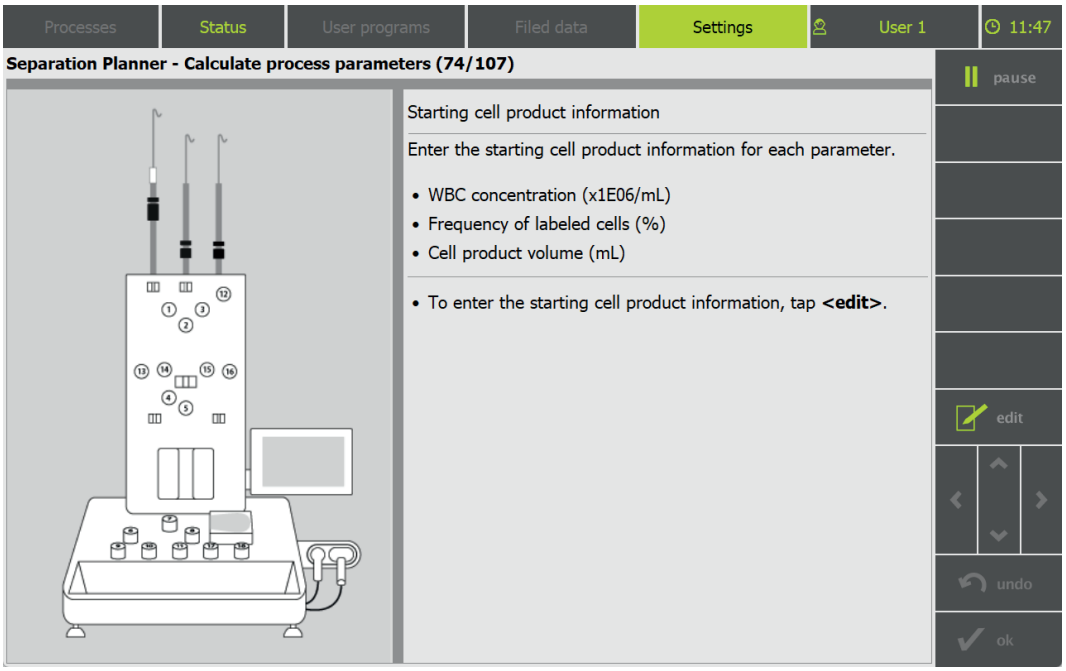
1. To start the separation planner, navigate to **Settings** > **Tools** > **Separation Planner** and tap **run**.
2. To choose a separation program from the list, use the navigation buttons ⌄ and ⌃ or tap on the program name. To continue, tap **ok**.



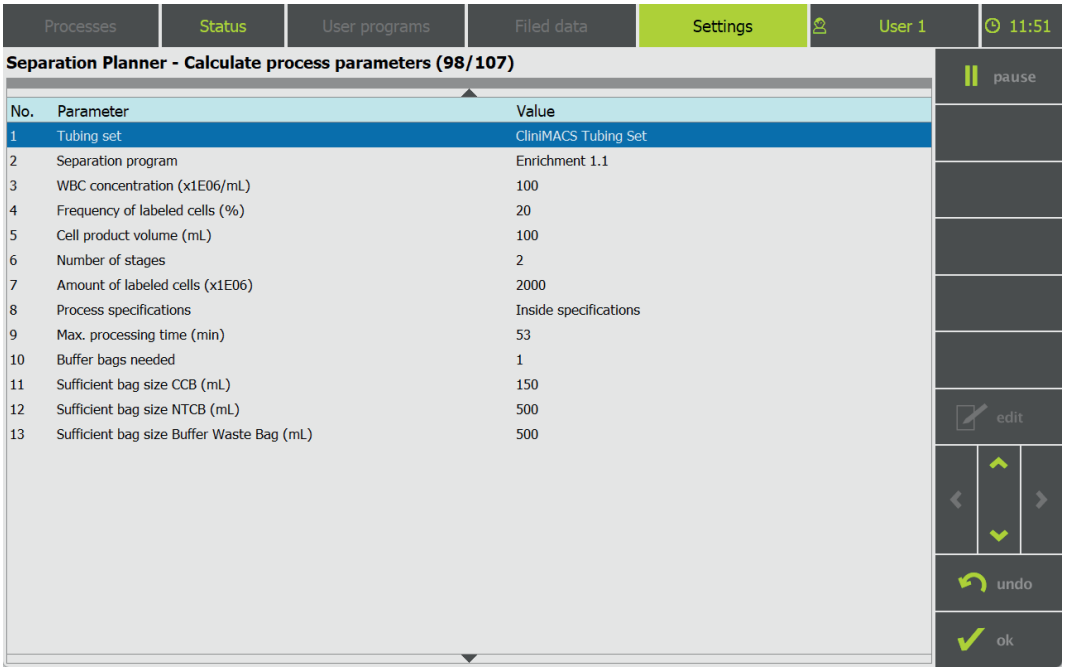
3. Choose a tubing set from the list. To continue, tap **ok** and confirm choice. To return to the list, tap **undo**.



4. To enter the required starting cell product parameters, tap **edit**. To change an entered parameter, tap **edit**. To continue tap **ok**.



5. Review the calculated process parameters. The estimated process parameters can be used for preparation of the planned separation procedure.



7.2.3 CD133 Selection 1/ CD133 Selection 2

CD133 Selection 1 and CD133 Selection 2 are optimized for the enrichment of CD133-positive cells.

The separation program CD133 Selection 1 must only be used in combination with the CliniMACS Tubing Set (REF 161-01). The separation program CD133 Selection 2 must only be used in combination with the CliniMACS Tubing Set LS (REF 162-01). Ensure that the tubing set is correct before the installation on the instrument.

Screen prompts and diagrams serving as procedure guides will appear on the screen.

- Perform and check each step according to the manual and software instructions before proceeding to the next step.
- To correct a mistake during data input, tap **undo** (refer to the CliniMACS Plus Software user manual for more details on the toolbar).

Choosing separation program CD133 Selection 1 or CD133 Selection 2

Depending on the application, choose CD133 Selection 1 or CD133 Selection 2 (see Figure 7.3).

The screenshots in the following sections may show either CD133 Selection 1 or CD133 Selection 2 in the title bar. Depending on the selected separation program, the program name shown in the software may differ from that shown in the screenshots in this user manual.

1. To select CD133 Selection 1/2 from the list, highlight the name of the program by using the navigation buttons ⌄ and ⌃ or by tapping on the program name.
2. To continue with the selected program, tap **run**.

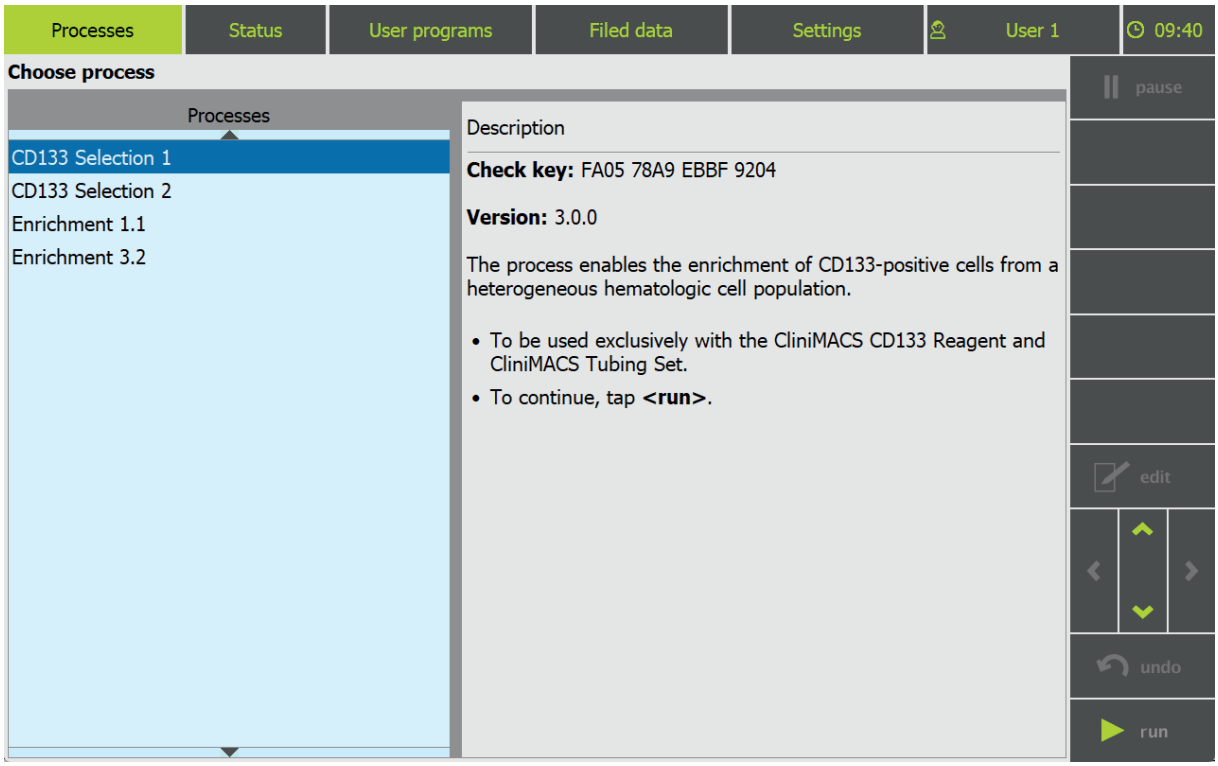


Figure 7.3: Choice of separation program CD133 Selection 1/2

Entering Run ID (optional)

The user can enter a Run ID for the process. To enter a Run ID, tap **edit**. To continue without any entry, tap **ok**.

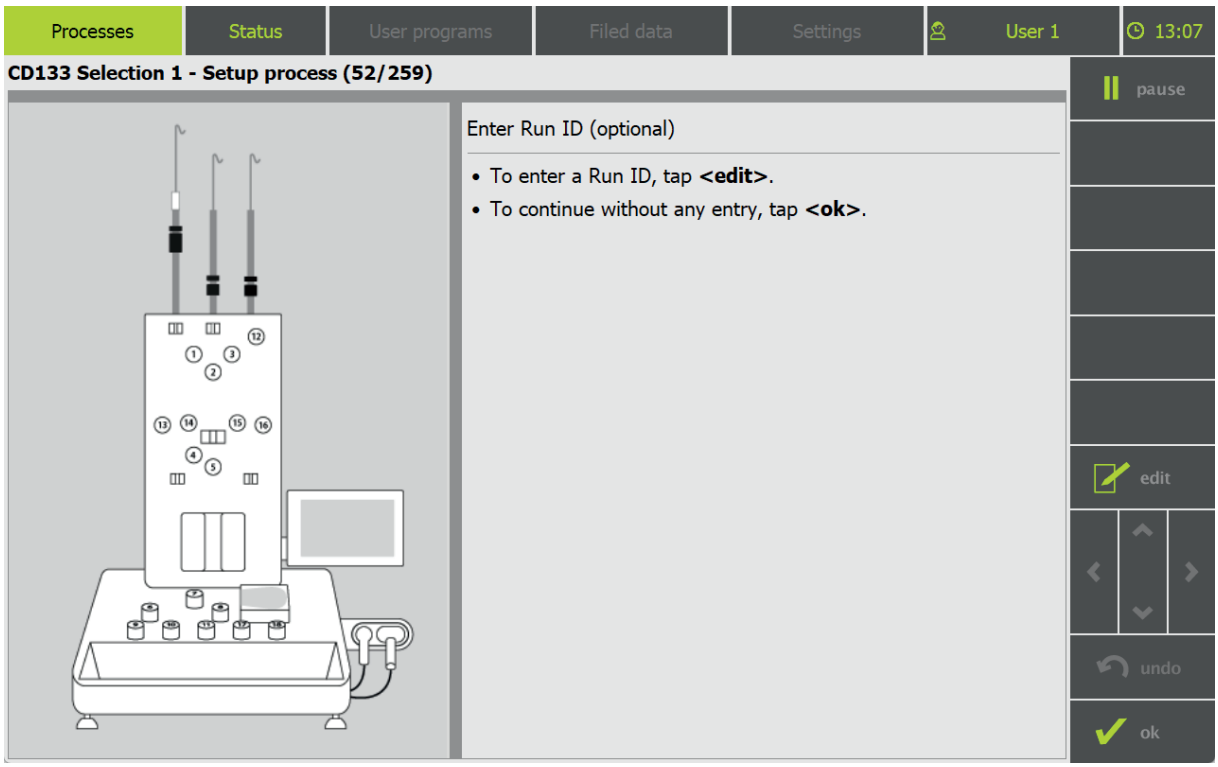


Figure 7.4: Entry of Run ID

Entering tubing set parameters

1. Use the barcode reader to scan the barcode label on the tubing set packaging.
2. Alternatively, enter the part number (P/N), LOT number, and use-by date manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.

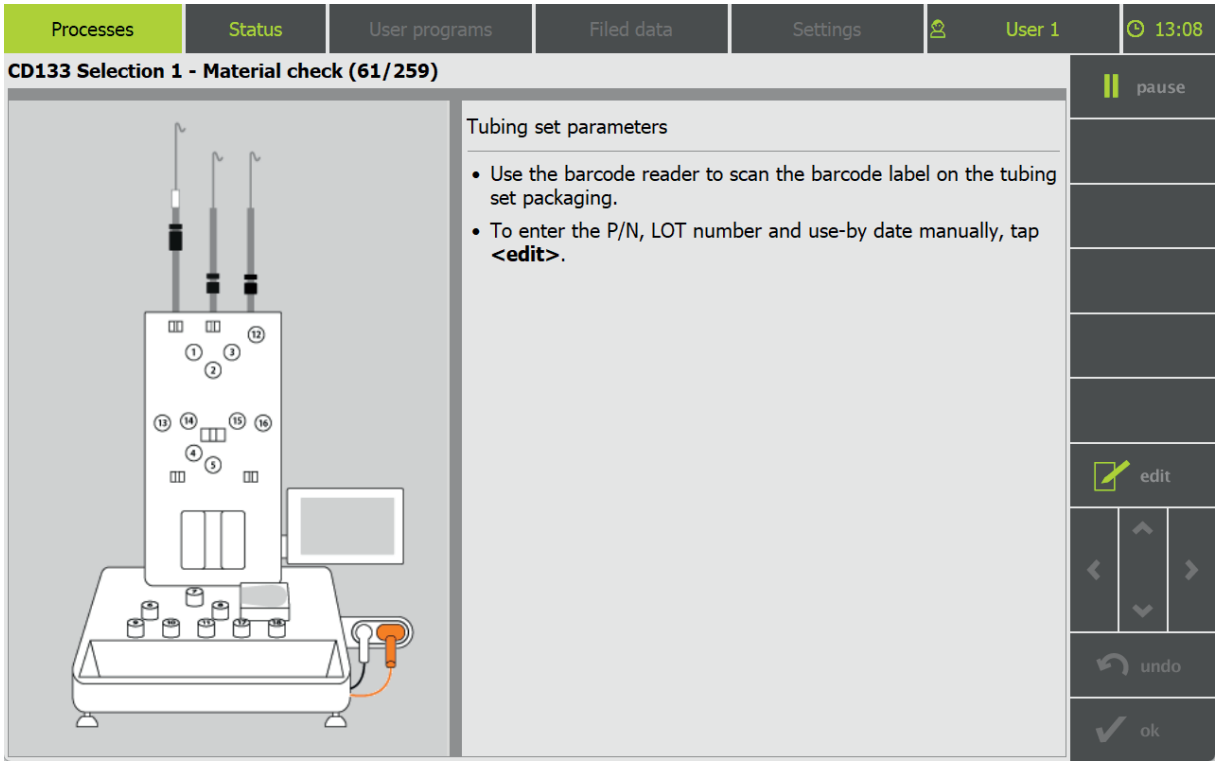


Figure 7.5: Entry of tubing set parameters

Entering starting cell product information (optional)

1. Use the barcode reader to scan the barcode label on the cell product.
2. Alternatively, enter the information manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm each entry, tap **ok**.

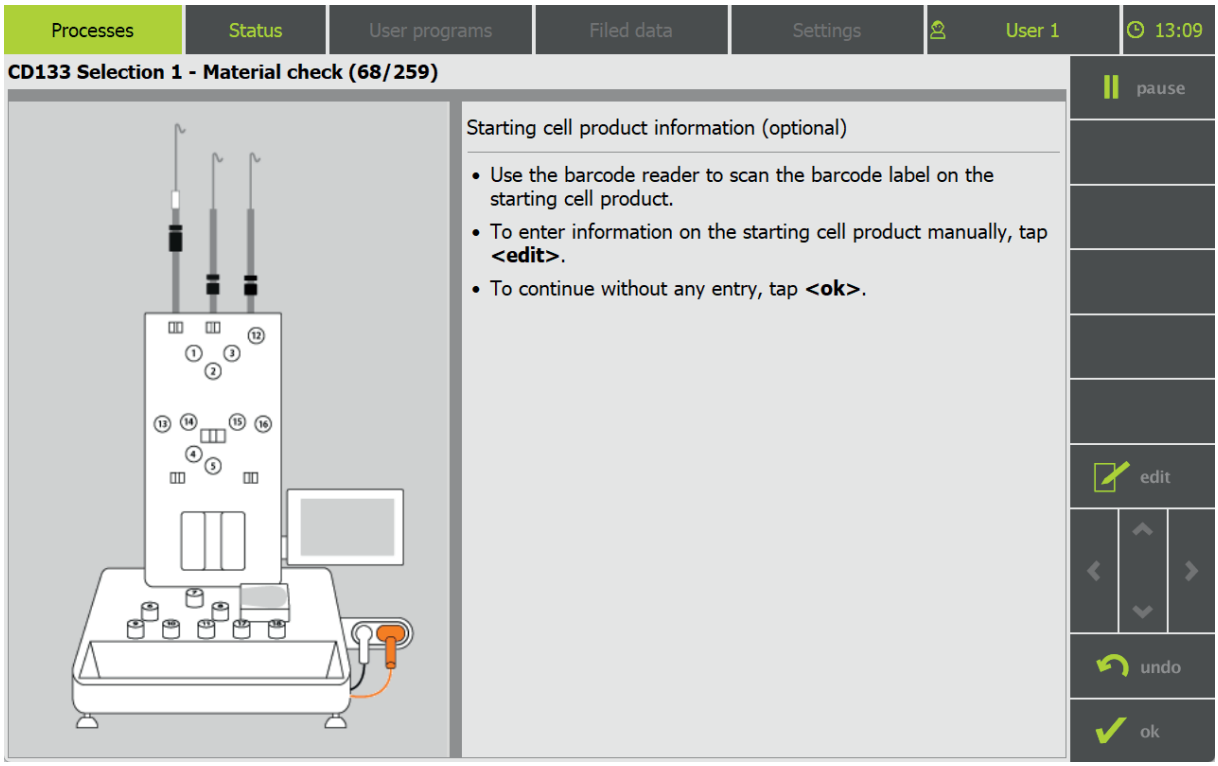


Figure 7.6: Entry of starting cell product information

Entering CliniMACS CD133 Reagent parameters

If CD133 Selection 1 has been started, enter the parameters for one reagent vial. If CD133 Selection 2 has been started, enter the parameters for two reagent vials.

1. Use the barcode reader to scan the barcode label on the CliniMACS CD133 Reagent.
2. Alternatively, enter the part number (P/N), LOT number, and use-by date manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm each entry, tap **ok**.

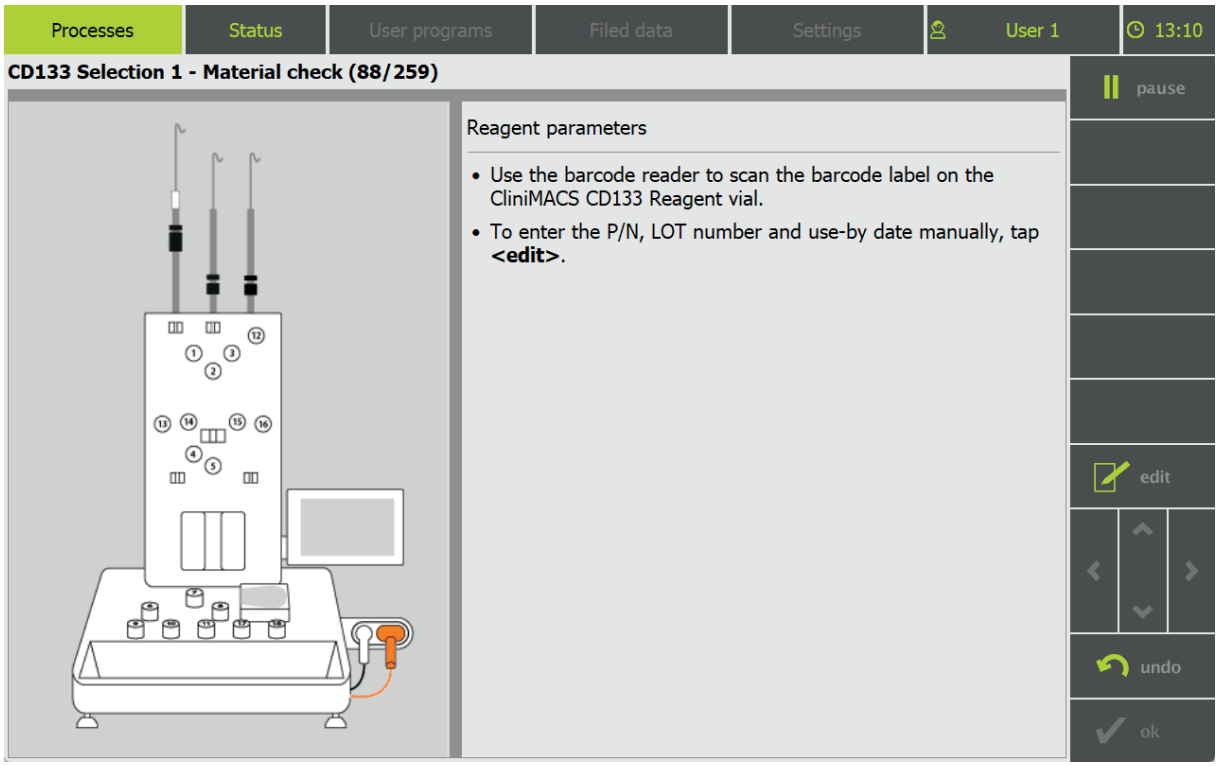


Figure 7.7: Entry of CliniMACS CD34 Reagent parameters

Entering process buffer parameters (optional)

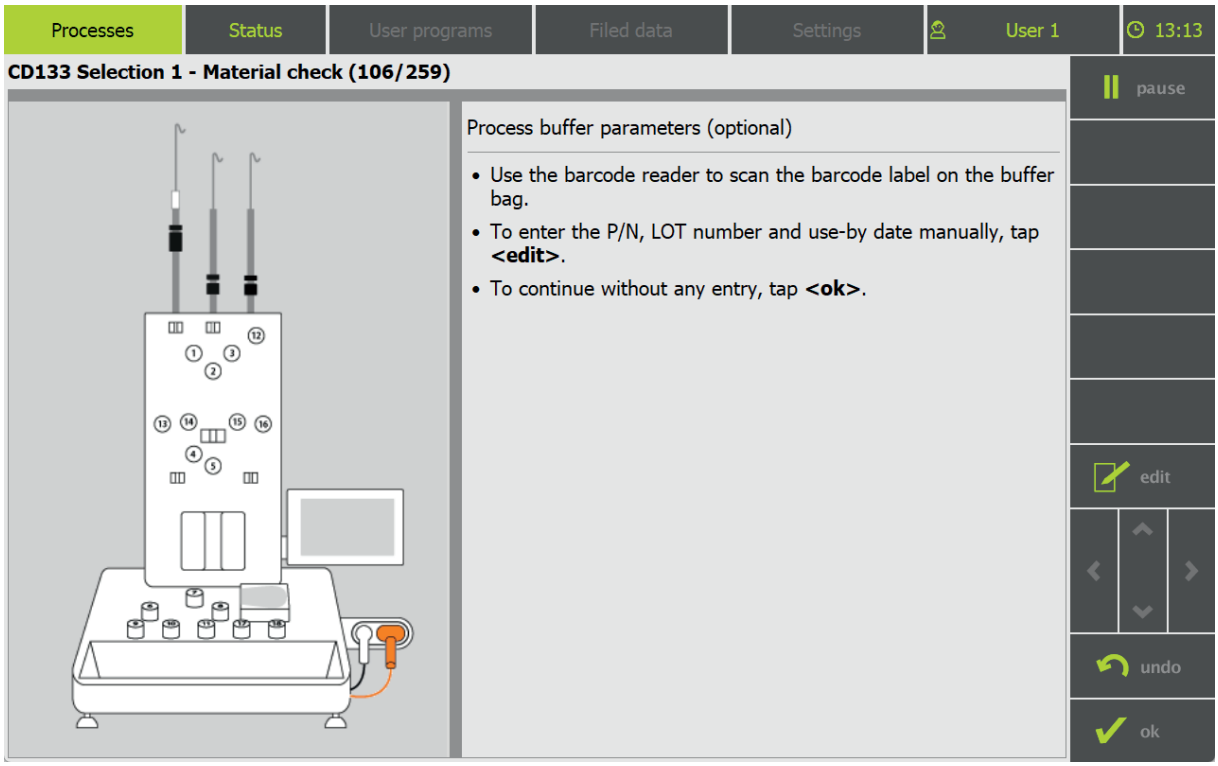


Figure 7.8: Entry of process buffer parameters

1. To enter the process buffer parameters, scan the barcode label on the buffer bag using the barcode reader.
2. Alternatively, enter the parameters manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
3. To continue without any entry, tap **ok**.

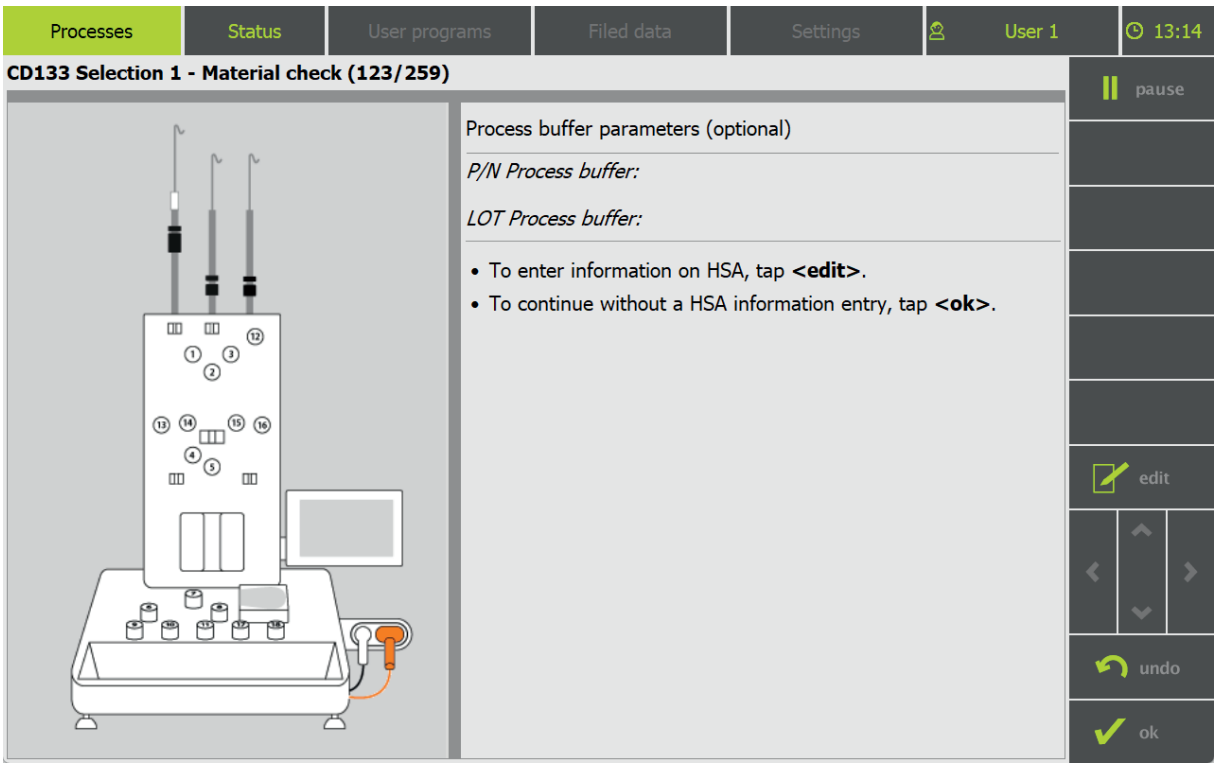


Figure 7.9: Entry of HSA information for the process buffer

Additionally, the user can enter HSA information for the process buffer.

1. To enter HSA information, tap **edit**. To continue without any entry, tap **ok**.

Entering information for additional materials (optional)

If multiple additional materials are used, repeat the following steps for each entry.

1. To enter the item name and information of the additional material, tap **edit**. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
2. To continue without any entry, tap **ok**.

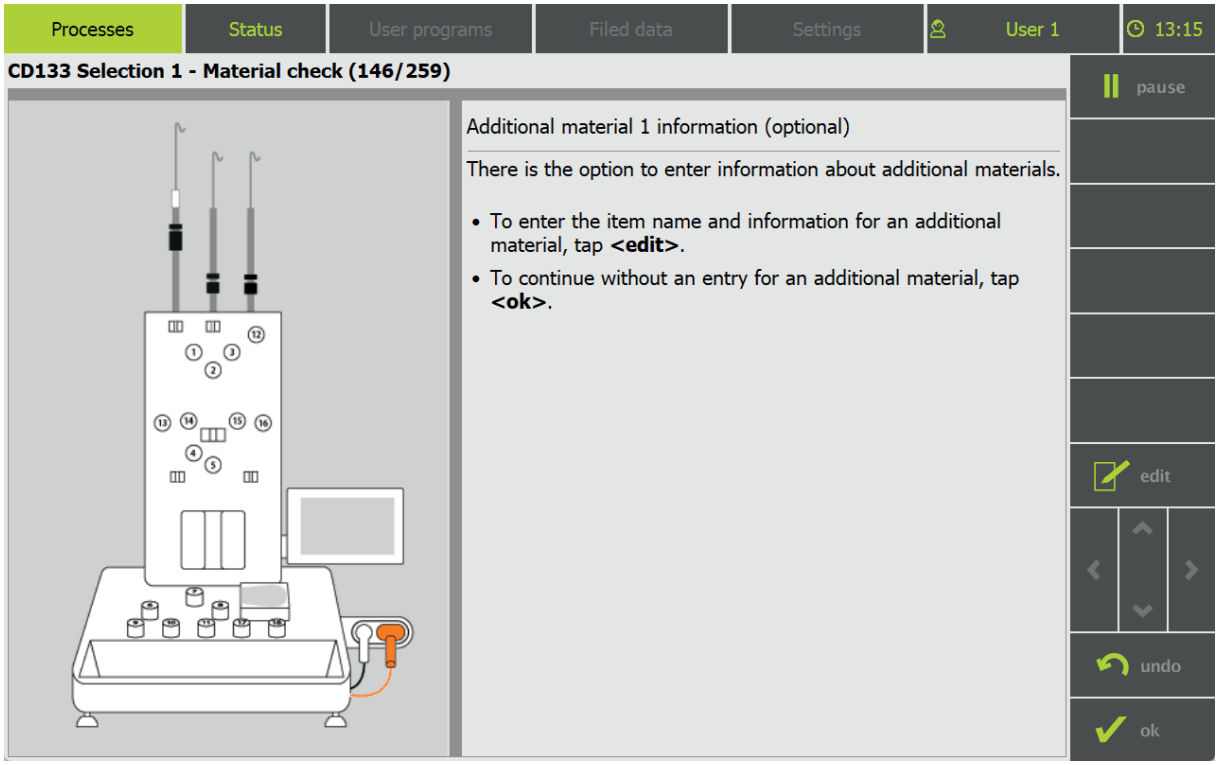


Figure 7.10: Entry of additional material information

Continue with the installation of the tubing set (see section 'Step 3: Installation of CliniMACS Tubing Sets').

7.2.4 Enrichment 1.1

The separation program Enrichment 1.1 must only be used in combination with the CliniMACS Tubing Set (REF 161-01) or the CliniMACS Tubing Set LS (REF 162-01). Ensure that the tubing set is correct before the installation on the instrument.

Screen prompts and diagrams serving as procedure guides will appear on the screen.

- Perform and check each step according to the manual and software instructions before proceeding to the next step.
- To correct a mistake during data input, tap **undo** (refer to the CliniMACS Plus Software user manual for more details on the toolbar).

IMPORTANT:
Before starting Enrichment 1.1, it is recommended to use the Separation Planner tool to plan and prepare for the actual separation run (see section 'Separation planner tool').

Choosing separation program Enrichment 1.1

1. To select Enrichment 1.1 from the list (see Figure 7.11), highlight the name of the program by using the navigation buttons ⌄ and ⌃ or by tapping on the program name.
2. To continue with the selected program, tap **run**.

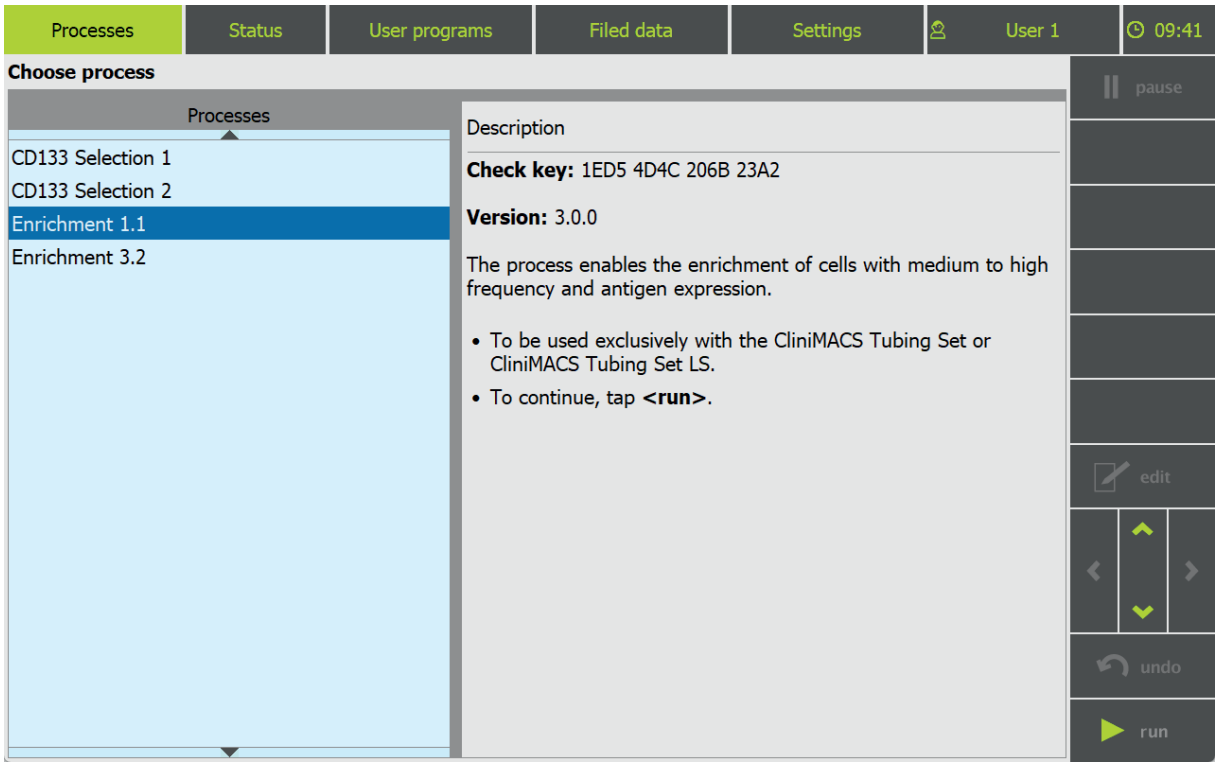


Figure 7.11: Choice of separation program Enrichment 1.1

Entering Run ID (optional)

The user can enter a Run ID for the process. To enter a Run ID, tap **edit**. To continue without any entry, tap **ok**.

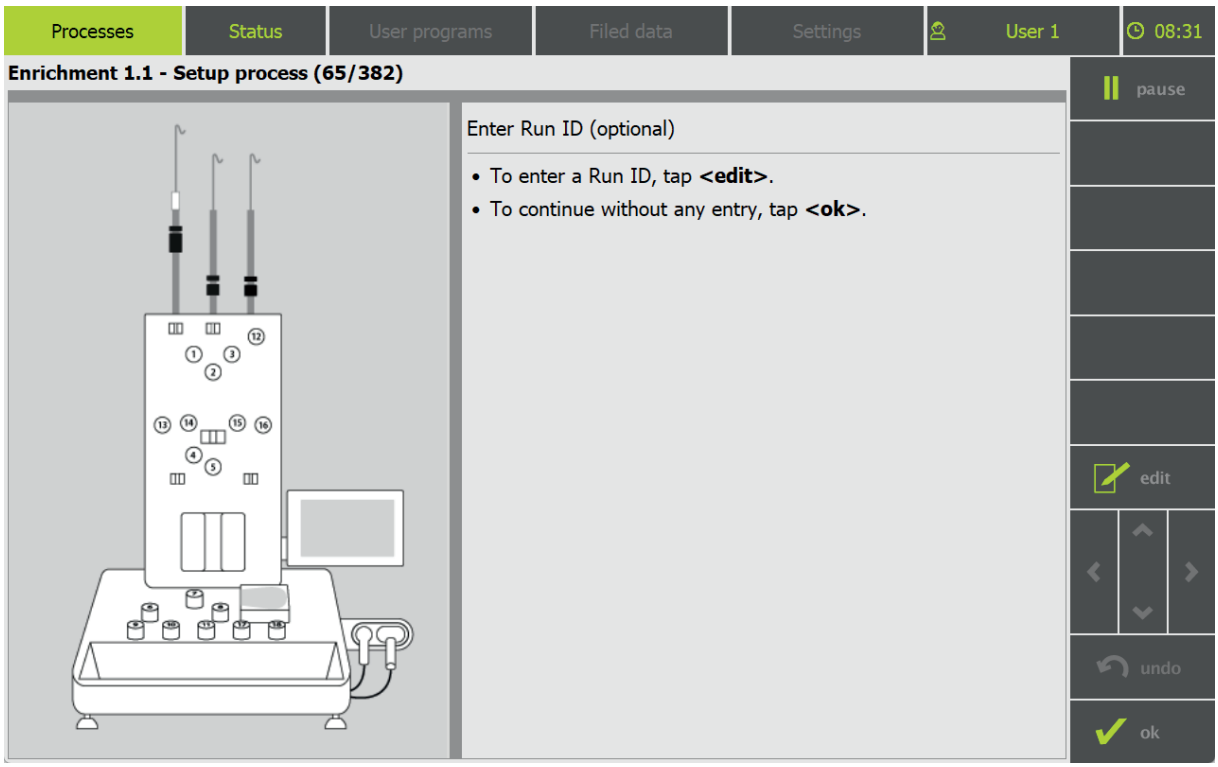


Figure 7.12: Entry of Run ID

Entering tubing set parameters

1. Use the barcode reader to scan the barcode label on the tubing set packaging.
2. Alternatively, enter the part number (P/N), LOT number, and use-by date manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.

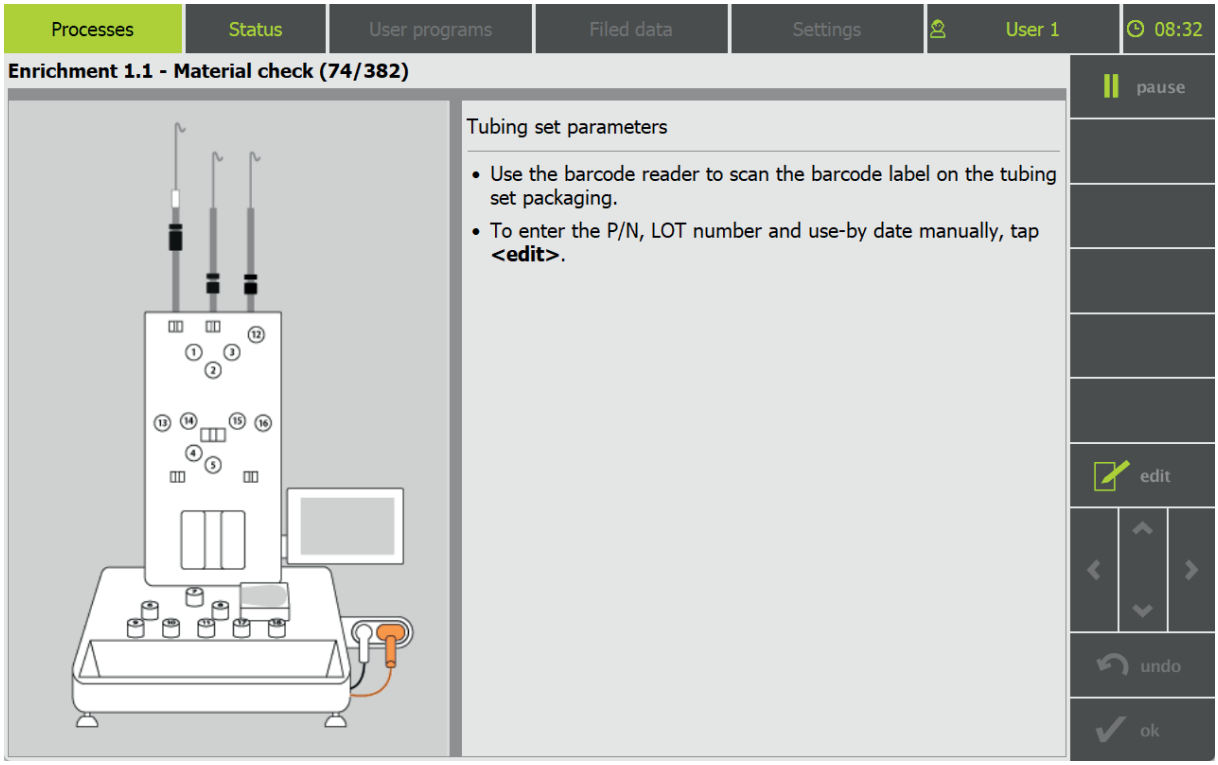


Figure 7.13: Entry of tubing set parameters

Entering starting cell product information (optional)

1. Use the barcode reader to scan the barcode label on the cell product.
2. Alternatively, enter the information manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm each entry, tap **ok**.

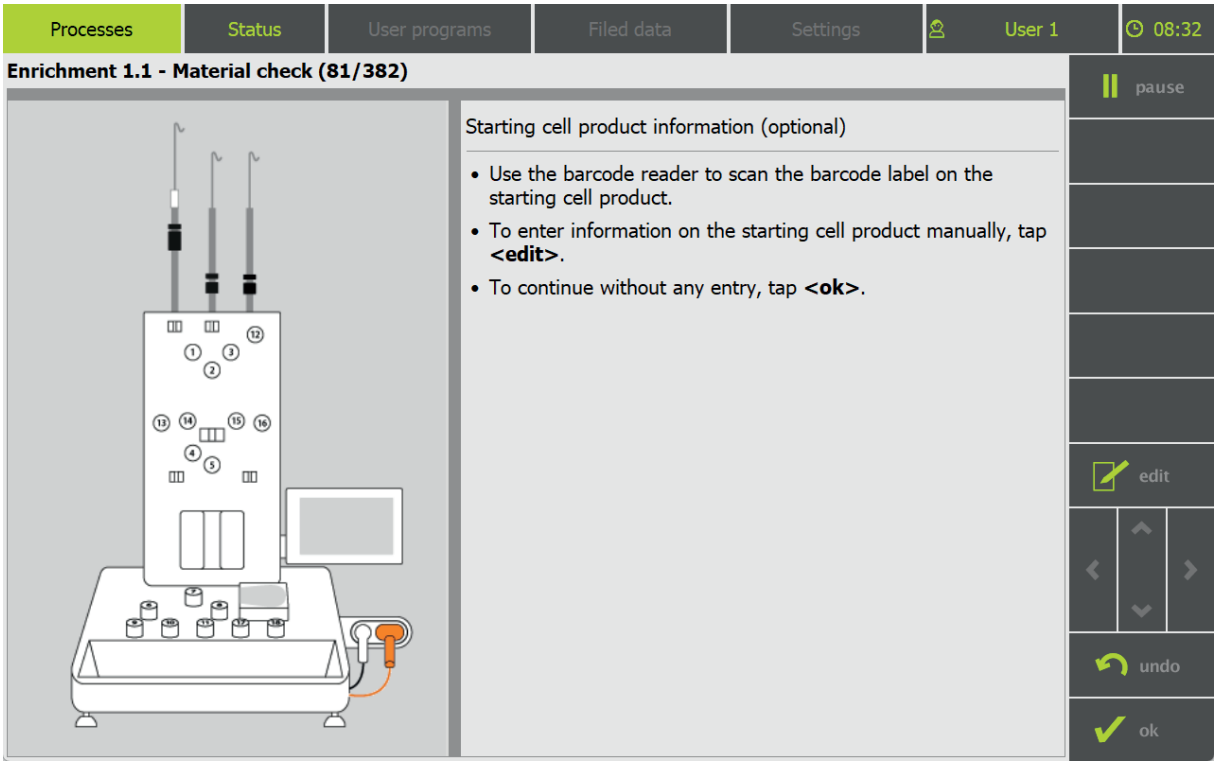


Figure 7.14: Entry of starting cell product information

Entering starting cell product parameters

Enter the starting cell product parameters (see Figure 7.15). The starting cell product parameters are necessary to adjust the separation to each individual cell product and to provide the user with important information about the required buffer and bag volumes.

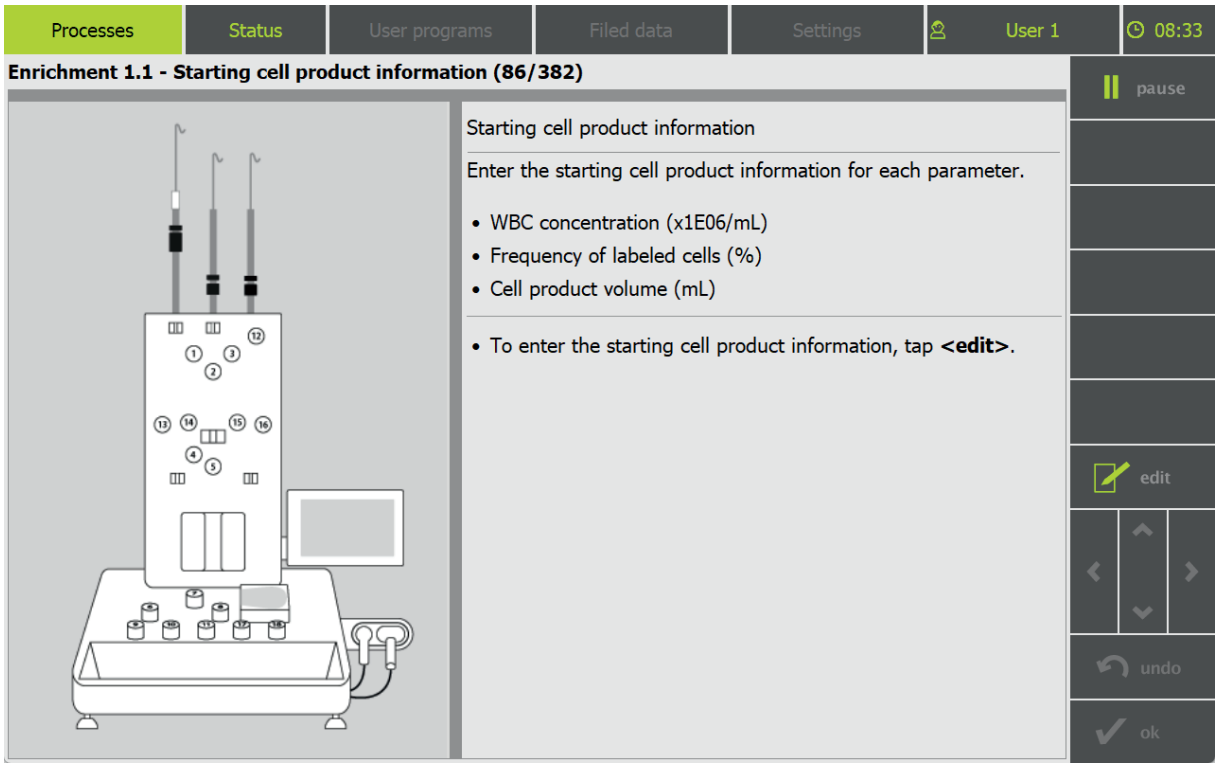


Figure 7.15: Entry of starting cell product parameters

⚠ CAUTION

Specifying wrong parameters may result in non-optimal cell product processing and may increase the risk of target cell loss.

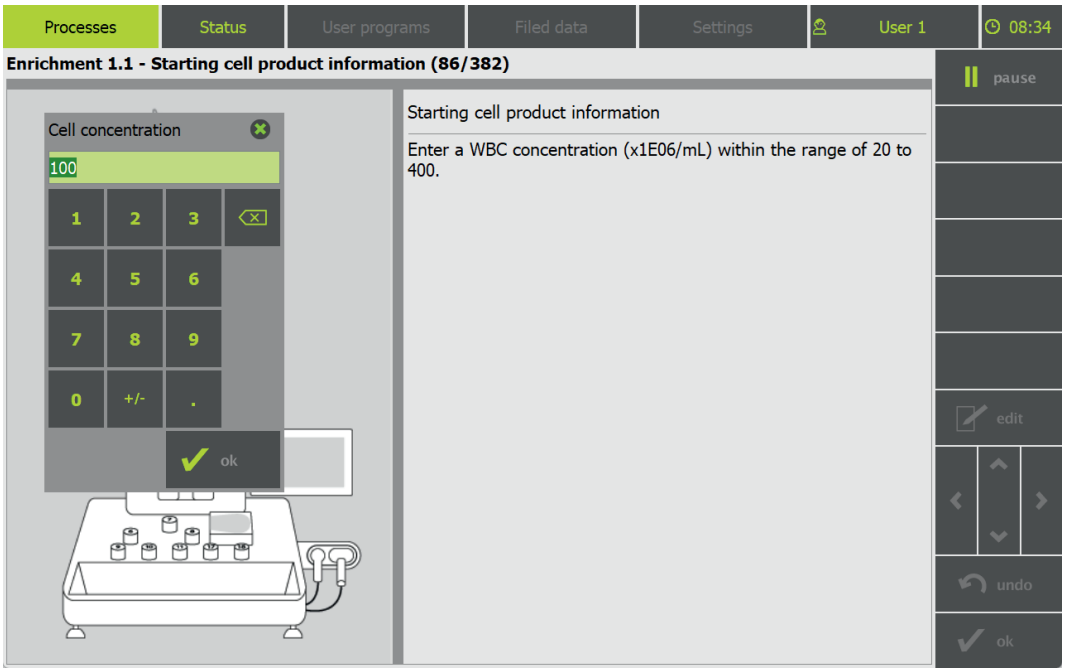
Entering values for WBC concentration and/or percentage of labeled cells which are lower than the actual cell product values may result in cell loss due to the system being overloaded. Entering a lower cell product volume may result in cell loss due to overloading or even to incomplete cell product processing.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

- Always ensure to enter the correct parameters.

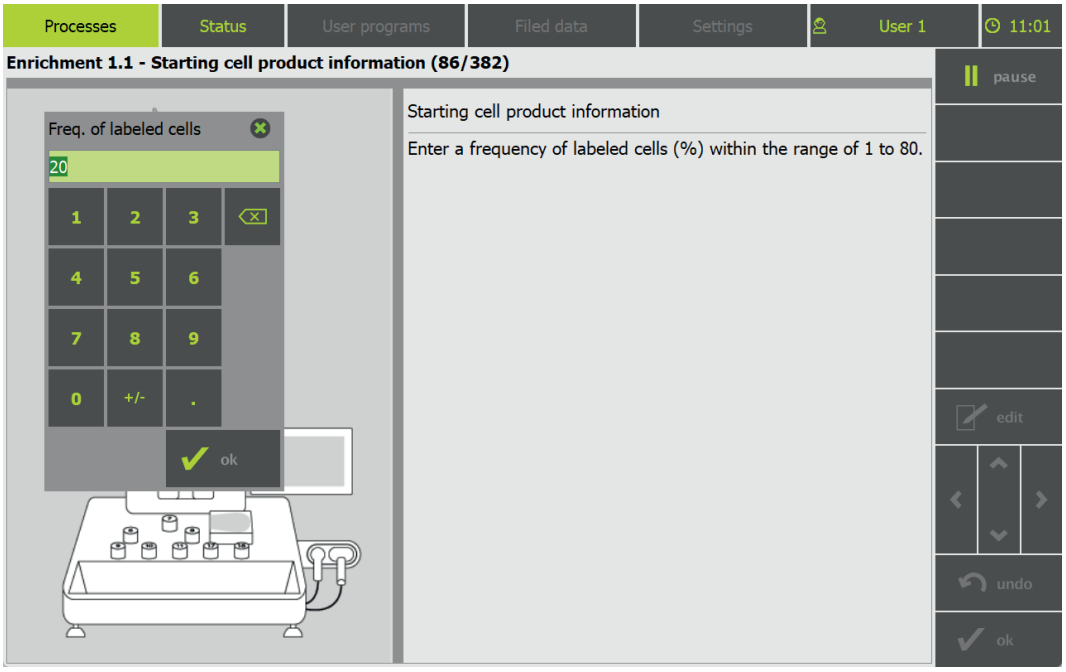
IMPORTANT:
All cell product parameters required refer to the cell product after the cell labeling and washing procedure. The allowed range shown on the right-hand side of the screen always takes into account the previous input. After each input, the software calculates and adjusts the accepted range of the next query.

To enter the starting cell product parameters, tap **edit**.

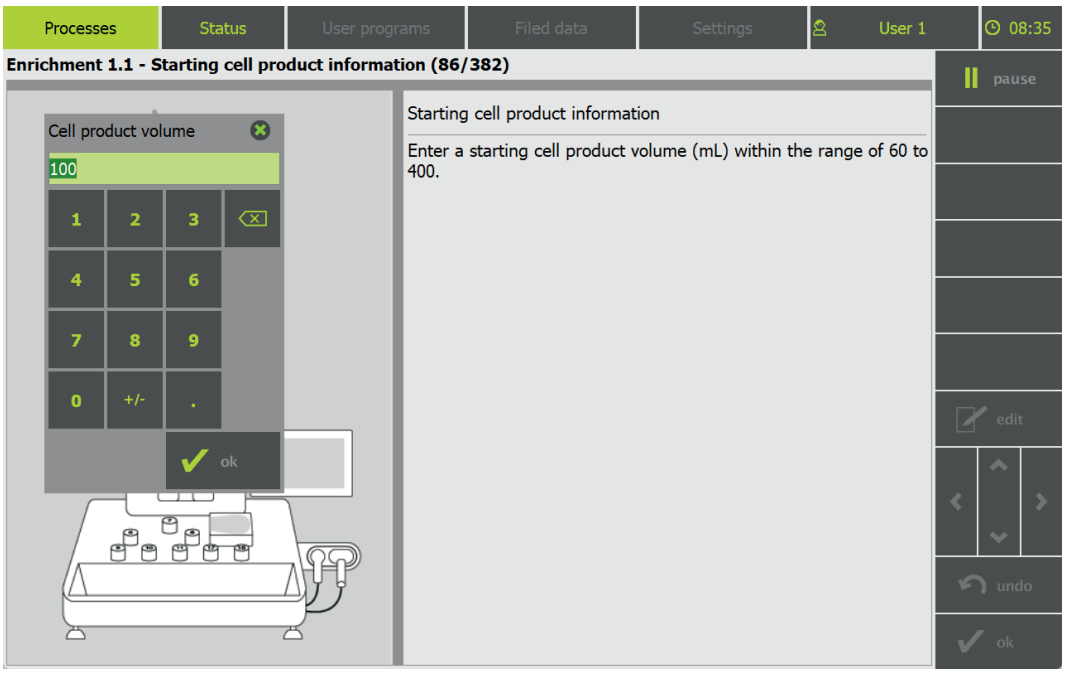
1. Enter the WBC concentration of the starting cell product. The allowed range is shown on the right-hand side of the screen. Ensure that the value entered reflects the value of the cell product. Otherwise cell product processing may not be optimal. To continue with the next input, tap **ok**.



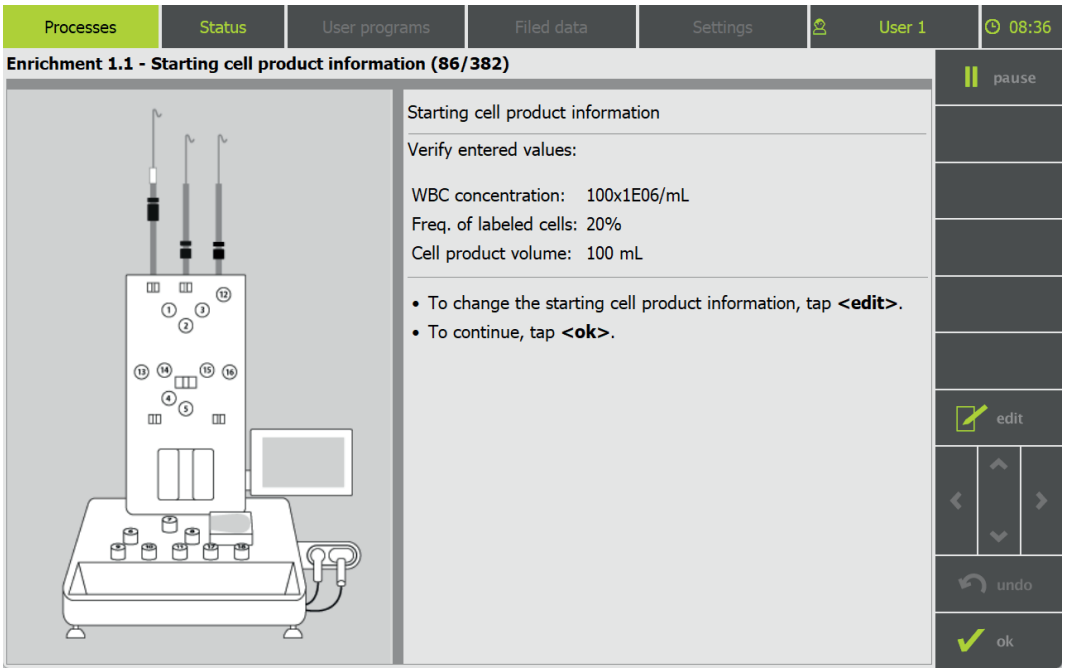
2. Enter the percentage of labeled cells. Ensure that the value entered reflects the value of the cell product. Otherwise cell product processing may not be optimal. To continue with the next input, tap **ok**.



3. Enter the volume of the cell product to be attached to the tubing set. Ensure that the value entered reflects the value of the cell product. Otherwise cell product processing may not be optimal. To continue, tap **ok**.



4. Verify the entered values. To correct an entry, tap **edit** and re-enter the values as described in steps 1 to 3. To continue, tap **ok**.



Checking the calculated cell number

After verifying the entered values, the CliniMACS Plus Software calculates the total number of labeled cells to check whether the separation capacity is sufficient.

If the result is within the system specifications, the screen shows Figure 7.16.

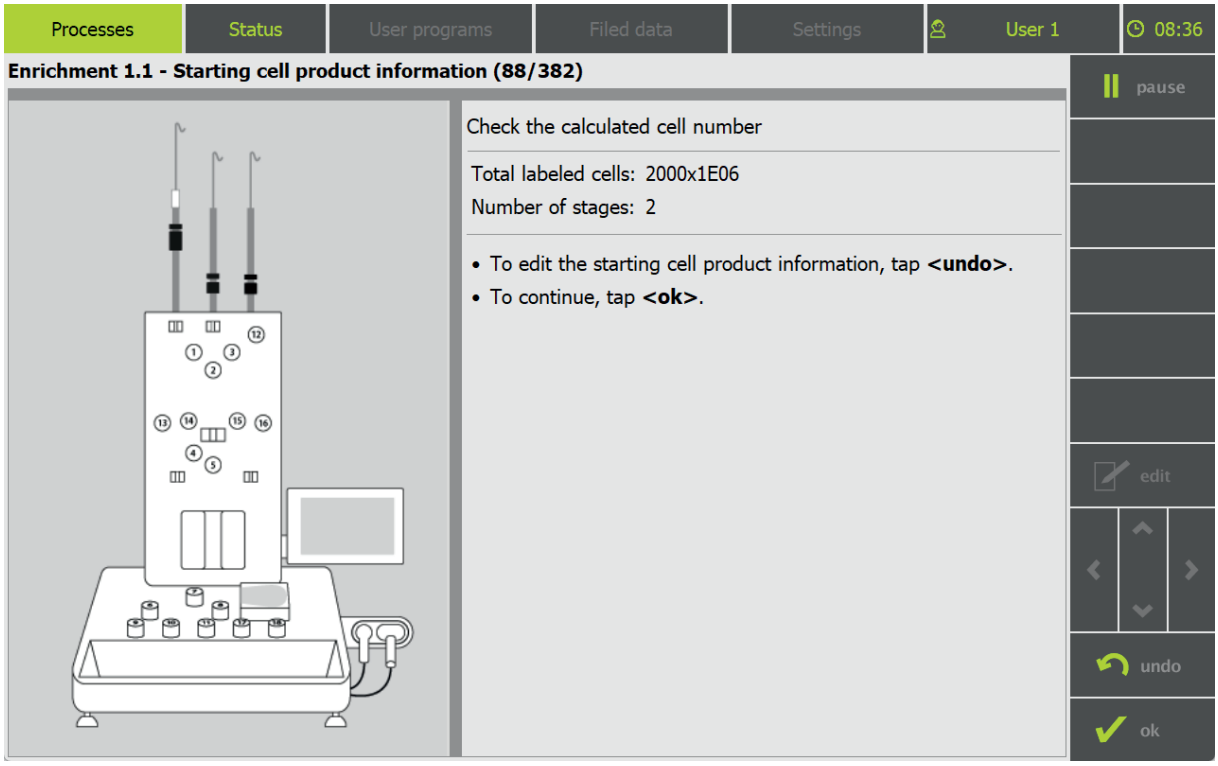


Figure 7.16: Calculation - Labeled cell number is within separation capacity

1. Ensure that the correct cell product parameters have been entered and the calculated number of cells is correct. To edit the entered values and return to Figure 7.15, tap **undo** > **edit**. To continue, tap **ok**.

If the result exceeds the standard separation capacity, the screen shows Figure 7.17.

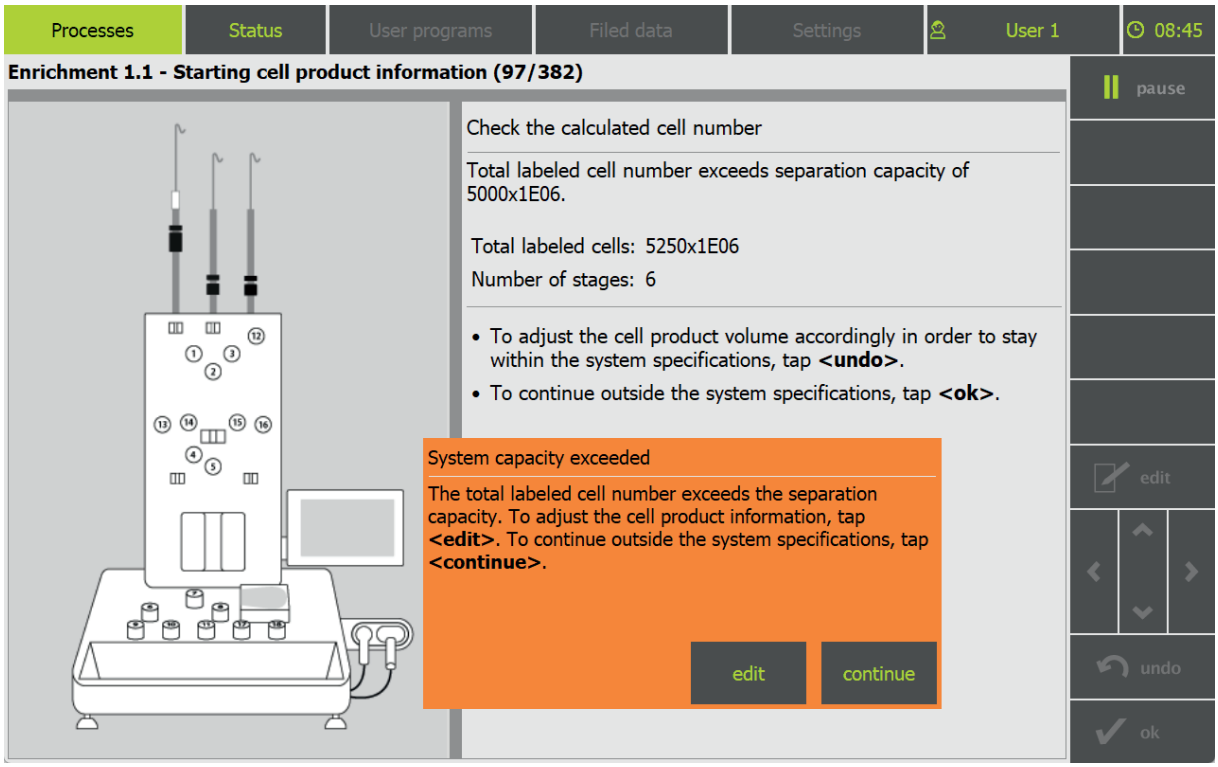


Figure 7.17: Calculation - Labeled cell number exceeds separation capacity

⚠ CAUTION

Risk of insufficient separation performance of labeled cells.

If the application specifications are exceeded, there is the risk of insufficient specific labeling.

- Use the required amount of cells and reagent as described for the respective application.

1. Ensure that the correct cell product parameters have been entered and the calculated number of cells is correct. To edit the entered values and return to Figure 7.15, tap **undo** > **edit**. To continue outside the system specifications, tap **ok** > **continue**.
2. To avoid overloading the system, split the cell product and determine the volume of each portion. To return to the previous screen (see Figure 7.15), tap **undo**.
    a. Enter the cell product parameters for the first aliquot of the cell product and continue with the separation of this portion.
    b. After the separation is finished, start a second separation for the second aliquot using a new tubing set.
    c. To continue, tap **ok**.

Checking the required buffer and bag volumes

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

The standard values are the following:

- Process buffer: 1000 mL
- Cell Collection Bag: 150 mL
- Negative Fraction Bag: 500 mL
- Buffer Waste Bag: 500 mL

If the calculated values are within the system specifications, the screen shows Figure 7.18. Prepare the required amount of buffer. Connect the appropriate bags to the tubing set when instructed.

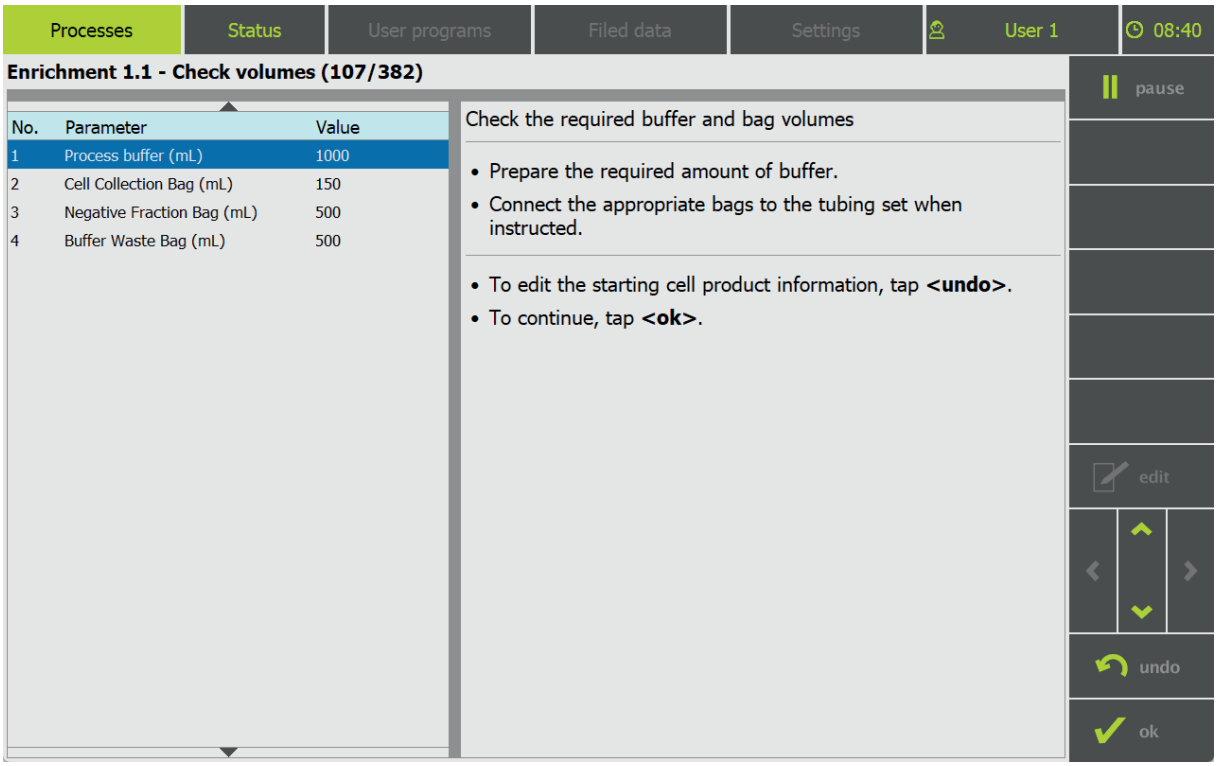


Figure 7.18: Check required buffer and bag volumes

If one of the calculated values exceeds these standard values, Figure 7.19 appears to inform about the amount of liquid that will be required and accumulated during the separation process. If replacement of bags is necessary, replace the bags delivered with the tubing set (capacity approximately 500 mL) by alternative bags of appropriate size.

- The bags originally attached to the tubing set are connected by luer connections.
- Bags that replace the original bags should have a female luer connector.

Standard bags can be connected by a Luer/Spike Interconnector.

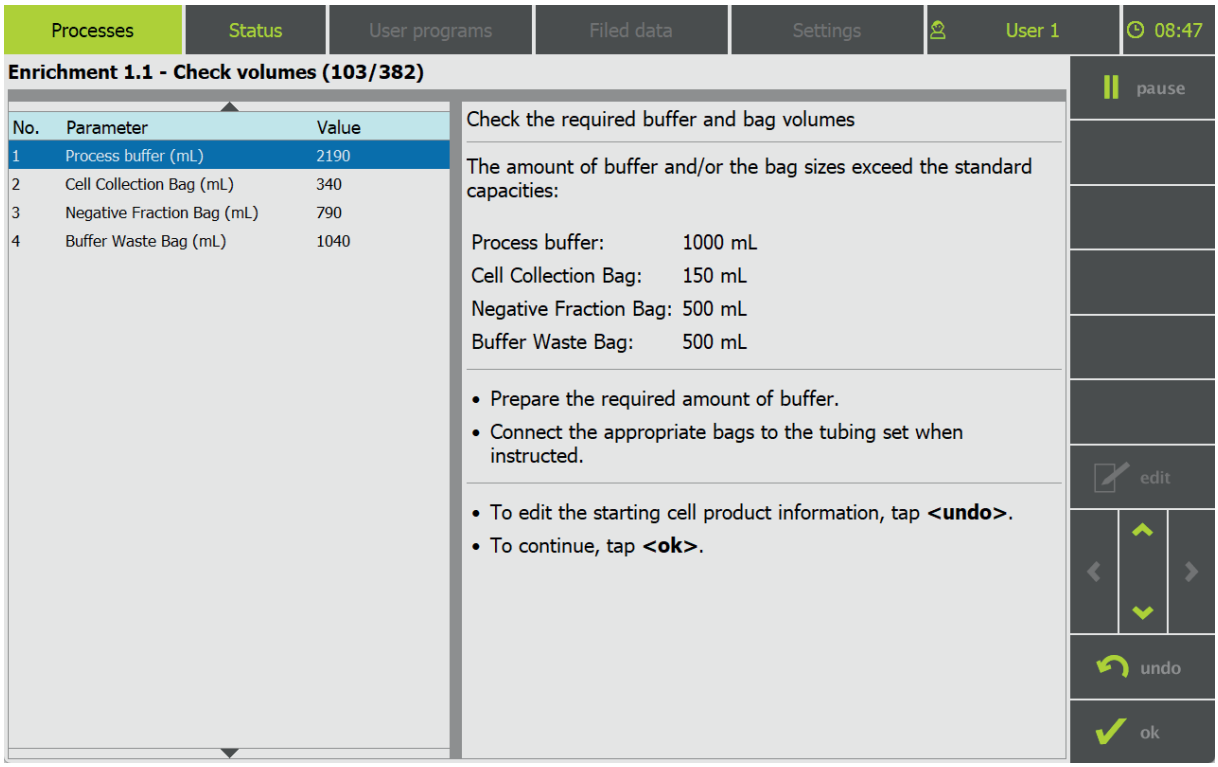


Figure 7.19: Separation capacity exceeded

⚠ WARNING

Risk of personal injury and infection.

Overloading the bag hangers can cause the instrument to tip over. Falling or damaged bags may become contaminated. Contaminated solution may lead to infection if used.

- Do not overload the bag hangers. The carrying capacity of a single bag hanger is 5 kg.

1. Ensure that the requested amount of buffer is available. DO NOT attach more than five liters of buffer on the bag hanger.
2. If necessary, open the tray of the tubing set under sterile conditions and replace the original bags with larger ones. Ensure unrestricted flow to these bags.
3. Attach a Cell Collection Bag of sufficient size to the free luer connector. If not specified in Figure 7.19, the maximum elution volume of the target cell fraction is 150 mL. Ensure that unrestricted flow to the Cell Collection Bag is possible.

IMPORTANT:

- Ensure that the required amount of buffer is available and the volume of the bags listed in Figure 7.19 is sufficient. Otherwise the performance of the separation will be compromised.
- Any modifications of the CliniMACS Tubing Sets should be performed under sterile conditions, for example, in the laminar flow hood.
- For further analysis or cell processing, note that the volumes listed in Figure 7.19 are not the exact volumes but volumes within the safety requirements.

4. Ensure that the Luer lock connections on the columns are undamaged. DO NOT tighten or manipulate the Luer lock connections on the columns.

To continue, tap **ok**.

Entering reagent parameters (optional)

1. To choose whether to enter reagent parameters or to continue without any entry, tap **ok**. A pop-up window appears.
2. Depending on the number of reagent vials used, tap **1 vial** or **2 vials**.
3. Use the barcode reader to scan the barcode label on the reagent vial.
4. Alternatively, enter the part number (P/N), LOT number, and use-by date manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
5. To continue without any entry, tap **continue**.

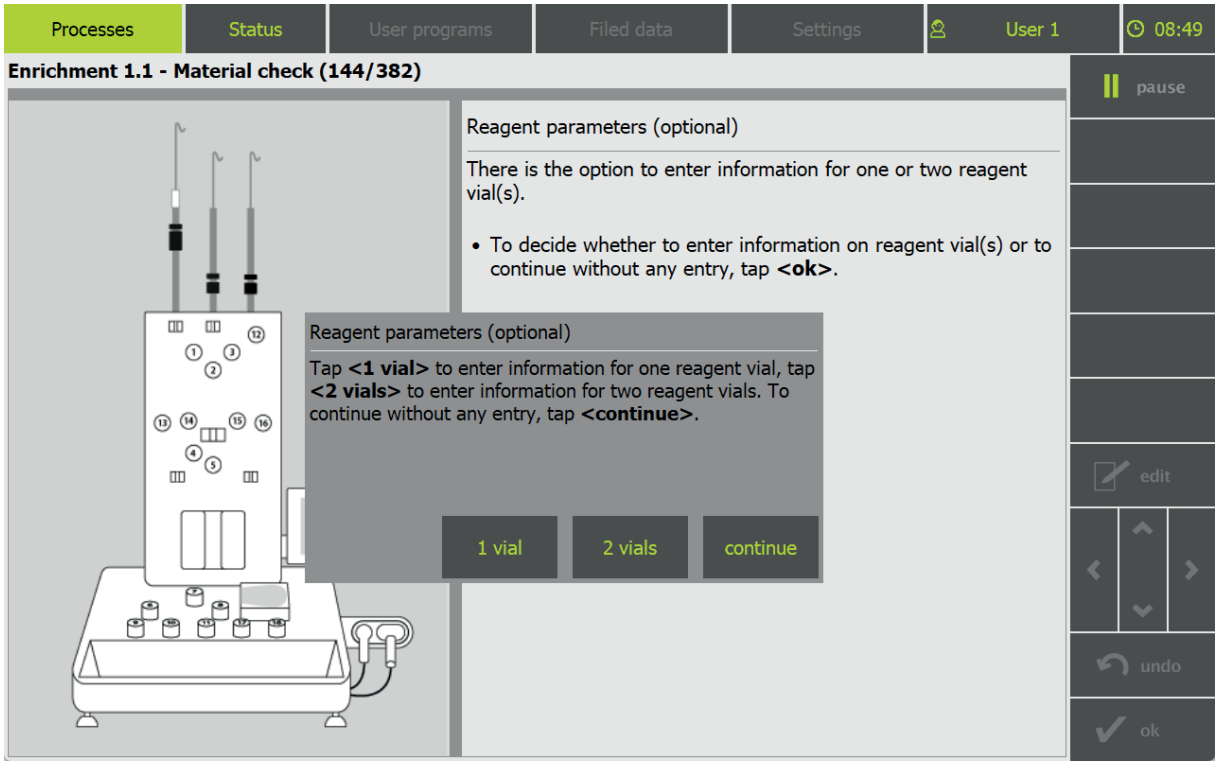


Figure 7.20: Choice of reagent parameter entry

Entering process buffer parameters (optional)

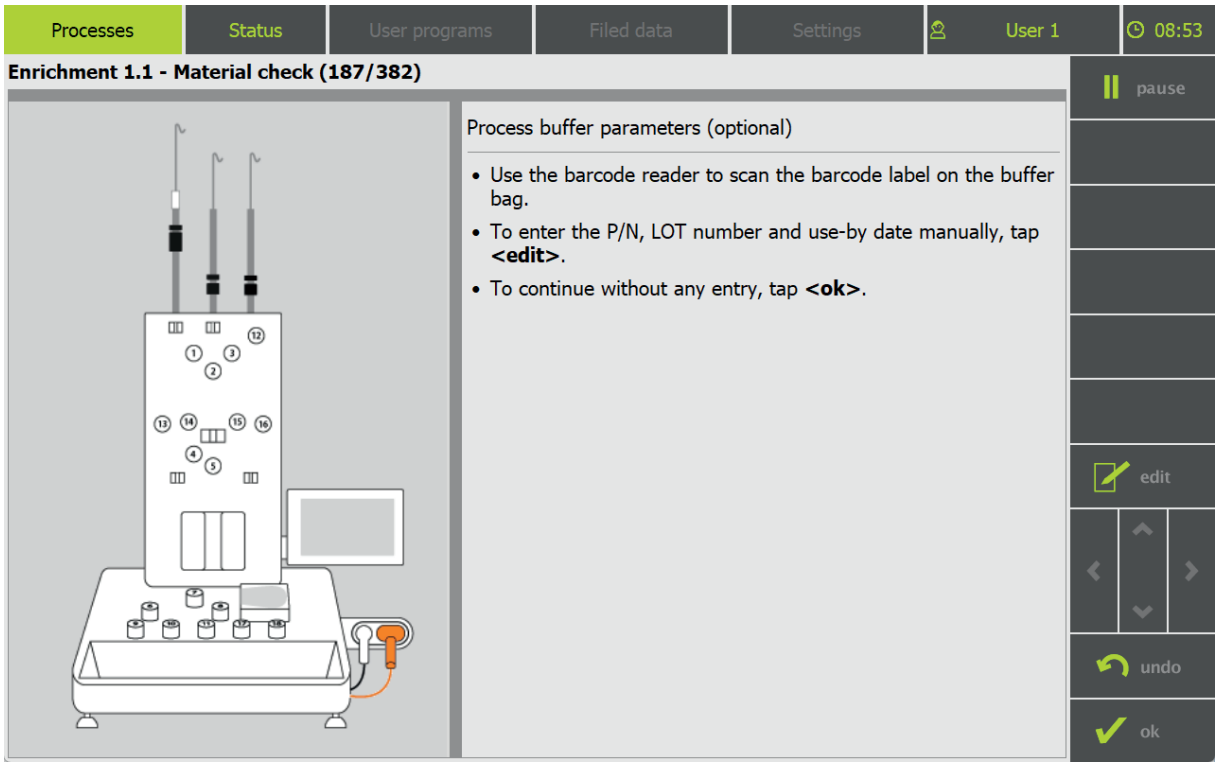


Figure 7.21: Entry of process buffer parameters

If more than one buffer bag is used, repeat the following steps for each buffer bag.

1. To enter the process buffer parameters, scan the barcode label on the buffer bag using the barcode reader.
2. Alternatively, enter the parameters manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
3. To continue without any entry, tap **ok**.

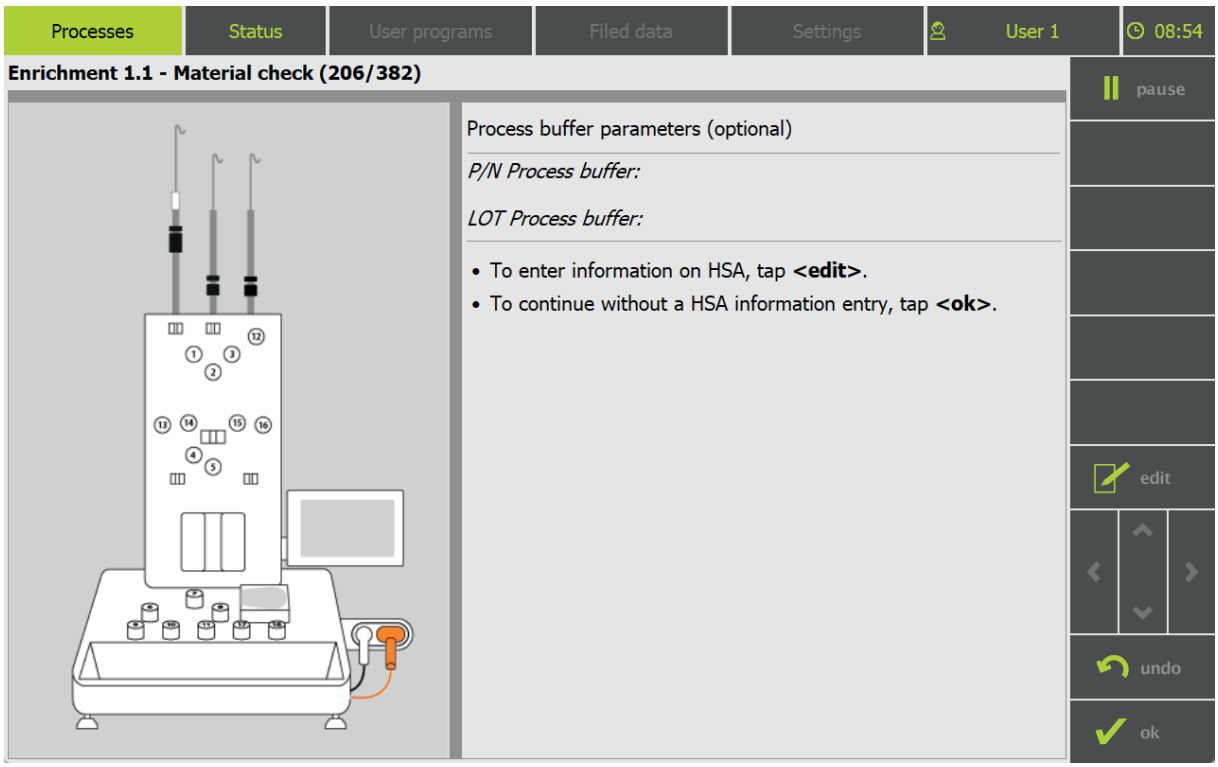


Figure 7.22: Entry of HSA information for the process buffer

Additionally, the user can enter HSA information for the process buffer.

1. To enter HSA information, tap **edit**. To continue without any entry, tap **ok**.

Entering information for additional materials (optional)

If multiple additional materials are used, repeat the following steps for each entry.

1. To enter the item name and information of the additional material, tap **edit**. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
2. To continue without any entry, tap **ok**.

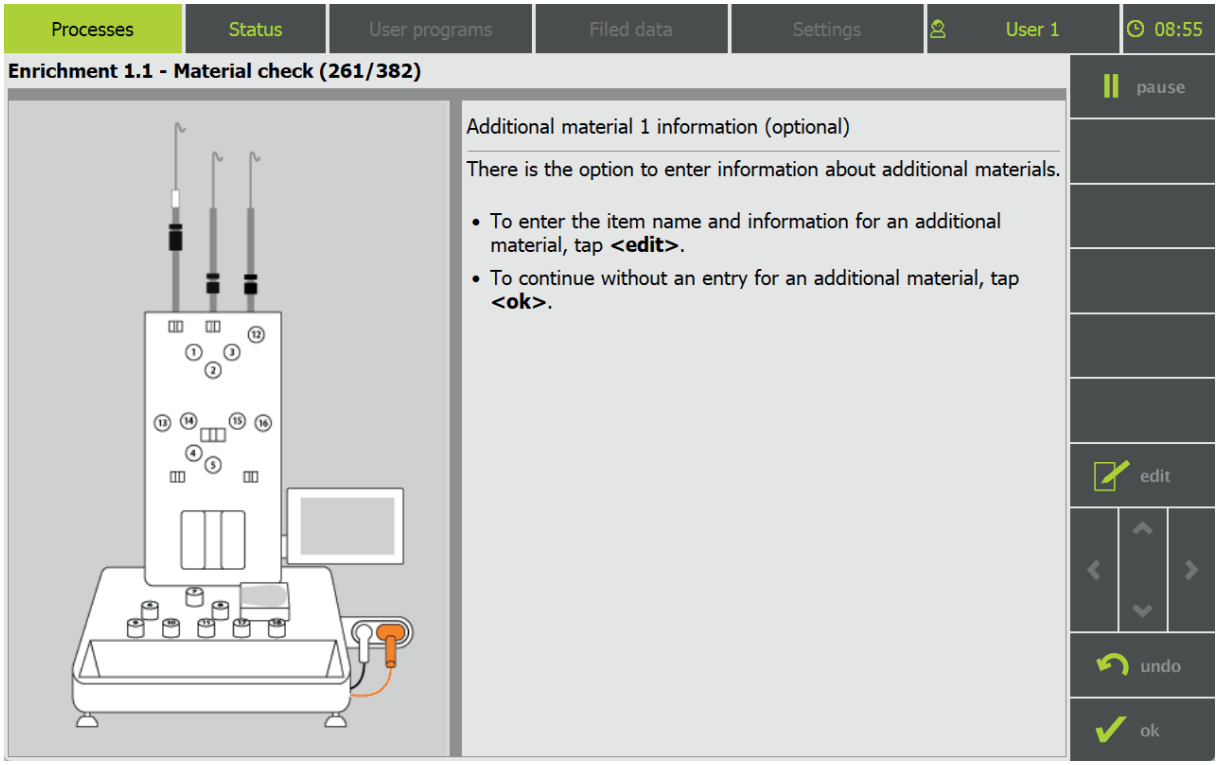


Figure 7.23: Entry of additional material information

Continue with the installation of the tubing set (see section 'Step 3: Installation of CliniMACS Tubing Sets').

7.2.5 Enrichment 3.2

The separation program Enrichment 3.2 must only be used in combination with the CliniMACS Tubing Set (REF 161-01) or the CliniMACS Tubing Set LS (REF 162-01). Ensure that the tubing set is correct before the installation on the instrument.

Screen prompts and diagrams serving as procedure guides will appear on the screen.

- Perform and check each step according to the manual and software instructions before proceeding to the next step.
- To correct a mistake during data input, tap **undo** (refer to the CliniMACS Plus Software user manual for more details on the toolbar).

Choosing of separation program Enrichment 3.2

1. To select Enrichment 3.2 from the list (see Figure 7.24), highlight the name of the program by using the navigation buttons ⌄ and ⌃ or by tapping on the program name.
2. To continue with the selected program, tap **run**.

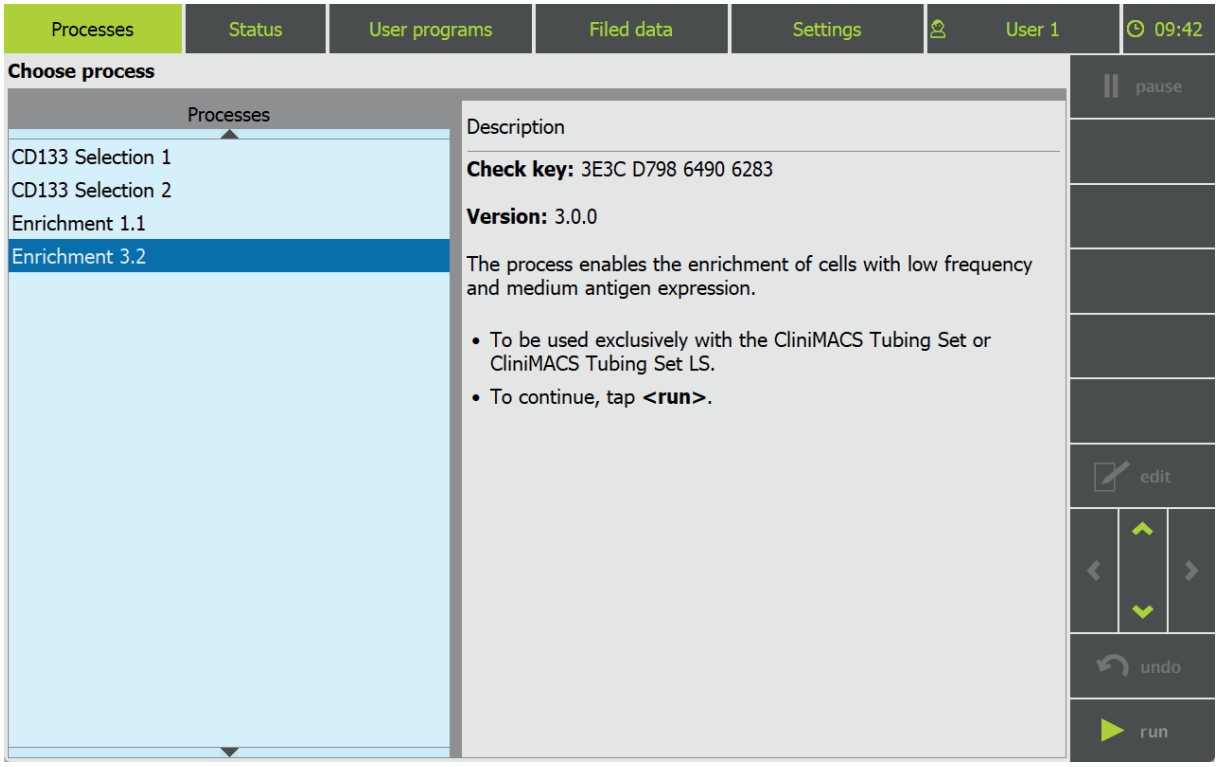


Figure 7.24: Choice of separation program Enrichment 3.2

Entering Run ID (optional)

The user can enter a Run ID for the process. To enter a Run ID, tap **edit**. To continue without any entry, tap **ok**.

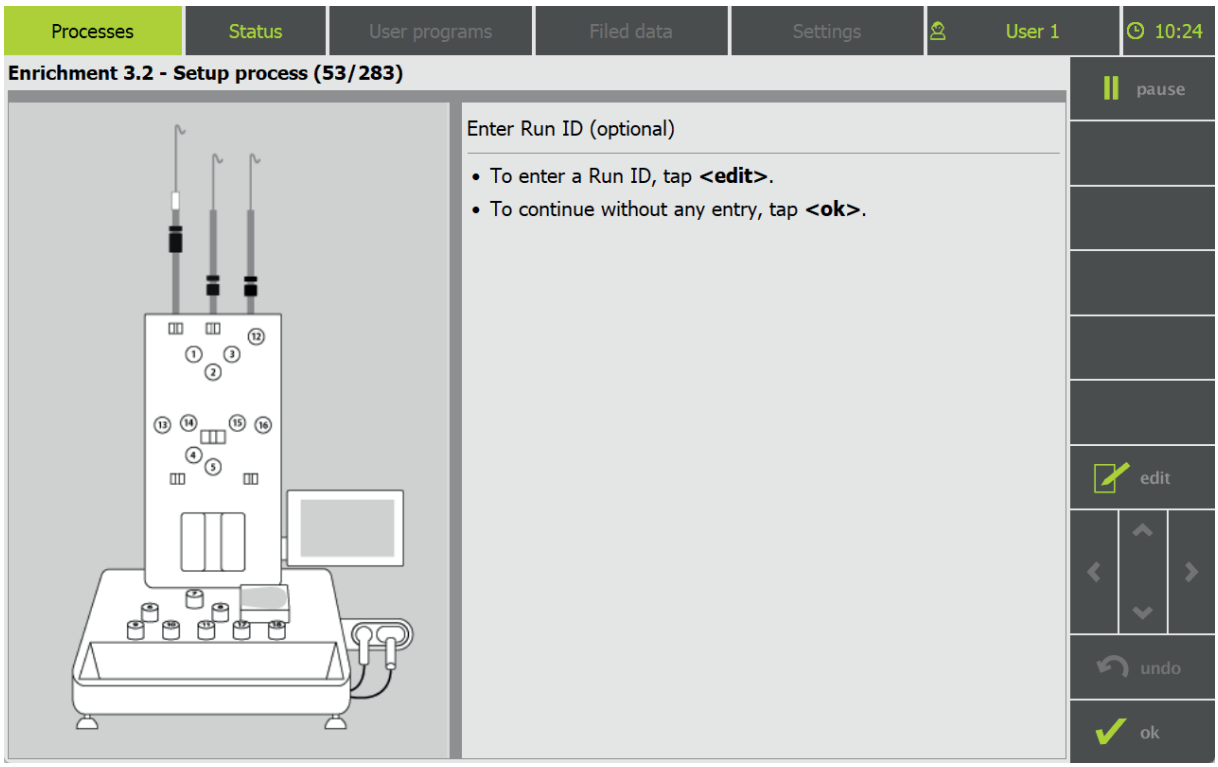


Figure 7.25: Entry of Run ID

Entering tubing set parameters

1. Use the barcode reader to scan the barcode label on the tubing set packaging.
2. Alternatively, enter the part number (P/N), LOT number, and use-by date manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.

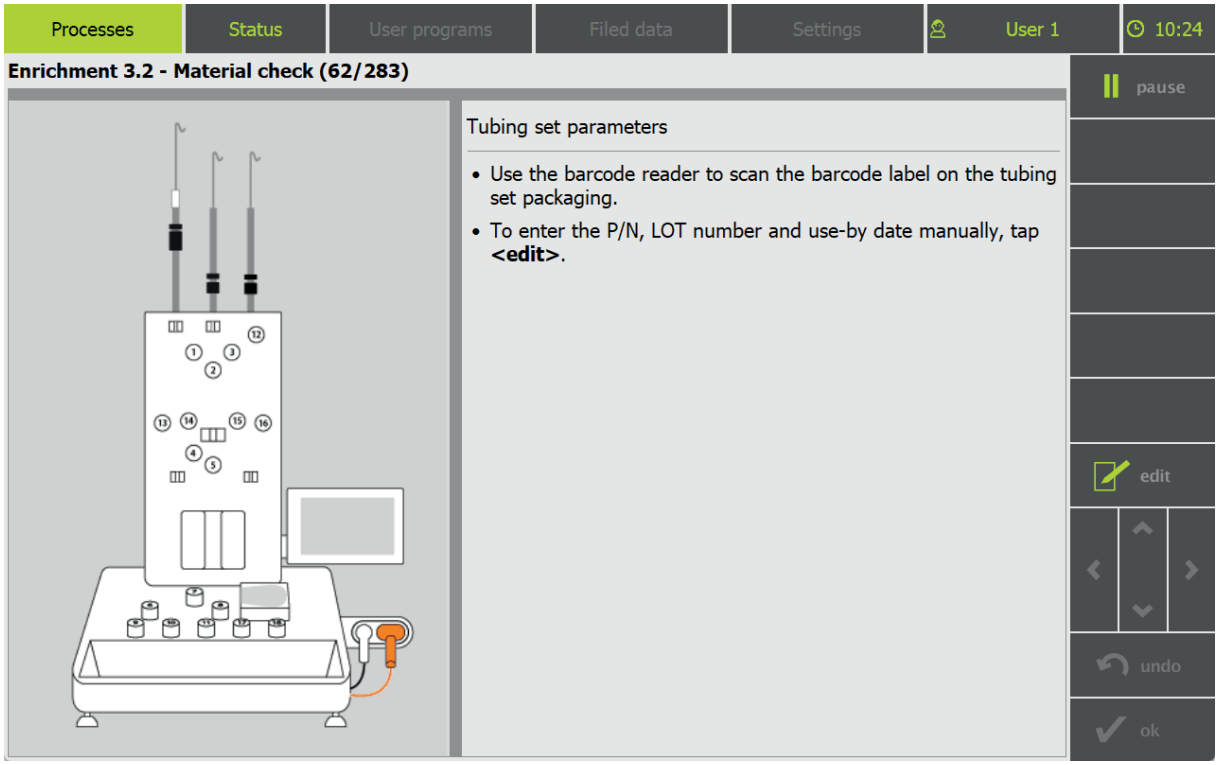


Figure 7.26: Entry of tubing set parameters

Entering starting cell product information (optional)

1. Use the barcode reader to scan the barcode label on the cell product.
2. Alternatively, enter the information manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm each entry, tap **ok**.

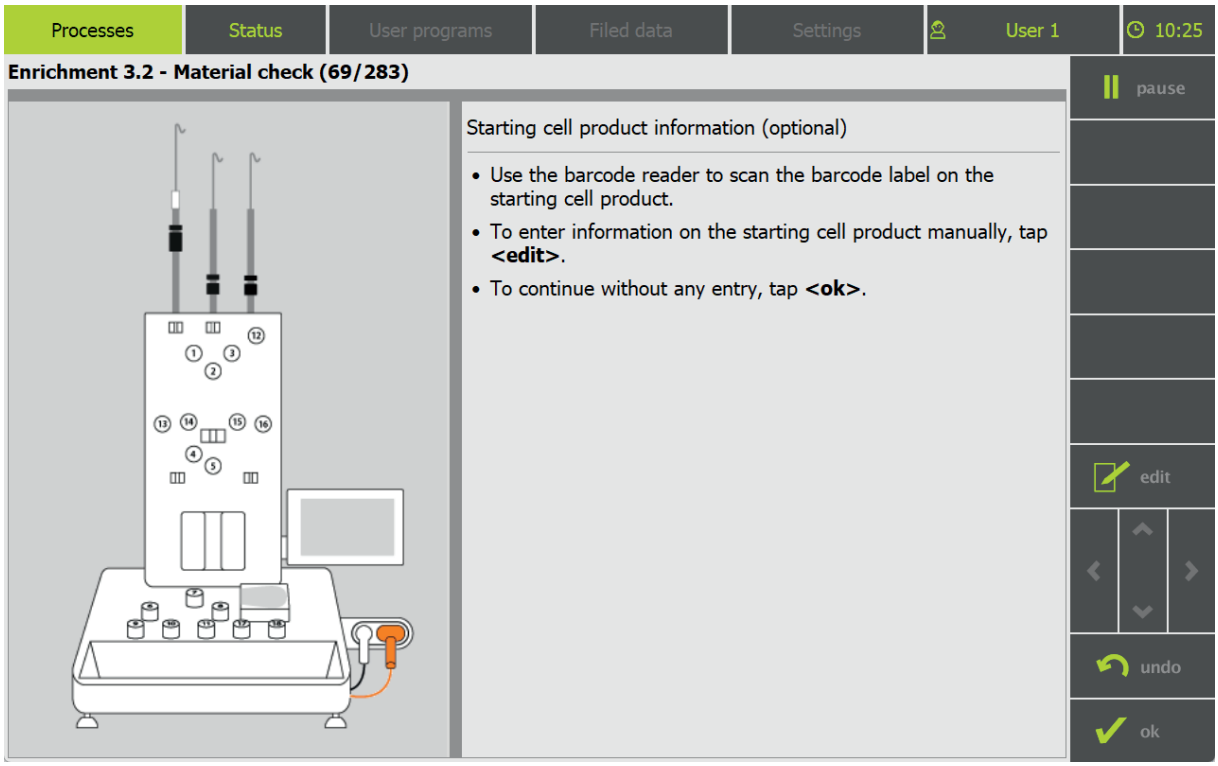


Figure 7.27: Entry of starting cell product information

Entering reagent parameters (optional)

1. To choose whether to enter reagent parameters or to continue without any entry, tap **ok**. A pop-up window appears.
2. Depending on the number of reagent vials used, tap **1 vial** or **2 vials**.
3. Use the barcode reader to scan the barcode label on the reagent vial.
4. Alternatively, enter the part number (P/N), LOT number, and use-by date manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
5. To continue without any entry, tap **continue**.

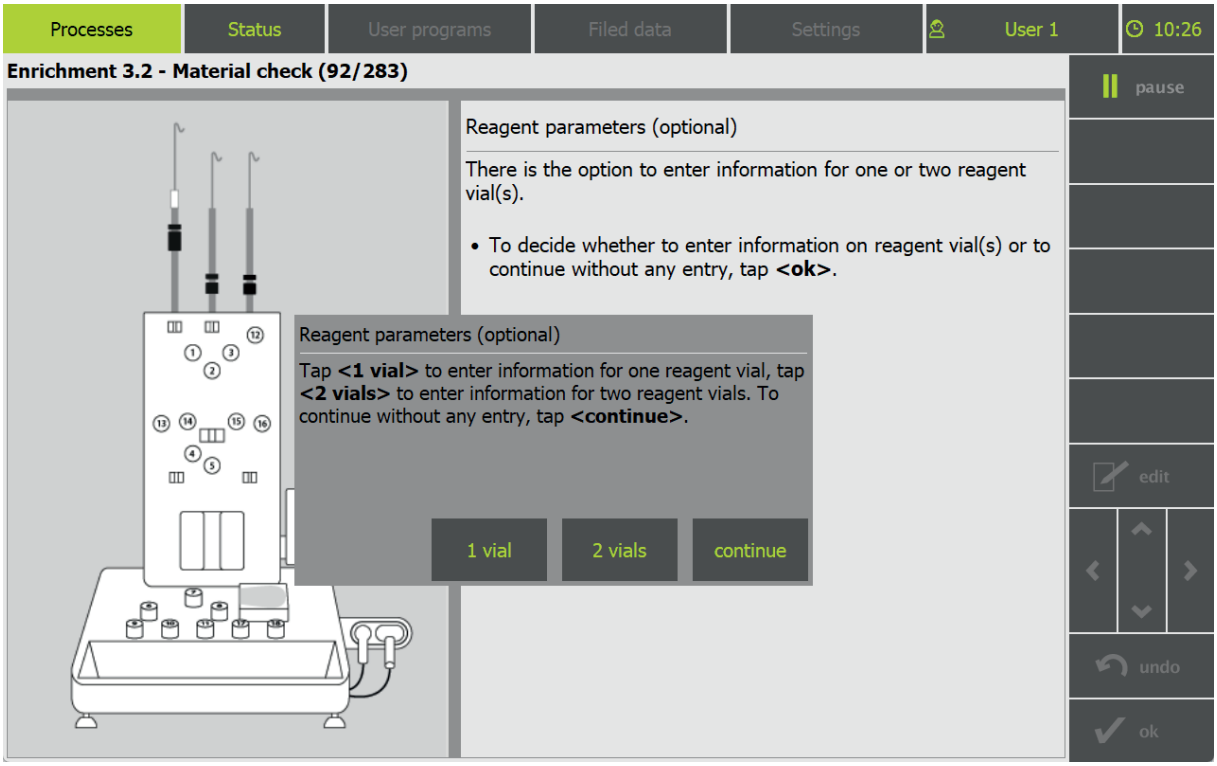


Figure 7.28: Choice of reagent parameter entry

Entering process buffer parameters (optional)

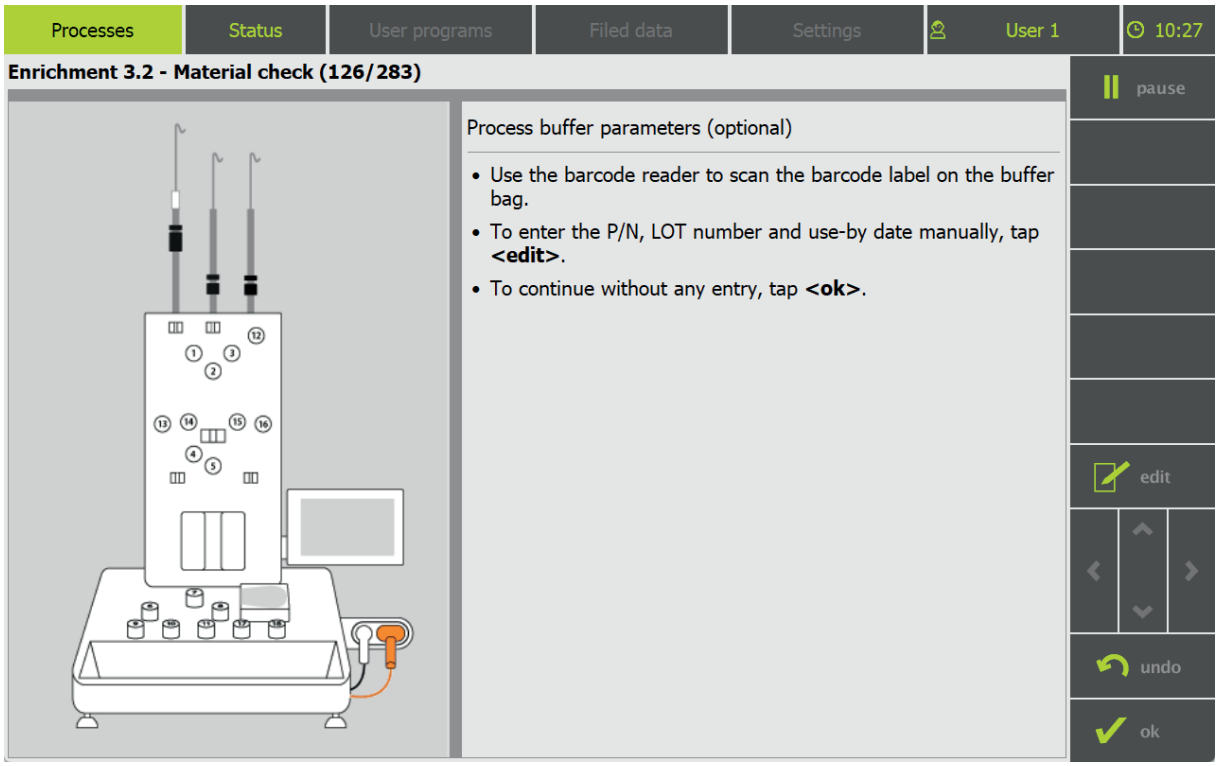


Figure 7.29: Entry of process buffer parameters

1. To enter the process buffer parameters, scan the barcode label on the buffer bag using the barcode reader.
2. Alternatively, enter the parameters manually by tapping **edit** and using the keyboard shown on the screen. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
3. To continue without any entry, tap **ok**.

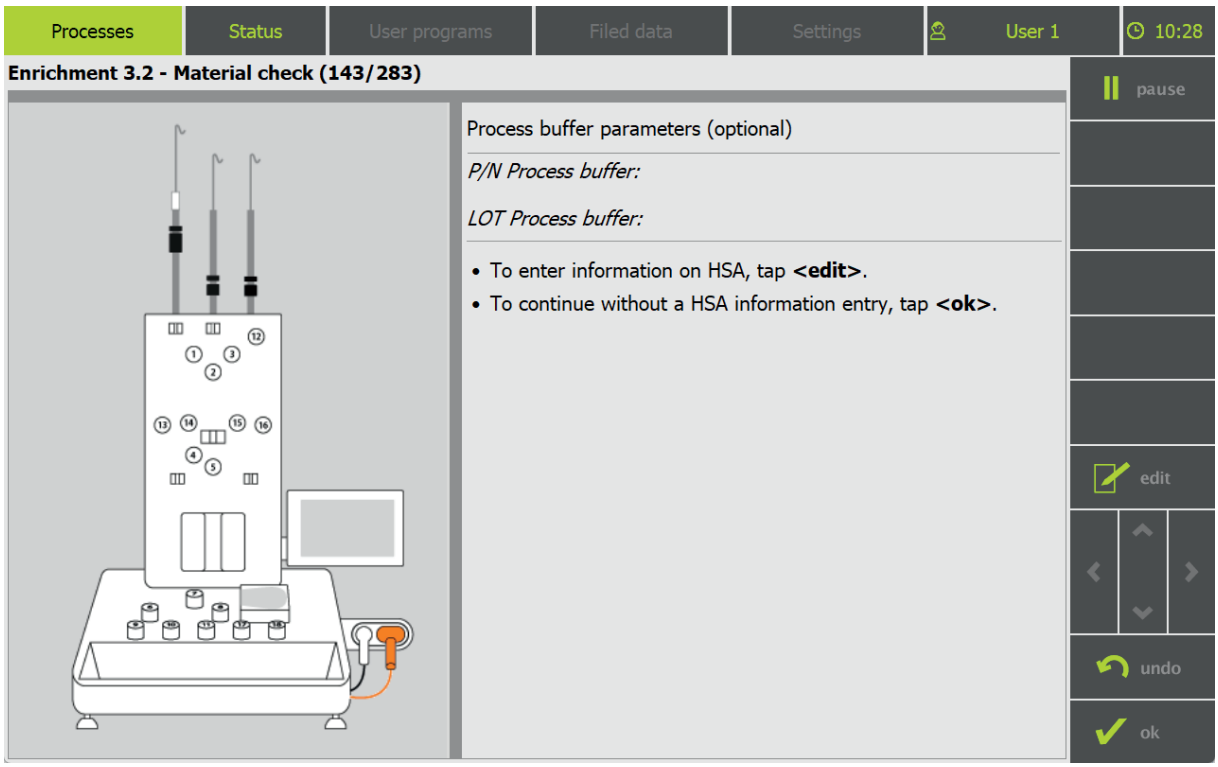


Figure 7.30: Entry of HSA information for the process buffer

Additionally, the user can enter HSA information for the process buffer.

1. To enter HSA information, tap **edit**. To continue without any entry, tap **ok**.

Entering information for additional materials (optional)

If multiple additional materials are used, repeat the following steps for each entry.

1. To enter the item name and information of the additional material, tap **edit**. An input field appears for each entry. To confirm the entry in the input field, tap **ok**.
2. To continue without any entry, tap **ok**.

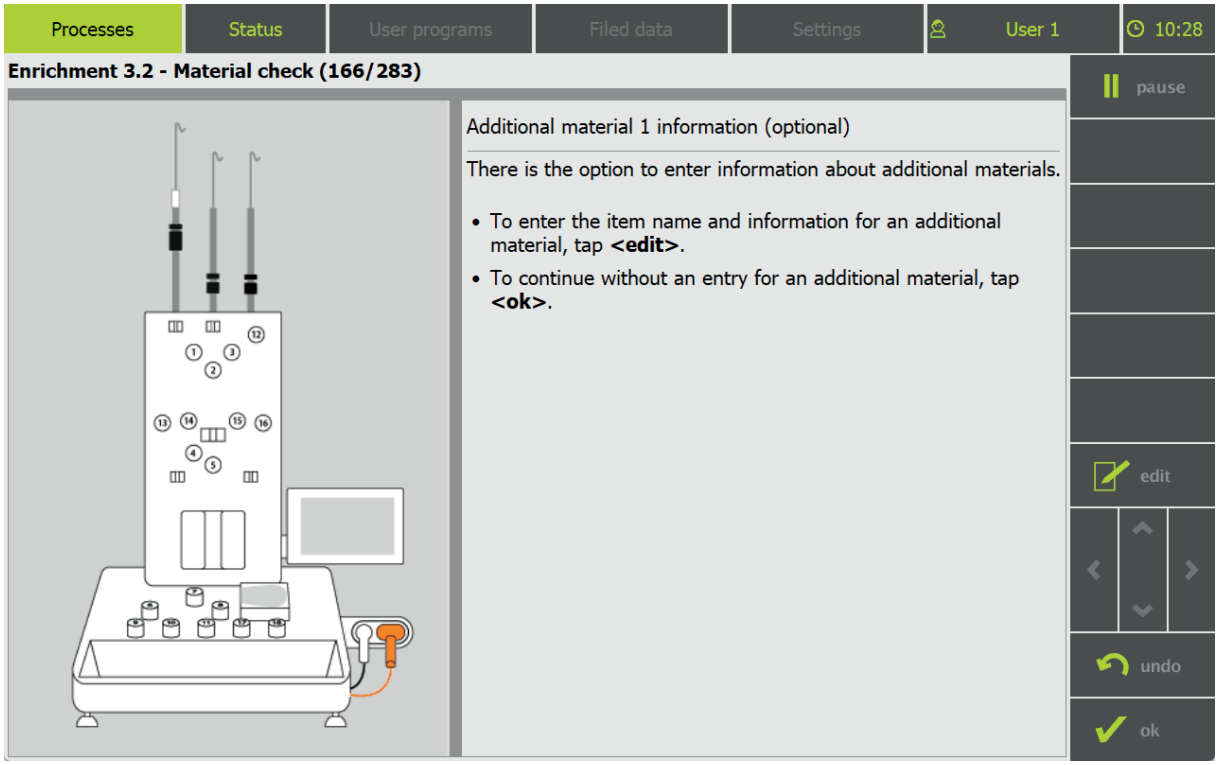


Figure 7.31: Entry of additional material information

Continue with the installation of the tubing set (see section 'Step 3: Installation of CliniMACS Tubing Sets').

7.3 Step 3: Installation of CliniMACS Tubing Sets

This chapter contains instructions and precautions for the installation of the CliniMACS tubing sets. Depending on the selected separation program, the program name shown in the software may differ from that shown in the screenshots in this user manual.

7.3.1 CliniMACS Tubing Set and CliniMACS Tubing Set LS

The CliniMACS Tubing Set and the CliniMACS Tubing Set LS are each provided in sealed, sterilized packages. Each tubing set is for single use and contains preassembled tubing and columns for one separation, providing a sterile fluid path (see Figure 7.32).

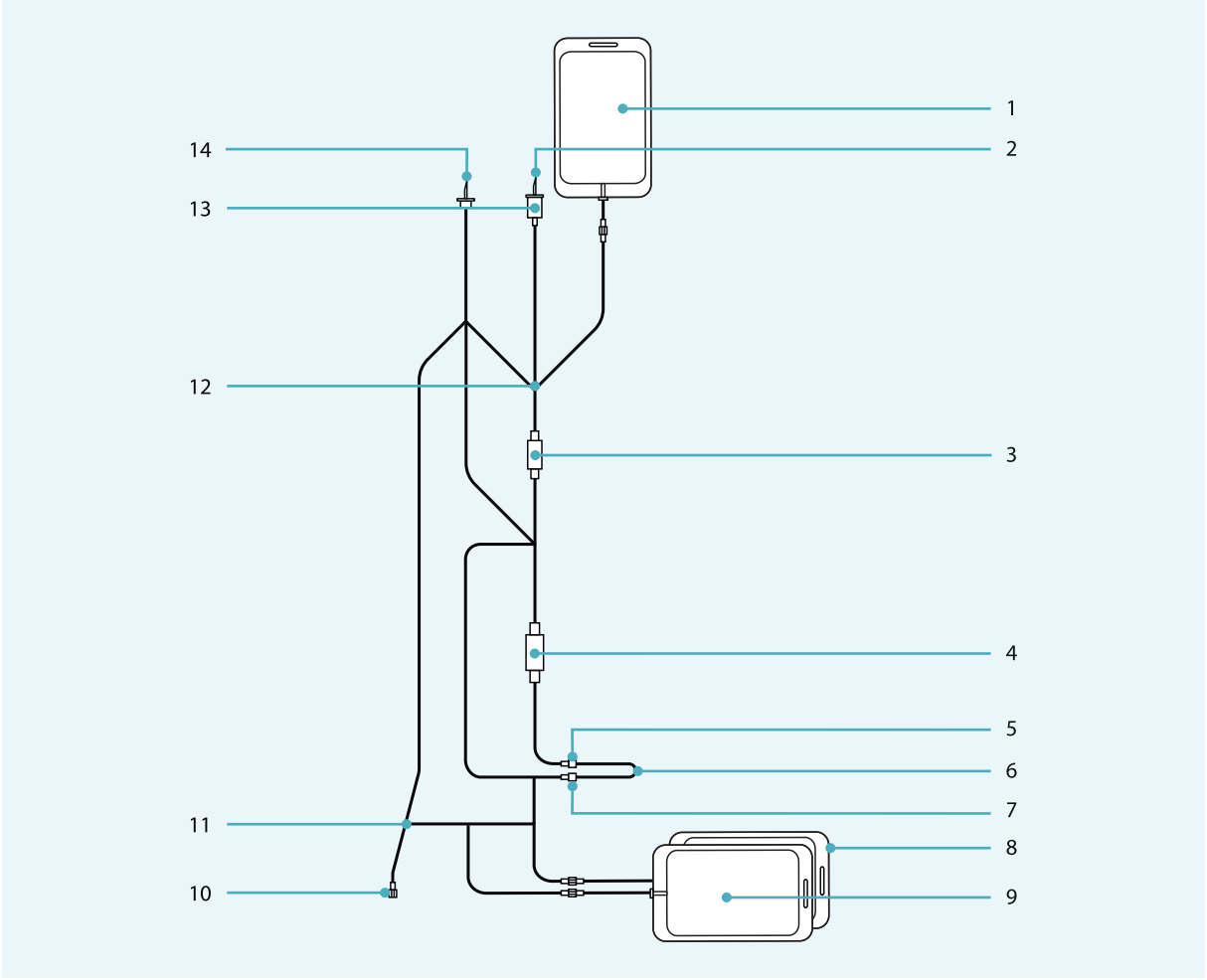


Figure 7.32: General construction of a CliniMACS Tubing Set (for example, CliniMACS Tubing Set)

1 Priming Waste Bag
2 Bubble trap spike
3 Pre-column
4 Separation column
5 Upper retaining ring
6 Pump tubing
7 Lower retaining ring
8 Negative Fraction Bag
9 Buffer Waste Bag
10 Luer connector
11 T-fitting
12 4-way fitting
13 Bubble trap
14 Buffer spike

Handling of preconnected process buffer and Cell Preparation Bag

After Step 2 is complete, the screen shows Figure 7.33. The instruction is shown on the right, and a diagram corresponding to the instruction on the left-hand side of the screen. The orange blinking features on the screen indicate the areas of attention.

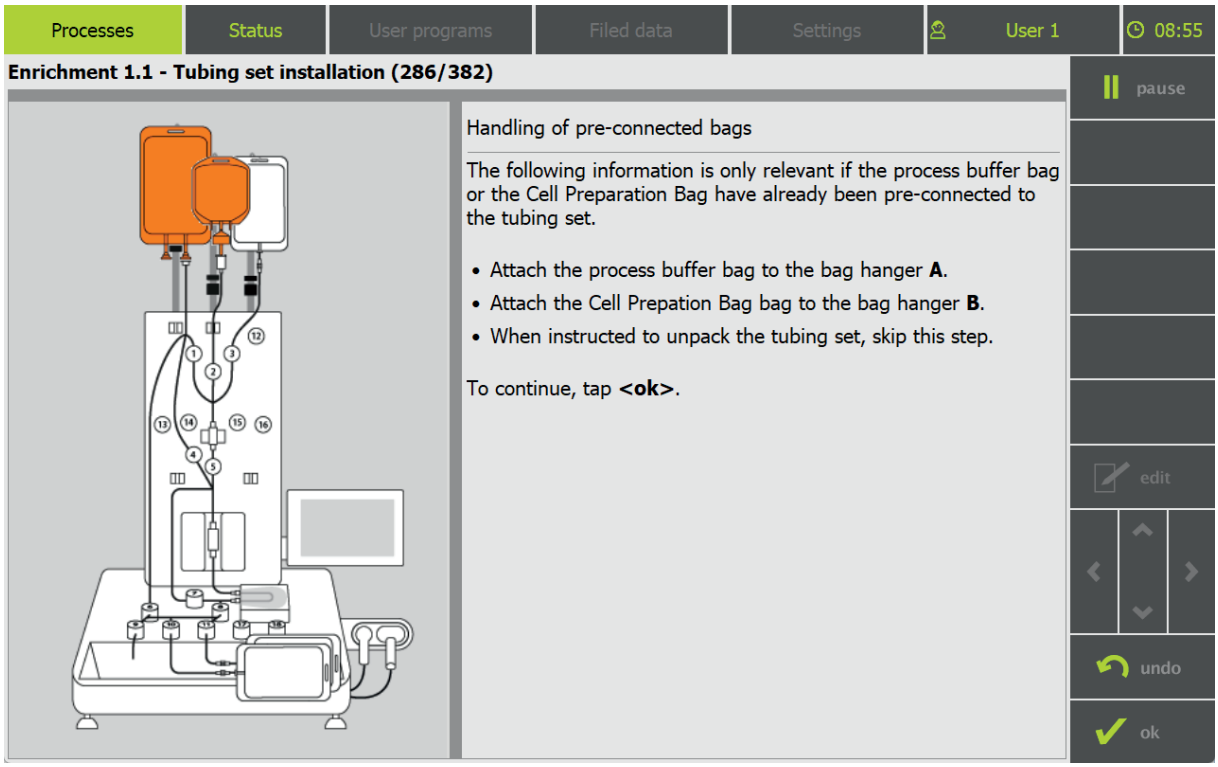


Figure 7.33: Handling of preconnected process buffer and Cell Preparation Bag

1. If the process buffer bag(s) or the Cell Preparation Bag are already connected to the tubing set, follow the instructions on the screen.
    a. Ensure that the clamps beneath the process buffer bag(s) and the Cell Preparation Bag are closed.
    b. Continue by tapping ok.
2. If the the bags are not preconnected to the tubing set, skip this step by tapping ok.

Unpacking the tubing set, connecting the Cell Collection Bag, attaching the Priming Waste Bag, and inserting the pre-column

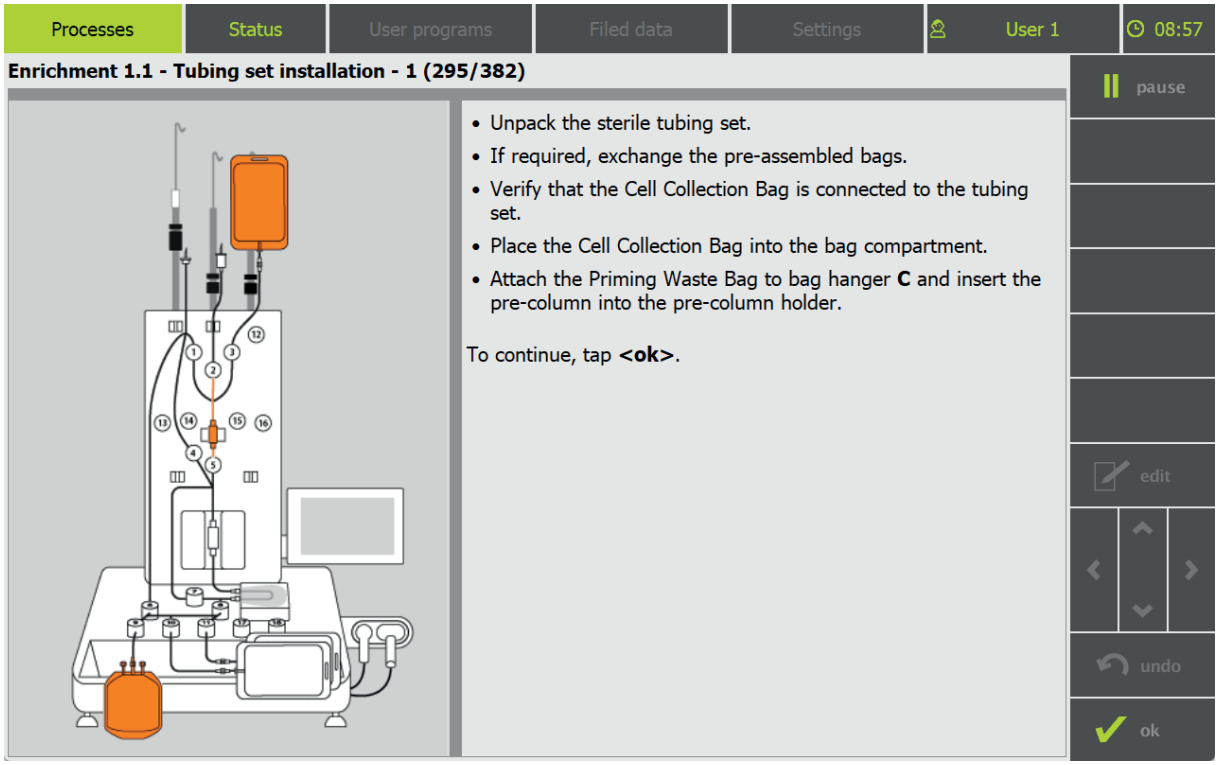


Figure 7.34: Unpacking of the tubing set, connection of the Cell Collection Bag, attachment of the Priming Waste Bag, and insertion of the pre-column

⚠ WARNING

Risk of contamination.

Damaged packaging indicates that the tubing set may no longer be sterile and therefore must not be used.

- Carefully inspect the packaging for damage, puncture or tears.
- Use the tubing set only if package is undamaged and sealed.

⚠ WARNING

Risk of injury.

If the separation column is inserted or removed while the magnet unit is switched on, there is the risk of personal injury.

- Only insert or remove the separation column when the magnet unit is switched off.

Unpacking tubing set

1. Unpack the sterile tubing set under sterile conditions (if not done in a clean room, for example, in a laminar flow hood).

2. CliniMACS Tubing Set LS: Before installing the tubing set, remove the pinch clamps used for the secure packaging of the columns. The pinch clamps are labeled with "Remove before assembly to CliniMACS Plus Instrument".
3. Ensure that the Luer lock connections to the bags are closed tightly. DO NOT tighten or manipulate the Luer lock connections on the columns.
4. If necessary, exchange the preassembled bags.

Connection of the Cell Collection Bag to the tubing set

1. Note the weight of the empty Cell Collection Bag(s).
2. In an aseptic environment, remove caps and attach the sterile Cell Collection Bag equipped with a Luer/Spike Interconnector to the tubing set before installing the tubing set onto the CliniMACS Plus Instrument.
3. Ensure that the original tubing of the Cell Collection Bag is either clamped or sealed off.
4. If more than one Cell Collection Bag is necessary for a separation, connect the bags using a plasma transfer set. Make sure that all connections are closed tightly.
5. Ensure that an unrestricted flow to the Cell Collection Bag is possible.
6. If the instrument is used in a clean room, continue with the installation of the tubing set according to the procedure in the following sections. If the instrument is not used in a clean room, install the tubing under sterile conditions as described in section 'Alternative installation of CliniMACS Tubing Sets'.

Attaching Priming Waste Bag and inserting pre-column

1. Ensure that the Cell Collection Bag is connected to the tubing set.
2. Attach the Priming Waste Bag to bag hanger C (see Figure 7.35).
3. Adjust the height of the bag hanger. Raise or lower the bag hanger to accommodate the height to the size of the Priming Waste Bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow, and that it is low enough to avoid the tubing or connections being stretched.
4. Place the pre-column into the pre-column holder (see Figure 7.35).

To continue, tap **ok**.

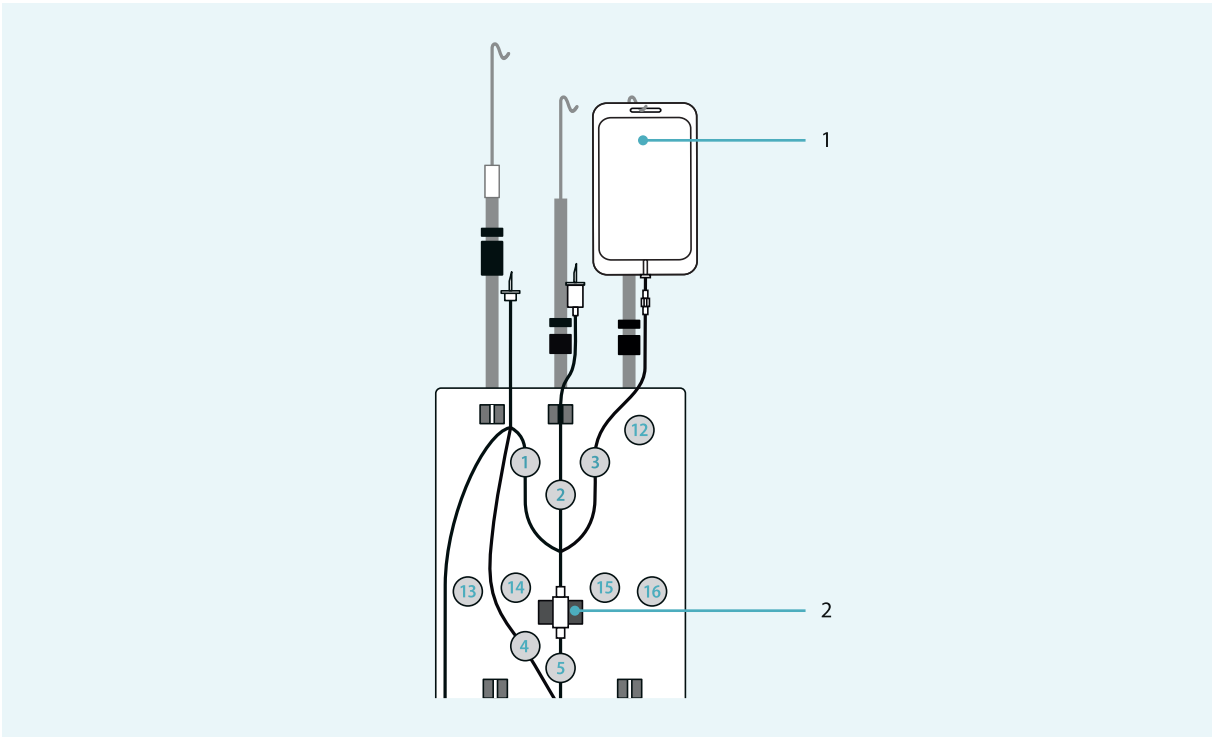


Figure 7.35: Attach Priming Waste Bag to bag hanger C

1 Priming Waste Bag

2 Pre-column holder

Inserting the separation column and loading valve 5

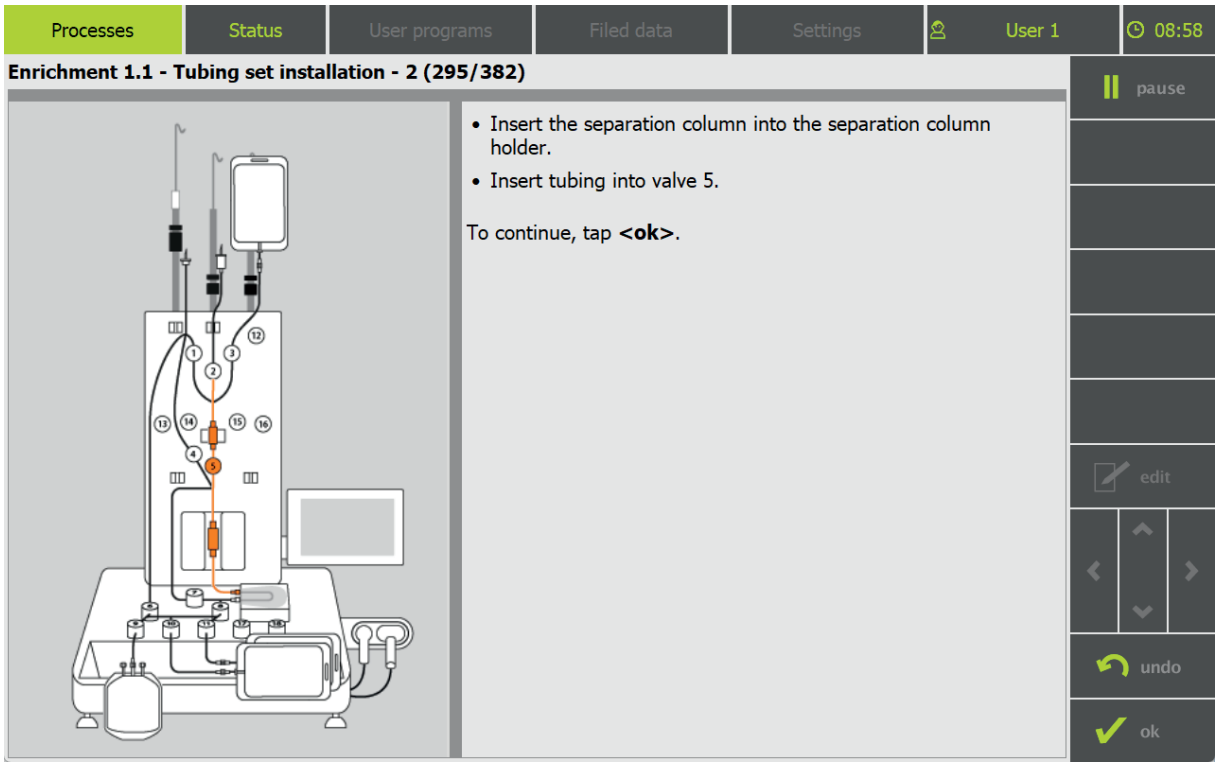


Figure 7.36: Insertion of separation column and loading of valve 5

⚠ WARNING

Risk of injury.

If the separation column is inserted or removed while the magnet unit is switched on, there is the risk of personal injury.

- Only insert or remove the separation column when the magnet unit is switched off.

The valve indicated in orange on the screen will open automatically to facilitate the tubing insertion.

1. Insert the separation column into the separation column holder (see Figure 7.37).
2. Insert the tubing into valve 5 (see Figure 7.38).

IMPORTANT:

- 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 that the tubing is correctly seated.
- Only insert the tubing set into open valves (when button is pushed inwards). If the tubing is inserted into a closed valve, the tubing will not fit correctly.
- To adjust the tubing after a valve closes, press the valve button to open the valve before pulling the tubing (see Figure 7.38).

To return to the previous screen (see Figure 7.34), tap undo. To continue, tap ok.

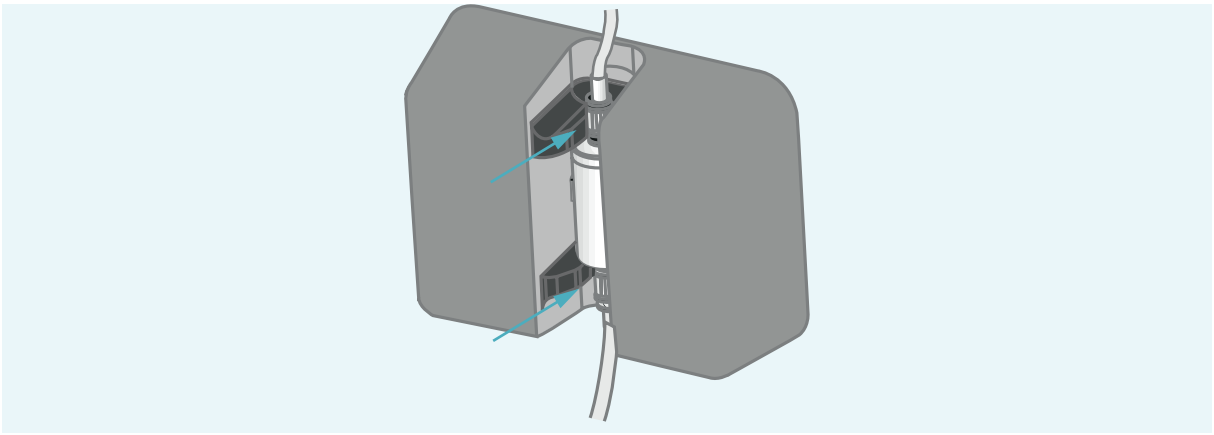


Figure 7.37: Separation column in separation column holder

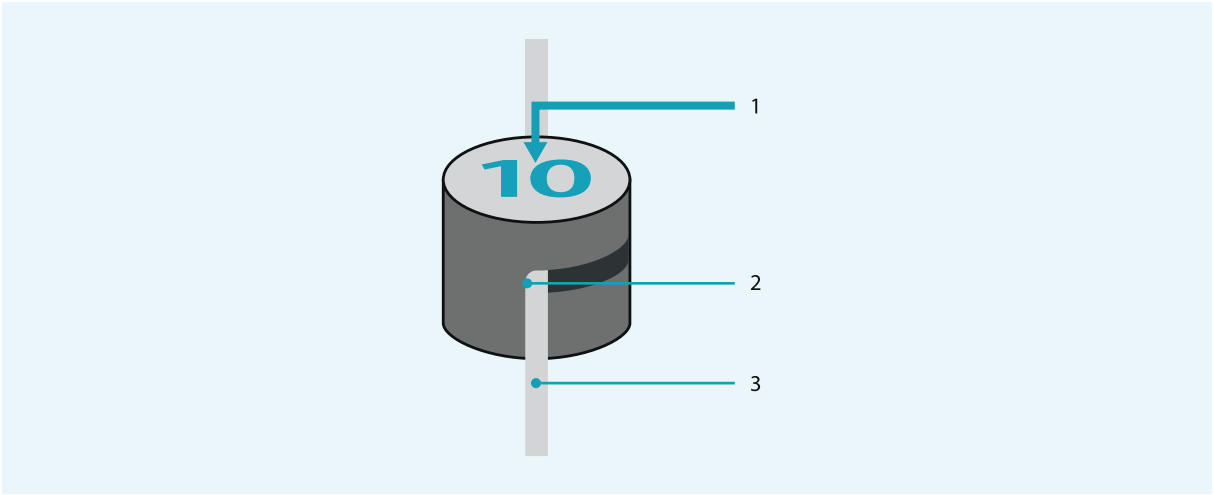


Figure 7.38: Correct insertion of the tubing into a valve

1 Press button to open valve
2 Inner end of slot
3 Tubing

Loading valves 1, 2, 3, and 4

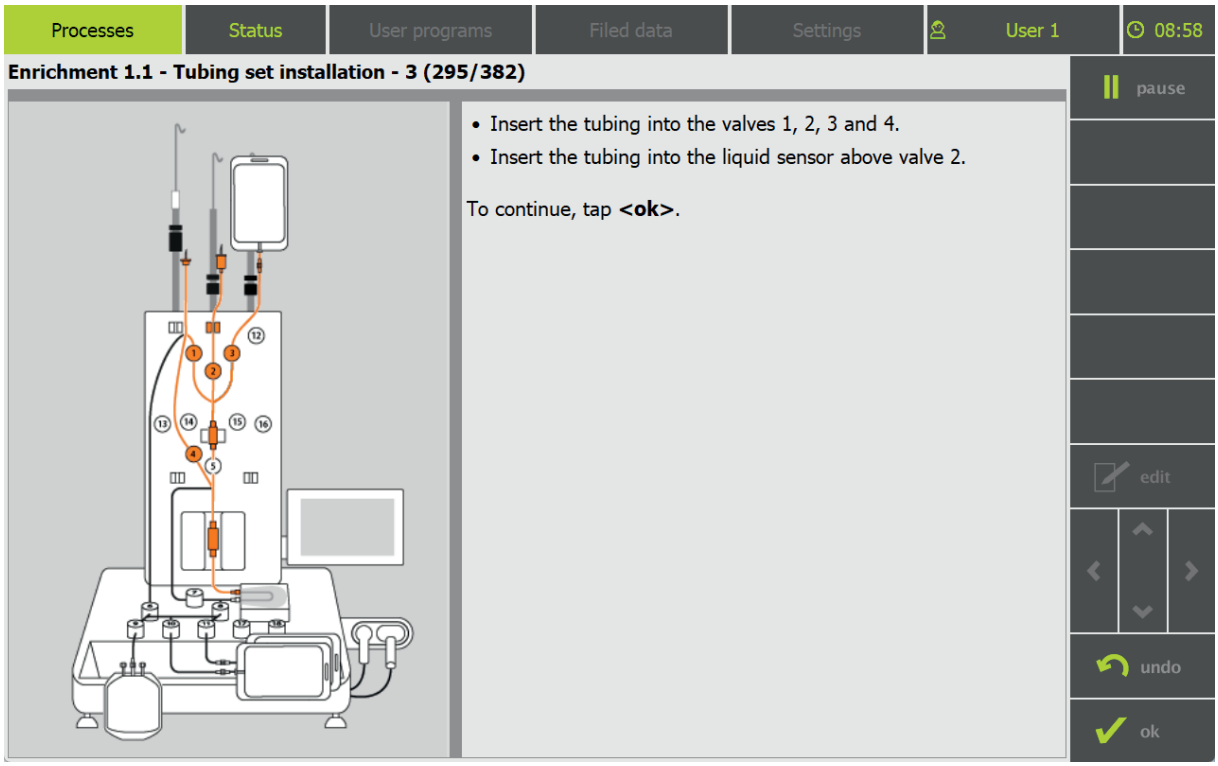


Figure 7.39: Insertion of tubing into valves 1, 2, 3, 4, and liquid sensor

1. Insert the tubing into valve 4. Ensure that the tubing is inserted correctly in the valve opening (see Figure 7.38). Pay particular attention to the area between valves 4 and 5 (see Figure 7.40) and keep the four-way fitting upright.
2. Insert the tubing into valve 1.
3. Position the 4-way fitting just below valve 2. Pay particular attention to the area below valve 2.
4. Insert the tubing into valves 2 and 3.
5. Insert the tubing into the liquid sensor between valve 2 and the bubble trap. Ensure that the tubing is inserted correctly into the sensor fitting.

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

To continue, tap **ok**.

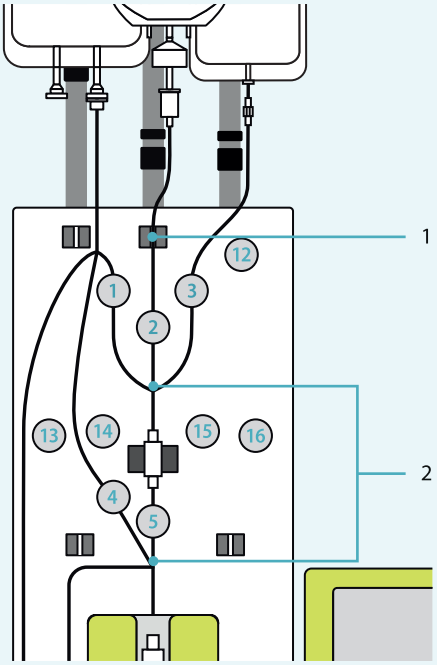


Figure 7.40: Tubing in valves - Liquid sensor and critical areas between valves 4 and 5 and below valve 2

1 Liquid sensor

2 Important areas between valves 4 and 5 and below valve 2

Inserting the pump tubing into the pump

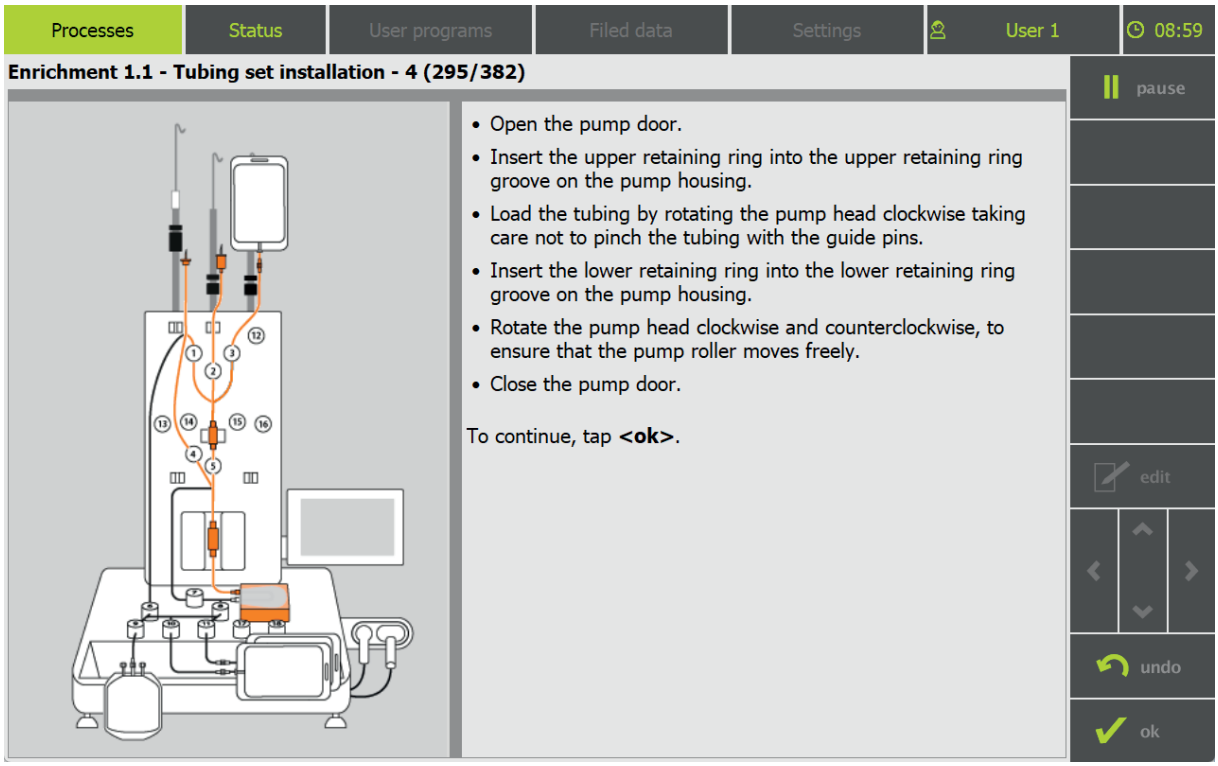


Figure 7.41: Insertion of the pump tubing into the pump

1. Open the pump door by lifting up at the left-hand edge.
2. Insert the upper retaining ring of the pump tubing into the retaining ring groove on the pump housing (see Figure 7.42).
3. Rotate the pump roller clockwise until the tubing is threaded between both sets of the tubing guide pins and fits snugly around the pump roller. Ensure that the tubing is not pinched at the end of the guide pins. If adjustment of the tubing inside the pump is necessary, remove the tubing by lifting the lower end and turning the pump roller counterclockwise.
4. Insert the lower retaining ring of the pump tubing into the retaining ring groove on the pump housing.
5. To ensure that the pump roller moves freely, rotate the pump roller clockwise and counterclockwise.
6. Close the pump door.

IMPORTANT:
If the pump door is opened during the cell separation program, the instrument run will immediately stop.

To continue, tap **ok**.

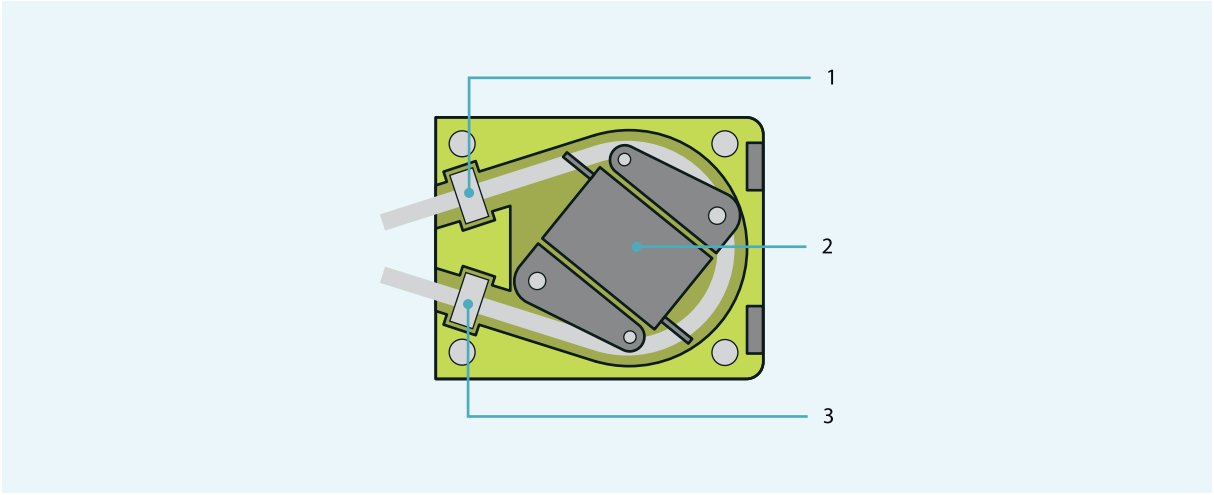


Figure 7.42: Insert pump tubing

1 Upper retaining ring
2 Pump roller
3 Lower retaining ring

Loading valves 7 and 8

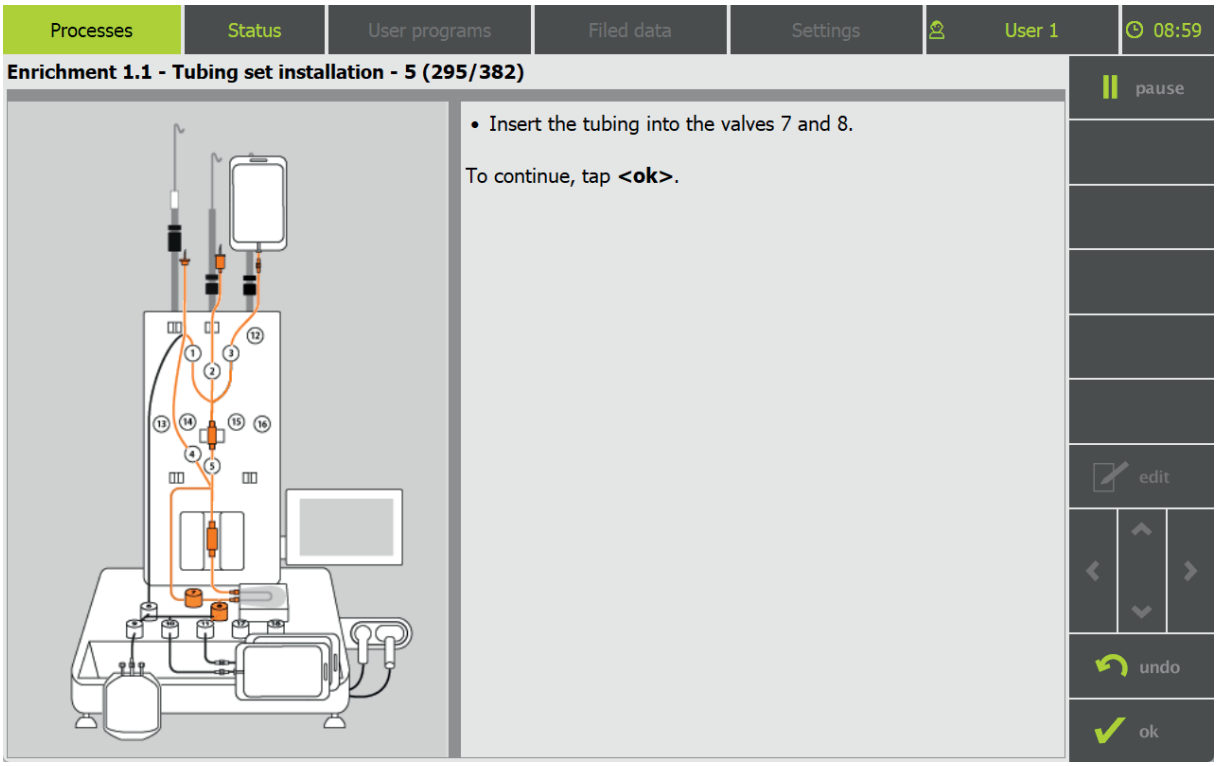


Figure 7.43: Loading of valves 7 and 8

Insert the tubing into the valves 7 and 8. To continue, tap ok.

Loading valves 6, 9, 10, and 11

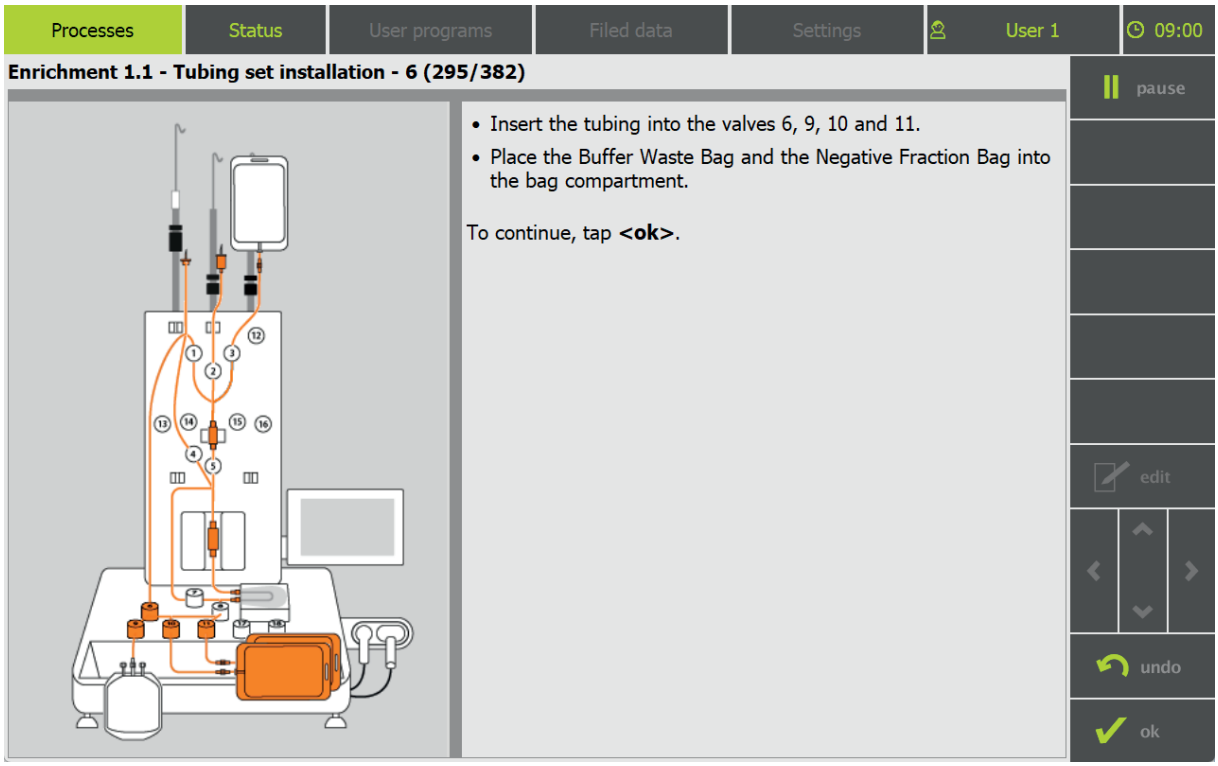


Figure 7.44: Load valves 6, 9, 10, and 11

1. Insert the tubing into valves 6, 9, 10, and 11.
2. Place the Negative Fraction Bag and the Buffer Waste Bag in the bag compartment.

To continue, tap **ok**.

Rechecking all tubing and attachments

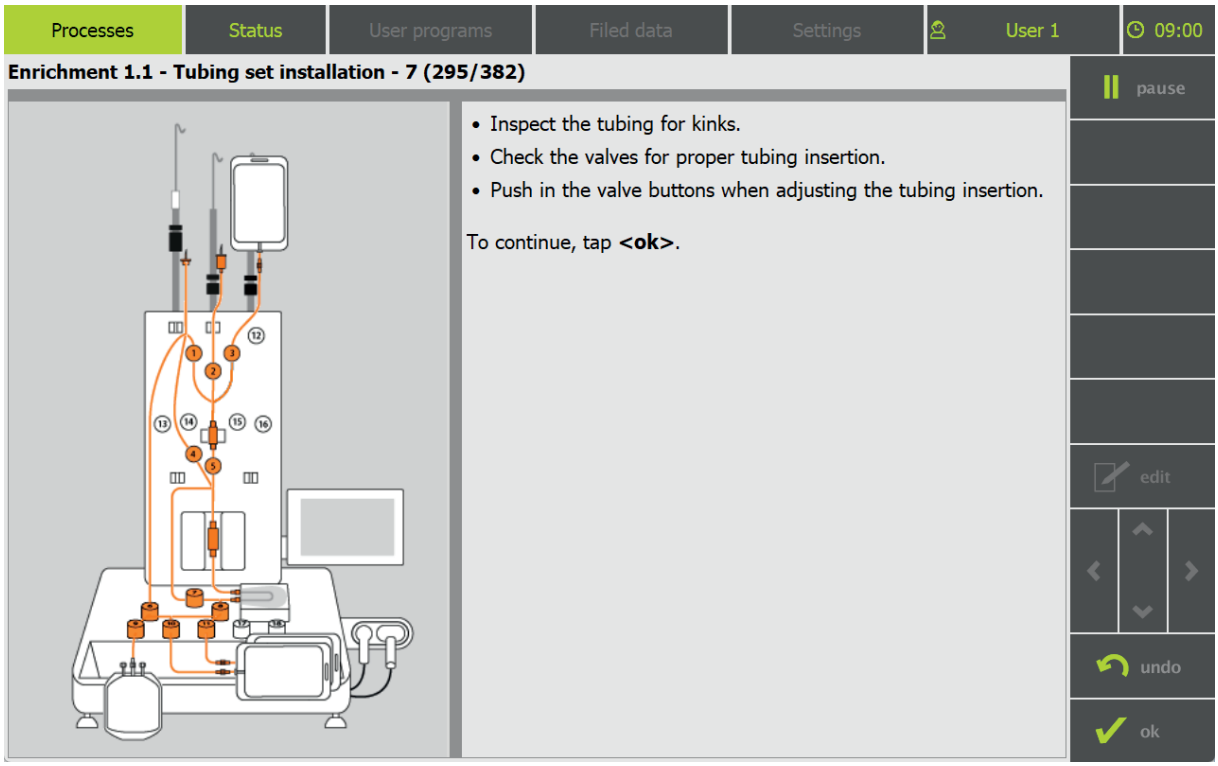


Figure 7.45: Recheck of all tubing and attachments

Before beginning the cell separation, recheck all tubing and attachments.

- Check all valves for proper tubing insertion. Ensure 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 and the area between the pump and valves 7 and 8 (see Figure 7.46) and between valves 4 and 5 (see Figure 7.40).

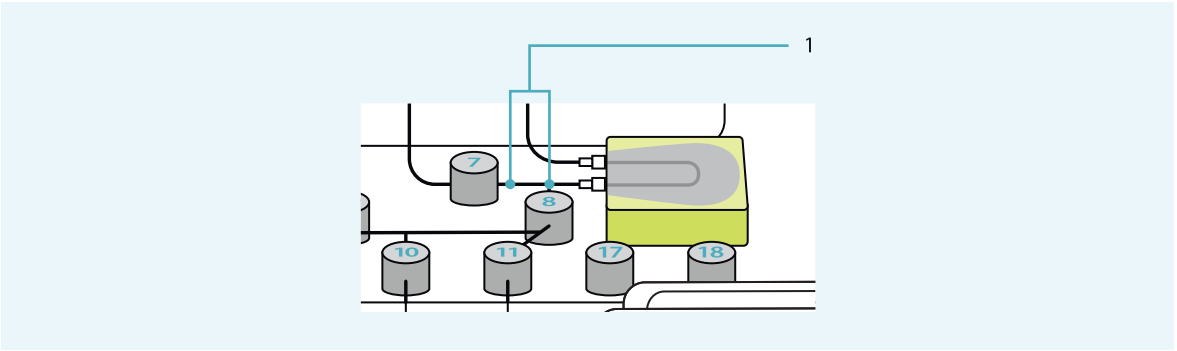


Figure 7.46: Tubing in valves - Critical area between valves 7 and 8

- If the tubing has to be adjusted after a valve is closed, do not pull the tubing without pressing the valve button to open the valve.
- If a tubing has been adjusted, it is necessary to press the corresponding valve buttons firmly twice.

To continue, tap **ok**.

Seating of valves

To ensure the proper fit of the tubing in the valves, the instrument will operate all of the valves in sequence twice. Watch and listen to ensure that all valves are working properly. If any valve does not operate correctly, refer to section 'Troubleshooting'. The magnet drive will also be tested during this check sequence.

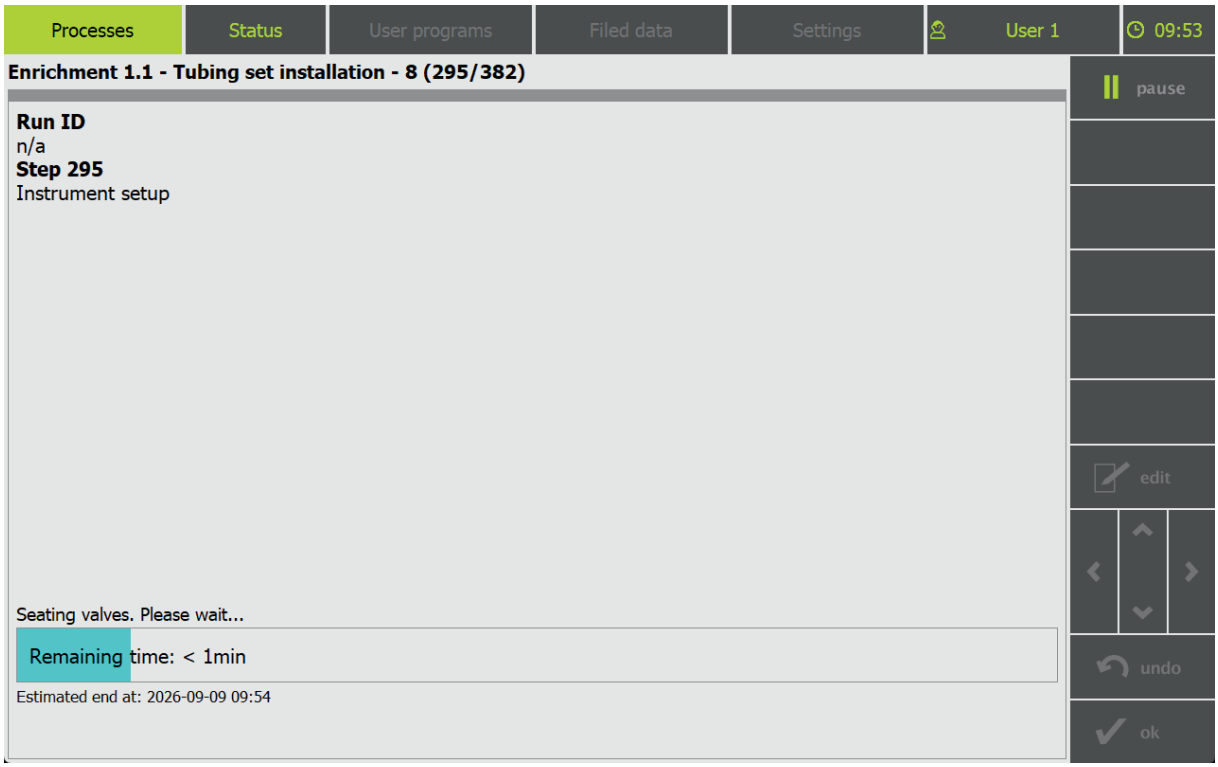


Figure 7.47: Seating of valves

Attaching the process buffer bag

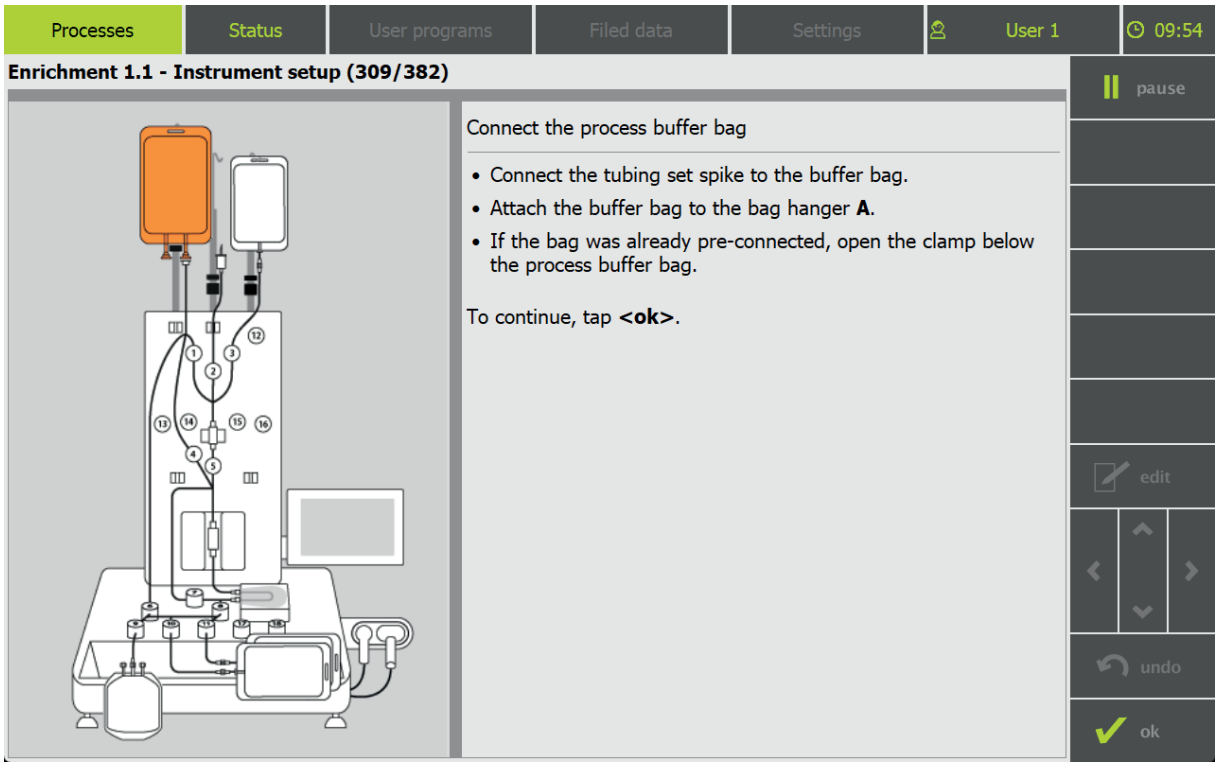


Figure 7.48: Attachment of the buffer bag

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

1. Using aseptic techniques, remove the cap from the buffer spike on the tubing set (see Figure 7.32) and connect it to the buffer bag.
   a. Ensure that the septum is punctured, allowing free flow of liquid.
   b. Gently squeeze the bag to confirm that the spike has penetrated the bag.
2. If more than one liter of buffer is necessary for a separation, connect two buffer bags using a plasma transfer set.
3. Attach the buffer bag to the buffer bag hook on the bag hanger A (see Figure 7.49).

⚠ WARNING

Risk of personal injury and infection.

Overloading the bag hangers can cause the instrument to tip over. Falling or damaged bags may become contaminated. Contaminated solution may lead to infection if used.

- Do not overload the bag hangers. The carrying capacity of a single bag hanger is 5 kg.

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

To continue, tap **ok**.

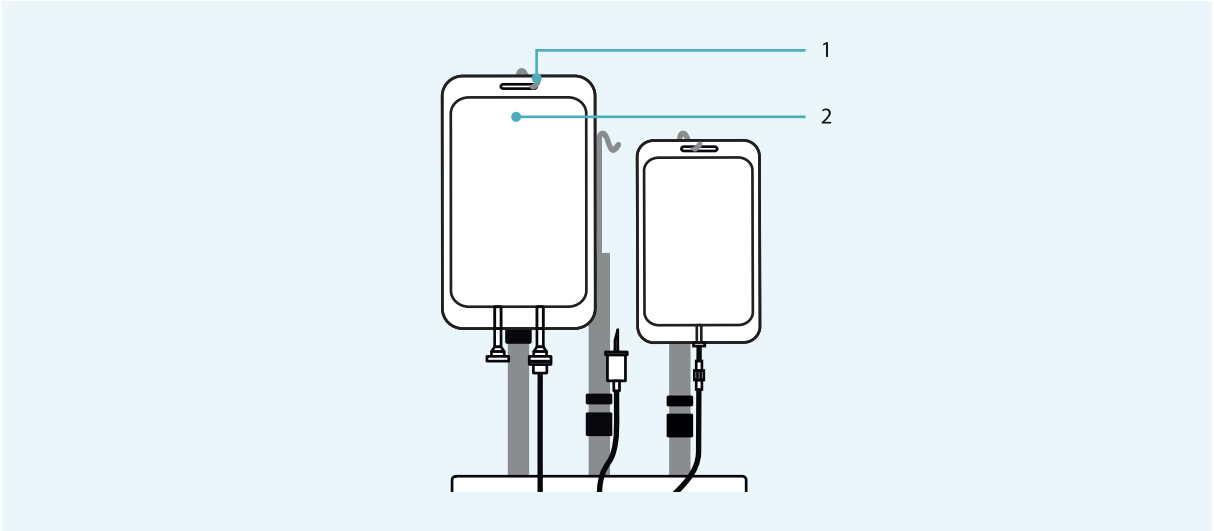


Figure 7.49: Attachment of the buffer bag to bag hanger A

1 Bag hanger hook

2 Process buffer

Priming of the tubing set

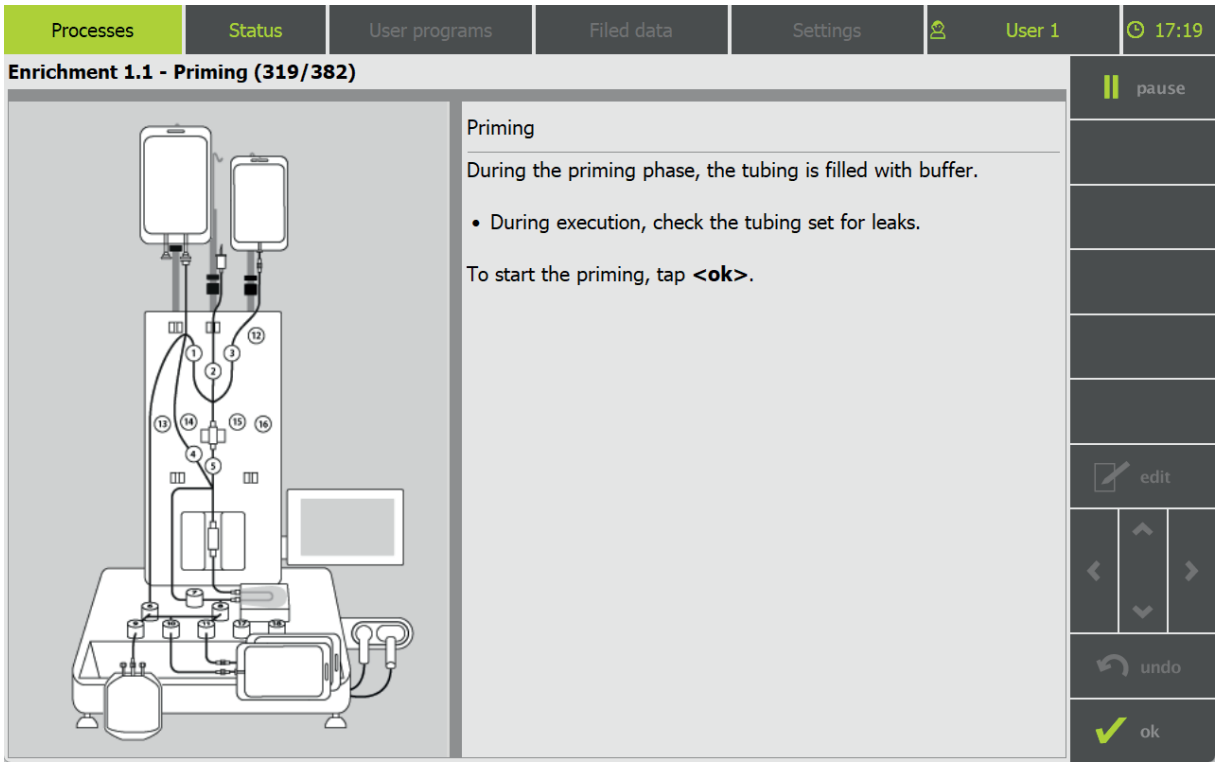


Figure 7.50: Priming of the tubing set

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

To start the priming, tap ok. The priming phase will take approximately 1 minute. The priming status is shown on the screen.

- During the priming phase, the tubing set is filled with the process buffer. The buffer will circulate 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 7.32).
- The priming cycles continue, repeating a series of steps.
- 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.

Final check of all tubing and attachments

After the priming is completed, the screen shows Figure 7.51.

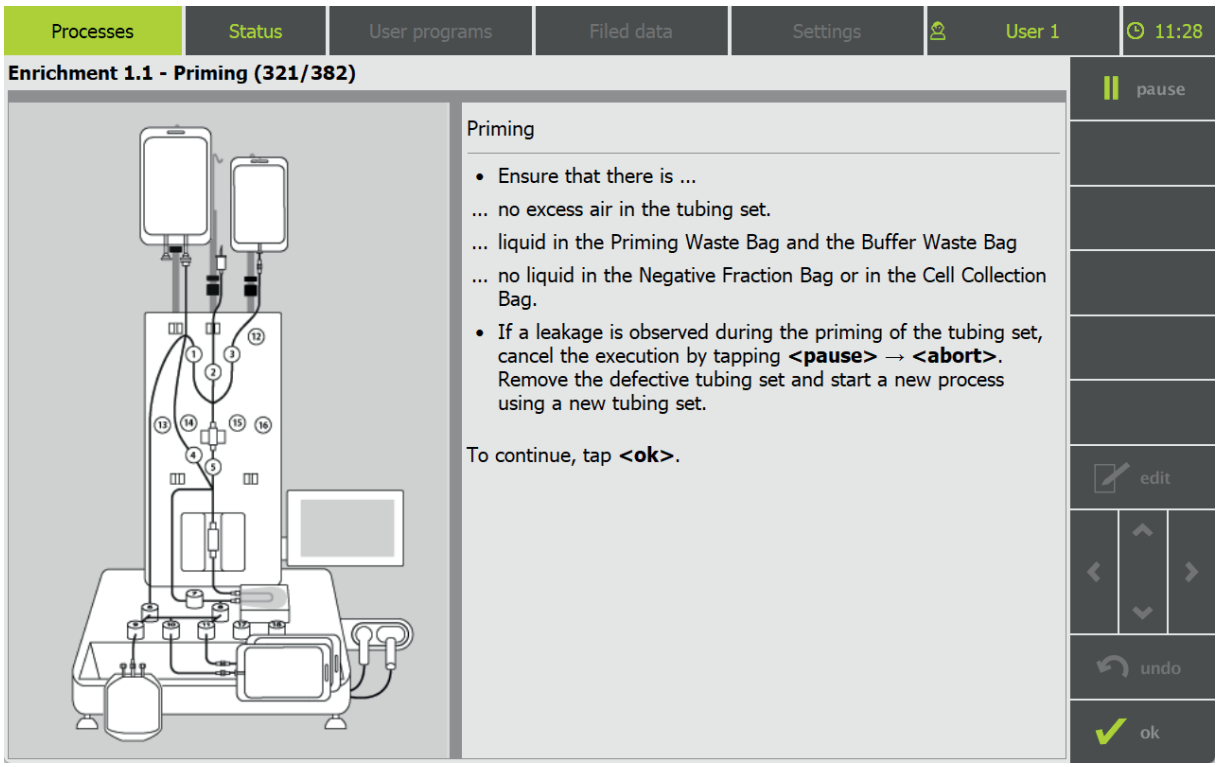


Figure 7.51: Final check of tubing

Before continuing the program, ensure the following:

- Liquid in all parts of the tubing set (except the pre-system filter)
- No excess air in the tubing set
- Fluid in the Priming Waste Bag and the Buffer Waste Bag
- No fluid in the Negative Fraction Bag or in the Cell Collection Bag

If leaks or malfunctions are observed, stop the run.

- To stop the run, tap **pause** > **abort**.
- If the tubing set is defective, remove the tubing set and start a new process using a new tubing set.

To continue, tap **ok**.

Integrity test

For additional safety, it is recommended to perform an integrity test to check the tubing set for leaks. The test sequence consists of two automated subsequences, which allow both the upper and the lower parts of the tubing set to be pressurized and observed separately. To continue with the integrity test, tap **ok** > **continue**. To skip the integrity test, tap **ok** > **skip**.

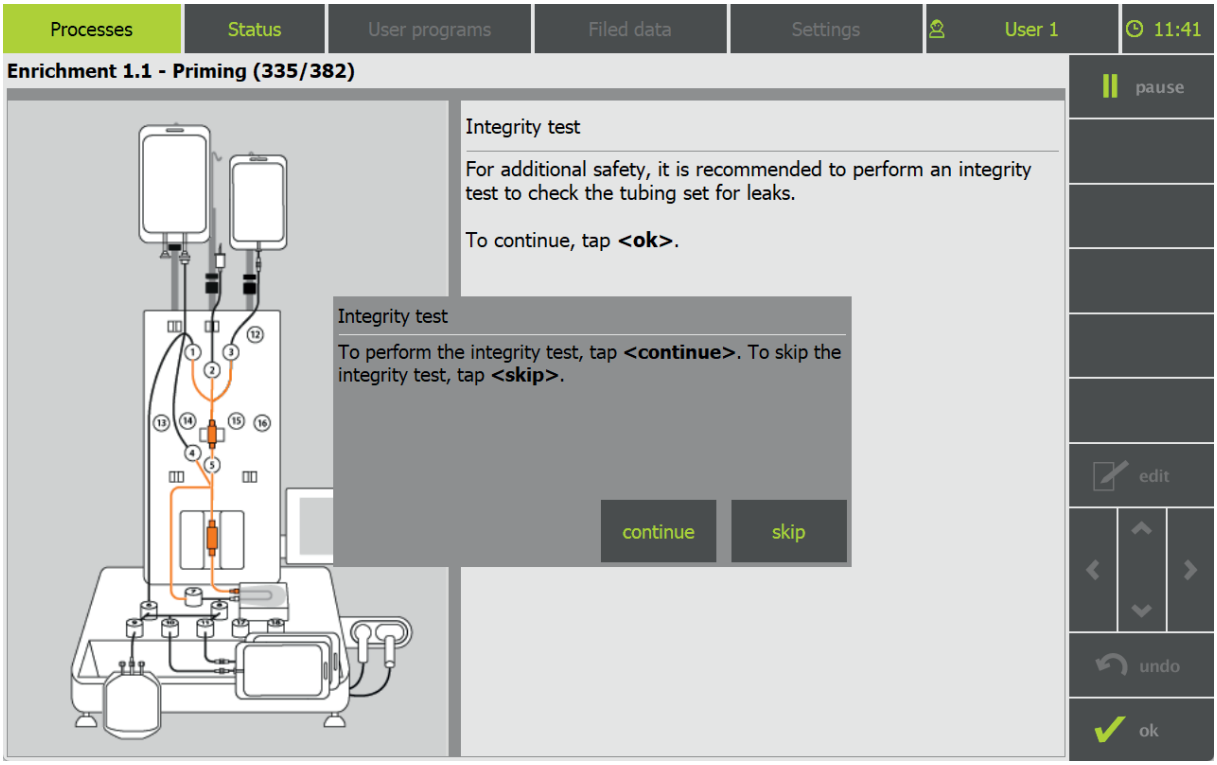


Figure 7.52: Integrity test

Integrity test for the upper part of the tubing set

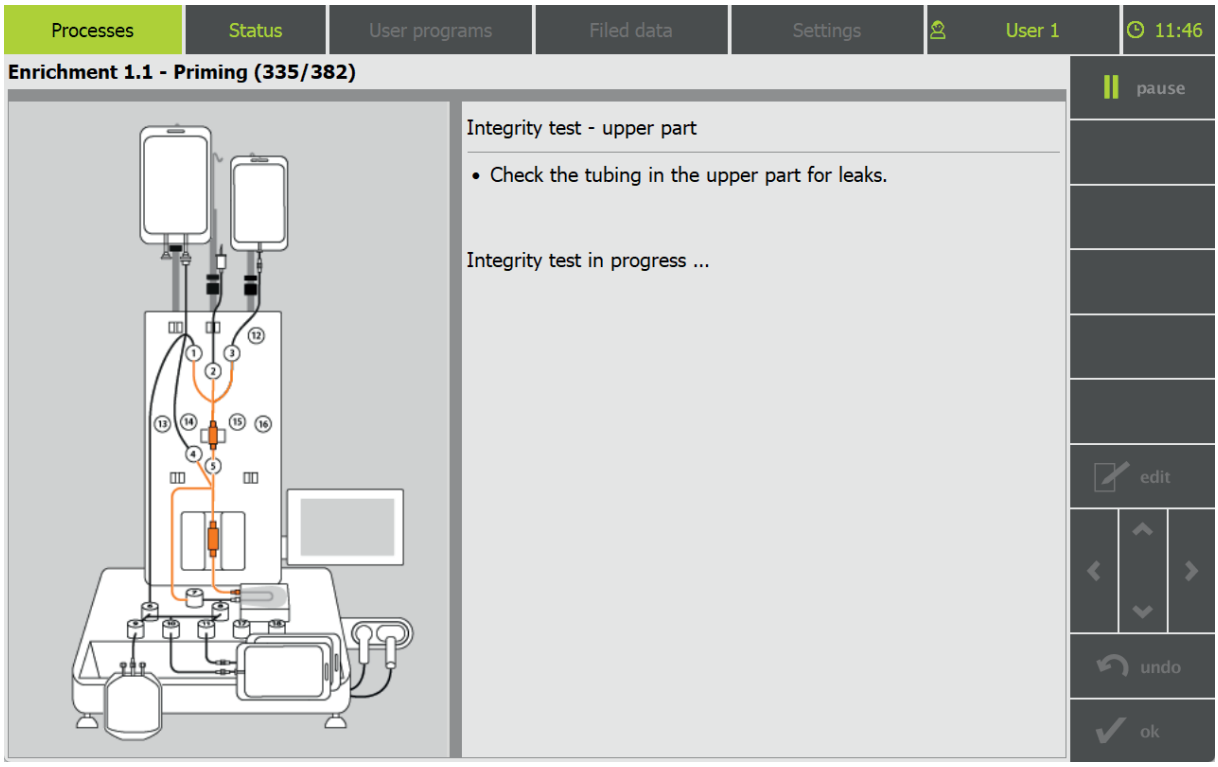


Figure 7.53: Integrity test for the upper part in progress

After tapping **continue**, the instrument starts the automated test sequence for the upper part of the tubing set (see Figure 7.53). Overpressure will be created and held for two minutes.

1. During this time, the user should watch all the connections above the upper pump tubing connection.

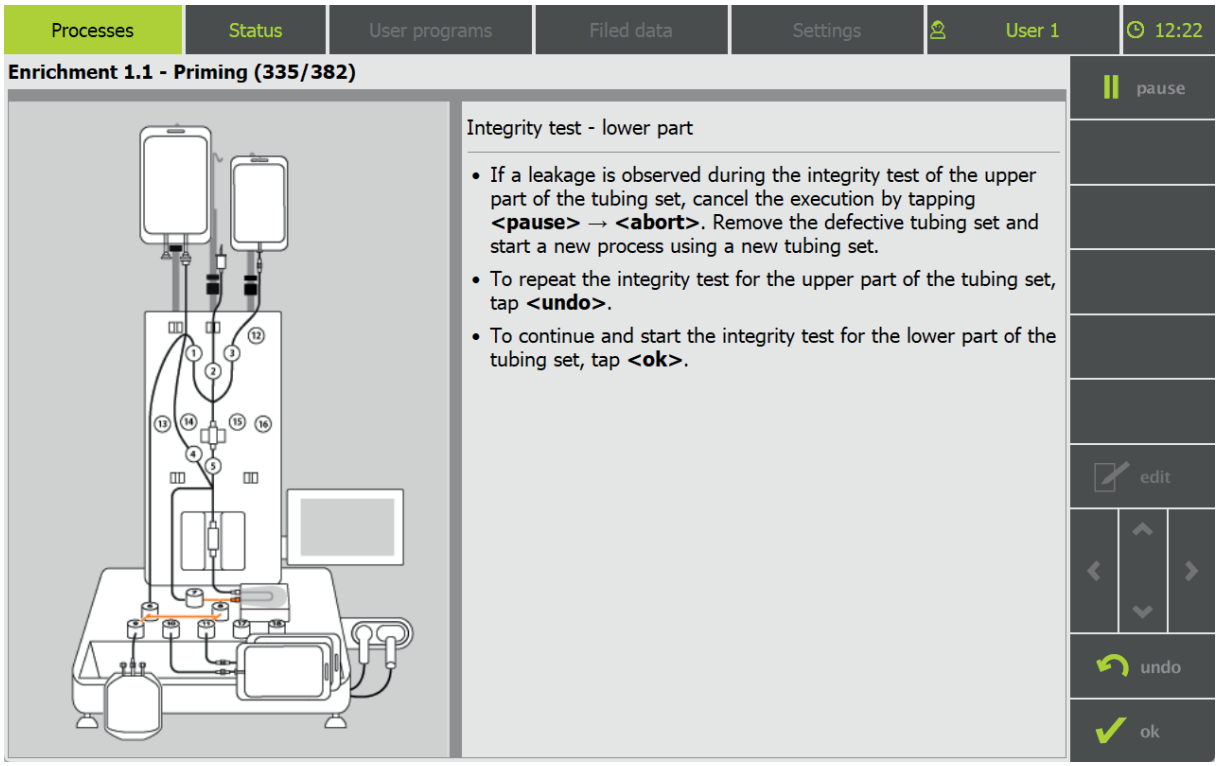


Figure 7.54: Integrity test for the upper part completed

After two minutes, the pressure is automatically released (see Figure 7.54).

1. Check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, stop the run and replace the defective tubing set with a new one.
    a. To stop the run, tap **pause** > **abort**.
    b. Remove the defective tubing set from the instrument and replace it with a new one. Start a new process with the new tubing set. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.
2. To repeat the integrity test of the upper part of the tubing set, tap **undo** > **repeat**. If no leaks are observed, continue with the integrity test of the lower part of the tubing set by tapping **ok**.

Integrity test for the lower part of the tubing set

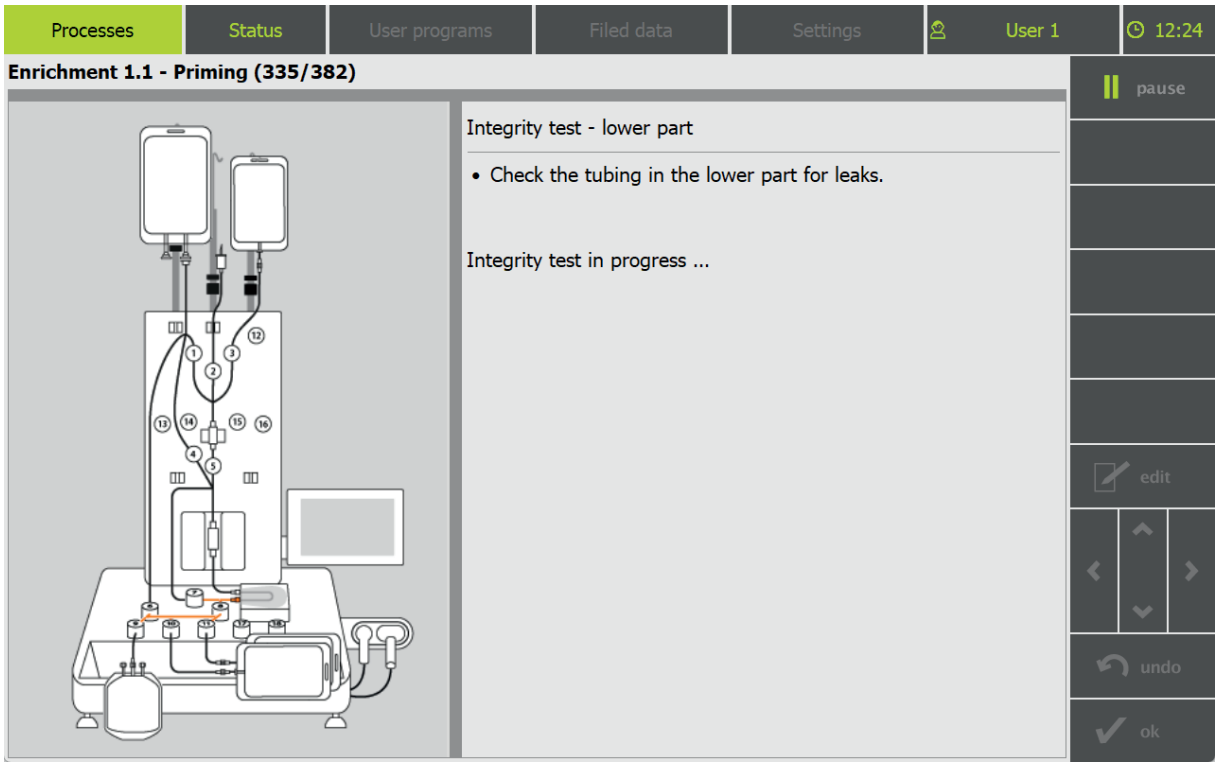


Figure 7.55: Integrity test for the lower part in progress

After tapping **ok**, the instrument starts the automated test sequence for the lower part of the tubing set (see Figure 7.55). Overpressure will be created and held for 30 seconds.

1. During this time, the user should watch the lower pump tubing connection and the T-fittings between valves 6, 8, 9, 10, and 11.

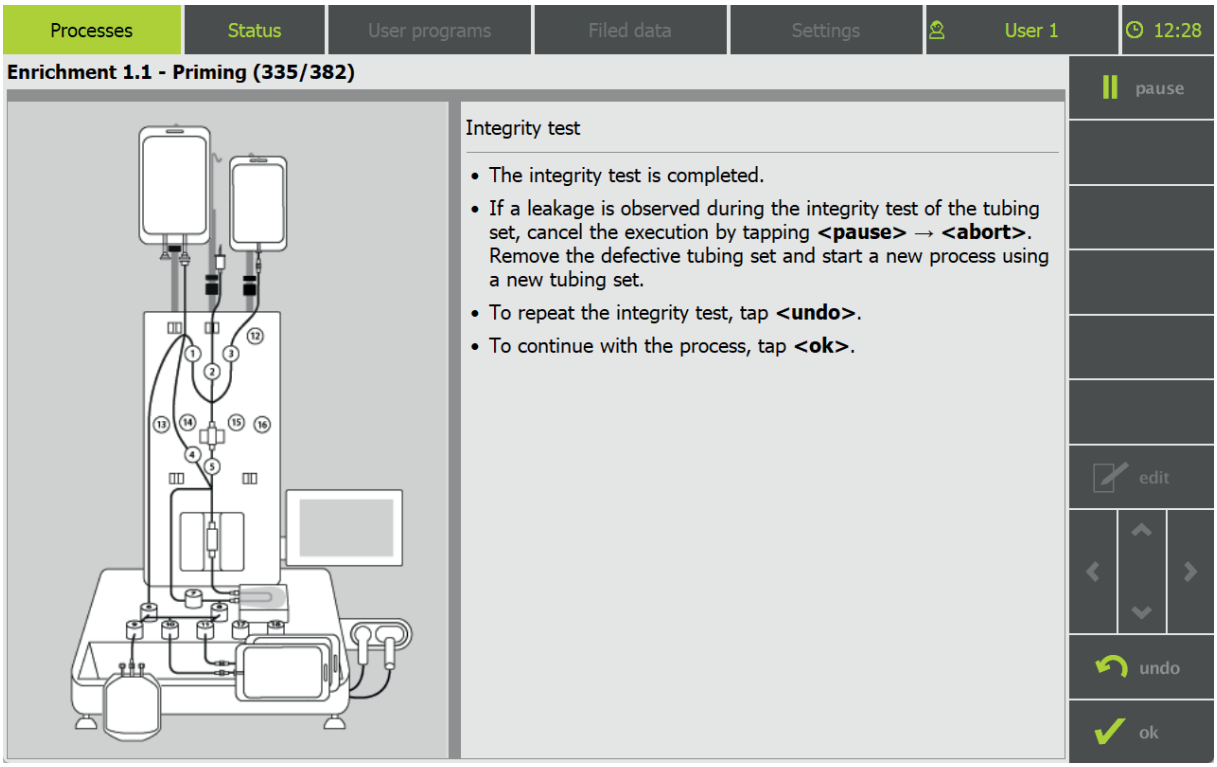


Figure 7.56: Integrity test for the lower part completed

After 30 seconds, the pressure is automatically released.

1. Check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, stop the run and replace the defective tubing set with a new one.
    a. To stop the run, tap **pause** > **abort**.
    b. Remove the defective tubing set from the instrument and replace it with a new one. Start a new process with the new tubing set. Contact Miltenyi Biotec Technical Support for instructions regarding the return of the defective tubing set.
2. To repeat the integrity test, tap **undo**. If no leaks are observed, continue with the process by tapping **ok**.

Connecting the Cell Preparation Bag

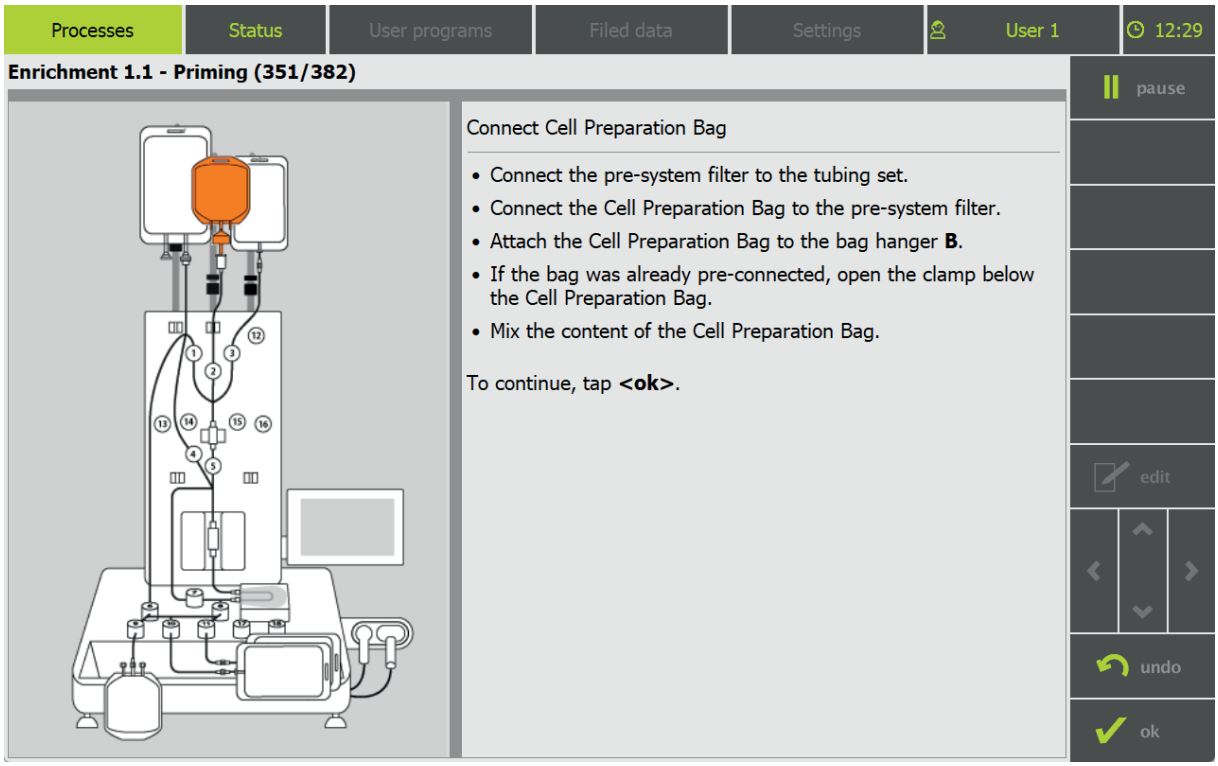


Figure 7.57: Connect Cell Preparation Bag

Connect the Cell Preparation Bag containing the labeled cell product to the tubing set.

1. Remove the cap from the bubble trap spike of the tubing set (see Figure 7.32).
2. Remove the cap from the blunt end of the pre-system filter. Firmly insert the spike into the pre-system filter (see Figure 7.59).
3. Remove the cap from the pre-system filter spike.
4. Spike the pre-system filter into an unused port of the Cell Preparation Bag ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.
5. Carefully check the connection between the pre-system filter and the tubing set to ensure that the connection is secure.
6. Hang the Cell Preparation Bag on the bag hanger B.
7. If the bag was already pre-connected, open the clamp below the Cell Preparation Bag.
8. Mix the content of the Cell Preparation Bag.
9. Adjust the bag hanger for the Cell Preparation Bag to hold the Cell Preparation Bag in an upright position.

To continue, tap ok.

Final check of the liquid sensor

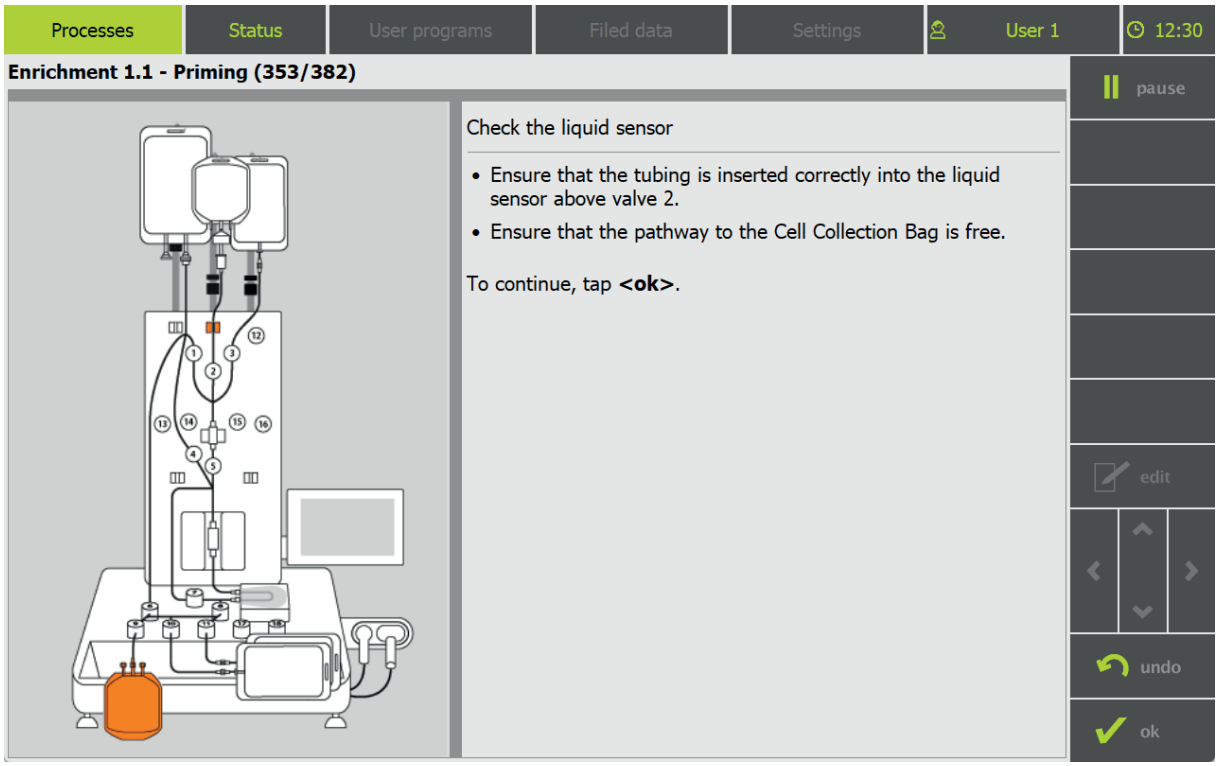


Figure 7.58: Final check of the liquid sensor

1. Check the liquid sensor tubing. Ensure that the tubing is properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure.
2. Ensure that the unrestricted flow to the Cell Collection Bag is possible (for example, open clamp below valve 9).

To continue with the separation (Step 4), tap **ok**.

Figure 7.59 shows the CliniMACS Plus Instrument with the CliniMACS Tubing Set, process buffer, Cell Preparation Bag, and Cell Collection Bag.

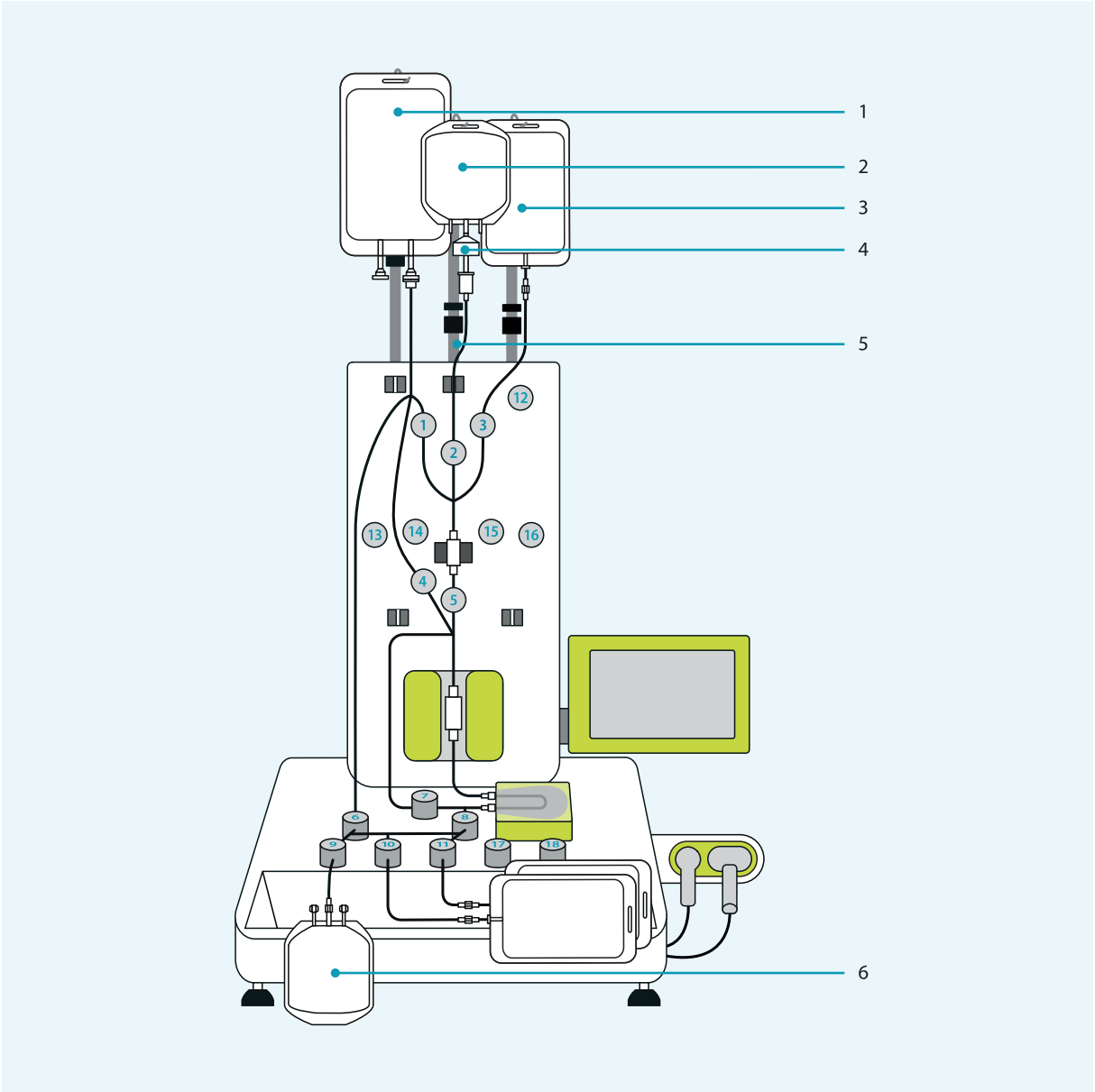


Figure 7.59: CliniMACS Plus Instrument with CliniMACS Tubing Set, process buffer, Cell Preparation Bag, and Cell Collection Bag

1 Process buffer
2 Cell Preparation Bag
3 Priming Waste Bag
4 Pre-system filter
5 Bag hanger B for Cell Preparation Bag
6 Cell Collection Bag

7.3.2 Alternative installation of CliniMACS Tubing Sets

The instructions in Step 3 and the screens shown by the CliniMACS Plus Instrument describe the installation of the CliniMACS tubing sets in a clean room.

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

The sterility of the cell separation process may be compromised during the attachment of the Cell Collection Bag, the process buffer, the pre-system filter, and the Cell Preparation Bag. To ensure that the system remains sterile, these components must be attached to the tubing set under sterile conditions (for example, laminar flow hood). When the components are attached to the tubing set before installation onto the CliniMACS Plus Instrument, the order of the instructions provided by the instrument and the instructions in Step 3 must be changed and further actions taken.

When operating the CliniMACS Plus Instrument outside a clean room, follow the following additional instructions, altering the instructions in Step 3.

Preparation for tubing set installation

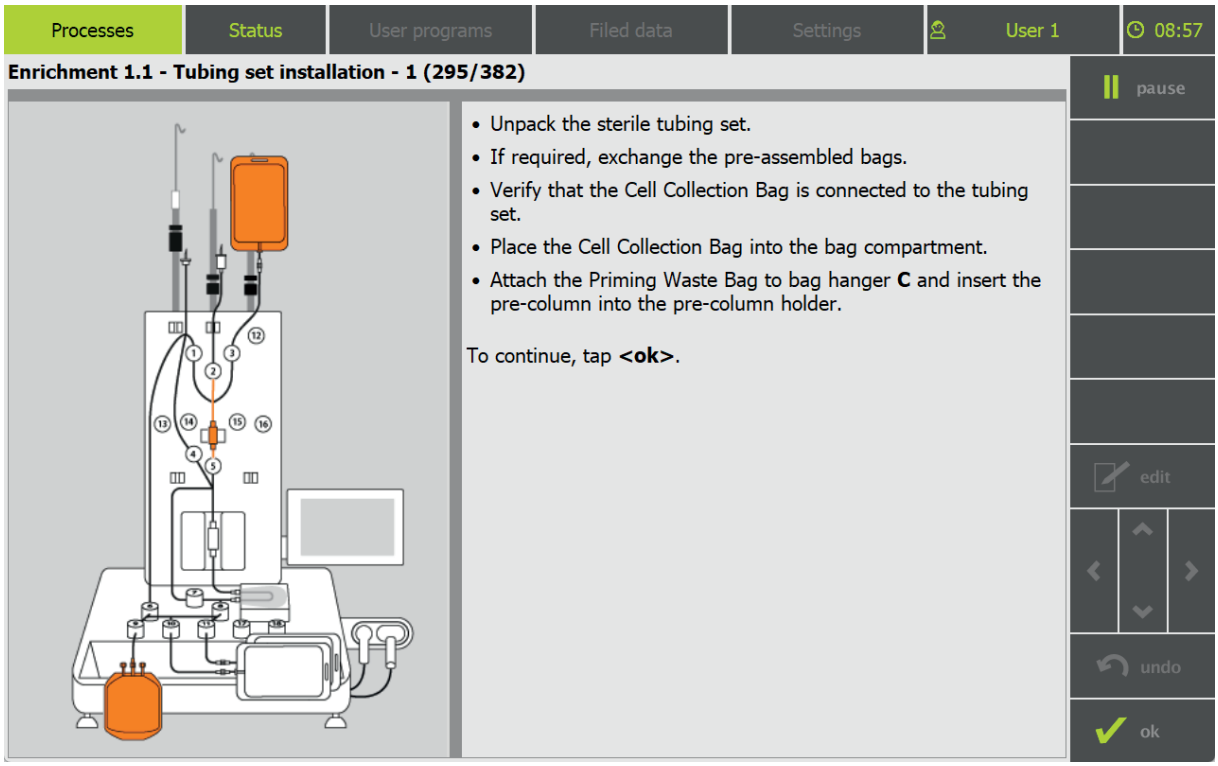


Figure 7.60: Unpacking the tubing set

As described, the Cell Collection Bag, the process buffer, the pre-system filter, and the Cell Preparation Bag must be attached to the tubing set before installing the tubing set onto the instrument under sterile conditions.

Unpack the tubing set under sterile conditions and attach the following components under sterile conditions as described in section 'Connection of the Cell Preparation Bag'.

Attachment of the process buffer

IMPORTANT:
To prevent the buffer from flowing into the tubing set prior to installation, clamp the tubing below the buffer spike with a locking forceps before connecting the process buffer to the tubing set (see clamp 1, Figure 7.63).

- Using aseptic techniques, remove the cap from the buffer spike on the tubing set and connect it to the process buffer bag.
- Ensure that the septum is punctured, allowing free flow of liquid.
- Gently squeeze the bag to ensure that the spike has penetrated the bag.

Attachment of the pre-system filter

Remove the cap from the spike of the bubble trap. Remove the cap from the lower opening of the pre-system filter.

Firmly insert the spike into the pre-system filter. DO NOT remove the top cap of the pre-system filter. Close the tubing just below the bubble trap using a locking forceps (see clamp 2 Figure 7.63). This prevents the prepared cell suspension in the Cell Preparation Bag from entering pre-system filter and tubing set.

Attachment of the 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 ensure that the spike has penetrated the bag.

Installation on the CliniMACS Plus Instrument

Hang the process buffer bag on bag hanger A, the Cell Preparation Bag on the bag hanger B, and the Priming Waste Bag on bag hanger C on the instrument.

To continue, tap **ok**. Refer to section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' for a detailed description of the following steps:

- **Attaching Priming Waste Bag and inserting the pre-column**
- **Inserting the separation column and loading valve 5**
- **Loading valves 1, 2, 3, and 4**
- **Inserting the pump tubing into the pump**
- **Loading valves 7 and 8**
- **Loading valves 6, 9, 10, and 11**
- **Rechecking all tubing and attachments**
- **Seating of valves**

Attachment of the CliniMACS PBS/EDTA Buffer

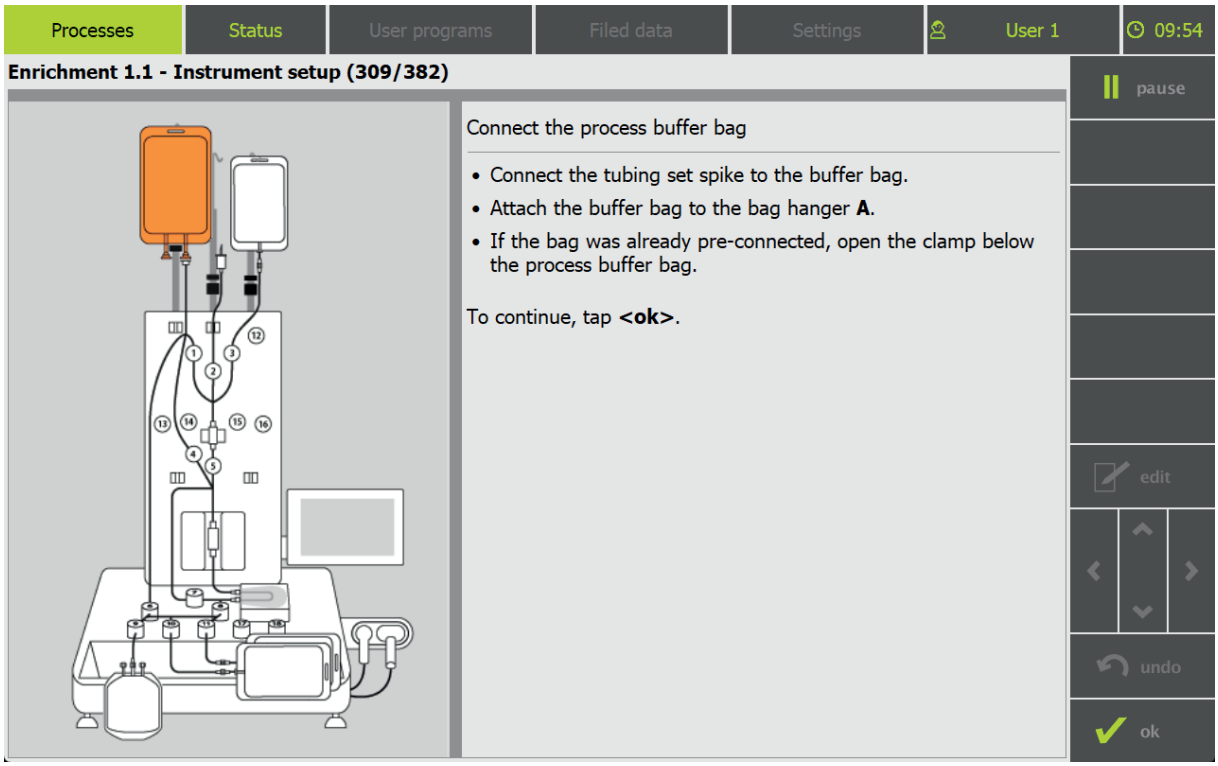


Figure 7.61: Attachment of process buffer bag

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

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

To continue, tap ok. Refer to section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' for a detailed description of the following steps:

- **Priming of the tubing set**
- **Final check of all tubing and attachments**
- **Integrity test**

Connect Cell Preparation Bag (and pre-system filter)

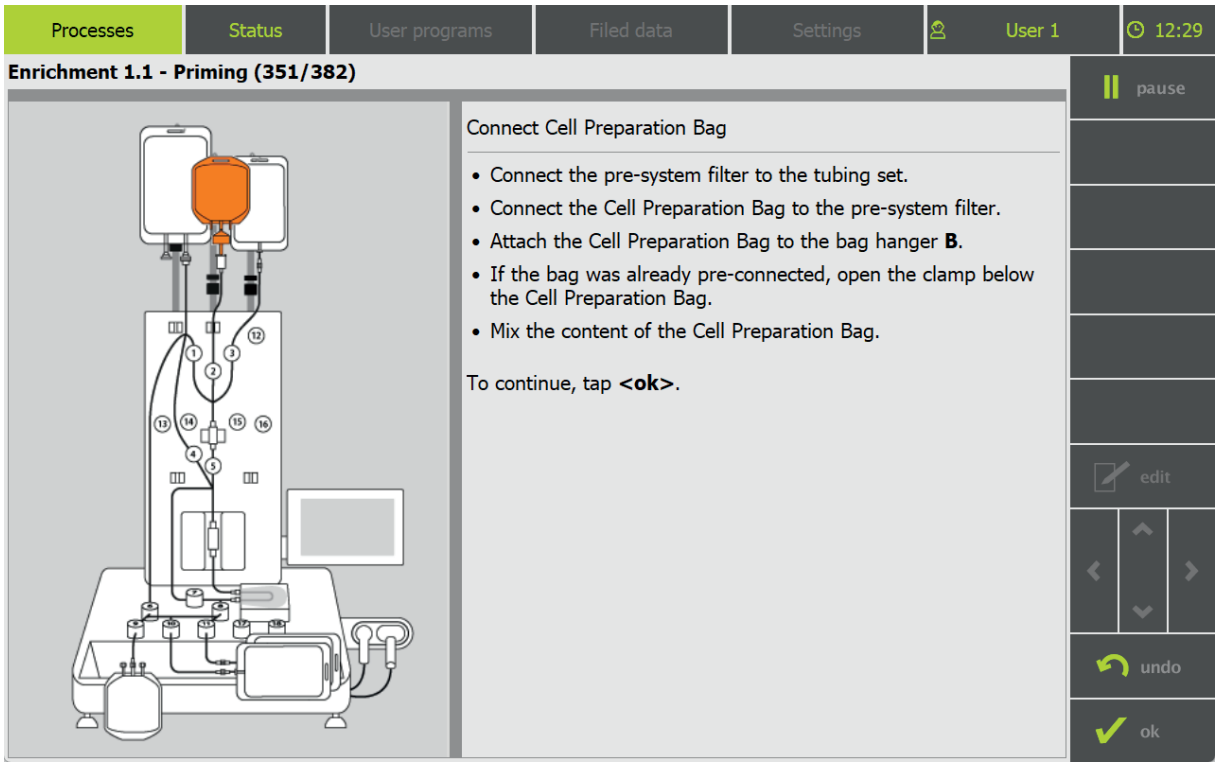


Figure 7.62: Connection of the Cell Preparation Bag

1. The Cell Preparation Bag and the pre-system filter were attached during 'Preparation for tubing set installation'.
2. Remove the locking forceps below the bubble trap (see Figure 7.63).

To continue, tap **ok**. Refer to section 'CliniMACS Tubing Set and CliniMACS Tubing Set LS' for a detailed description of the following step:

- **Final check of the liquid sensor**

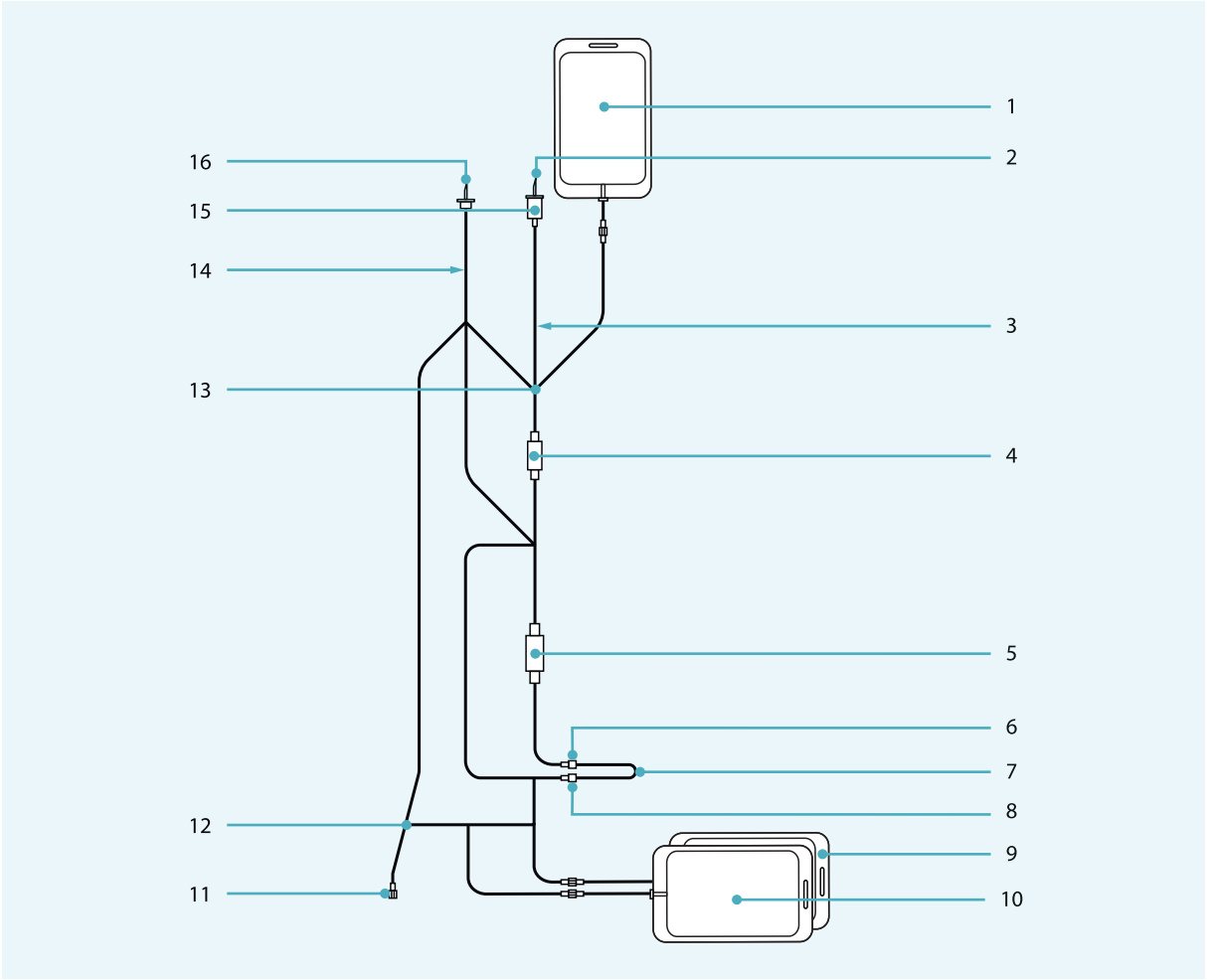


Figure 7.63: General construction of a CliniMACS Tubing Set (for example, CliniMACS Tubing Set) with clamping positions 1 and 2

1 Priming Waste Bag
2 Bubble trap spike
3 Clamp 2 here
4 Pre-column
5 Separation column
6 Upper retaining ring
7 Pump tubing
8 Lower retaining ring
9 Negative Fraction Bag
10 Buffer Waste Bag
11 Luer connector
12 T-fitting
13 4-way fitting
14 Clamp 1 here
15 Bubble trap
16 Buffer spike

7.4 Step 4: CliniMACS Plus Separation

7.4.1 CD133 Selection 1/2

Starting the separation program

Once the final check is completed, the CliniMACS Plus Instrument is ready to begin the separation (see Figure 7.64).

IMPORTANT:
The screen may show a different program name depending on the chosen separation program (CD133 Selection 1 or CD133 Selection 2).

1. To start the separation program, tap **ok**. The instrument will automatically perform the chosen separation procedure. At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter.
2. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.
3. To view the current step of the separation procedure, tap **Status** in the menu bar. The name of the current step is shown on the right-hand side of the screen.

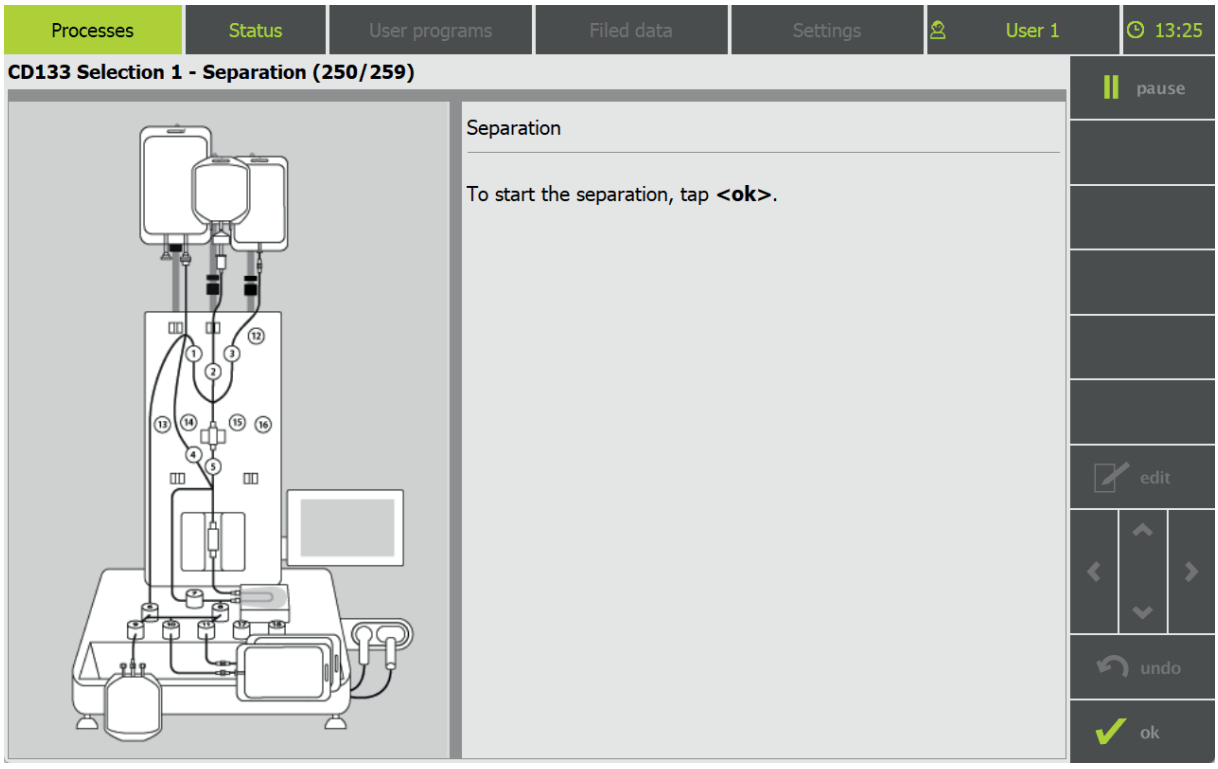


Figure 7.64: Starting the separation

The following section 'Separation procedure' describes the individual steps of the separation procedure. If a warning or error message appears during the separation process, refer to chapter 'Troubleshooting' for information on corrective actions.

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to remove remaining air from the pre-system filter.

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

Column wash I

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

Release of cells I

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

Reloading of cells I

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

Column wash II

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

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

The cells are released and reapplied on the separation column for a second time in order to remove any unlabeled cells that unspecifically 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 CD133 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 cells are released from the separation column and collected in the attached Cell Collection Bag.

Disconnecting bags and removing tubing set

When the separation is completed, the screen shows Figure 7.65.

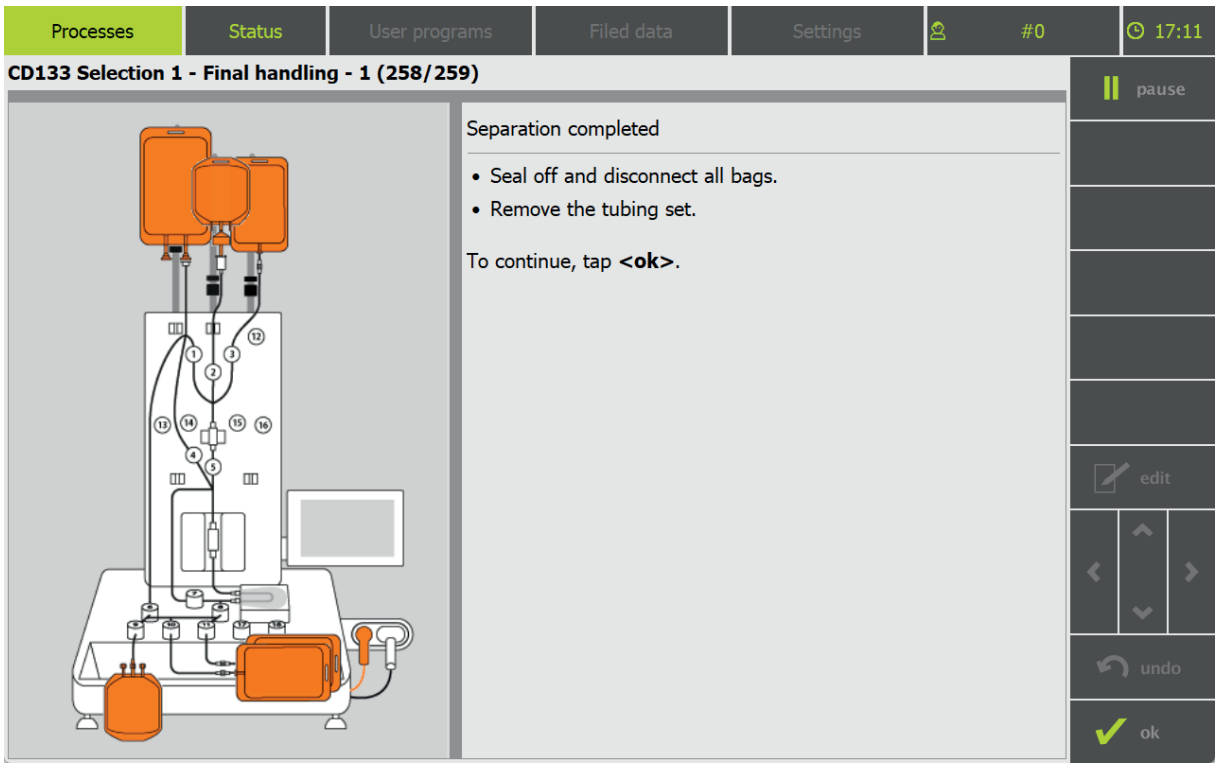


Figure 7.65: Separation completed - Sealing off bags and removing the tubing set

⚠ WARNING

Risk of infection and loss of cell product.

Multiple seals made closer than the recommended distance may result in rupture of the sealed tubing segment and leakage of the cell product. Leakage may expose the user to biological material, potentially resulting in infection, and may lead to loss of the cell product.

- Maintain a minimum distance of at least 1 cm between adjacent seals.

1. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.66).
    a. Make three hermetic seals in the tubing directly below valve 9, with a distance of at least 1 cm between each seal.
    b. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
2. Weigh the filled Cell Collection Bag. Record the weight.
    a. 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.
    b. Record the weight.
3. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, for example, 0.5 mL, and retain for analysis.

4. Using the MACS TubeSealer, seal off the tubing above the luer lock of the Negative Fraction Bag.
    a. Make three hermetic seals in the tubing, with a distance of at least 1 cm between each seal.
    b. Sever the center seal to disconnect the Negative Fraction Bag.
5. Disconnect the Buffer Waste Bag in the same way.
6. Remove the Negative Fraction Bag and Buffer Waste Bag.
7. Keep all bags until final analysis of all cells has been accomplished.
8. Remove the tubing set in the following order:
    a. Beginning with valves 6, 9, 10, and 11, and working upwards, press on the valves and release the tubing from the liquid sensor and from the valves.
    b. Release the columns from the column holders.
    c. Dispose the tubing set as a biohazard, according to standard hospital procedures.

The target cells can now be processed in accordance with clinical protocols. To continue, tap **ok**.

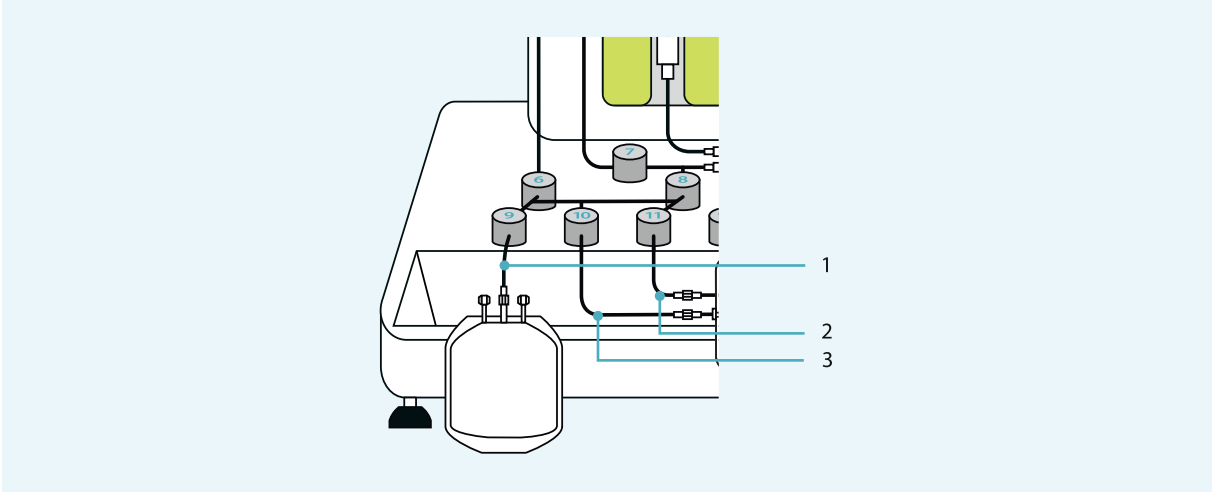


Figure 7.66: Sealing off bags

1 Seal off Cell Collection Bag
2 Seal off Negative Fraction Bag
3 Seal off Buffer Waste Bag

Shutting down and cleaning the instrument

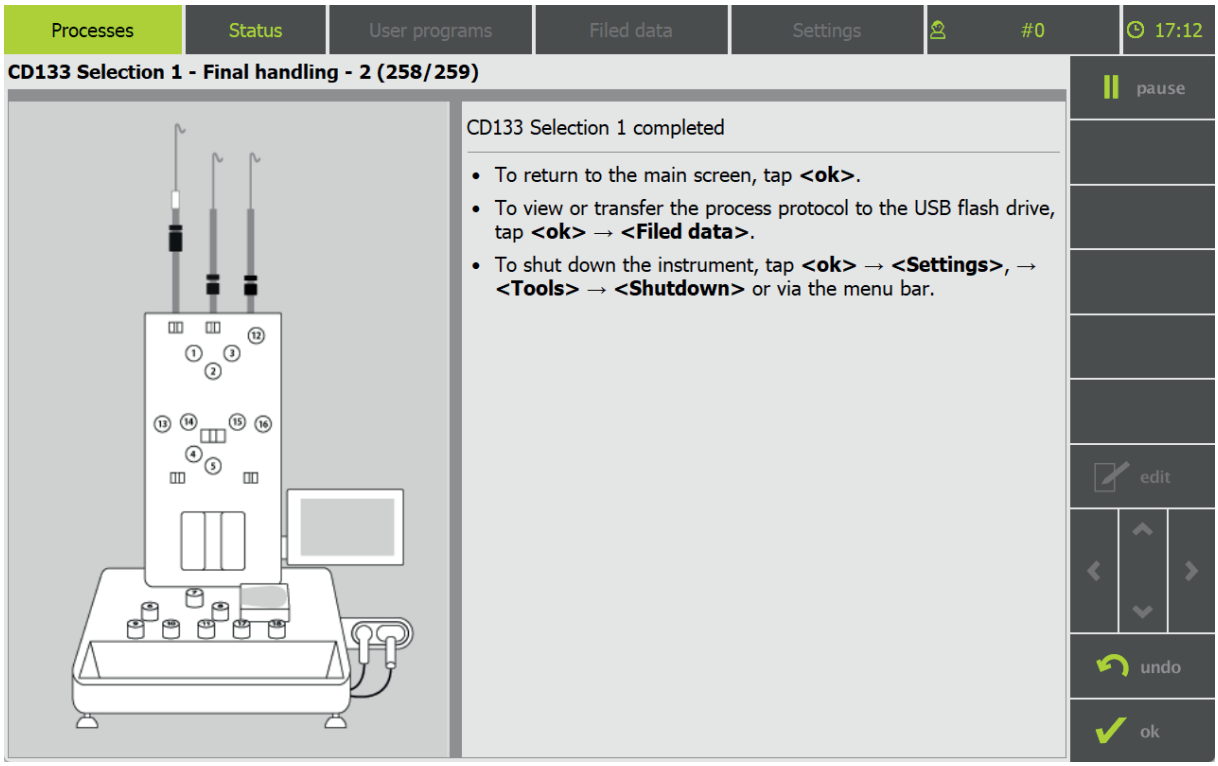


Figure 7.67: Completion of the separation program CD34 Selection 1/2

1. After the completion of the separation program, the user can choose one of the following options:
   - To return to the main screen, tap ok.
   - To view the process protocol or to transfer it to a storage device, tap ok > Filed data.
   - To shut down the instrument, tap ok > Settings > Tools > Shutdown.
2. Clean the instrument according to cleaning instructions as described in the CliniMACS Plus Instrument user manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

⚠ CAUTION

Risk of reduced quality of target cells.

The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised.

- Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- purity (depletion efficiency) and recovery of target cells

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

7.4.2 Enrichment 1.1

Starting the separation program

Once the final check is completed, the CliniMACS Plus Instrument is ready to begin the separation (see Figure 7.68).

1. To start the separation program, tap **ok**. The instrument will automatically perform the chosen separation procedure. At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter.
2. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.
3. To view the current step of the separation procedure, tap **Status** in the menu bar. The name of the current step is shown on the right-hand side of the screen.

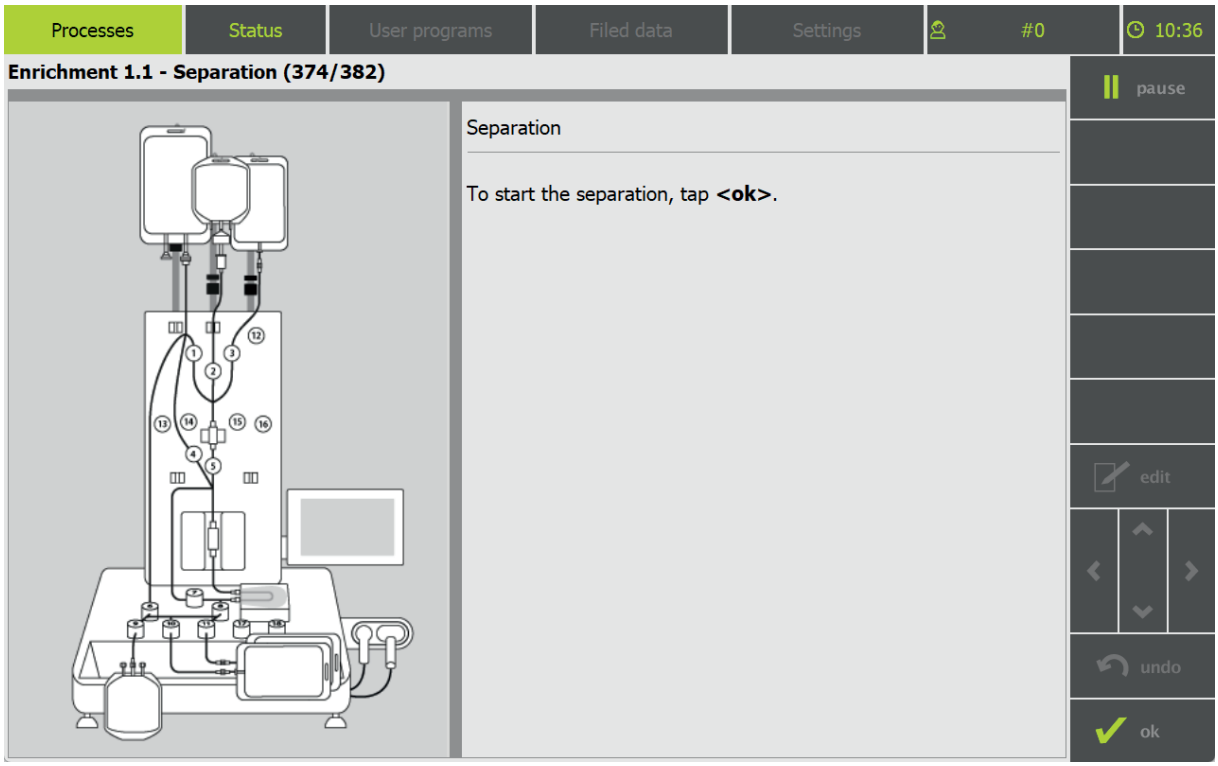


Figure 7.68: Starting the separation

The following section 'Separation procedure' describes the individual steps of the separation procedure. If a warning or error message appears during the separation process, refer to chapter 'Troubleshooting' for information on corrective actions.

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to remove remaining air from the pre-system filter.

Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set and loads the cell product via the pre-column on the separation column. The magnetically labeled cells (target cells) are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and are collected in the Negative Fraction Bag.

If the number of magnetically labeled cells (calculated by the CliniMACS Plus Software) exceeds the binding capacity of the separation column, the separation program automatically loads and separates the cell product in suitable portions ("staged loading"). The loading will be stopped when the capacity of the separation column is reached, and the separation program will proceed to the next step of the separation. After the last step (final elution of the cells) has been completed, the next portion of the sample will be loaded onto the column and processed accordingly.

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.

Release of cells

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

Reloading of cells

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

Column wash II

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

Final elution of the cells

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

Disconnecting bags and removing tubing set

When the separation is completed, the screen shows Figure 7.69.

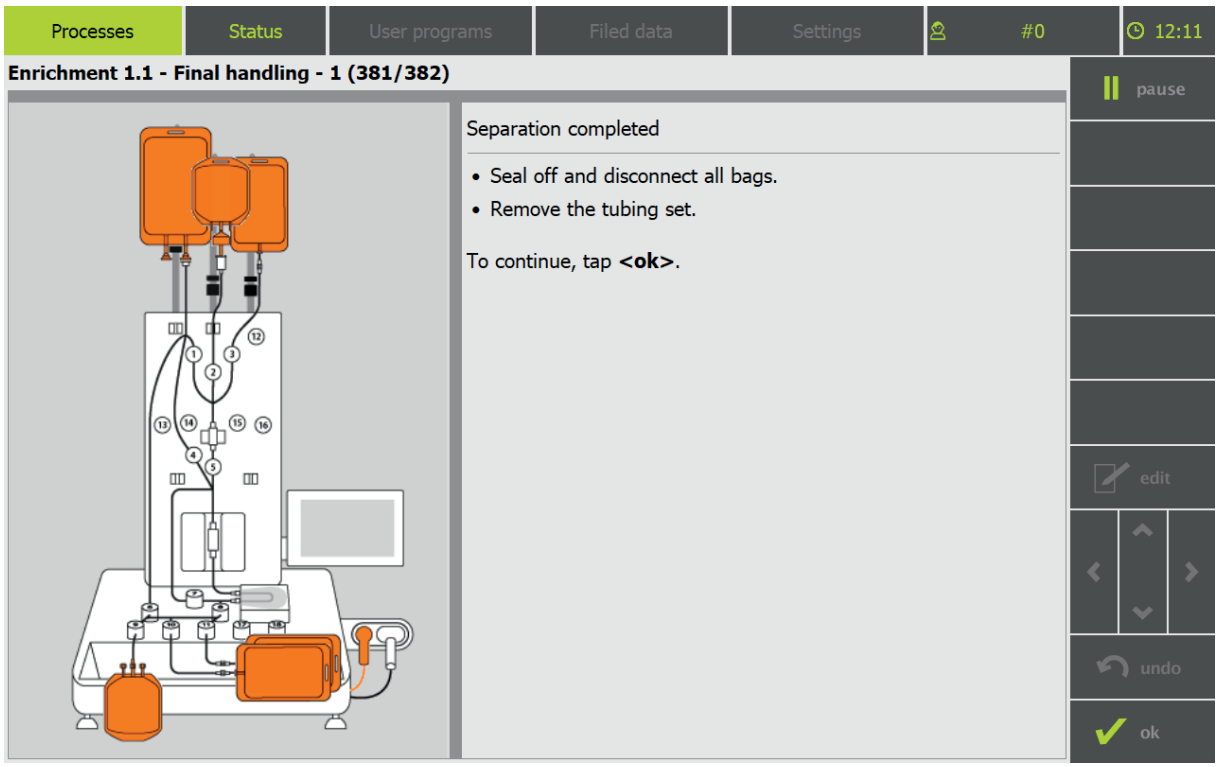


Figure 7.69: Separation completed - Sealing off bags and removing the tubing set

⚠ WARNING

Risk of infection and loss of cell product.

Multiple seals made closer than the recommended distance may result in rupture of the sealed tubing segment and leakage of the cell product. Leakage may expose the user to biological material, potentially resulting in infection, and may lead to loss of the cell product.

- Maintain a minimum distance of at least 1 cm between adjacent seals.

1. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.70).
    a. Make three hermetic seals in the tubing directly below valve 9, with a distance of at least 1 cm between each seal.
    b. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
2. Weigh the filled Cell Collection Bag. Record the weight.
    a. 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.
    b. Record the weight.
3. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, for example, 0.5 mL, and retain for analysis.

4. Using the MACS TubeSealer, seal off the tubing above the luer lock of the Negative Fraction Bag.
    a. Make three hermetic seals in the tubing, with a distance of at least 1 cm between each seal.
    b. Sever the center seal to disconnect the Negative Fraction Bag.
5. Disconnect the Buffer Waste Bag in the same way.
6. Remove the Negative Fraction Bag and Buffer Waste Bag.
7. Keep all bags until final analysis of all cells has been accomplished.
8. Remove the tubing set in the following order:
    a. Beginning with valves 6, 9, 10, and 11, and working upwards, release the tubing from the liquid sensor and from the valves by pressing on the valves.
    b. Release the columns from the column holders.
    c. Dispose the tubing set as a biohazard, according to standard hospital procedures.

The target cells can now be processed in accordance with clinical protocols. To continue, tap **ok**.

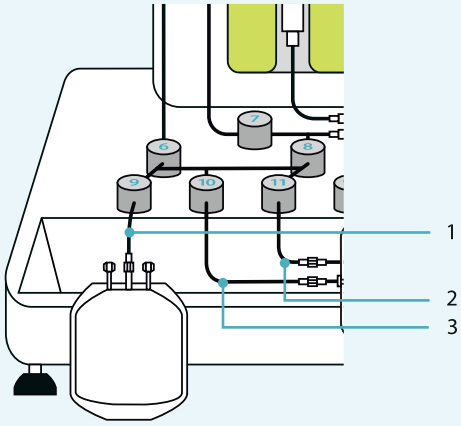


Figure 7.70: Seal off bags

1 Seal off Cell Collection Bag
2 Seal off Negative Fraction Bag
3 Seal off Buffer Waste Bag

Shutting down and cleaning the instrument

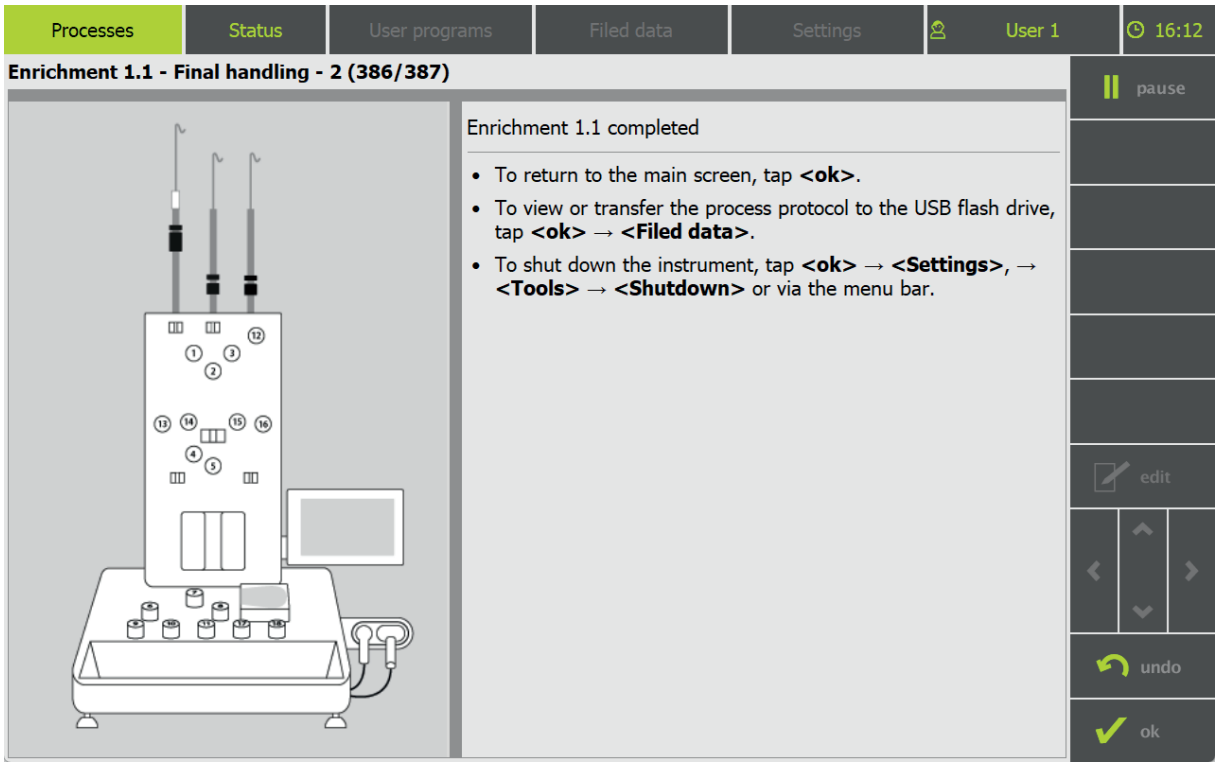


Figure 7.71: Completion of the separation program Enrichment 1.1

1. After the completion of the separation program, the user can choose one of the following options:
   - To return to the main screen, tap ok.
   - To view the process protocol or to transfer it to a storage device, tap ok > Filed data.
   - To shut down the instrument, tap ok > Settings > Tools > Shutdown.
2. Clean the instrument according to cleaning instructions as described in the CliniMACS Plus Instrument user manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

⚠ CAUTION

Risk of reduced quality of target cells.

The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised.

- Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- purity (depletion efficiency) and recovery of target cells

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

7.4.3 Enrichment 3.2

Starting the separation program

Once the final check is completed, the CliniMACS Plus Instrument is ready to begin the separation (see Figure 7.72).

1. To start the separation program, tap **ok**. The instrument will automatically perform the chosen separation procedure. At the beginning of the separation, buffer is pumped upwards towards the Cell Preparation Bag to fill the pre-system filter.
2. Tap the side of the filter gently several times to remove any bubbles which might be trapped in the filter.
3. To view the current step of the separation procedure, tap **Status** in the menu bar. The name of the current step is shown on the right-hand side of the screen.

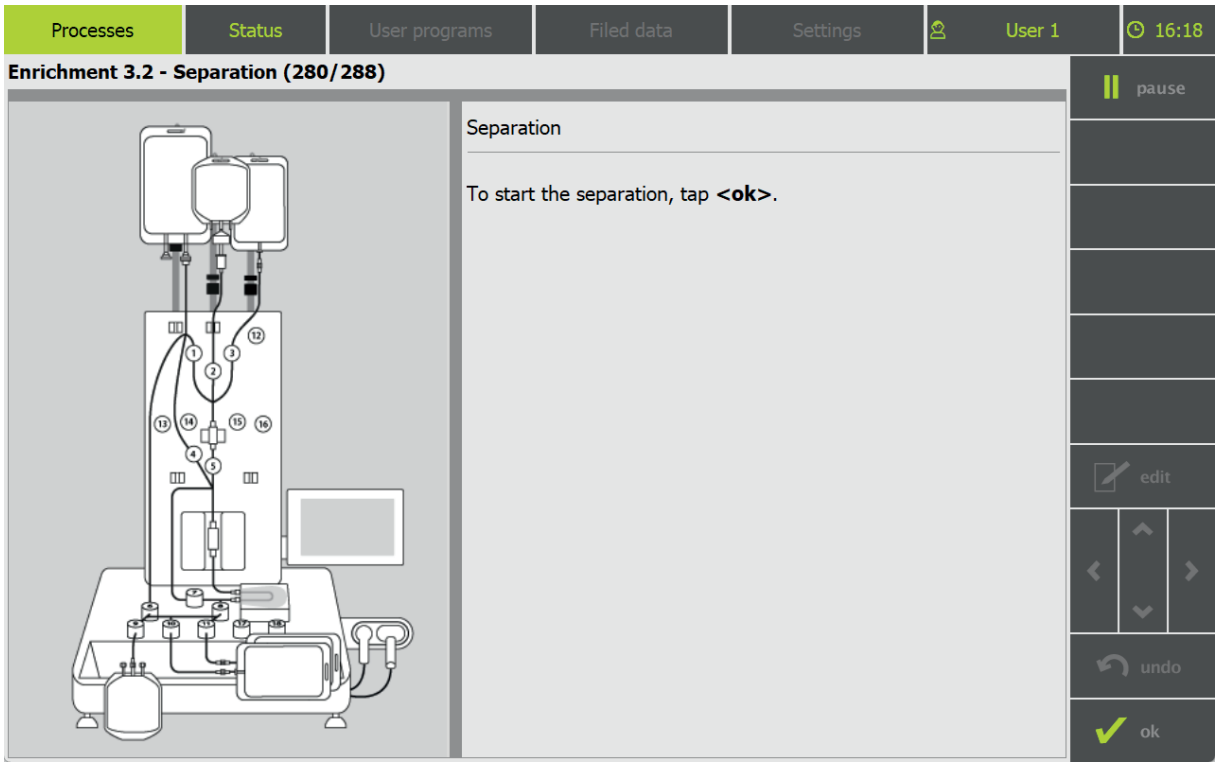


Figure 7.72: Starting the separation

The following section 'Separation procedure' describes the individual steps of the separation procedure. If a warning or error message appears during the separation process, refer to chapter 'Troubleshooting' for information on corrective actions.

Separation procedure

Loading cells

The separation procedure starts with the filling of the pre-system filter to remove remaining air from the pre-system filter. Then the separation of the labeled cells begins. The pump draws the contents of the Cell Preparation Bag into the tubing set and loads the cell product via the pre-column on the separation column. The magnetically labeled cells (target cells) are retained in the separation column, placed in the magnetic field, while the unlabeled cells (non-target cells) are passed through and are 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.

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 onto the separation column.

Column wash II

The 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 onto the separation column for a second time in order to remove any unlabeled cells sticking unspecifically to the column matrix. Afterwards the separation column is washed again.

Final elution of the cells

The magnet is moved into the "OFF" position and the magnetically labeled cells (target cells) are released and collected in the Cell Collection Bag.

Disconnecting bags and removing tubing set

When the separation is completed, the screen shows Figure 7.73.

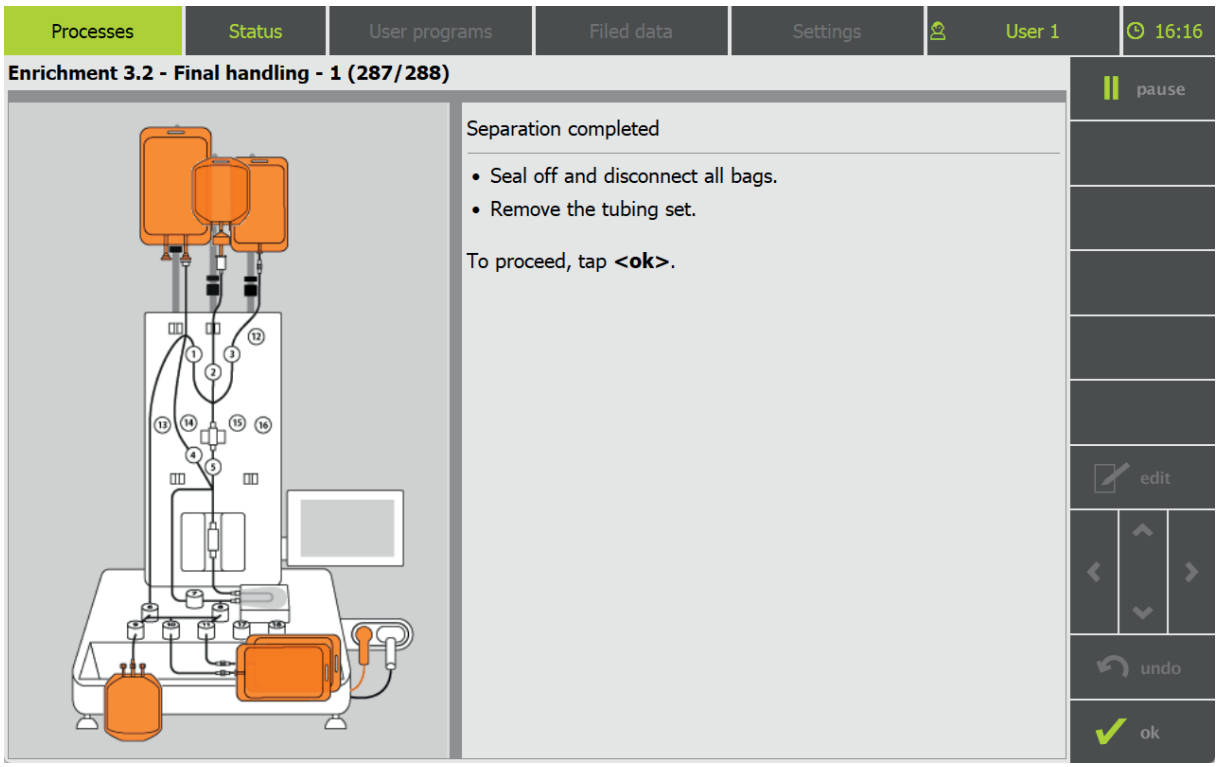


Figure 7.73: Separation completed - Sealing off bags and removing the tubing set

⚠ WARNING

Risk of infection and loss of cell product.

Multiple seals made closer than the recommended distance may result in rupture of the sealed tubing segment and leakage of the cell product. Leakage may expose the user to biological material, potentially resulting in infection, and may lead to loss of the cell product.

- Maintain a minimum distance of at least 1 cm between adjacent seals.

1. Clamp or seal the tubing above the luer lock connecting the Cell Collection Bag to the tubing set (see Figure 7.74).
    a. Make three hermetic seals in the tubing directly below valve 9, with a distance of at least 1 cm between each seal.
    b. Carefully sever the middle seal to disconnect the Cell Collection Bag from the tubing set.
2. Weigh the filled Cell Collection Bag. Record the weight.
    a. 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.
    b. Record the weight.
3. Mix the target cell suspension thoroughly by rotating the bag. Take a suitable amount, for example, 0.5 mL, and retain for analysis.

4. Using the MACS TubeSealer, seal off the tubing above the luer lock of the Negative Fraction Bag.
    a. Make three hermetic seals in the tubing, with a distance of at least 1 cm between each seal.
    b. Sever the center seal to disconnect the Negative Fraction Bag.
5. Disconnect the Buffer Waste Bag in the same way.
6. Remove the Negative Fraction Bag and Buffer Waste Bag.
7. Keep all bags until final analysis of all cells has been accomplished.
8. Remove the tubing set in the following order:
    a. Beginning with valves 6, 9, 10, and 11, and working upwards, press on the valves and release the tubing from the liquid sensor and from the valves.
    b. Release the columns from the column holders.
    c. Dispose the tubing set as a biohazard, according to standard hospital procedures.

The target cells can now be processed in accordance with clinical protocols. To continue, tap **ok**.

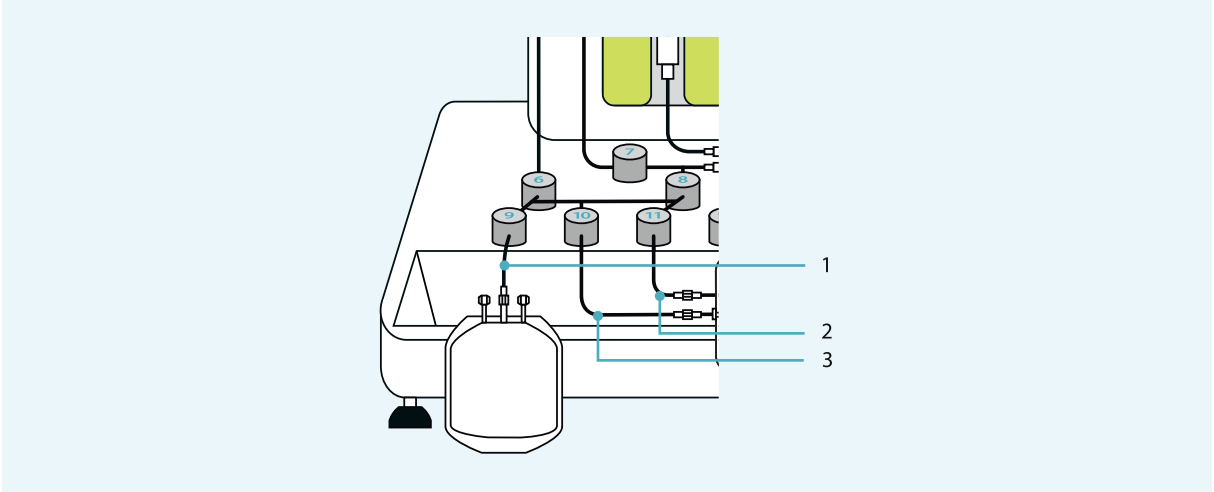


Figure 7.74: Seal off bags

1 Seal off Cell Collection Bag
2 Seal off Negative Fraction Bag
3 Seal off Buffer Waste Bag

Shutting down and cleaning the instrument

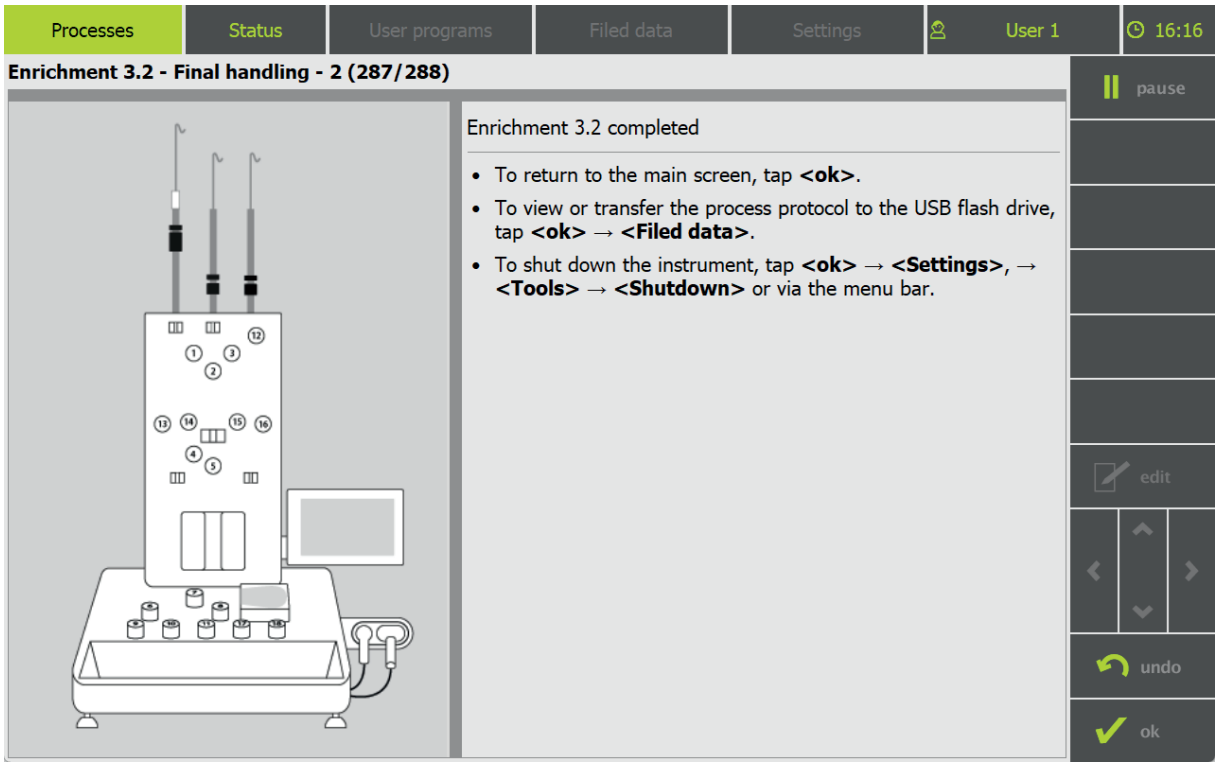


Figure 7.75: Completion of the separation program Enrichment 3.2

1. After the completion of the separation program, the user can choose one of the following options:
   - To return to the main screen, tap ok.
   - To view the process protocol or to transfer it to a storage device, tap ok > Filed data.
   - To shut down the instrument, tap ok > Settings > Tools > Shutdown.
2. Clean the instrument according to cleaning instructions as described in the CliniMACS Plus Instrument user manual. Follow the standard procedures for the treatment of infectious material.

Analysis of cells

⚠ CAUTION

Risk of reduced quality of target cells.

The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised.

- Examine the target cells regarding quality and quantity according to their intended use.

This must include the following parameters:

- total number of leukocytes
- viability and total number of target cells
- purity (depletion efficiency) and recovery of target cells

It is also recommended to determine the total number of leukocytes and the viability of the non-target cell fraction. The list is an example and other tests should be included based on the intended use and clinical protocols. Record the analysis data.

8

Troubleshooting

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

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

8.1 Miltenyi Biotec Technical Support

In any case of instrument malfunction or a process failure that cannot be corrected by the user, contact the Miltenyi Biotec Technical Support team:

+49 2204 8306-3803

technicalsupport@miltenyi.com

Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

8.2 Error and warning messages

The instrument and software can have malfunctions. Error and warning messages are shown on the screen and marked as such. If the error or warning cannot be corrected by the user, record the number shown in the message panel and contact Miltenyi Biotec Technical Support as soon as possible. One possible error message and warning message is shown in Figure 8.1 and Figure 8.2.

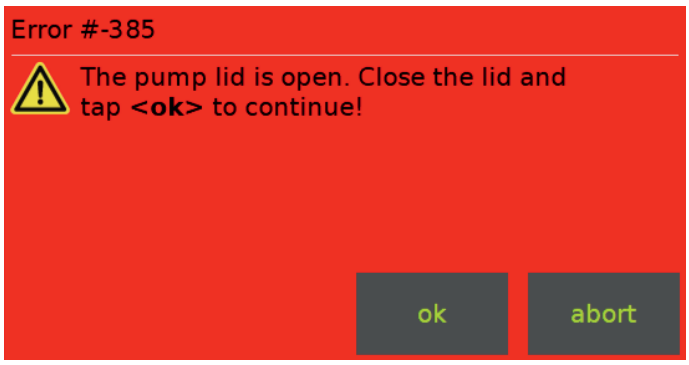


Figure 8.1: Error message

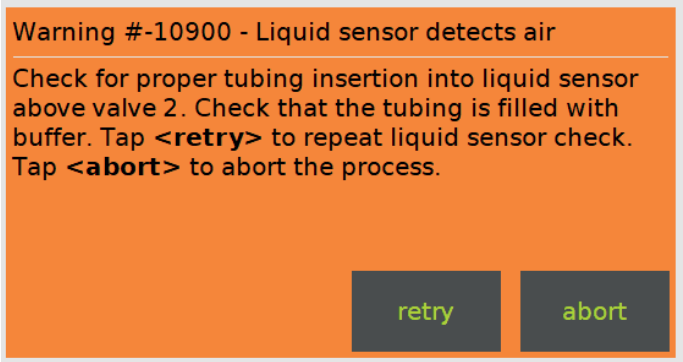


Figure 8.2: Warning message

8.3 MACS TubeSealer

For troubleshooting information about possible unexpected events related to tube sealing with the MACS TubeSealer, refer to chapter 'Troubleshooting' of the MACS TubeSealer user manual.

8.4 Barcode scanning

A barcode label cannot be scanned correctly.

Incorrect material scanned

- Ensure that the material is specified for the current separation program.
- Use the barcode reader to scan the barcode label again.

Barcode scanner does not scan the barcode label

- Make sure that the barcode label is clean, undamaged, and fully visible to the barcode reader.
- Use the barcode reader to scan the barcode label again.
- If the barcode cannot be scanned, enter the information manually.

Barcode is missing

To enter the material information manually, tap edit and enter the information in the input fields shown on the screen.

8.5 Preparation of the leukapheresis product

The cell product received is diluted

Ideally, magnetic labeling is performed in diluted leukapheresis product. Adjust the leukapheresis product to a final dilution of approximately 1:3.

- If the cell product received is more diluted than 1:3, or if the user does not exactly know the concentration of plasma in the cell product, add immunoglobulin to the cell product before the addition of the reagent. The recommended concentration of immunoglobulin in the labeling volume is 1.5 mg/mL.
- It is important to have a certain amount of immunoglobulin in the cell product during the labeling in order to minimize non-specific binding of the reagent.

The number of target cells in the leukapheresis product is low

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

Poor viability of cells in the leukapheresis product

The leukapheresis product may have been harvested, stored, or transported inappropriately.

To ensure better sample quality, the preparation and separation of the leukapheresis product should be performed immediately after leukapheresis.

- Keep the leukapheresis product at a leukocyte concentration of less than 0.2×10^9 per mL.
- If necessary, dilute the leukapheresis product with autologous plasma.
- The leukapheresis product should not be older than 24 hours when starting the labeling and separation procedure.
- If the leukapheresis product has to be stored, for example, overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).

8.6 CliniMACS Plus Instrument and CliniMACS Tubing Sets

8.6.1 Loading and priming of the tubing set

IMPORTANT:
Contact Miltenyi Biotec Technical Support for instructions on returning a defective tubing set.

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

The valves are designed to work properly once the tubing is inserted.

- Press the valve manually to open it.
- Watch the valve carefully during the seating of valves.

If the valve does not depress during the seating of valves, see 'Valve does not depress during seating of valves'.

Valve does not depress during seating of valves

Ensure that tubing is correctly inserted. Ensure that the valves are cleaned thoroughly. Any valve that is contaminated by fluid has to be exchanged.
Contact Miltenyi Biotec Technical Support.

Buffer is leaking from tubing set during priming

Tubing set is defective.

1. Abort the program by tapping **pause** > **abort**.
2. Remove the defective tubing set.
3. Restart the separation program using a new tubing set and sufficient new buffer.

Excessive air occurs in tubing set after priming

Buffer bag is not spiked properly.

1. Abort the program by tapping **pause** > **abort**.

2. Remove the defective tubing set.
3. Restart the separation program using a new tubing set and sufficient new buffer.

Ensure that the septum of the buffer bag is properly punctured.

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

Tubing set is not installed correctly.
If the tubing set is not installed correctly or the valves are not functioning properly, liquid can leak behind the valves. This may result in a poor performance of the CliniMACS Plus separation.

1. Abort the program by tapping **pause** > **abort**.
2. Remove the defective tubing set.
3. Restart the separation program using a new tubing set and sufficient new buffer.

Pump motor stalls during priming

Pump tubing is not inserted correctly.

1. Abort the program by tapping **pause** > **abort**.
2. Restart the separation program.
3. During the tubing set installation, clamp the tubing below the process buffer with a locking forceps and correct the position of the pump tubing.
4. Before starting the priming sequence, remove the locking forceps.

8.7 Automated cell separation

8.7.1 Emergency program (to be used with enrichment programs only)

If a run terminates irreversibly before the target cells are eluted from the separation column, the **Emergency Program** can be run to elute the cells from the separation column. The Emergency program is designed only for use with all enrichment programs together with either a CliniMACS Tubing Set (REF 161-01) or a CliniMACS Tubing Set LS (REF 162-01).

IMPORTANT:

- The Emergency program must not be used with any depletion programs.
- The Emergency program will elute approximately 75 mL of fluid. Ensure that a suitable Cell Collection Bag is connected to the tubing set.

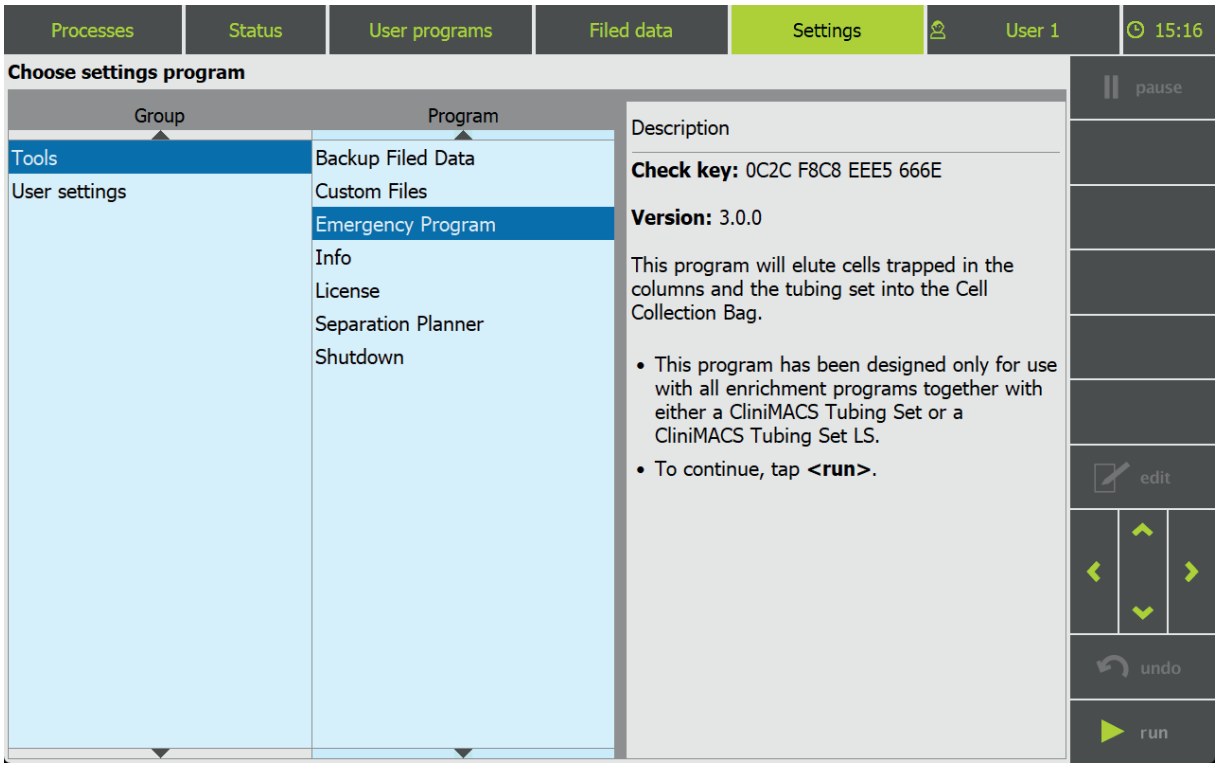


Figure 8.3: Selection of Emergency Program

1. Navigate to the emergency program by tapping **Settings** > **Tools** > **Emergency Program**. To start with the elution of the trapped cells, tap **run**.
    a. The magnet will be withdrawn from the magnetic separation unit. Refer to the CliniMACS Plus Instrument user manual for more information on the magnetic separation unit.
    b. Check if the magnet is withdrawn by holding a small magnetizable item to the magnetic separation unit. If the magnet is not withdrawn, or if there is an ongoing power failure, contact Miltenyi Biotec Technical Support.
2. Transfer the eluted cells collected in the Cell Collection Bag to a new 600 mL transfer bag.
3. Pool the remaining cells in the Cell Preparation Bag with the cells eluted by the emergency program.
4. Start a new separation procedure using a new tubing set and sufficient new buffer.

8.7.2 Unexpected events: All separation programs

Run is aborted before completion of cell separation program

- Pump door opened: For safety reasons, the separation in progress will automatically stop if the pump door is opened during the instrument run. An error message appears on the screen (see Figure 8.4).

  Close the pump door and tap retry.

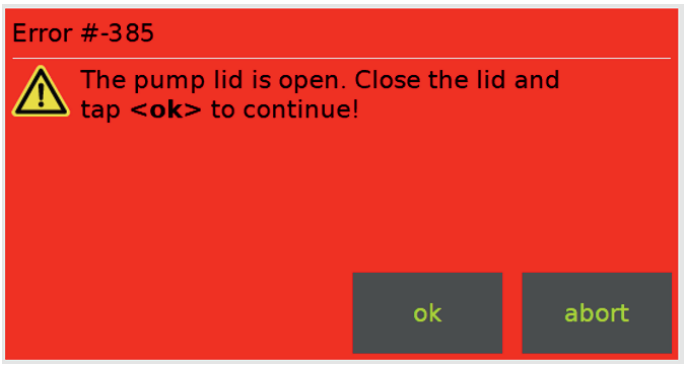


Figure 8.4: Error message no. 385

- Power failure: A power failure results in the termination of the CliniMACS Plus separation. Once terminated, the cell separation will not resume even if the power supply is restored. Contact Miltenyi Biotec Technical Support.
  - **Enrichment programs:** Recover as much of the cell product as possible from the tubing set by running the Emergency program (see section 'Emergency program (to be used with enrichment programs only)').

Liquid sensor detects air

If the liquid sensor is not able to detect liquid in the tubing, warning message no. 10900 will appear during the starting phase of the cell loading process. As the pre-system filter is rinsed with buffer prior to the cell loading, there must be liquid in the tubing at this point.

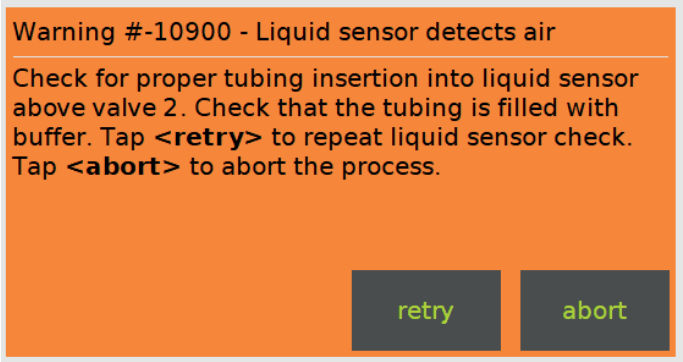


Figure 8.5: Warning message no. 10900 - Liquid sensor detects air

Check the following points:

1. Is the tubing inserted correctly into the liquid sensor above valve 2? If not, do so.
2. Is the tubing filled with buffer? If not, see point 3.

3. If the tubing is not filled with buffer, proceed as follows:
    a. Inspect the tubing for kinks blocking the buffer flow upwards into the pre-system filter and Cell Preparation Bag.
    b. Adjust the position of the tubing set. If necessary, raise or lower the bag hangers using the bag hanger clamps.
    c. Adjust the position of the tubing in the valves. To alter the position of the tubing, open the valve by manually pressing the button.
    d. Ensure that the tubing is not kinked, twisted, or taut.
4. Is the Cell Preparation Bag spiked properly? Ensure that the pre-system filter spike penetrates the septum of the Cell Preparation Bag port.
5. Is the tubing set completely filled with buffer? If not, ensure that the buffer spike of the tubing set penetrates the buffer bag. For corrective action, see 'Loading and priming of the tubing set'.

After the corrective action, continue the separation by tapping **retry**. If warning message no. 10900 appears again after each of the possible causes listed above have been ruled out, the liquid sensor may be defect. Contact Miltenyi Biotec Technical Support.

Cells move to wrong part of tubing set. Liquid is leaking past valves

- Tubing set is not properly inserted.
  If the instrument run is ongoing, tap **pause** and clamp the affected tubing with a locking forceps. Depress the appropriate valve by pushing the valve in and adjust the tubing. Remove the locking forceps and tap **resume** to continue the separation.
- Valve is not functioning properly.
  Tap **pause**. Clamp the affected tubing with a locking forceps. Depress the valve manually several times to unstick the stuck valve. Remove the locking forceps and continue the separation by tapping **resume**. If the valve cannot be unstuck, contact Miltenyi Biotec Technical Support.
- Wrong separation program used.
  Check the screen for the name of the program currently used. Abort the run by tapping **pause** > **abort** and immediately contact Miltenyi Biotec Technical Support.

Magnet does not move

Magnet drive is not functioning due to a failure.

An error message appears on the screen (see section 'Error and warning messages'). Record the number of the error message and contact Miltenyi Biotec Technical Support.

Pump motor stalls during cell separation

Pump tubing is not inserted correctly, so the pump might be unable to rotate. In this case, the error shown in Figure 8.6 will appear on the screen.

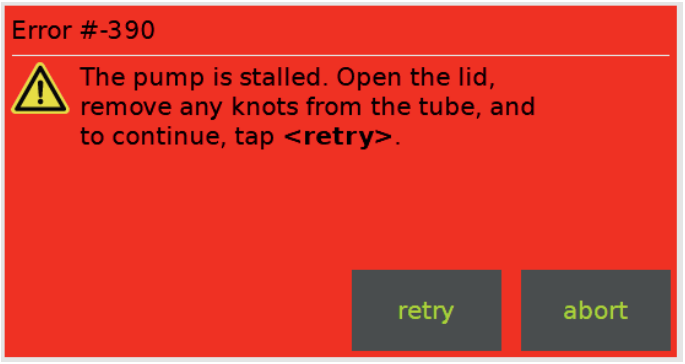


Figure 8.6: Error message no. 390

1. Carefully remove the pump tubing from the pump.
2. Remove any knots from the pump tubing and ensure that the tubing is not damaged by incorrect insertion.
    a. If the pump tubing is leaking, save the cells retained on the column by clamping the tubing above and below the separation column, then contact Miltenyi Biotec Technical Support.
    b. If the pump tubing is not damaged, it can be reinserted into the pump housing.
3. To continue with the separation, tap retry.

If the pump tubing is flat and collapsed, the separation column may be clogged. In this case, contact Miltenyi Biotec Technical Support.

Cell loading does not stop even though the Cell Preparation Bag and the pre-system filter are empty

The liquid sensor may not function correctly if the tubing surface inside the sensor is wet.

1. Tap pause to pause the separation.
2. Remove tubing from the liquid sensor.
3. Dry the tubing and the contact area using a lint-free cloth.
4. Reinsert the tubing into the liquid sensor and tap resume.

If the liquid sensor cannot be activated, skip cell loading and continue the separation by tapping Skip cell load > skip.

IMPORTANT:

- The Skip cell load function is not available for Depletion 3.1.
- Skipping the cell loading cannot be undone.

8.7.3 Unexpected events: Enrichment programs only

Run is aborted before completion of cell separation program

- Pump door opened: For safety reasons, the separation in progress will automatically stop if the pump door is opened during the instrument run. An error message appears on the screen (see Figure 8.7).

  Close the pump door and tap **retry**.

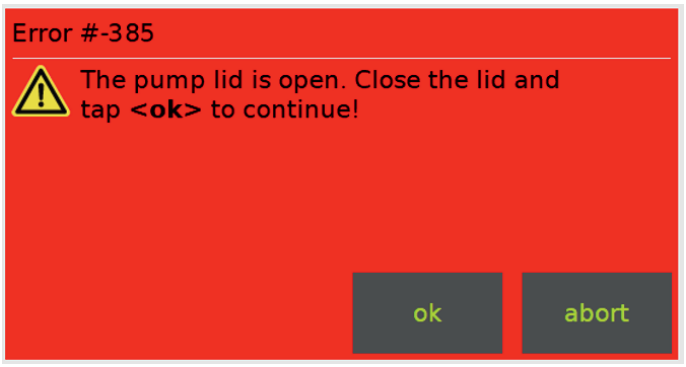


Figure 8.7: Error message no. 385

- Power failure: A power failure results in the termination of the CliniMACS Plus separation. Once terminated, the cell separation will not resume even if the power supply is restored. Recover as much of the cell product as possible from the tubing set by running the Emergency program (see section 'Emergency program (to be used with enrichment programs only)').

8.7.4 Unexpected events: CD133 Selection 1/2

Cell loading stopped before complete cell product is loaded onto the columns

The pre-system filter is clogged due to a large amount of cell debris or incomplete filling of the filter.

The continued pumping created a vacuum which led to the generation of air bubbles, thus triggering the liquid sensor. As a result, the separation process continued with the column washes before the complete cell product could be loaded. A part of the cell product remains in the Cell Preparation Bag after the separation is finished.

IMPORTANT:
It is not possible to restart the cell loading once the loading sequence has stopped.

1. Allow the CliniMACS Plus Instrument to finish the separation program. DO NOT abort the current run, as this may result in unnecessary cell loss.
2. After the separation procedure is finished, filter the remaining cell product with a 200 µm in-line blood filter and transfer it into a new transfer bag.
3. Immediately perform a second separation using a new tubing set and sufficient new buffer.

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

The pre-column is clogged due to large amount of cell debris in the Cell Preparation Bag.

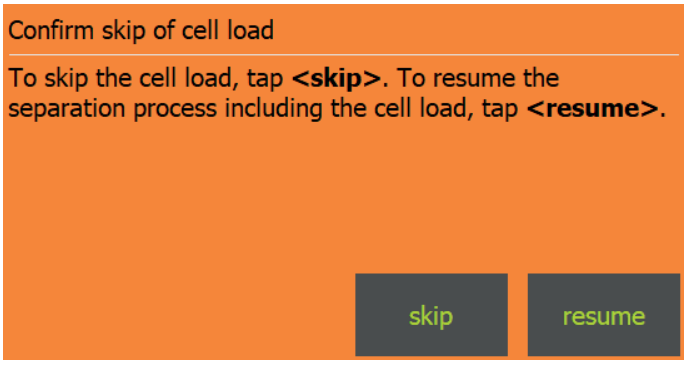
It is necessary to skip the loading of the remaining cell product manually and to continue the separation with the cells that have already been loaded onto the system.

IMPORTANT:
Skipping the cell loading cannot be undone.

1. To skip the cell loading process, tap **Skip cell load** on the right-hand side of the screen.

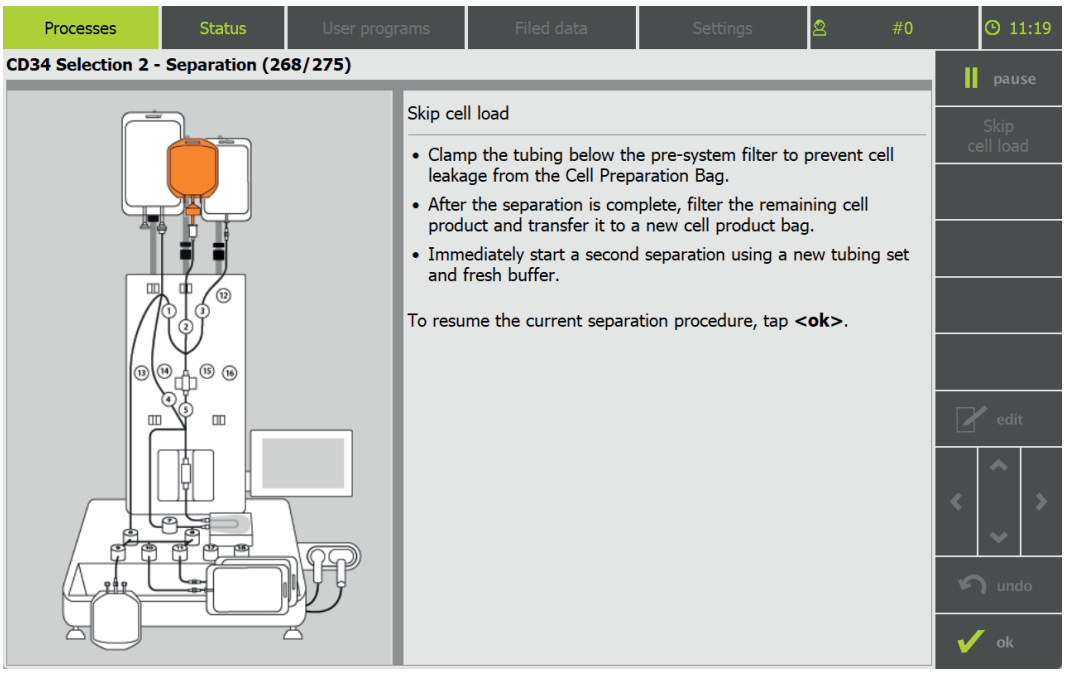


2. An orange pop-up window will appear for confirmation. To stop the cell loading and continue with the column washes, tap **skip**.



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

5. Immediately perform a second separation with a new tubing set and sufficient new buffer.



8.7.5 Unexpected events: Enrichment 1.1

Cell loading stopped before complete cell product is loaded onto the columns

The pre-system filter is clogged due to a large amount of cell debris or incomplete filling of the filter.

The continued pumping created a vacuum which led to the generation of air bubbles, thus triggering the liquid sensor. As a result, the separation process continued with the column washes before the complete cell product could be loaded. A part of the cell product may remain in the Cell Preparation Bag after the separation is finished.

IMPORTANT:
It is not possible to restart the cell loading once the loading sequence has stopped.

1. Allow the CliniMACS Plus Instrument to finish the separation program. DO NOT abort the current run, as this may result in unnecessary cell loss.
2. Determine the total cell number, percentage of target cells, and the remaining cell product volume in the Cell Preparation Bag. Enter actual cell product parameters during the parameter input (Step 2).
3. After the separation procedure has been finished, filter the remaining cell product with a 200 µm in-line blood filter and transfer it into a new transfer bag.
4. Immediately perform a second separation with a new tubing set and sufficient new buffer.

Wrong cell product parameter values were entered

The entered cell product volume was lower than the actual volume in the attached Cell Preparation Bag.

1. Allow the CliniMACS Plus Instrument to finish the separation program. DO NOT abort the current run, as this may result in unnecessary cell loss.
2. Determine the total cell number, percentage of target cells, and the remaining cell product volume in the Cell Preparation Bag. Enter actual cell product parameters during the parameter input (Step 2).

3. Process remaining cell product with a new tubing set, a new pre-system filter, and sufficient new buffer.

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

The locking forceps next to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked.

Tap **pause** and open the locking forceps next to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and tap **retry** to restart the elution sequence.

8.7.6 Unexpected events: Enrichment 3.2

Cell loading stopped before complete cell product is loaded onto the columns

The pre-system filter is clogged due to a large amount of cell debris or incomplete filling of the filter.
The continued pumping created a vacuum which led to the generation of air bubbles, thus triggering the liquid sensor. As a result, the separation process continued with the column washes before the complete cell product could be loaded. A part of the cell product remains in the Cell Preparation Bag after the separation is finished.

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

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

The locking forceps next to the Cell Collection Bag is not open during the elution sequence. Therefore, the pathway to the Cell Collection Bag is blocked.

Tap **pause** and open the locking forceps next to the Cell Collection Bag. Open the pump door and check whether the pump tubing is inserted correctly. Close the pump door and tap **retry** to restart the elution sequence.

8.8 Cell separation performance

8.8.1 Unexpected events: Enrichment programs only

The yield of target cells is low

- Target cells were poorly labeled with the reagent:
  - Reagent is expired. Check the use-by date. DO NOT use any reagent after the use-by date.
  - Reagent was not stored properly. Check the storage temperature. DO NOT use any reagent that has been stored improperly (see instructions for use of the reagent).
  - Recommended labeling procedure has not been followed. Refer to cell product preparation and separation procedure sections in the user manual.

- Cells were lost during the preparation steps.
  - Cells were removed with the supernatant into Plasma Waste Bag and Wash Waste Bags due to incomplete sedimentation or too early resuspension of the cells, for example, when the bag was removed from the centrifuge. Compare leukocyte content of the unlabeled cell product and the labeled cell product. Check the centrifugation settings for proper centrifugation. Determine cell counts from all waste bags.
  - Buffer did not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5% (w/v), (see section 'CliniMACS Materials required' of the respective application).
  - Centrifuge settings were suboptimal. Check the centrifugation settings.
  - Centrifuge imbalance or use of brake or asymmetrical loading of centrifuge.
- Analysis was incorrect.
  - Sampling error occurred. Check cell suspension for clumped or settled cells. Confirm that representative samples have been taken and repeat analysis.
  - Staining error occurred. Check flow cytometry reagents. Repeat staining.
  - Flow cytometer settings were improper. Check the instrument settings.

The purity of target cells is low.

- The cell product was stored inappropriately. Preparation and separation of the cell product should be performed immediately after leukapheresis. Keep the cell product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the cell product with autologous plasma. The cell product should not be older than 24 hours when starting the labeling and separation procedure. If the cell product has to be stored, for example, overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).
- The magnetic labeling protocol has not been followed (for example, incorrect volumes during magnetic labeling). Follow the instructions given for the magnetic labeling (see Step 1 in the section of the respective application). For troubleshooting purposes, determine the leukocyte subsets (B cells, T cells, monocytes, granulocytes as well as platelets) contaminating the target cell fraction and contact Miltenyi Biotec Technical Support for advice.
- High numbers of granulocytes contaminated the start product (suboptimal apheresis setting). Dying granulocytes will then bind the CliniMACS Reagent non-specifically which may lead to decreased purity of the target cells.
- Valve malfunction occurred. Eluted target cell fraction has been contaminated by part of the non-target fraction or buffer waste fraction. Inspect the tubing placement within the valves to ensure proper functioning. Assess target cell content of the non-target cell fraction and buffer waste. If necessary, pool the target and non-target cell fraction, reduce to a suitable volume, and repeat the separation with a new tubing set and sufficient new buffer.

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

- Buffer does not contain HSA. Supplement the buffer with HSA to a final concentration of 0.5% (w/v), (see section 'CliniMACS Materials required' of the respective application).

- The cell product may have been stored inappropriately. The preparation and separation of the cell product should be performed immediately after leukapheresis. Keep the cell product at a leukocyte concentration of less than 0.2×10^9 per mL. If necessary, dilute the leukapheresis with autologous plasma. The cell product should not be older than 24 hours when starting the labeling and separation procedure. If the cell product has to be stored, for example, overnight, it should be kept at controlled room temperature (+19 °C to +25 °C [+66 °F to +77 °F]).
- Incomplete cell product loading due to clogging of separation column, pre-system filter, or pre-column. Check the total cell number and depletion efficiency of the remaining cells.

8.8.2 Unexpected events: CD133 Selection 1/2 and Enrichment 1.1

The purity of the target cells is low

Elution from the separation column was incomplete.

- Separation program was aborted. Check screen for error message. Continue with section 'Run is aborted before completion of cell separation program'.
- Pump failure or valve failure occured. Recover cells from the tubing set following the emergency program described in section 'Emergency program (to be used with enrichment programs only)'. 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.
- Capacity of reagent and/or tubing set was exceeded. Refer to capacity limit of the relevant application.
- Tubing is not properly inserted. Check all valves for proper tubing insertion.

8.8.3 CD133 Selection 1/2

The purity of the target cells is low

Non-target cells were retained.

- Mobilization of target cells was poor. Very low number of target cells has occurred. Therefore, a low number of contaminating non-target cells (for example, granulocytes, monocytes, platelets) may lead to decreased purity.
- Insufficient plasma or immunoglobulins were present during magnetic labeling. Follow the instructions given for the magnetic labeling (see Step 1 of the respective application).
  If a final concentration of about 30% autologous plasma in the cell product during magnetic labeling cannot be guaranteed, add immunoglobulin to the cell product. A final concentration of 1.5 mg/mL is recommended for the efficient blocking of non-specific reagent binding during magnetic labeling.

8.8.4 Unexpected events: Enrichment 1.1

The yield of the target cells is low

Incorrect cell product parameter input. Cross-check the parameter input with the analysis results.

9

Legal notes

9.1 Limited warranty

Except as stated in a specific warranty statement, which may accompany this Miltenyi Biotec product, or unless otherwise agreed in writing by a duly authorized Miltenyi Biotec representative, Miltenyi Biotec's warranty for products purchased directly from Miltenyi Biotec shall be subject to the terms and conditions of sale under which it was provided to you by the respective Miltenyi Biotec sales organization. These terms and conditions are available on request or at www.miltenyibiotec.com. The applicable terms and conditions of sale may vary by country and region. Nothing herein should be construed as constituting an additional warranty.

For products purchased from third-party retailers or resellers (e.g., purchased from an Authorized Distributor of Miltenyi Biotec), different terms and conditions may apply.

To determine the warranty that came with your product, see your packing slip, invoice, receipt or other sales documentation. Some components of a product combination you purchased may have a shorter warranty than that listed on your packing slip, invoice, receipt or other sales documentation (e.g., goods subject to shelf life and obsolescence).

Miltenyi Biotec's warranty for this product only covers product issues caused by defects in material or workmanship during normal use. It does not cover product issues caused by any other reason, including but not limited to product issues due to use of the product in a manner other than specifically described in this manual, for example: inappropriate or improper use; incorrect assembly or installation by an operator or a third-party; reasonable wear and tear; negligent or incorrect operation, handling, storage, servicing, or maintenance; non-adherence to the operating instructions; unauthorized modification of or to any part of this product; or use of inappropriate consumables, accessories, or work materials.

Miltenyi Biotec's warranty does not cover products sold AS IS or WITH ALL FAULTS or consumables. Nothing herein should be construed as constituting an additional warranty.

Miltenyi Biotec must be informed immediately if a claim is made under such warranty. If a material or manufacturing defect occurs within the warranty period Miltenyi Biotec will take the appropriate steps to restore the full usability of the instrument.

Limitation on damages

Miltenyi Biotec shall not be liable for any incidental or consequential damages for breach of any express or implied warranty or condition on this product.

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10

Glossary

10.1 Glossary of symbols

An overview of symbols used for the CliniMACS Plus System is provided in the CliniMACS Plus Instrument user manual. The glossary of symbols depicts the symbols used in the user manuals and labeling of the CliniMACS Products.

10.2 Glossary of terms

Apheresis	The method of collecting blood in which whole blood is withdrawn, a desired component selected and retained, and the remainder of the blood returned to the donor
Bag compartment	Compartment of the CliniMACS Plus Instrument in which the Negative Fraction Bag and Buffer Waste Bag are placed
Bag hanger	Support on the CliniMACS Plus Instrument to mount the Cell Preparation Bag, Non-Target Cell Bag, Priming Waste Bag, Reapplication Bag, and buffer bag
Bone marrow aspirate	Sample of bone marrow removed by the insertion of a needle into the bone and pulled into a syringe
Buffer Waste Bag	Waste bag to collect buffer during cell separation using the CliniMACS Plus Instrument
CD133 antigen	The CD133 antigen is a 5-transmembrane cell surface antigen with a molecular weight of 117 kD and is expressed on 30–60% of all CD34-positive cells including CD34 bright cells
CD14 antigen	The CD14 antigen is a 53-55 kD cell membrane glycoprotein expressed on monocytes. The CD14 antigen is known as a receptor for complex of LPS and LPS binding protein (LBP)
CD271 antigen	The CD271 antigen is a member of the NCAM-family (Neural Cell Adhesion Molecule-family) which is expressed within the hematopoietic system on NK, NKT cells. The CD271 antigen, also known as low-affinity nerve growth factor receptor, is expressed on mesenchymal stromal cells (MSC) and can be used for the direct enrichment of these cells from bone marrow aspirate.

CD62L antigen	The human CD62L antigen is a 74 kDa glycoprotein and a member of the selectin family of cell surface molecules. The CD62L antigen, also known as L-selectin, LECAM-1, or LAM-1, binds a series of glycoproteins including CD34, GlyCAM- 1, and MAdCAM-1. CD62L is important for homing of naive lymphocytes via endothelial venules to peripheral lymph nodes and Peyer's patches. The CD62L antigen also contributes to the recruitment of leukocytes from the blood to areas of inflammation. Most hematopoietic cells express CD62L, including most peripheral blood B cells, T cells, monocytes, granulocytes, and some myeloid cells from bone marrow, and thymocytes. CD62L is continuously endoproteolytically cleaved from the cell surface of neutrophils and lymphocytes. Proteolysis is accelerated, for example, after antigenic activation of T cells
Cell Collection Bag	Bag in which the purified target cells are accumulated after separation
Cell Preparation Bag	Bag into which cell product is transferred and in which magnetic labeling and washing of cells are performed
CliniMACS CD133 Reagent	Reagent for magnetic labeling of cells expressing the CD133 antigen
CliniMACS CD14 Reagent	Reagent for magnetic labeling of cells expressing the CD14 antigen
CliniMACS CD271 Reagent	Reagent for magnetic labeling of cells expressing the CD271 antigen
CliniMACS CD62L Reagent	Reagent for magnetic labeling of cells expressing the CD62L antigen
CliniMACS PBS/EDTA Buffer	Buffer used for cell preparation and cell separation with the CliniMACS Plus System: PBS (phosphate buffered saline), supplemented with 1 mM EDTA, pH 7.2. Before use, CliniMACS PBS/EDTA Buffer must be supplemented with pharmaceutical grade HSA to a final concentration of 0.5% (weight/volume, i.e., 5 g HSA per liter buffer)
CliniMACS Plus Instrument	Magnetic cell separation instrument based on the MACS Technology
CliniMACS Tubing Set, CliniMACS Tubing Set LS	Set of tubing, connectors, columns, and bags through which the magnetically labeled cell suspension is processed and in which the magnetic cell separation takes place
EDTA	Ethylene-diamine-tetra-acetic acid
×g	Multiples of the earth's gravitational acceleration
Hematopoietic progenitor cells	Progenitor cells of lymphoid, myeloid, and erythroid lineages
HSA	Human serum albumin
Human serum albumin	Pharmaceutical grade HSA approved in the country of the user is necessary as a buffer supplement when used with the CliniMACS Plus System
IgG	Immunoglobulin G
In-bag procedure	Sterile processing and transferring the cells in the closed environment of transfer bags

Labeling	Reaction of cells with magnetic labeling reagent, e.g., CliniMACS CD34 Reagent to CD34-positive cells
Leukapheresis	Apheresis collecting leukocytes
Liquid sensor	Component of the CliniMACS Plus Instrument that detects liquid in the tubing
Luer connector	Screw coupling, part of the tubing set
Luer/Spike Interconnector	Transfer set with piercing pin (spike) and syringe adapter (female luer) for plasma or fluids
MACS TubeSealer	Heat sealer used for sealing PVC and EVA tubes of the tubing sets installed on the instrument
Magnetic antibody	A super-paramagnetically labeled antibody
MDC	Myeloid dendritic cells
Monoclonal antibodies	A single type of antibody that is directed against a specific epitope (antigen, antigenic determinant) and is produced by a single clone of B cells or a single hybridoma cell line, which is formed by the fusion of a lymphocyte cell with a myeloma cell. Some myeloma cells synthesize single antibodies naturally
Negative Fraction Bag	Bag of the CliniMACS Tubing Set and CliniMACS Tubing Set LS containing the non-target cell fraction
Orbital rotator	Device used to mix cell product during the reaction with CliniMACS Reagents
PBMC	Peripheral Blood Mononuclear Cell
Peristaltic pump	Tubing pump used in the CliniMACS Plus Instrument to control the flow rate of fluid in the tubing set
Plasma extractor	Device used to extract liquid from the Cell Preparation Bag after cell washing
Plasma transfer set	Transfer set with two piercing pins (spikes) for plasma or fluids
Plasma Waste Bag	Waste bag to collect excess plasma prior to the labeling procedure
Pre-column	First column in the CliniMACS Tubing Set and the CliniMACS Tubing Set LS, serves as filter to trap cells having non-specific interactions with the column matrix
Pre-column holder	Support mounted on the CliniMACS Plus Instrument that holds the pre-column in place
Pre-system filter	40 µm filter device between Cell Preparation Bag and precolumn used to trap clumps and cell debris
Priming	Step prior to cell separation in which buffer is flushed through the tubing set
Priming Waste Bag	Bag in which buffer from priming step is collected
Pump safety switch	Sensor that prevents pump operation when the pump door is open

Retaining ring	Part of a tubing set that enables the pump tubing to remain in its proper location
rpm	Revolutions per minute
Sampling site coupler	Injection port, e.g., for removal of samples or addition of CliniMACS Reagents to the Cell Preparation Bag
Separation column	Column in which magnetically labeled cells are separated when exposed to the magnetic field
Separation column holder	Molded guides in the magnet housing that holds the separation column in place
Separation program	Software program designed for the enrichment or depletion of magnetically labeled cell subsets from a mixed cell population. Choose from a menu of separation programs depending on the intended separation
Separation reagent	Reagent for magnetic labeling of cells, e.g., CliniMACS CD34 Reagent
T-fitting	T-shaped fitting on a tubing set where three tubing meet
Transfer bag	Bag with a tubing and a spike at the end
Wash Waste Bag	Collection bag in which the wash supernatant is collected by separation from the sedimented cell suspension after centrifugation steps during sample preparation
WBC	White blood cells
Starting cell product	Cell-containing product used as starting material for the CliniMACS Plus separation process, e.g., leukapheresis harvest, PBMCs, or bone marrow

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