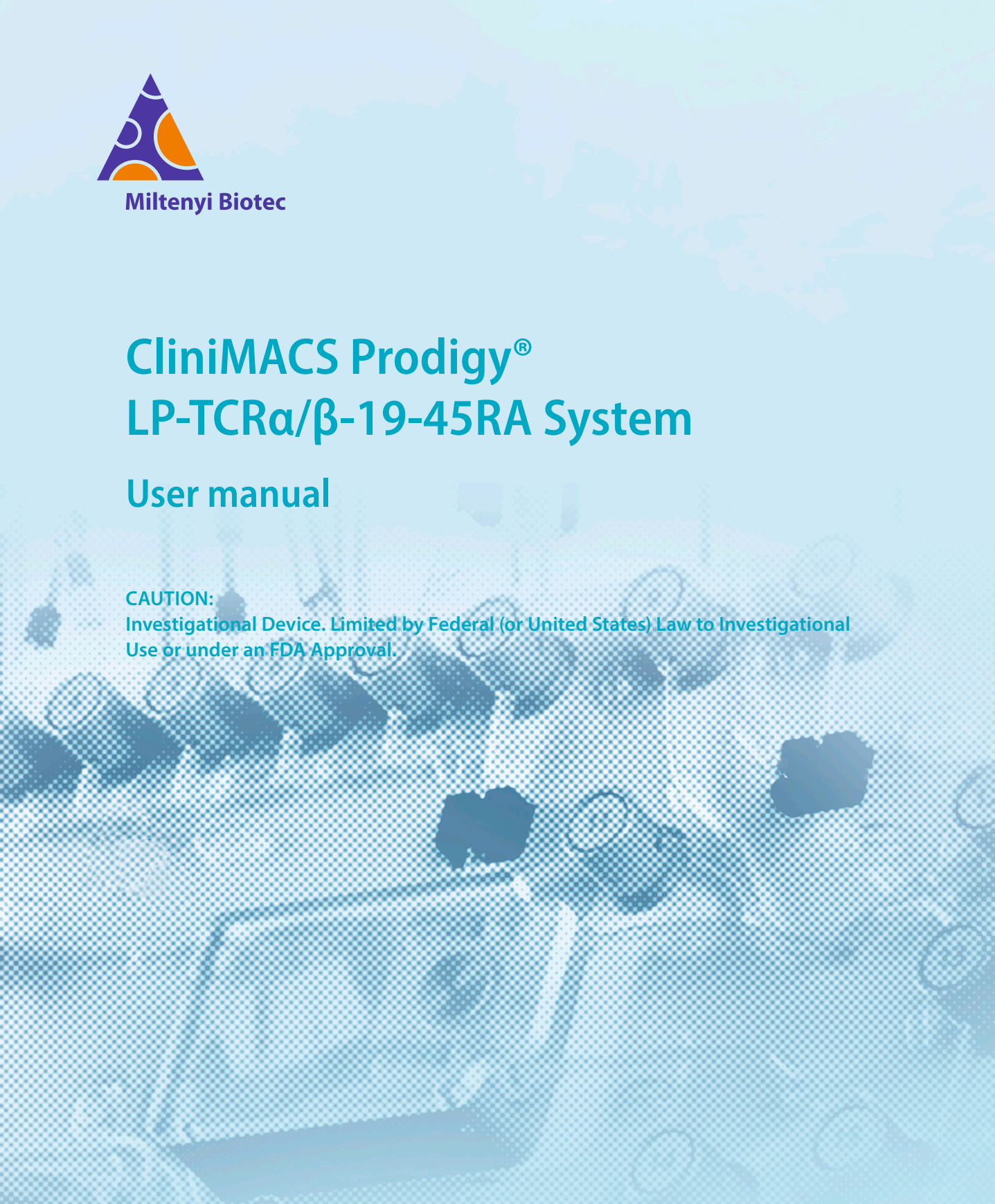
Miltenyi Biotec

CliniMACS Prodigy®
LP-TCRα/β-19-45RA System

User manual

CAUTION:
Investigational Device. Limited by Federal (or United States) Law to Investigational Use or under an FDA Approval.



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

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CliniMACS Prodigy®
LP-TCRα/β-19-45RA System

User manual

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

For U.S. distributed by:
Miltenyi Biotec Inc
2303 Lindbergh Street
Auburn, CA 95602
USA

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

www.miltenyibiotec.com

Essential information

This user manual provides information for the use of the CliniMACS Prodigy LP-TCRα/β-19-45RA System. For further details on other processes running on the CliniMACS Prodigy, refer to the CliniMACS Prodigy user manual for the respective application. Instructions, warnings, precautions, and other important information for the use of the instrument as well as warnings and precautions concerning the handling of biohazardous materials and starting cell product are described in the CliniMACS Prodigy user manual (Instrument). For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Prodigy LP-TCRα/β-19-45RA System components, refer to the instructions for use provided for the respective component.

⚠ WARNING

The operation of the CliniMACS Prodigy System must be performed by trained operators only. Before putting the system into operation, carefully read and understand the safety information, warnings, precautions, and instructions for proper operation of the CliniMACS Prodigy provided in the instructions for use of the CliniMACS Prodigy System components (including, without limitation, the safety information in the CliniMACS Prodigy user manual (Instrument), chapter 3 "Important safety information") and in any safety-related recommendations issued by Miltenyi Biotec. Pay special attention to all warnings displayed on the instrument or provided with consumables and accessories. The operator must adhere to all instructions and procedures at all times during the operation of the instrument, confirming that all safety information, warnings, precautions, and instructions are observed. Failure to follow the safety information, warnings, precautions, and instructions contained in the instructions for use can result in instrument malfunction, property damage, loss of target cells, personal injury, and/or death.

Retain the instructions for use 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 for all personnel responsible for installation, operation, and maintenance.

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1 Introduction

1.1 General information

The CliniMACS Prodigy LP-TCRα/β-19-45RA System including the CliniMACS Prodigy, the CliniMACS TCRα/β-Biotin and CliniMACS TCRα/β-Biotin XL, the CliniMACS Anti-Biotin Reagent and CliniMACS Anti-Biotin Reagent XL, the CliniMACS CD19 Reagent and CliniMACS CD19 Reagent XL, the CliniMACS CD45RA Reagent XS, the CliniMACS Prodigy TS 320, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* depletion of human TCRα/β, CD19, and CD45RA-expressing cells from leukapheresis products.

The LP-TCRab-19-45RA Depletion application includes five use cases:

Use case 1: Depletion of CD45RA-positive cells (LP-45RA Depletion process)

Use case 2: Depletion of TCRα/β-positive cells (LP-TCRab Depletion process)

Use case 3: Depletion of TCRα/β-positive and CD19-positive cells (LP-TCRab-19 Depletion process)

Use case 4: Depletion of TCRα/β-positive and CD45RA-positive cells (LP-TCRab-45RA Depletion process)

Use case 5: Depletion of TCRα/β-positive, CD19-positive and CD45RA-positive cells (LP-TCRab-19-45RA Depletion process)

Figure 1.1 illustrates the different use cases with the depletion sequences included. Use case 1 performs a CD45RA depletion. Use case 2 executes a TCRα/β depletion, whereas use case 3 enables a simultaneous TCRα/β and CD19 depletion. Use case 4 performs a CD45RA depletion followed by a TCRα/β depletion. Use case 5 combines a CD45RA depletion followed by a simultaneous TCRα/β and CD19 depletion.

Pre-process, intermediate process and post process include preparative handling steps for the use case selected.

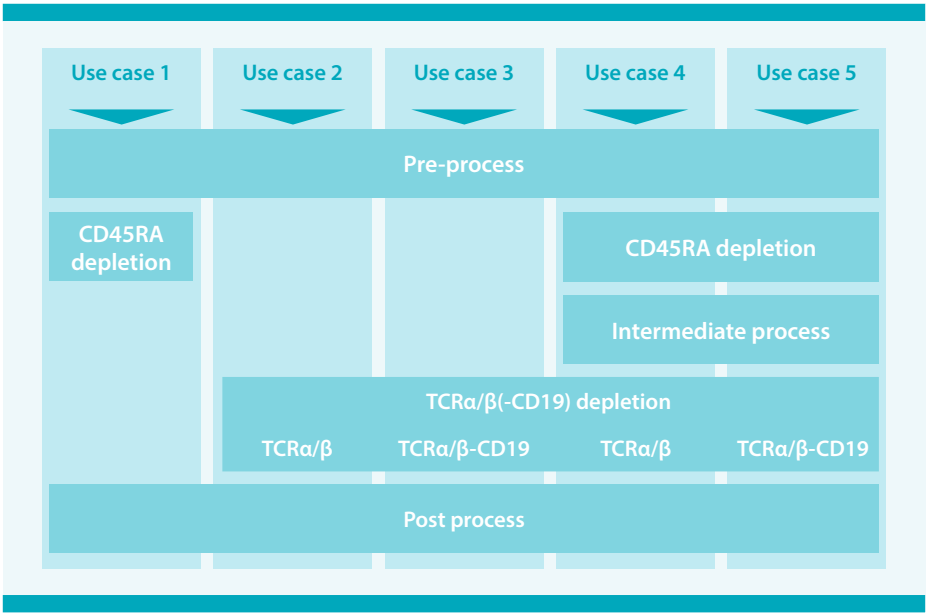


Figure 1.1: Use cases LP-TCRab-19-45RA Depletion

All TCRα/β(-CD19) depletion-including use cases (i.e., use case 2–5) can be performed either as normal-scale (NS) or large-scale (LS) procedures.

Use cases 4 and 5 are combining two depletion parts and start after initial preparations (pre-process) with a CD45RA depletion. Therefore, a defined part of the leukapheresis product is magnetically labeled with CliniMACS CD45RA Reagent XS. Subsequently, the magnetically labeled cells are depleted in a continuous flow separation sequence.

At the end of the CD45RA depletion part the first target cell product, depleted of CD45RA-positive cells, is provided for further use. The instrument is prepared for the second depletion part (intermediate process). In this second depletion part the remaining leukapheresis product is either depleted of TCRα/β-positive cells (use case 4) or simultaneously depleted of TCRα/β and CD19-positive cells (use case 5).

To do so, the cell product is incubated with the CliniMACS TCRα/β-Biotin (or CliniMACS TCRα/β-Biotin XL) first. In the subsequent magnetic labeling step the cell product is then simultaneously labeled with CliniMACS Anti-Biotin Reagent (or CliniMACS Anti-Biotin Reagent XL), use case 4, and CliniMACS CD19 Reagent (or CliniMACS CD19 Reagent XL), use case 5. Subsequently, the magnetically labeled cells are depleted in a continuous flow separation process.

At the end of this second depletion part the second target cell product, depleted of TCRα/β (use case 4) and additionally CD19-positive cells (use case 5), is provided for further use.

Figure 1.2 illustrates the split of the starting cell product for the two depletion parts of the LP-TCRab-19-45RA Depletion application.

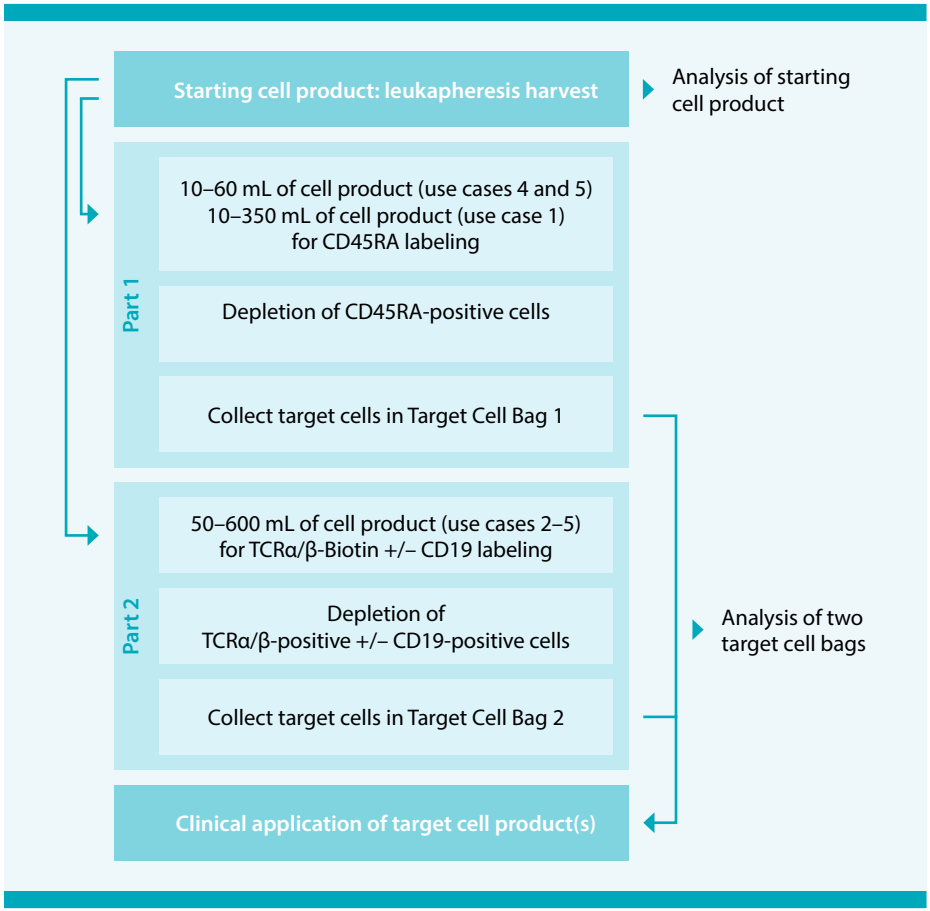


Figure 1.2: Process overview CliniMACS Prodigy LP-TCRab-19-45RA Depletion

1.2 Separation process

The depletion procedure of TCRα/β, CD19, and CD45RA-expressing cells with the CliniMACS Prodigy LP-TCRα/β-19-45RA System is automated and requires only minimal preparation steps:

- Install the CliniMACS Prodigy TS 320 on the instrument.
- Attach process buffer, infusion solution, rinsing solution (for use cases 4 and 5 only).
- Enter separation-related cell product information after priming of the tubing set.
- Transfer the starting cell product into the Application Bag.
- Transfer the CliniMACS Reagents into the dedicated reagent bags.

For detailed information regarding the preparation steps required see section 6.1 ´Initialization of the CliniMACS Prodigy´ to section 6.5.3 ´Connection of CliniMACS Reagents´.

The process can be paused at a specified time in order to make the TCRα/β-(CD19)-depleted cell product available according to specific logistic needs. For detailed information, see section 6.5.4 ´Process end time (storage option)´.

Once the process has been started, subsequent steps are automated and only minimal further handling is required. The target cells (TCRα/β(-CD19)-negative and/or CD45RA-negative cells) are provided in infusion solution in separate bags, ready for further use. Samples for quality control can be taken at the beginning of the process, after CD45RA depletion, before TCRα/β(-CD19) depletion, and at the end of the entire process.

Depletion process	Capacity total WBC	Capacity labeled cells
Use case 1		
LP-45RA	6×10^9	2.4×10^9
Use case 2		
LP-TCRab (NS)	60×10^9	24×10^9
LP-TCRab (LS)	120×10^9	48×10^9
Use case 3		
LP-TCRab-19 (NS)	60×10^9	24×10^9 (TCRα/β) 10×10^9 (CD19)
LP-TCRab-19 (LS)	120×10^9	48×10^9 (TCRα/β) 15×10^9 (CD19)
Use case 4		
LP-TCRab-45RA (NS)	6×10^9 (CD45RA depletion) 60×10^9 (TCRα/β/CD19 depletion)	2.4×10^9 (CD45RA) 24×10^9 (TCRα/β)
LP-TCRab-45RA (LS)	6×10^9 (CD45RA depletion) 120×10^9 (TCRα/β depletion)	2.4×10^9 (CD45RA) 48×10^9 (TCRα/β)
Use case 5		
LP-TCRab-19-45RA (NS)	6×10^9 (CD45RA depletion) 60×10^9 (TCRα/β/CD19 depletion)	2.4×10^9 (CD45RA) 24×10^9 (TCRα/β) 10×10^9 (CD19)
LP-TCRab-19-45RA (LS)	6×10^9 (CD45RA depletion) 120×10^9 (TCRα/β/CD19 depletion)	2.4×10^9 (CD45RA) 48×10^9 (TCRα/β) 15×10^9 (CD19)

Table 1.1: Overview of use case-related process specifications

As depicted in Table 1.1 the process capacities for the different use cases are as follows:

- **Normal-scale TCRα/β(-CD19) depletion** (use case 2/3 and 4/5)
  Up to 24×10^9 TCRα/β-positive cells (and up to 10×10^9 CD19-positive cells) from up to 60×10^9 WBC
- **Large-scale TCRα/β(-CD19) depletion** (use case 2/3 and 4/5)
  Up to 48×10^9 TCRα/β-positive cells (and up to 15×10^9 CD19-positive cells) from up to 120×10^9 WBC
- **CD45RA depletion** (use case 1 and 4/5)
  Up to 2.4×10^9 CD45RA-positive cells from 6×10^9 total WBC

IMPORTANT

The process duration for the different use cases depends on the number of WBC, the number of labeled cells and therefore on the number of stages required for the depletion sequences.

2
Glossary

2.1 Graphical depiction

The following chart depicts the panels used in this user manual to inform the user about potential risks if the outlined warnings and precautions are not followed. The hazard level classifies the hazard, as described below. The level, type, and source of the hazard, as well as potential consequences, prohibitions, and measures are indicated as follows. Icons on the left side specify the risk.

⚠ 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 information considered important, but not hazard-related (e.g., messages relating to property damage).

IMPORTANT

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

2.2 Glossary of symbols and terms

An overview of symbols and terms used for the CliniMACS Prodigy System is provided in the CliniMACS Prodigy user manual (Instrument). The glossary of symbols depicts the symbols used for labeling of the CliniMACS Products.

3 Materials required

The following section outlines the materials and consumables that are required for use of the CliniMACS Prodigy LP-TCRα/β-19-45RA System.

3.1 System components

The following CliniMACS Materials are required:

CliniMACS Materials	Quantity
CliniMACS Prodigy	1
CliniMAC S TCRα/β-Biotin	1
CliniMACS TCRα/β-Biotin XL	1
CliniMACS Anti-Biotin Reagent	2
CliniMACS Anti-Biotin Reagent XL	1
CliniMACS CD19 Reagent	2
CliniMACS CD19 Reagent XL	1
CliniMACS CD45RA Reagent XS	1
CliniMACS Prodigy TS 320	1
CliniMACS PBS/EDTA Buffer, 3 L	3–4

Table 3.1: CliniMACS Materials required

Table 3.1 lists up all possible system components. Refer to Table 3.4 for an overview of concrete quantitative material requirements for all use cases and scales available.

⚠ CAUTION

Risk of process failure. If an incorrect reagent variant is used, the process cannot be executed. Follow the instructions and pay particular attention to use the appropriate CliniMACS Reagent variant applicable for the use case and scale selected (e.g., CliniMACS TCRα/β-Biotin vs. CliniMACS TCRα/β-Biotin XL).

Only use the CliniMACS Materials mentioned in Table 3.1 in section 3.1 ´System components´ for the LP-TCRab-19-45RA Depletion application.
See section 1.1 ´General information´ for an overview of the different use cases of the LP-TCRab-19-45RA Depletion application.

All large-scale TCRα/β(-CD19) procedures of the LP-TCRab-19-45RA Depletion application mandatorily require the use of CliniMACS XL reagent variants, whereas all normal-scale TCRα/β(-CD19) procedures require the use of the standard reagent variants. All use cases which are including a CD45RA depletion require the use of CliniMACS CD45RA Reagent XS.

See Table 3.4 in 3.4 ´Summary of required materials´ of this user manual for an overview of quantitative material requirements of all use cases available.

3.2 Accessories

In addition to the system components, further accessories are required (see Table 3.2).

CliniMACS Accessories	Quantity
Transfer Set Coupler/Coupler	2–7

Table 3.2: CliniMACS Accessories required

3.3 Additional materials and equipment

In addition to the system components and the accessories, further materials and equipment are required.

Materials and equipment
Human serum albumin (HSA), pharmaceutical grade, to be added to the CliniMACS PBS/EDTA Buffer and to the infusion solution to a final concentration of 0.5% (w/v)
Appropriate Luer Lock syringes (10 mL, 50 mL) and hypodermic 20 gauge needles
Transfer bag(s) 1,000 mL
3-way tube adapter (optional)
Infusion solution (e.g., sodium chloride solution, for infusion), preferably in bags
Rinsing solution (aqua bidest. for injection use), preferably in bags
Uninterruptable power supply unit (optional)
Sterile docking device
Cell counter
Flow cytometer

Table 3.3: Additional materials and equipment required

IMPORTANT

This protocol requires the use of components, accessories, and materials, which are not part of the CliniMACS Prodigy LP-TCRα/β-19-45RA System (e.g., human serum albumin (HSA)). Therefore, either components, e.g., infusion solution, rinsing solution and HSA, which are registered as drugs in the country of the user must be used or, if not available as registered drugs, all risks arising from these components must be evaluated by the user before application of the target cell product(s) to humans. Special attention should be given to the stability of the target cell product(s) in the chosen infusion solution.

3.4 Summary of required materials

The relevant quantities of system components and additional materials needed for the different use cases and scales of the LP-TCRab-19-45RA Depletion application are specified in Table 3.4 below.

Item	Use case								
	1	2		3		4		5	
		NS	LS	NS	LS	NS	LS	NS	LS
CliniMACS Materials									
CliniMACS Prodigy TS 320 (pc)	1	1	1	1	1	1	1	1	1
CliniMACS TCRα/β-Biotin (vial)	–	1	–	1	–	1	–	1	–
CliniMACS TCRα/β-Biotin XL (vial)	–	–	1	–	1	–	1	–	1
CliniMACS Anti-Biotin Reagent (vial)	–	2	–	2	–	2	–	2	–
CliniMACS Anti-Biotin Reagent XL (vial)	–	–	1	–	1	–	1	–	1
CliniMACS CD19 Reagent (vial)	–	–	–	2	–	–	–	2	–
CliniMACS CD19 Reagent XL (vial)	–	–	–	–	1	–	–	–	1
CliniMACS CD45RA Reagent XS (vial)	1	–	–	–	–	1	1	1	1
CliniMACS PBS/EDTA Buffer (3 L bags)	1	3	≤9 stages: 3 >9 stages: 4	≤9 stages: 3 >9 stages: 4	≤9 stages: 3 >9 stages: 4	3	≤9 stages: 3 >9 stages: 4	≤9 stages: 3 >9 stages: 4	≤9 stages: 3 >9 stages: 4
Additional materials									
Infusion solution (L)	1	2	2	2	2	2	2	2	2
Rinsing solution (L)	–	–	–	–	–	0.5	0.5	0.5	0.5

Table 3.4: Overview of the quantities of CliniMACS Materials and additional materials required

4

Warning and precautions

At all times, local working area safety instructions, policies, standards regarding good manufacturing practice, health, safety, and prevention of accidents must be adhered to.

Instructions, warnings, precautions, and other important information for the use of the CliniMACS Prodigy as well as warnings and precautions concerning the handling of biohazardous materials and starting cell product are described in the CliniMACS Prodigy user manual (Instrument). For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Prodigy LP-TCRα/β-19-45RA System components, refer to the instructions for use provided for the respective component.

Any serious incident that has occurred in relation to this product should be reported to Miltenyi Biotec B.V. & Co. KG – using the contact information provided – and the competent authority of the member state in which the user of this product is established.

5 Preparative steps

The depletion of TCRα/β, CD19, and CD45RA-expressing cells with the CliniMACS Prodigy LP-TCRα/β-19-45RA System is automated and requires only a few preparative steps.

⚠ WARNING

Risk of infection. Preparative steps that are not performed in a sterile environment carry the risk of infection. Perform all preparative steps in a sterile environment (e.g., laminar flow hood) using aseptic techniques.

5.1 Testing of the starting cell product

⚠ WARNING

Risk of infection. Depending on the biological material used, contact may lead to severe personal injury or death. Always wear personal safety equipment in accordance with warnings and precautions, in particular if biohazardous material is or has been used.

Depending on the use case selected, the starting leukapheresis product needs to be tested for the following parameters:

- WBC concentration ($\times 10^6$/mL), all use cases
- Frequency of TCRα/β (alternatively CD3)-positive cells (%), use cases 2–5
- Frequency of CD19 (alternatively CD20)-positive cells (%), use cases 3 and 5
- Frequency of CD45RA-positive cells (%), use case 1, 4, and 5
- Cell product volume (mL), all use cases

The results received must be entered in the software during the setup of the process.

For testing of the starting cell product a fluorochrome staining for CD45, CD45RA, TCRα/β (CD3) and CD19 (CD20) is recommended. The percentage of all CD45RA-positive cells is used for parameter input related to the CD45RA depletion part. The frequency of CD3-positive cells can be determined instead of TCRα/β frequency. As CD20 staining (instead of CD19 staining) is required for analytical process monitoring, CD20 frequency can also be used for parameter input related to the TCRα/β(-CD19) depletion part. Contact Miltenyi Biotec Technical Support for analysis support, if required.

A dead cell exclusion must not be performed to determine WBC concentration and frequency of labeled cells. Depending on the use case selected, all TCRα/β-positive (CD3-positive), CD45RA-positive, and CD19-positive (CD20-positive) cells are to be included irrespective of potential non-specific binding of fluorochrome antibodies.

Based on the cell parameter input, total WBC and labeled cell numbers are calculated and the number of required loading stages for the depletion sequence(s) is automatically determined.

⚠ CAUTION

Risk of reduced quality of target cells. Wrong cell parameter input can lead to reduced quality of target cells. The cell parameter input must exactly reflect the actual values of the leukapheresis product to be processed. Make sure that no leukocyte population is excluded from the analysis.

5.2 Preparation of process buffer

Supplement the required amount (see Table 3.4 in section 3.4 ´Summary of required materials´) of CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (w/v), i.e., add 25 mL of 20% HSA per liter of buffer. The HSA-supplemented buffer is henceforth referred to as "process buffer".

IMPORTANT

HSA is not a component of this system. Use only pharmaceutical grade HSA approved in your country. Carefully read the package insert of the HSA used; in particular the section regarding hypersensitivity reactions and the risk of infection associated with HSA as a blood-derived product. Evaluate all risks arising from this material.

5.3 Preparation of infusion solution

Supplement the required amount (see Table 3.4 in section 3.4 ´Summary of required materials´) of suitable solution for infusion (e.g., NaCl, for infusion) with HSA to a final concentration of 0.5% (w/v), i.e., add 25 mL of 20% HSA per liter of infusion solution. Follow the HSA-related statements in the **IMPORTANT** paragraph of section 5.2 ´Preparation of process buffer´ for preparation of the infusion solution. The HSA-supplemented solution is henceforth referred to as "infusion solution".

If the infusion solution container is not suitable for connection to the CliniMACS Prodigy TS 320, then transfer the infusion solution volume required into a suitable transfer bag prior to connection.

If more than one bag of infusion solution is required, connect the bags to each other with a plasma transfer set or an appropriate adapter, e.g., 3-way tube adapter. Make sure that the item used for connection is clamped prior to interconnection.

5.4 Preparation of rinsing solution

500 mL aqua bidest., for injection use, are required for use cases 4 and 5 of the LP-TCRab-19-45RA Depletion application. This solution is henceforth referred to as “rinsing solution”.

If the rinsing solution container is not suitable for connection to the CliniMACS Prodigy TS 320, then transfer the rinsing solution into a suitable transfer bag prior to connection.

6

Separation process

6.1 Initialization of the CliniMACS Prodigy

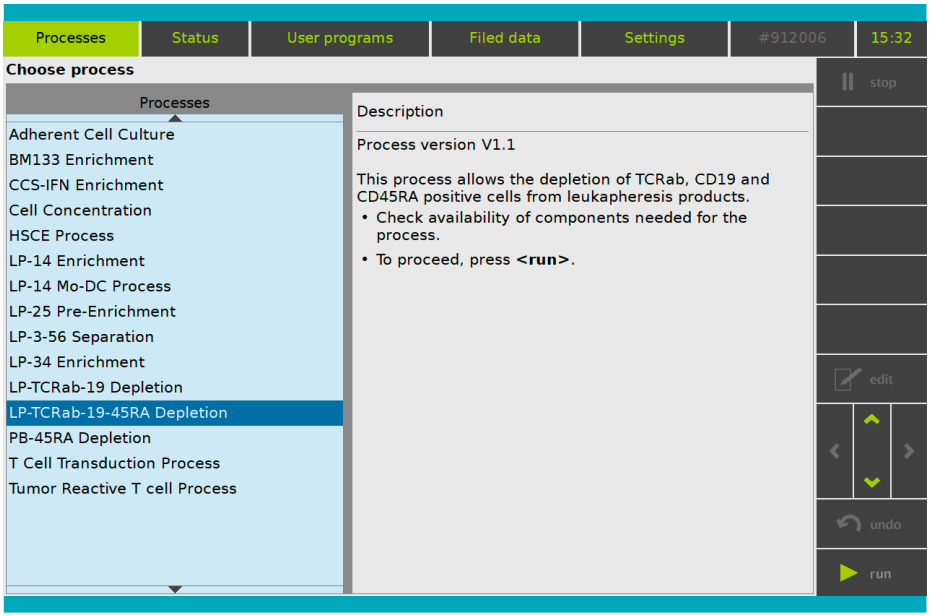
Switch on the CliniMACS Prodigy with the power switch located on the rear side of the instrument.

Note: A graphic user interface showing screens with instructions and pictures will guide through the entire procedure. Parts of the text presented on the screen depictions in the user manual may differ slightly from the screens shown on the instrument. Use the touchscreen functionality and buttons on the menu and tool bar to operate the CliniMACS Prodigy. For details, refer to the CliniMACS Prodigy user manual (instrument).

After the instrument initialization phase is completed, the main screen is shown. Select "LP-TCRab-19-45RA Depletion" in the Processes tab. Tap <run> to confirm the selection.

⚠ CAUTION

Risk of process failure. If alarm signals are not recognized and the required actions are not executed or confirmed, there is the risk of process failure. Make sure that the user recognizes optical and acoustical alarm signals at any time during the process.



Screen 6.1: Selection of the LP-TCRab-19-45RA Depletion process

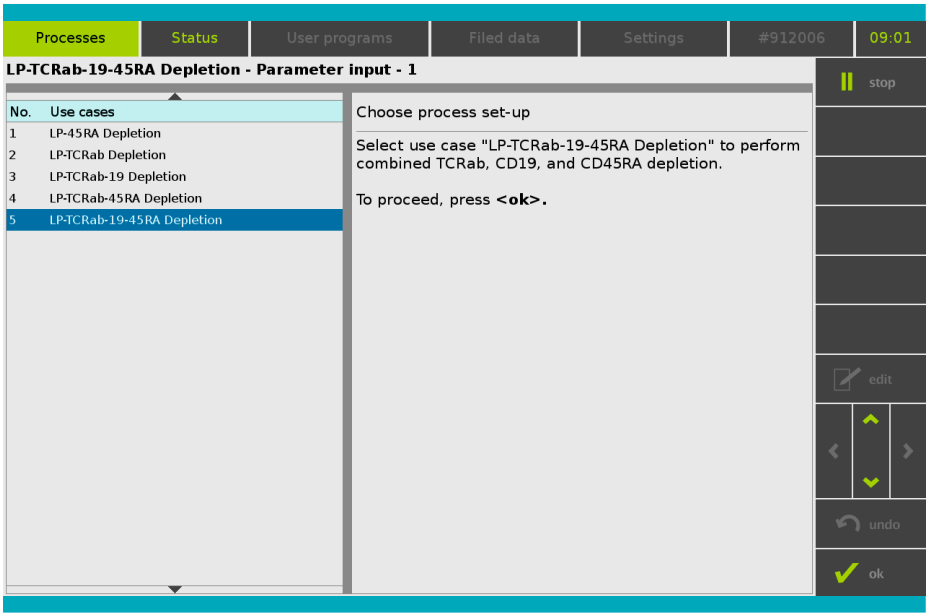
⚠ CAUTION

Risk of process failure. If the incorrect drive shaft is installed on the instrument, the LP-TCRab-19-45RA Depletion application is not executed. The LP-TCRab-19-45RA Depletion application is designed for the use with the CliniMACS Prodigy TS 320 only. The CliniMACS Prodigy TS 320 contains a large CentriCult™ Chamber requiring a specific drive shaft. Make sure the drive shaft is installed by Miltenyi Biotec Instrument Service before using the tubing set.

Any CliniMACS Prodigy with SN 490 or lower needs the installation of the new drive shaft. Instruments with serial numbers higher than 490 are already equipped with the new drive shaft.

Contact Miltenyi Biotec Technical Support if a drive shaft exchange is required.

After process initialization five different use cases for the LP-TCRab-19-45RA Depletion application are shown. Select the desired use case and tap <ok> to confirm the selection.



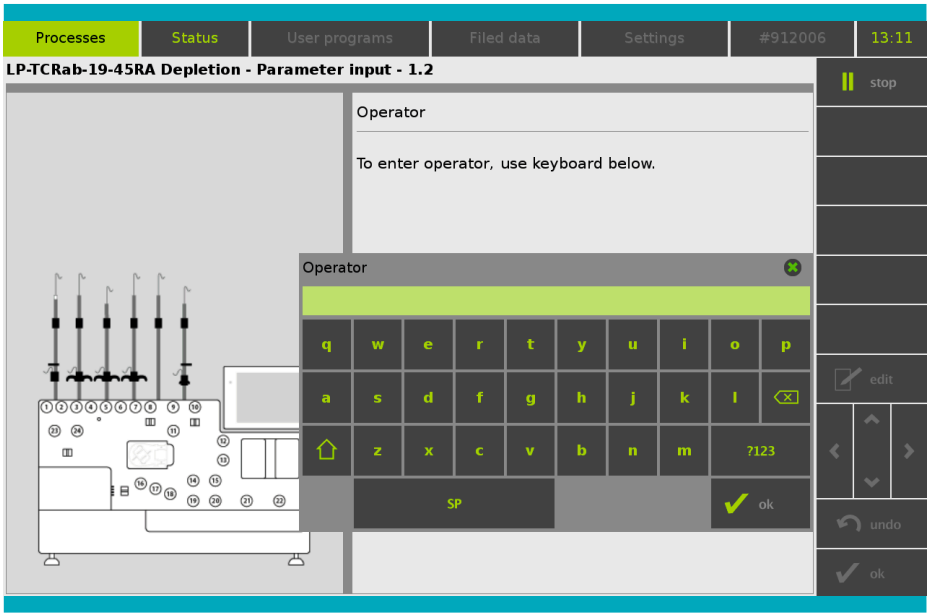
Screen 6.2: Use case selection

Subsequent to the use case confirmation, the software initializes the selected subprocess.

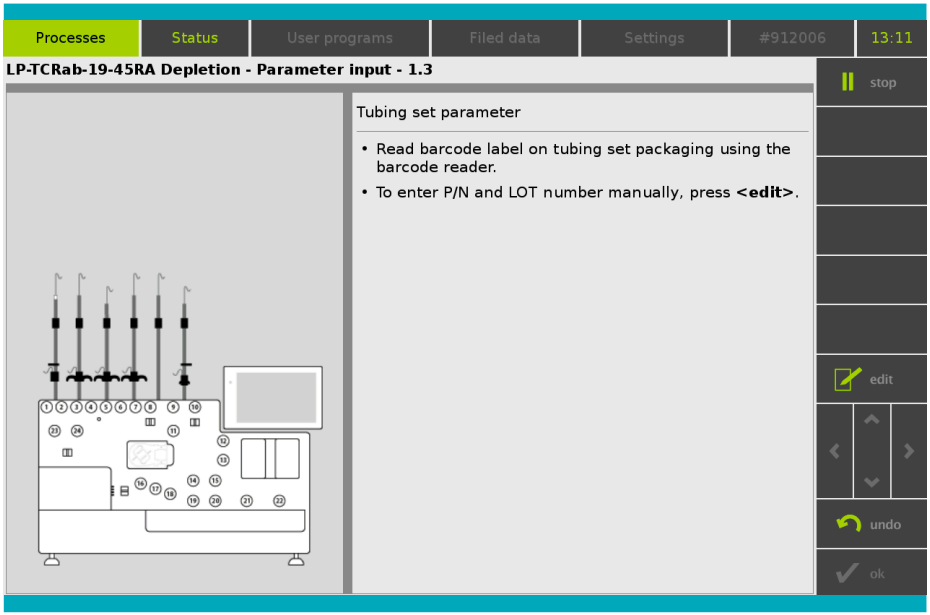
6.2 Parameter input

Multiple parameters must be entered for the LP-TCRab-19-45RA Depletion application either with the barcode reader connected to the CliniMACS Prodigy or with the keyboard shown on the screen. Tap <ok> to confirm each entry. All entered parameters are saved in the process-related protocol.

Within the next steps of the process (see Screen 6.3 and Screen 6.4) enter information related to the operator and the CliniMACS Prodigy TS 320. For the manual entry of capital letters tap <⇧>, for space tap <SP> for numbers tap <?123>, and for backspace tap <⌫>.



Screen 6.3: Entry of operator-related information



Screen 6.4: Entry of CliniMACS Prodigy TS 320 part number (P/N) and LOT number (LOT)

6.3 Tubing set installation

6.3.1 Components of the CliniMACS Prodigy TS 320

The components of the CliniMACS Prodigy TS 320 are pre-assembled on a mounting sheet and provided in a sealed and sterilized package. All components of the tubing set are illustrated in Figure 6.1.

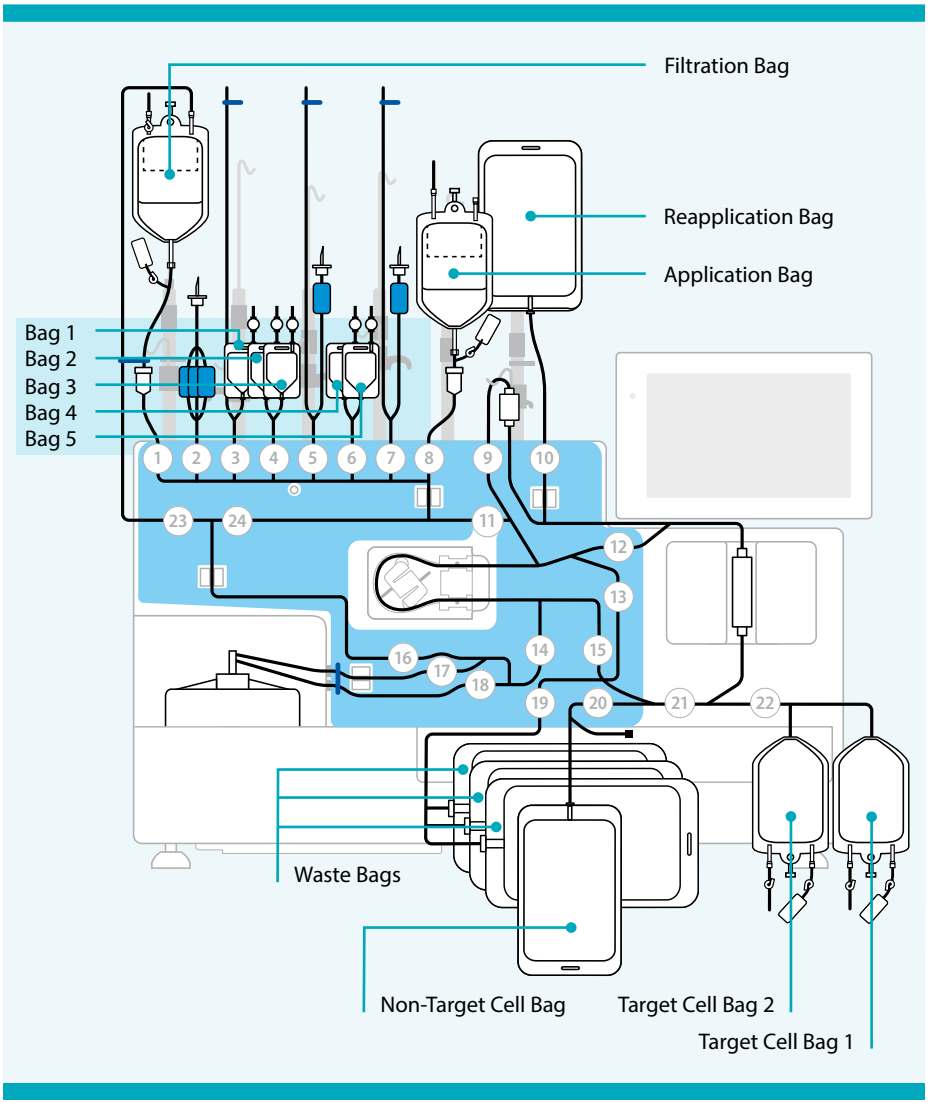


Figure 6.1: Components of the CliniMACS Prodigy TS 320

6.3.2 Installation steps

This section guides step by step through the installation of the CliniMACS Prodigy TS 320 (see Screen 6.5 to Screen 6.24).

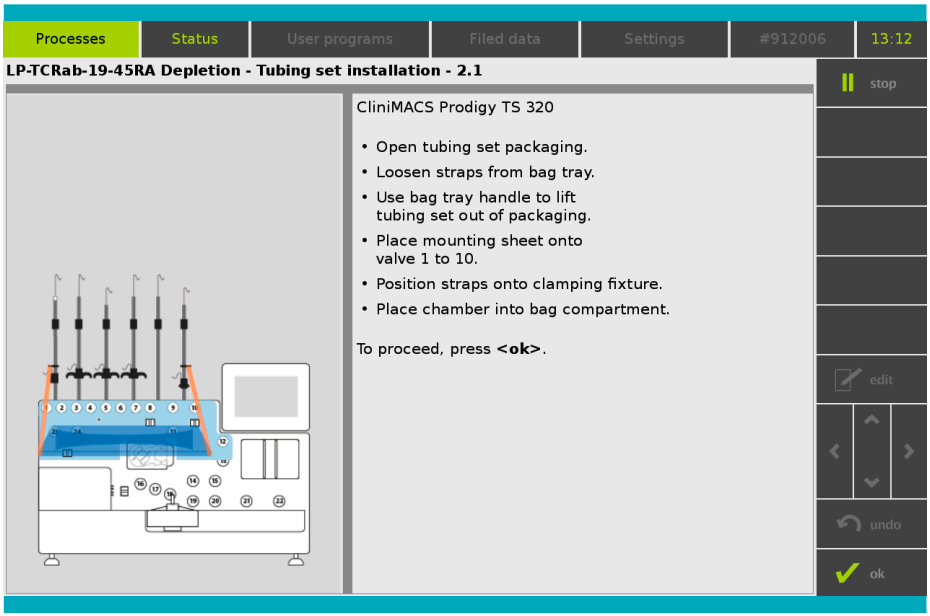
Each screen shows the verbal instructions supported by an informative image. Blinking and orange features in an image indicate areas of attention. At any step during the installation tap <undo> to return to a previous step.

NOTICE

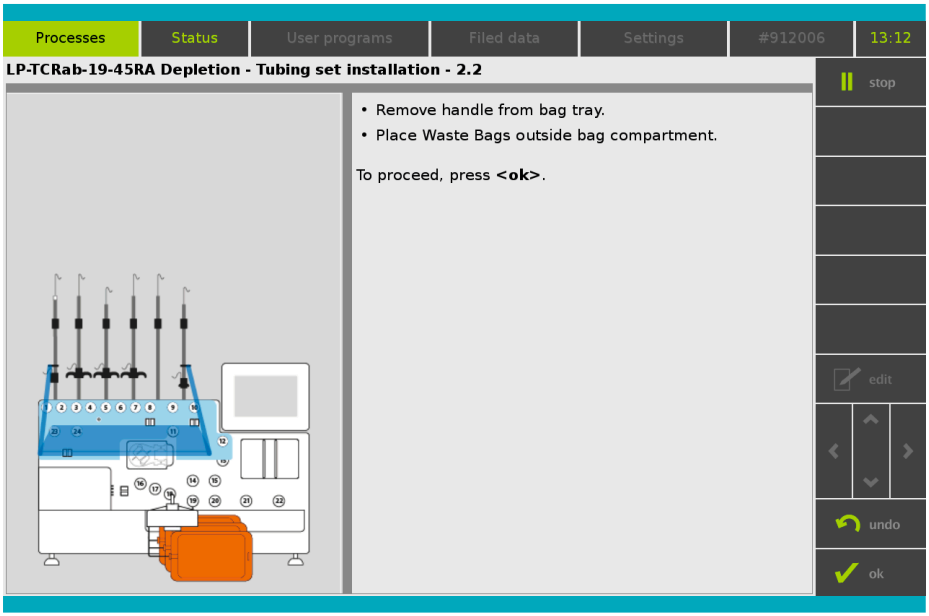
Risk of damage to the instrument. Overloading the bag hangers can damage the instrument. The carrying capacity of a single bag hanger is 5 kg. Do not overload the bag hangers.

⚠ 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.



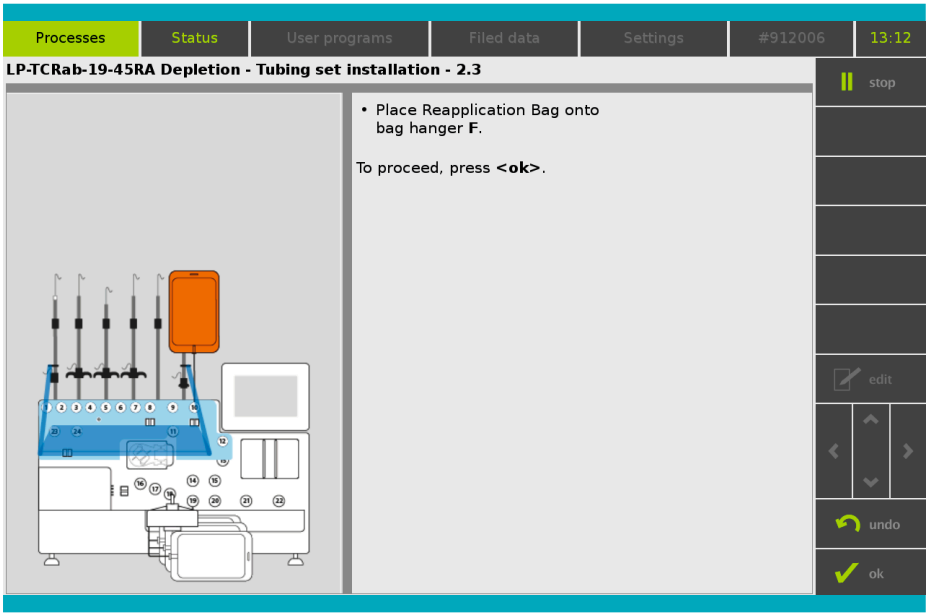
Screen 6.5: Installation of bag tray and mounting sheet onto the instrument



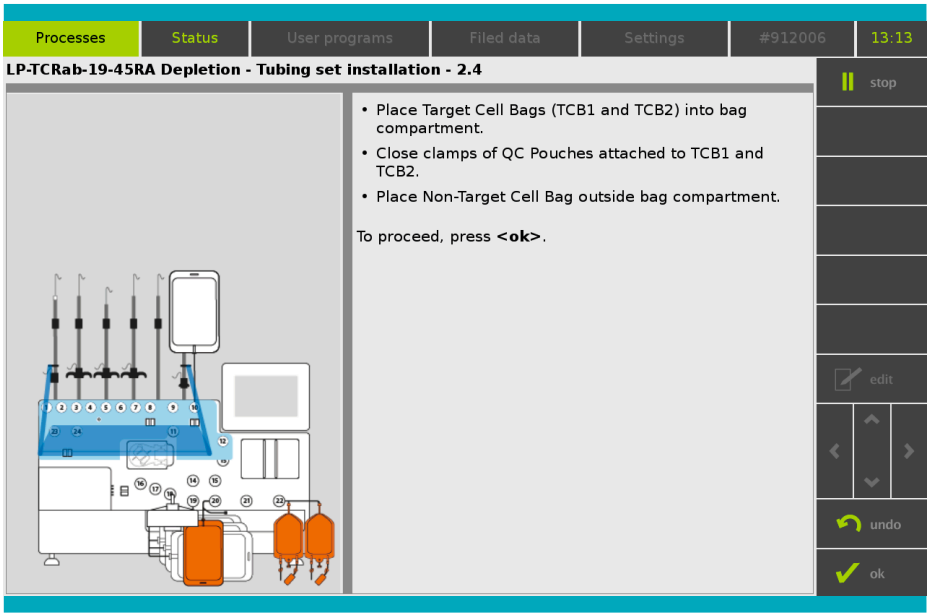
Screen 6.6: Positioning of the three Waste Bags

IMPORTANT

The Waste Bags must be safely placed outside of the bag compartment on a plain surface next to or below the CliniMACS Prodigy.



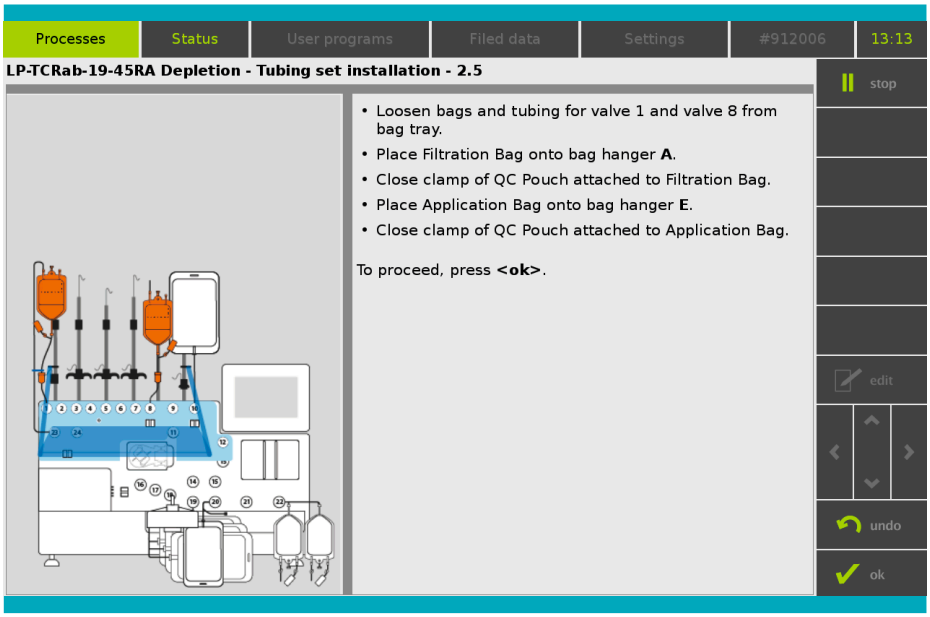
Screen 6.7: Positioning of the Reapplication Bag



Screen 6.8: Positioning of Target Cell Bags (TCB1 and TCB2) and Non-Target Cell Bag

IMPORTANT

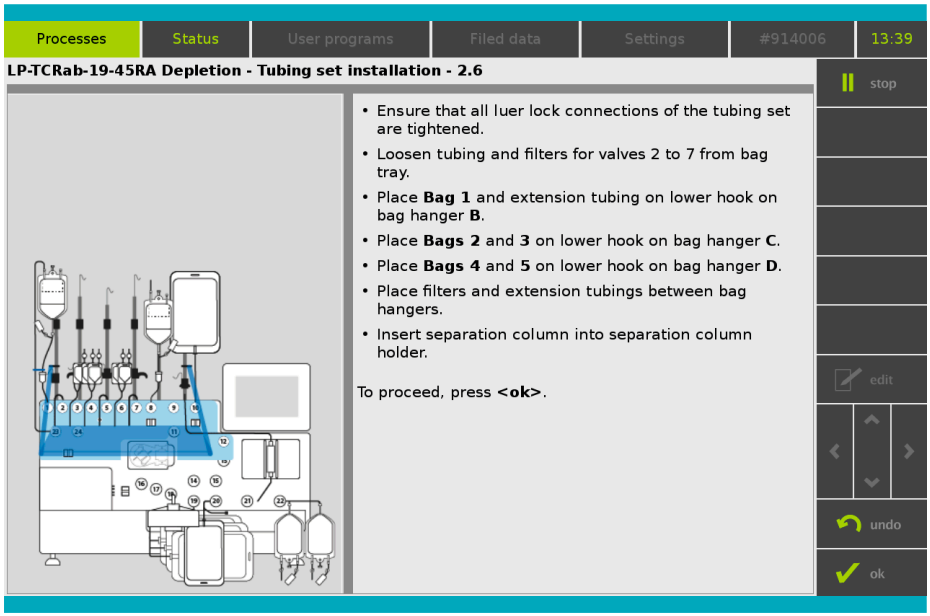
Whereas both Target Cell Bags (TCB) must be placed in the bag compartment, take particular care that the Non-Target Cell Bag (NTCB) is safely placed outside of the bag compartment on a plain surface next to or below the CliniMACS Prodigy.



Screen 6.9: Positioning of Filtration Bag and Application Bag

IMPORTANT

Use the center eyelet to position Application Bag and Filtration Bag upright on the respective bag hanger. Make sure that the clamps on the QC Pouches attached to Filtration Bag, Application Bag, and Target Cell Bags are closed.



Screen 6.10: Attachment of Bags 1 – 5 and insertion of separation column

The reagent bags must be placed on the lower hooks of the corresponding bag hangers. Table 6.1 presents an overview of the different reagent bags, the respective CliniMACS Reagent to be filled in and their positioning at the instrument. Bag 5 must not be used for any of the use cases available.

Reagent	Bag no.	Bag hanger
CliniMACS TCRα/β-Biotin (or CliniMACS TCRα/β-Biotin XL)	1	B
CliniMACS Anti-Biotin (or CliniMACS Anti-Biotin XL)	2	C
CliniMACS CD19 Reagent (or CliniMACS CD19 Reagent XL)	3	C
CliniMACS CD45RA Reagent XS	4	D
Not used	5	D

Table 6.1: Positioning of CliniMACS Reagents

Figure 6.2 shows how to insert the separation column into the column holder of the magnet unit.

⚠ 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.

Contact Miltenyi Biotec Technical Support for assistance if required.

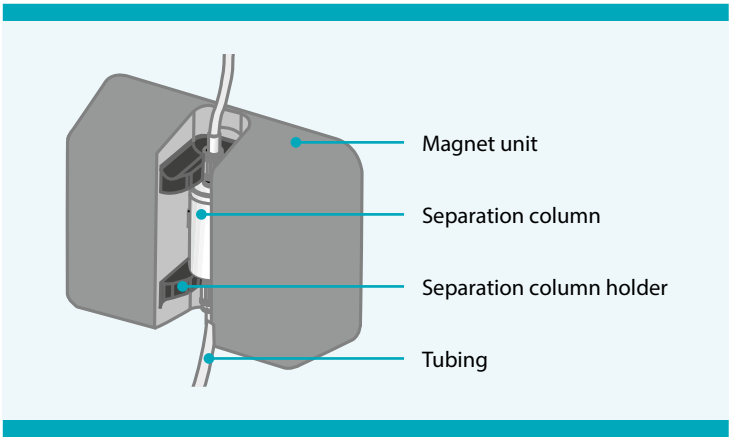
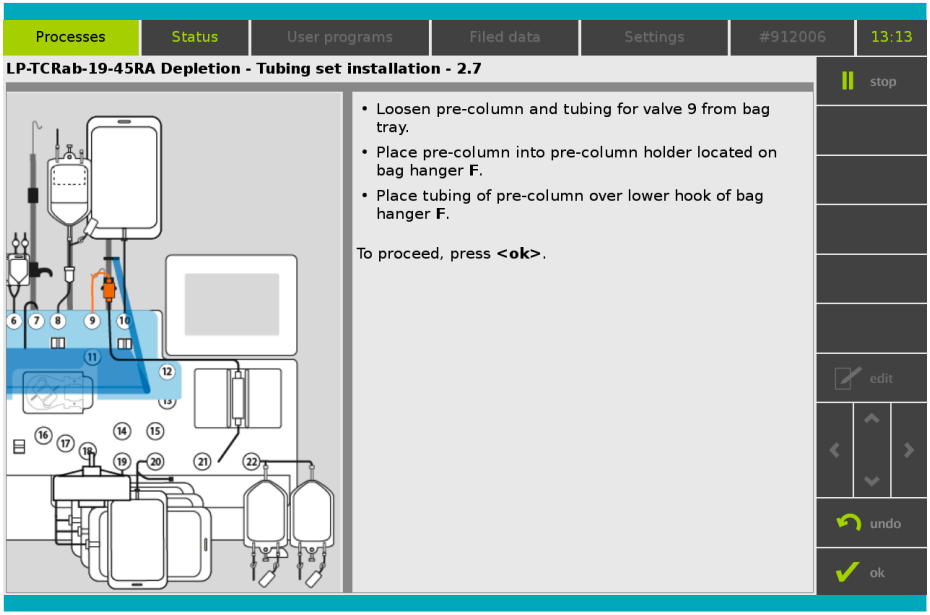


Figure 6.2: Separation column inserted into magnet unit



Screen 6.11: Insertion of pre-column

Figure 6.3 details how to place the pre-column tubing over the lower hook on bag hanger (F) and which pre-column tubing must be inserted in valve 9.

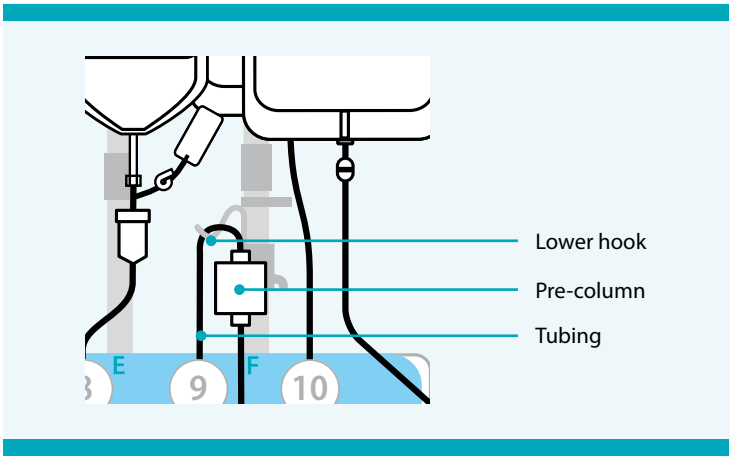
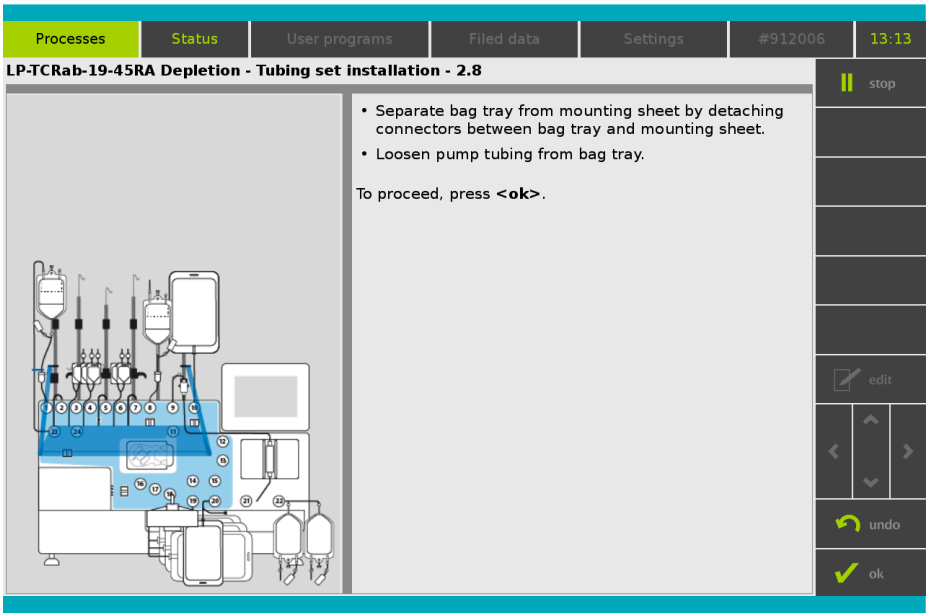
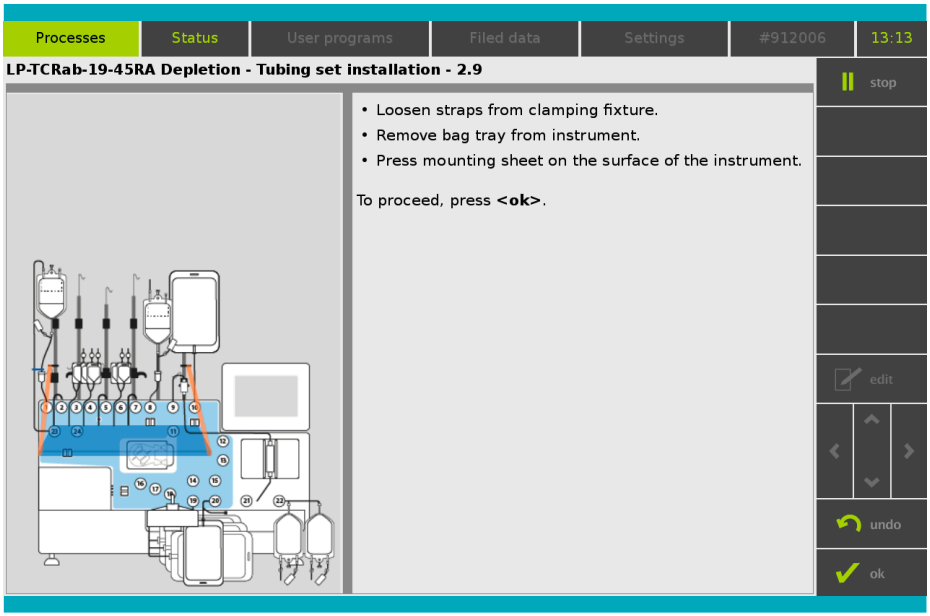


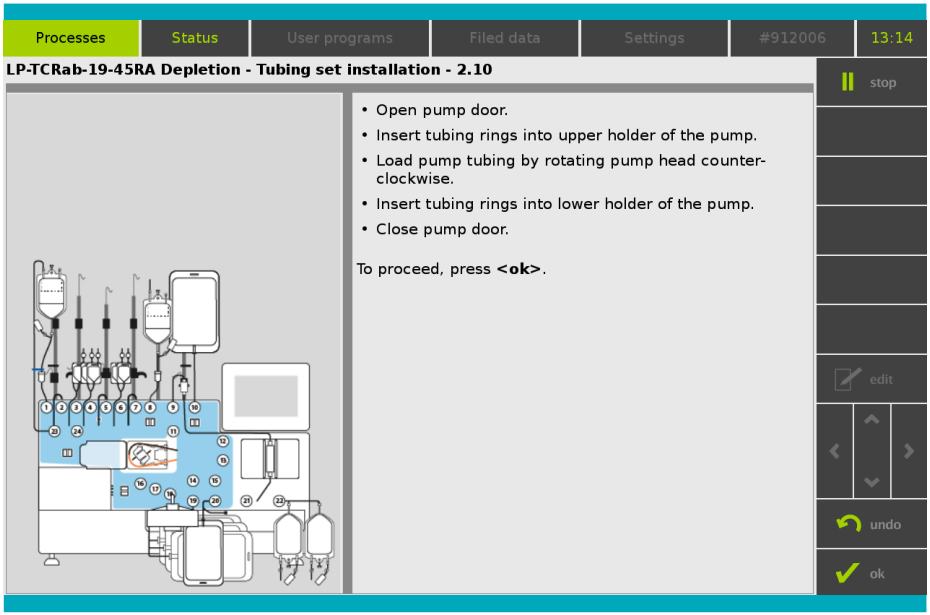
Figure 6.3: Guidance of pre-column tubing



Screen 6.12: Separation of bag tray from mounting sheet



Screen 6.13: Removal of bag tray from instrument



Screen 6.14: Insertion of pump tubing into pump

Figure 6.4 shows how to insert the pump tubing into the pump.

1. Insert the upper tubing rings in the intended cavities.
2. Rotate the pump head counterclockwise until the tubing is threaded between both sets of tubing guide pins of the pump head.
3. Then insert the lower tubing rings in the intended cavities.
4. Make sure that the pump tubing is not pinched between the guide pins.
5. Turn the pump head clockwise and counterclockwise to make sure the pump head moves freely.

To unload the tubing remove the lower end of the tubing from the cavities while turning the pump head clockwise until the tubing is released.

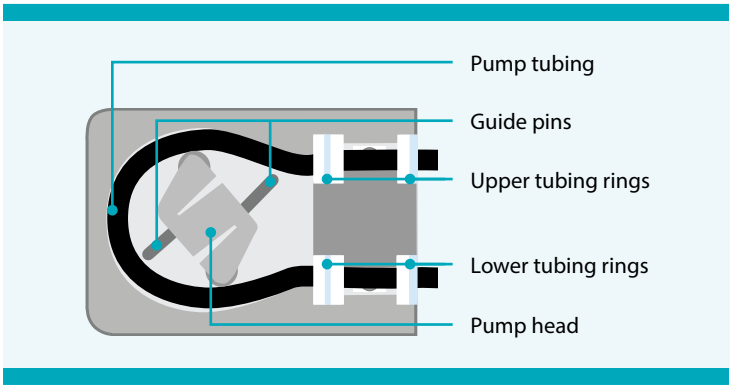
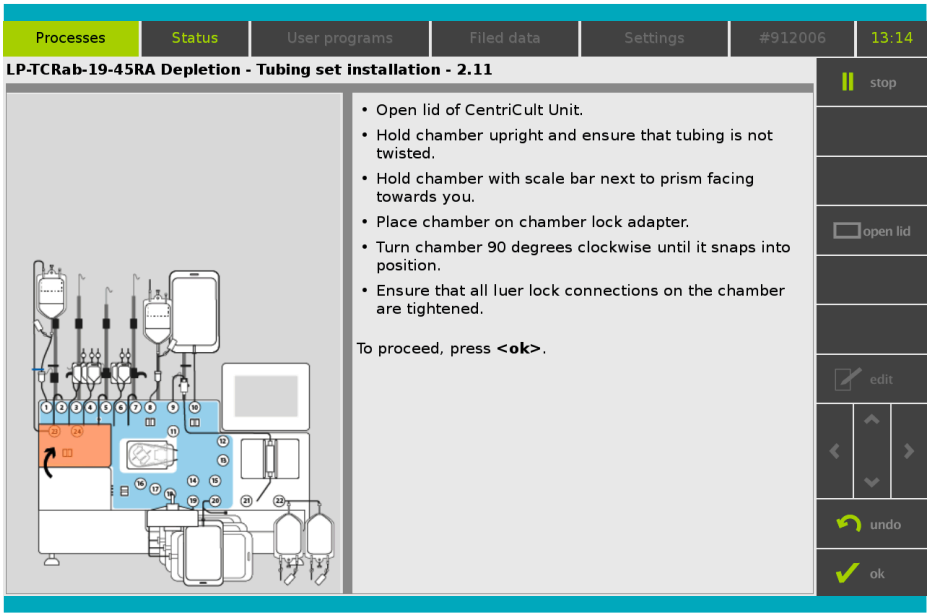


Figure 6.4: Pump tubing inserted into pump



Screen 6.15: Installation of chamber in CentriCult Unit

Figure 6.5 shows the components of the CentriCult Unit.

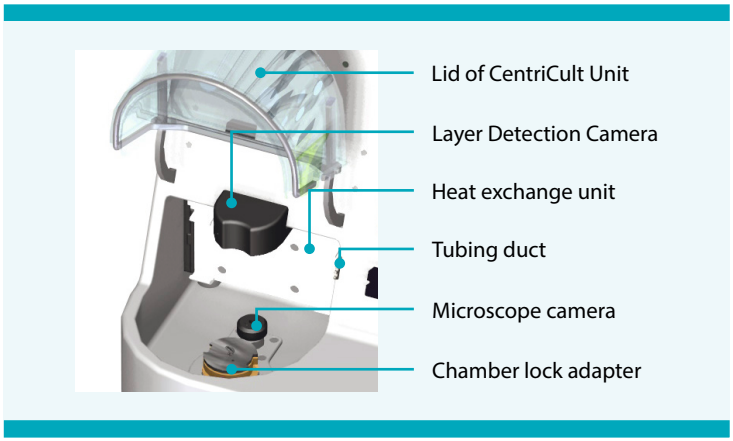


Figure 6.5: Components of CentriCult Unit

The CentriCult Unit lid unlocks automatically during this installation step. If the lid does not unlock automatically, tap <open lid> on the menu bar.

IMPORTANT

The chamber has a scale bar on its lid. This scale bar must point towards the user when placed on the chamber lock adapter. Before closing the lid, make sure that no tubing is pinched by the lid.

NOTICE

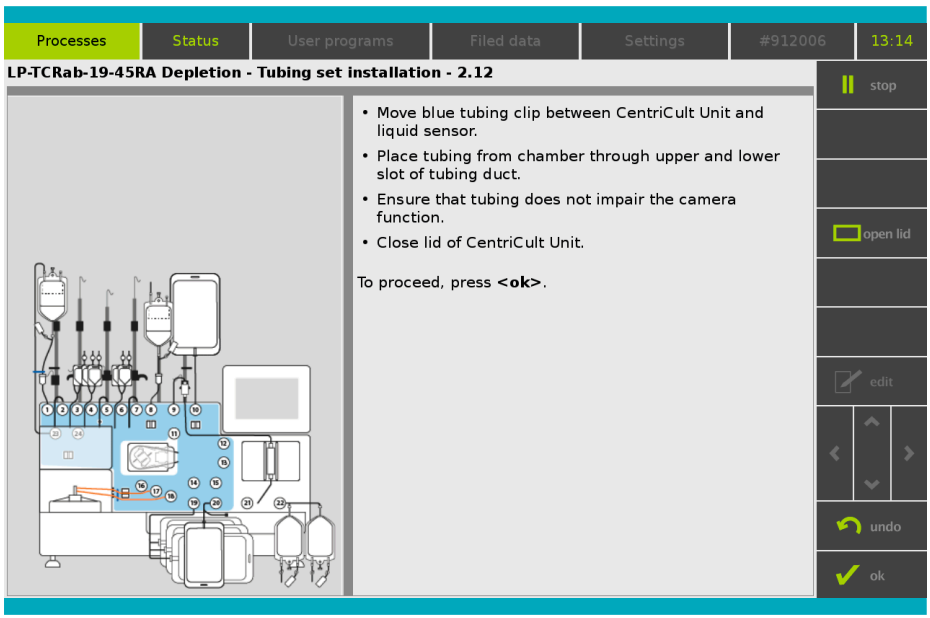
Risk of process delay. An incorrectly installed chamber tubing can cause a process delay. Before positioning the chamber onto the chamber lock adapter confirm that the tubing attached to the chamber is in a parallel position and not twisted or bent. The chamber must not be detachable from the chamber lock adapter once it is snapped into position.

If a twist or bending of the chamber tubing is recognized after the chamber is already locked, follow the instructions in the **IMPORTANT** paragraph connected to Screen 6.21 and correct the tubing position during that step.

⚠ CAUTION

Risk of process failure. An incorrectly installed chamber can cause a process failure. Make sure that the chamber is tightly locked in position. Do not use the tubing set if the chamber cannot be installed properly.

Also refer to important drive shaft requirements specified in section 6.1 ´Initialization of the CliniMACS Prodigy´.



Screen 6.16: Insertion of chamber-associated tubing

Figure 6.6 illustrates how to insert the two chamber tubing lines through the tubing duct.

Make sure that the tubing is not twisted or pinched by the lid of the CentriCult Unit.

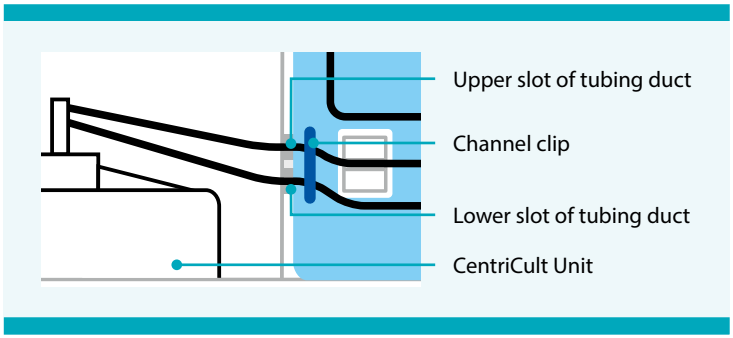
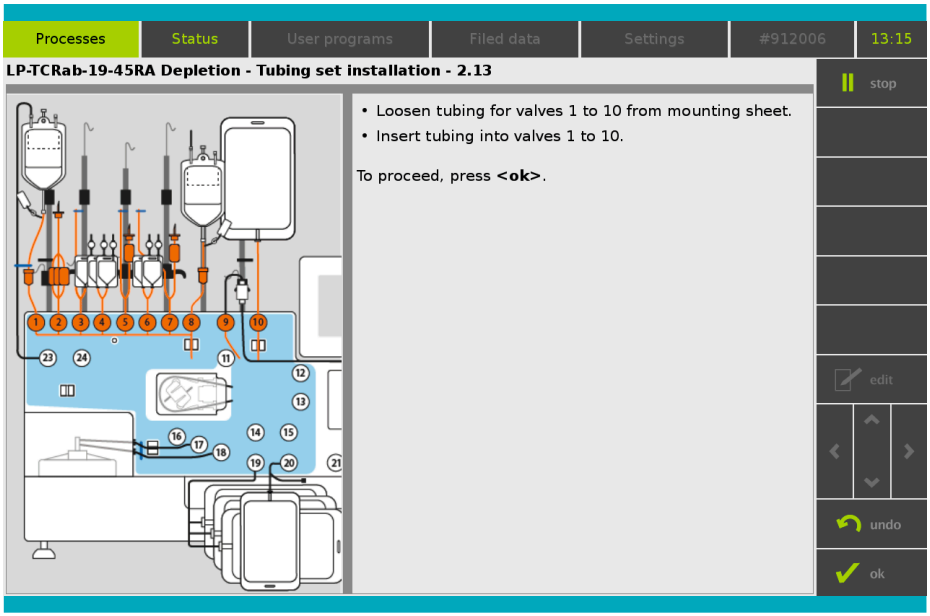


Figure 6.6: Guidance of tubing through tubing duct



Screen 6.17: Insertion of tubing into valves 1 – 10

Figure 6.7 shows how to insert the tubing into a valve correctly. Only insert the tubing into open valves (when the valve button is pressed down). Otherwise, the tubing will not fit correctly and will not be opened and closed properly during the process. If a tubing position has to be adjusted, press the valve button to open the valve before adjustment.

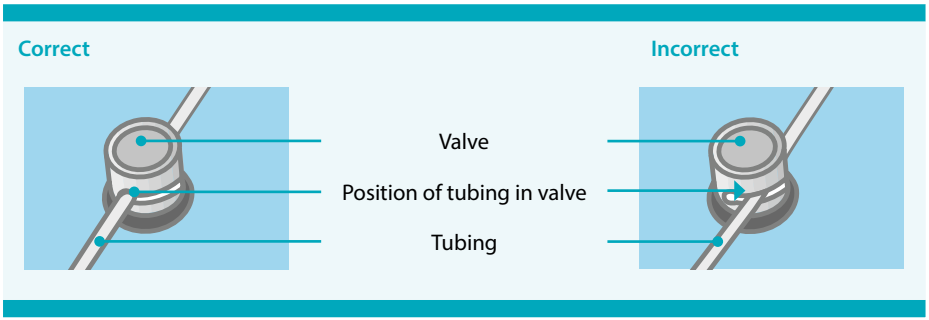
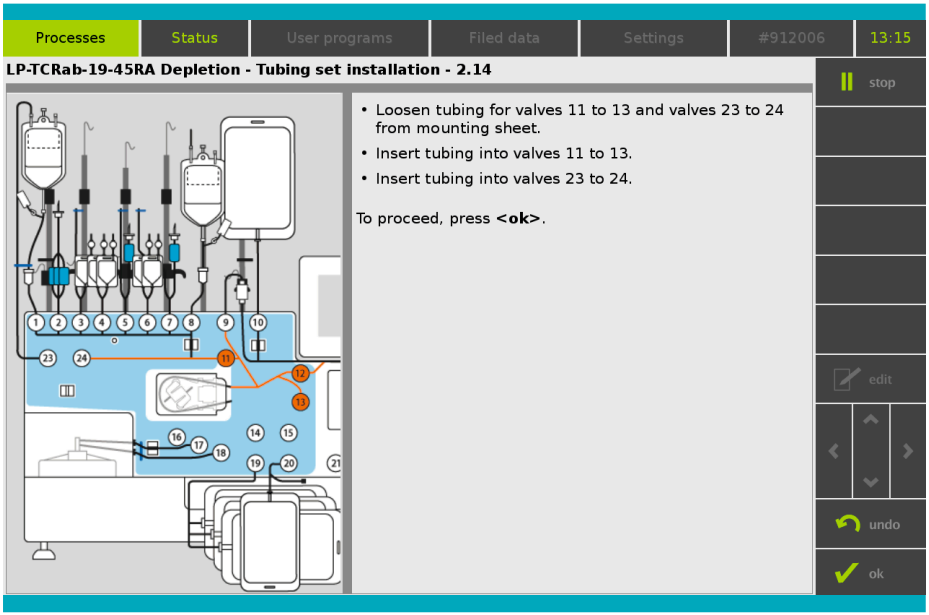


Figure 6.7: Insertion of tubing into valves



Screen 6.18: Insertion of tubing into valves 11 – 13 and 23 – 24

Figure 6.8 details the correct tubing insertion for valve 12 and 13.

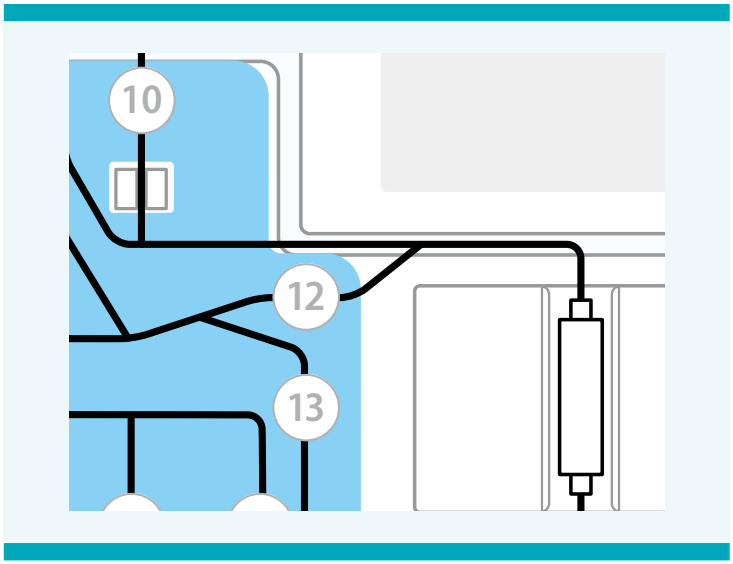
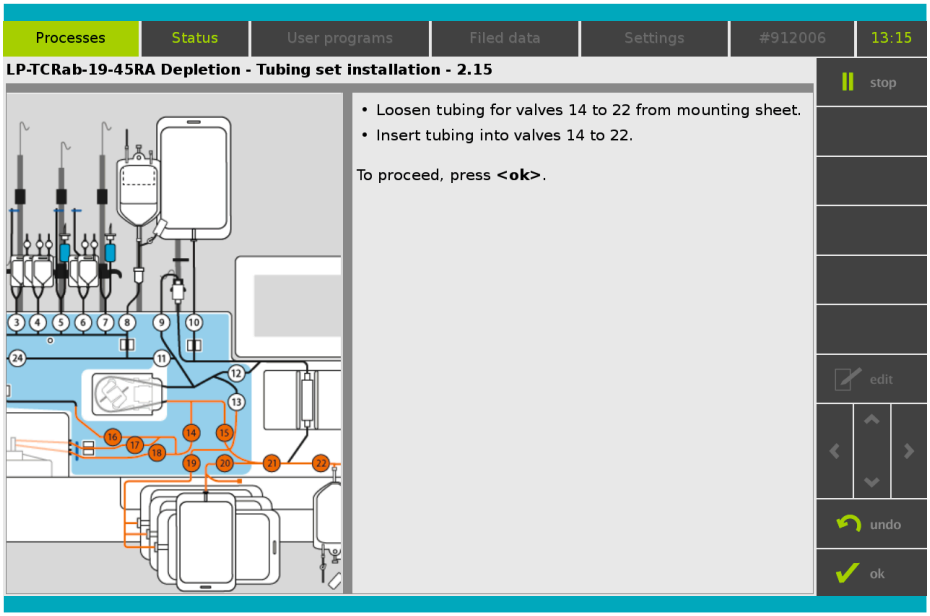
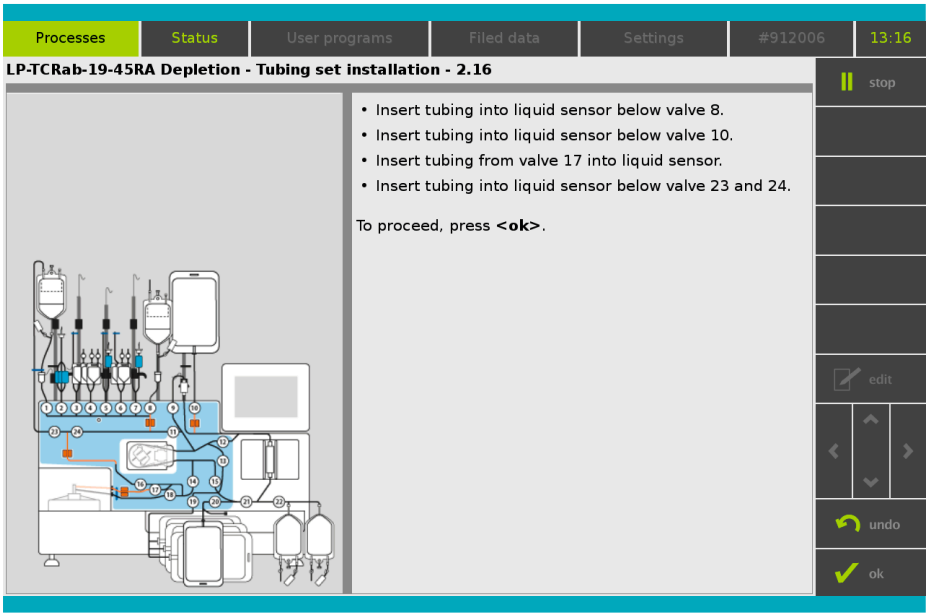


Figure 6.8: Tubing positioning for valves 12 and 13



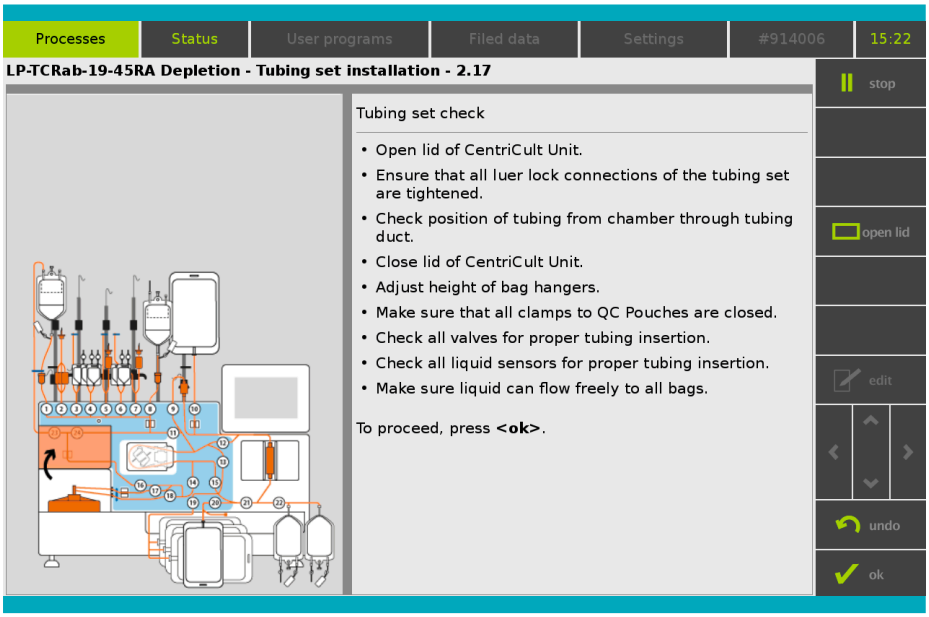
Screen 6.19: Insertion of tubing into valves 14 – 22



Screen 6.20: Insertion of tubing into liquid sensors

IMPORTANT

To ensure a proper operation of the liquid sensor, both, the liquid sensor and the outside of the tubing must be dry. If any liquid is present on the liquid sensor, dry the area with a soft, lint-free cloth.



Screen 6.21: Final check of tubing set installation

IMPORTANT

Review all tubing for kinks or severe bending that could restrict the flow of liquid through the tubing set and verify the tightness of all Luer Lock connections of the tubing set. If a tubing position has to be adjusted, press the valve button to open the respective valve beforehand (see Figure 6.7).
Furthermore, it is possible to remove and re-insert the chamber at this step, (e.g., if the chamber tubing has been twisted during installation).

The CentriCult Unit lid unlocks automatically during this process step. If the lid does not unlock automatically, tap **<open lid>** on the tool bar.

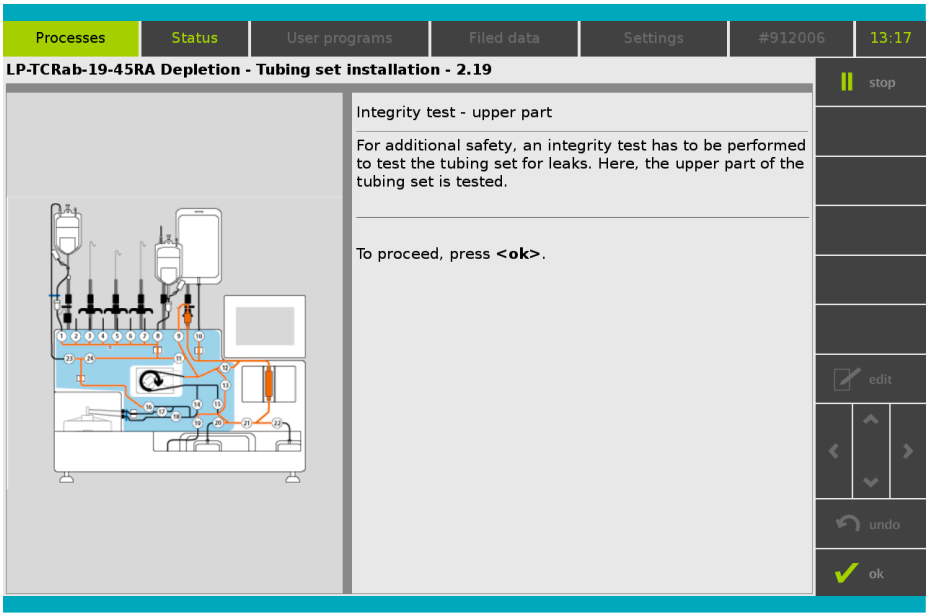
If necessary, raise or lower the bag hangers to adjust the height. Make sure that the bags are positioned high enough to prevent severe bending of the tubing and low enough to avoid tubing or connections being stretched. In addition, verify the tightness of all Luer Lock connections on the tubing set.

In case the lid cannot be opened, contact Miltenyi Biotec Technical Support.

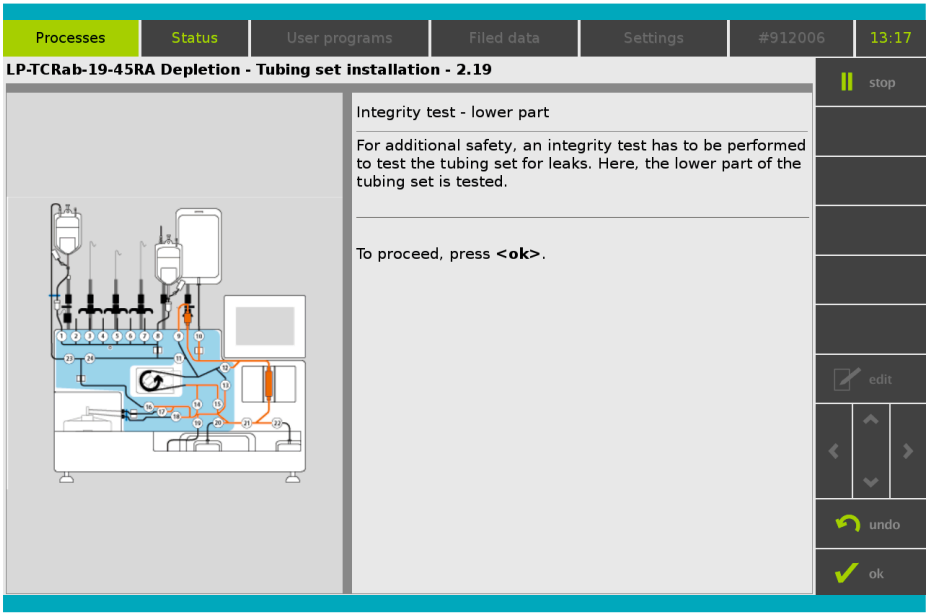
When the tubing set installation is completed, all valves are automatically opened and closed twice to verify proper functionality. In addition, the forward and backward movement of the magnet is tested. If a valve or the magnet does not operate correctly, contact Miltenyi Biotec Technical Support.

IMPORTANT

For additional safety, an integrity test must be performed to test the tubing set for potential leaks. The test consists of two automated sequences that apply controlled overpressure separately to the upper and lower part of the tubing set.



Screen 6.22: Integrity test for the upper part of the tubing set



Screen 6.23: Integrity test for the lower part of the tubing set

Follow the instructions on the screen to start the integrity test for the upper and lower part of the tubing set.

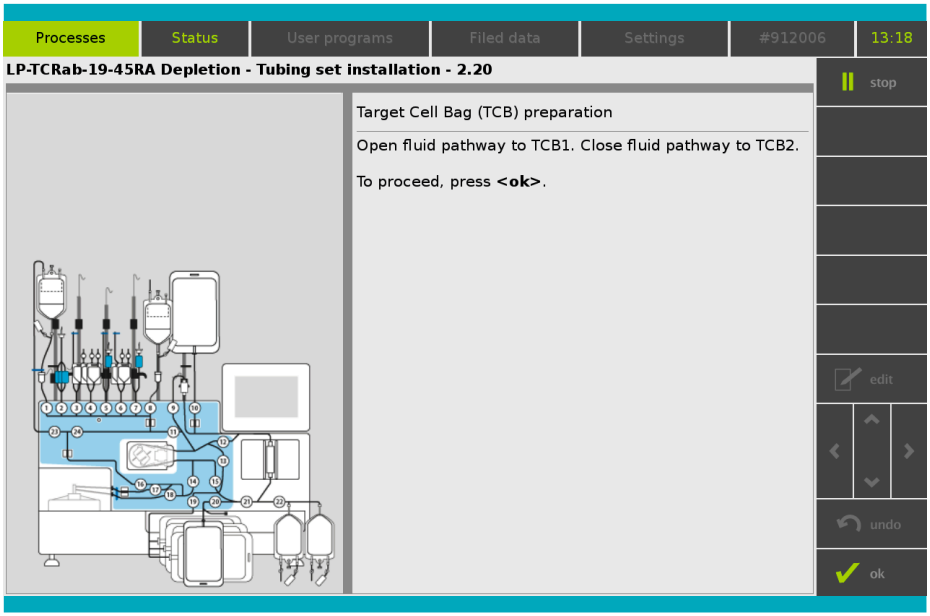
⚠ WARNING

Risk of contamination. A failed integrity test can indicate a potential leakage in the tubing set, which carries the risk of contamination. If a tubing set fails the integrity test, carefully verify that all tubing is correctly inserted into the appropriate valves. Repeat the integrity test after verification of proper tubing set installation. Do not use the tubing set if it is not possible to pass the upper and lower integrity test.

Contact Miltenyi Biotec Technical Support for assistance, if required.

After successful completion of the integrity test, open or close the fluid pathways to the two Target Cell Bags with the small integrated clamps as instructed on the screen.

As an example, Screen 6.24 shows the instructions for use case 5.



Screen 6.24: Preparation of TCB1 and TCB2

6.4 Tubing set priming

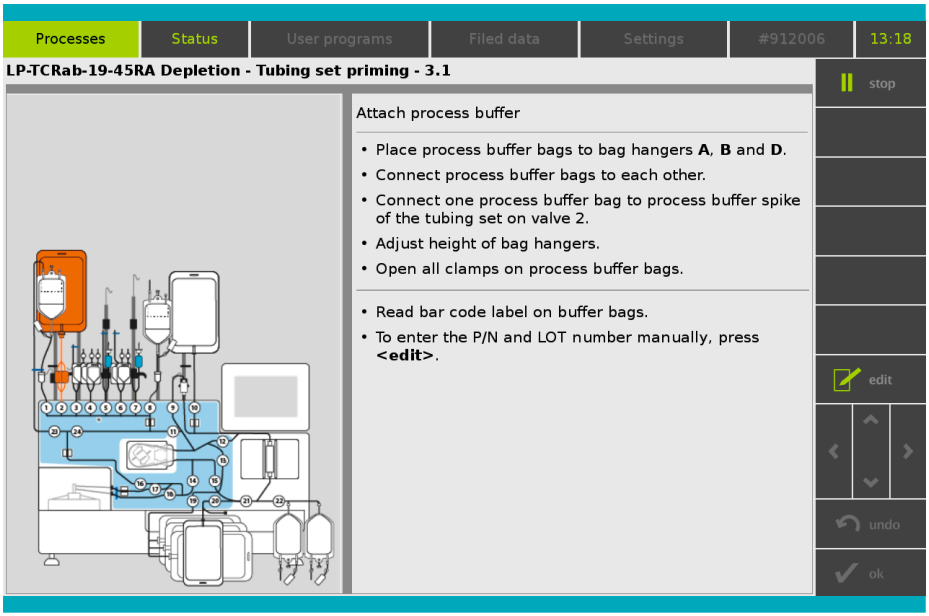
6.4.1 Connection of process buffer, infusion solution, and rinsing solution

IMPORTANT

- See Table 3.4 in section 3.4 ´Summary of required materials´ for case-dependent requirements of infusion solution and rinsing solution.
- Note that use cases 1, 2, and 3 do not require rinsing solution.
- See chapter 5 ´Preparative steps´ for the preparation of process buffer, infusion solution, and rinsing solution.

Screen 6.25 to Screen 6.31 guide through priming preparations and priming phase.

Note: Only the process buffer bag that is connected to the tubing set is shown on the screens. For a better graphical overview, the other buffer bags are not shown, but their handling is described in detail in the on-screen instructions.

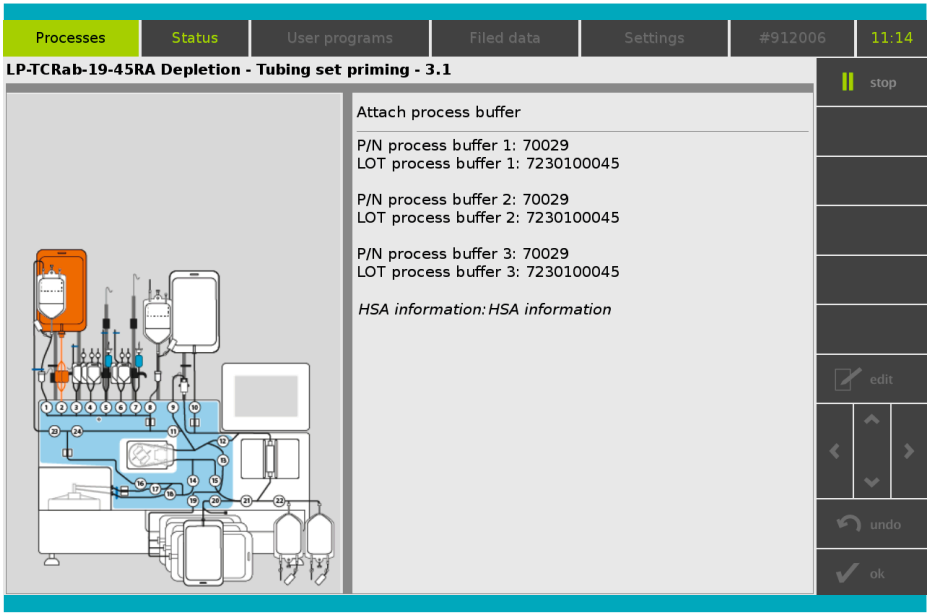


Screen 6.25: Connection and positioning of process buffer bags

IMPORTANT

- Three or four process buffer bags are required for use cases 2–5. Three bags must be connected for the priming sequence already. Place bags as indicated on the screen. Connect the bags to each other with plasma transfer sets. Close the clamps at the plasma transfer sets prior to interconnection. The bag placed on bag hanger B is then connected to the buffer spike of the tubing set (see Screen 6.25). Open clamps after process buffer connections are completed.
- Use case 1: Only one process buffer bag is required for LP-45RA Depletion. The bag must be connected to the buffer spike of the tubing set and placed to bag hanger B.
- Use cases 2–5: Adjust the height of bag hanger A and D to the highest position possible. To ensure optimal liquid flow, position the outlet port of the process buffer bag at bag hanger B a bit lower than the process buffer bags at A and D. Take particular care not to stretch the triple sterile filters at the buffer line and make sure that the triple sterile filters are in a vertical position after connection. After height adjustment, open all clamps at the interconnections between the process buffer bags.

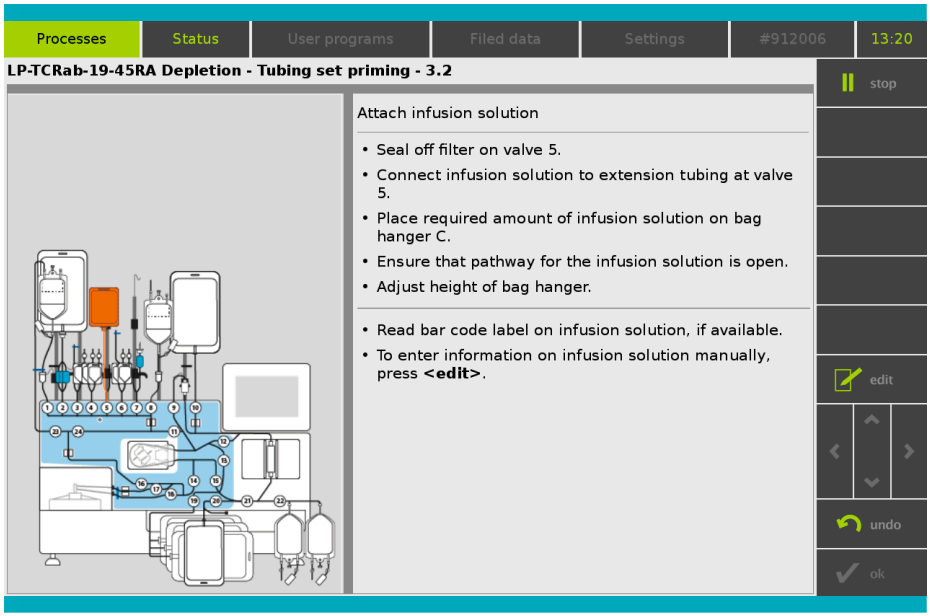
Depending on the TCRα/β(-CD19) cell parameter input (see Screen 6.38 to Screen 6.43), the software will automatically determine and indicate if a fourth process buffer bag is required (see Screen 6.46).



Screen 6.26: Input of part number (P/N) and lot number (LOT) of the process buffer bags and HSA information

Read the barcode label on the process buffer bags with the barcode reader. Alternatively, tap <edit> to enter part numbers (P/N) and lot number (LOT) manually with the keyboard shown on the screen.

Tap <edit> to enter information regarding the HSA.



Screen 6.27: Connection of infusion solution

Figure 6.9 depicts the connection of infusion solution to the tubing line at valve 5. The tubing line at valve 5 is split to two branches. One branch has a sterile filter and ends up in a spike connector. The other branch is a sealed extension tubing designed for sterile welding. The extension tubing (line without the sterile filter) must be used for the infusion solution connection:

- Seal off the tubing with the sterile filter close to the branching.
- Weld the infusion solution bag to the extension tubing.

Open the pathway to the infusion solution bag(s) as instructed on the screen.

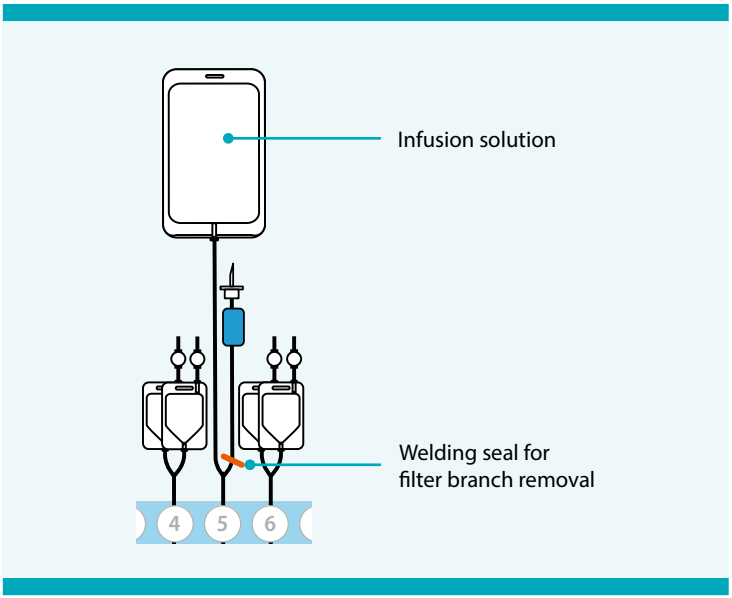
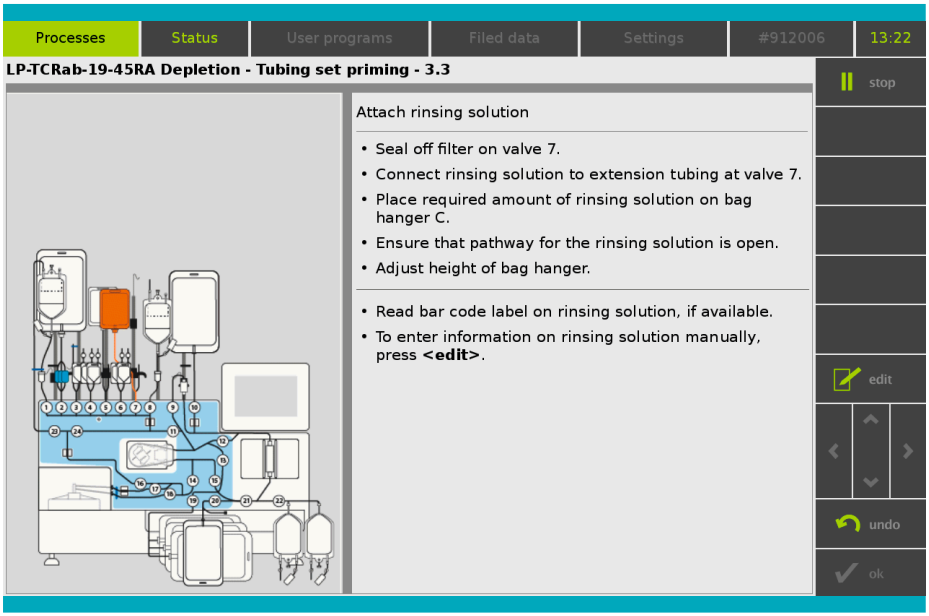


Figure 6.9: Connection of infusion solution

If the infusion solution cannot be welded to the extension tubing at valve 5 directly, use e.g., a plasma transfer set for sterile connection of infusion solution bag and extension tubing. If more than one infusion solution bag needs to be connected, split the extension tubing line (e.g., by a 3-way adapter) or connect one bag and use suitable adapters like plasma transfer set(s) for interconnection of the other bag(s).

Read the barcode label on the infusion solution with the barcode reader. Alternatively, tap **<edit>** to enter product-specific information manually with the keyboard shown on the screen.



Screen 6.28: Connection of rinsing solution

Figure 6.10 depicts the connection of rinsing solution to the tubing line at valve 7. The tubing line at valve 7 is split to two branches. One branch has a sterile filter and ends up in a spike connector. The other branch is a sealed extension tubing designed for sterile welding. The extension tubing (line without the sterile filter) must be used for rinsing solution connection:

- Seal off the tubing with the sterile filter close to the branching.
- Weld the rinsing solution bag to the extension tubing.

Open the pathway to the rinsing solution bag as instructed on the screen.

If the rinsing solution cannot be welded to the extension tubing at valve 7 directly, use, e.g., a plasma transfer set for sterile welding of the rinsing solution bag and the extension tubing.

Read the barcode label on the rinsing solution bag with the barcode reader. Alternatively, tap <edit> to enter product-specific information manually with the keyboard shown on the screen.

If necessary, raise or lower the hook to adjust the height of bag hanger C.

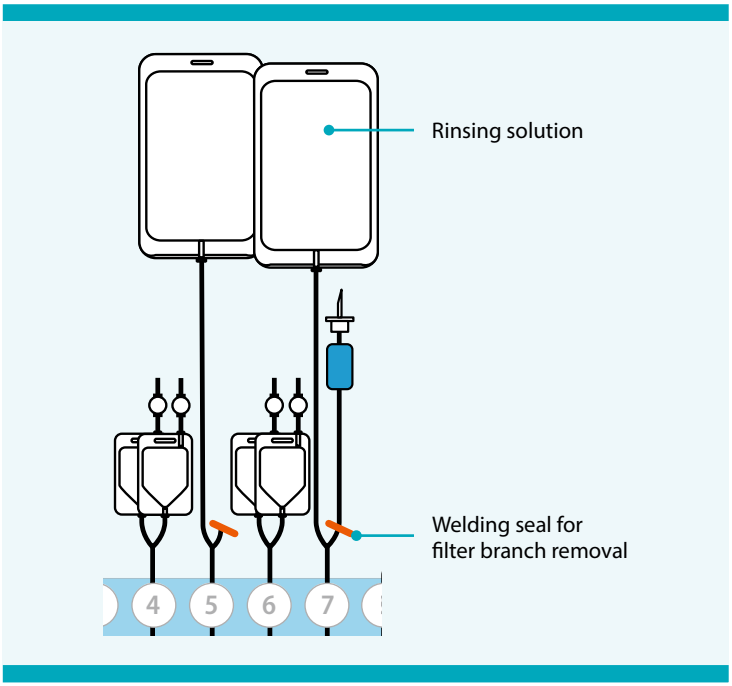
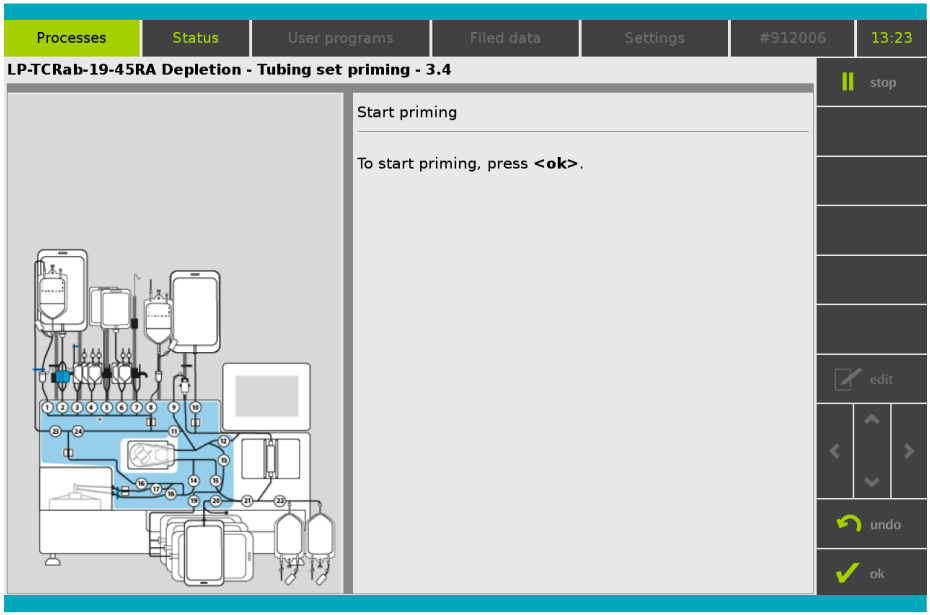


Figure 6.10: Connection of rinsing solution

If infusion and rinsing solution cannot be positioned safely together on bag hanger C, then hang the rinsing solution on bag hanger F. In this case, place the Reapplication Bag over the rinsing solution bag.

6.4.2 Priming phase



Screen 6.29: Start of the priming phase

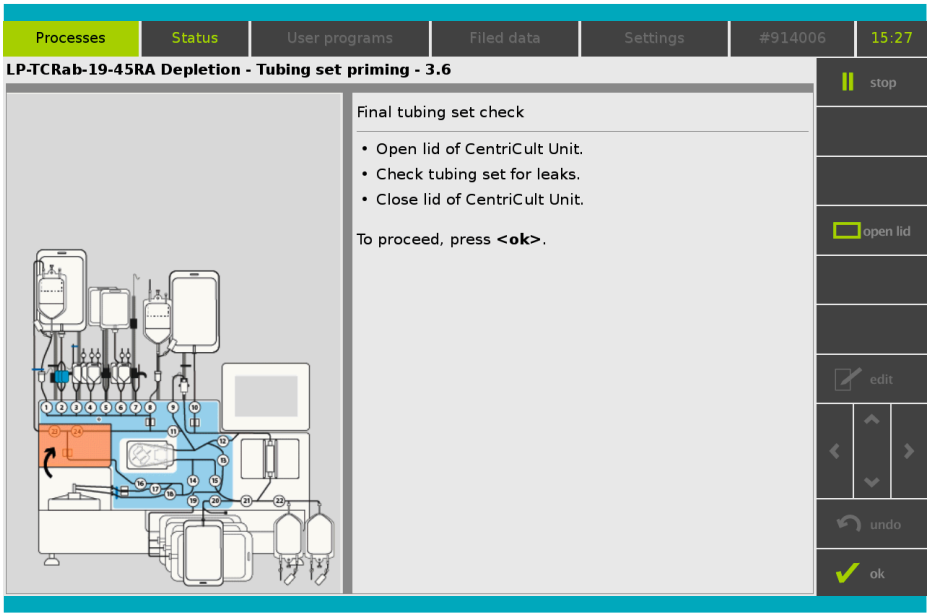
During the priming phase, the liquid flow of the connected solutions is verified and the tubing set is filled with process buffer.

When the priming phase is completed, check all bags, tubing, fittings, valves, chamber and columns for the appearance of leaks or the presence of kinks which may block fluid pathways.

IMPORTANT

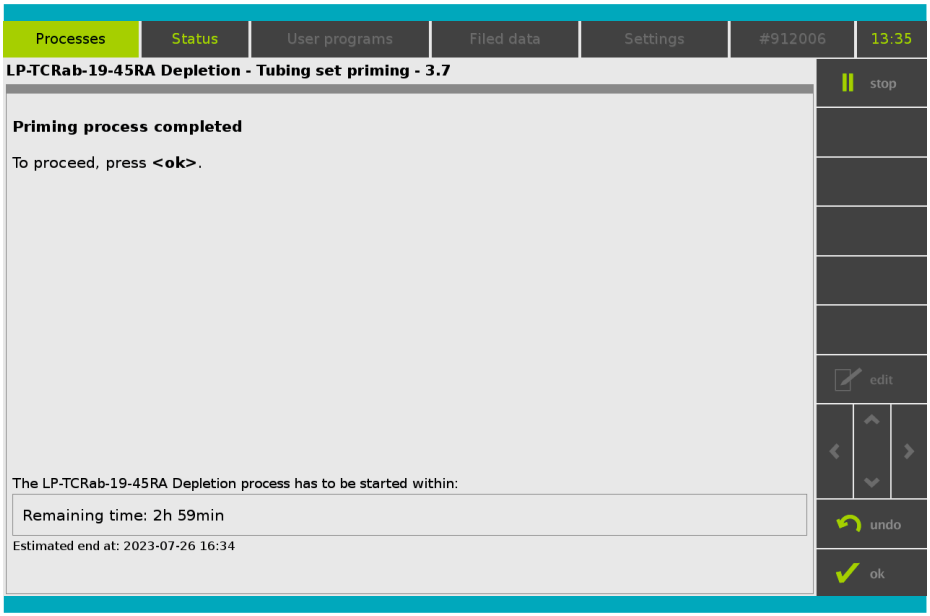
If leaks are observed, the tubing set must not be used. In this case, restart the process using a new tubing set, process buffer, infusion solution and rinsing solution.

The CentriCult Unit lid unlocks automatically during this process step. If the lid does not unlock automatically, tap **<open lid>** on the tool bar. In case the lid cannot be opened, contact Miltenyi Biotec Technical Support.



Screen 6.30: Final tubing set check after priming phase

Screen 6.31 is shown when the priming phase is completed.



Screen 6.31: End of priming phase

After priming, connect reagents and starting cell product. Make sure that all materials required are available for the separation process.

Before proceeding with the separation process, make sure that the starting cell product, analyzed as described in section 5.1 ´Testing of the starting cell product´ is available.

6.5 Preparation for the automated depletion process

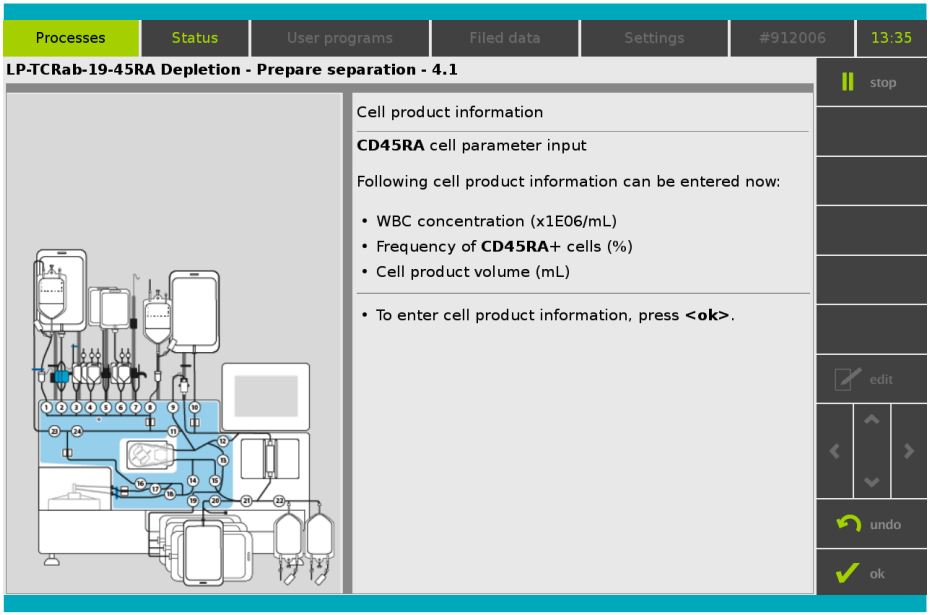
This section describes exemplarily the handling steps required for the most comprehensive use case 5, LP-TCRab-19-45RA Depletion.
Important information regarding divergent handling steps for the other use cases is highlighted in the respective handling step.

6.5.1 Cell parameter input

IMPORTANT

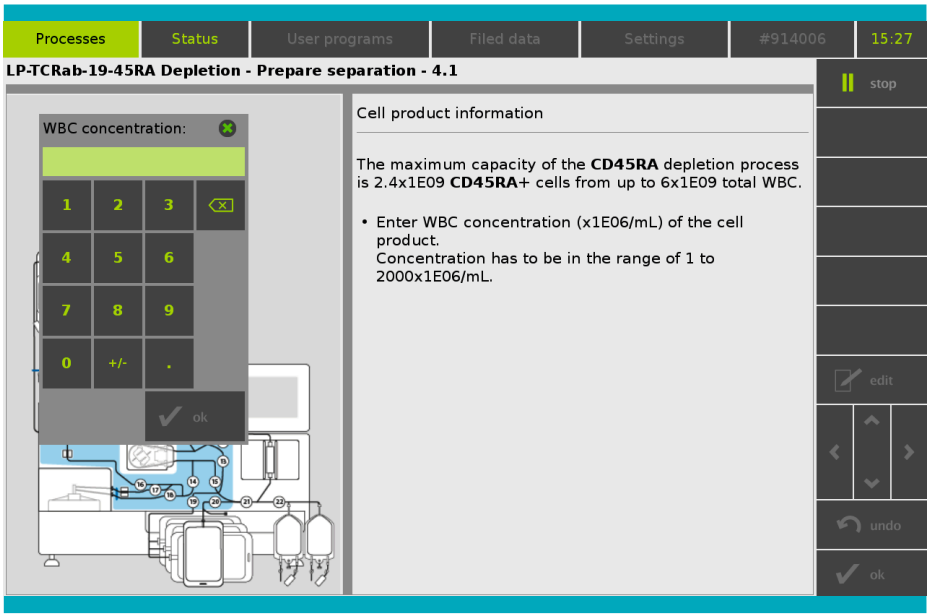
- Process scale and duration depend on the numbers of WBC and labeled cells within in the starting cell product. The input of respective cell product-specific parameters (i.e., WBC concentration, frequency of labeled cells and cell product volume) is always required during instrument setup. See section 5.1 ´Testing of the starting cell product´for details on analytical testing required.
- The required cell parameter input is related to the depletion parts included in the use case selected, e.g., use case 1 (CD45RA depletion only) requires cell product information related to CD45RA depletion but not cell product information related to TCRα/β(-CD19) depletion. For details on use cases and related process specifications, see Table 1.1 in section 1.1 ´General information´.

Cell parameter input for CD45RA depletion



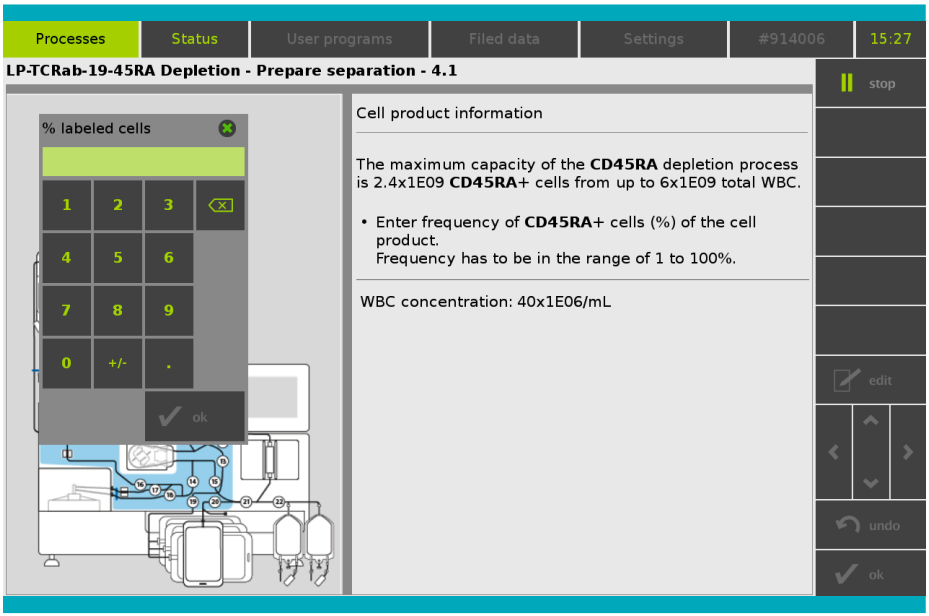
Screen 6.32: Cell product information input screen for the CD45RA depletion part

Enter the WBC concentration of the starting cell product that will be used for the CD45RA depletion part. The acceptable input range is shown on the screen.



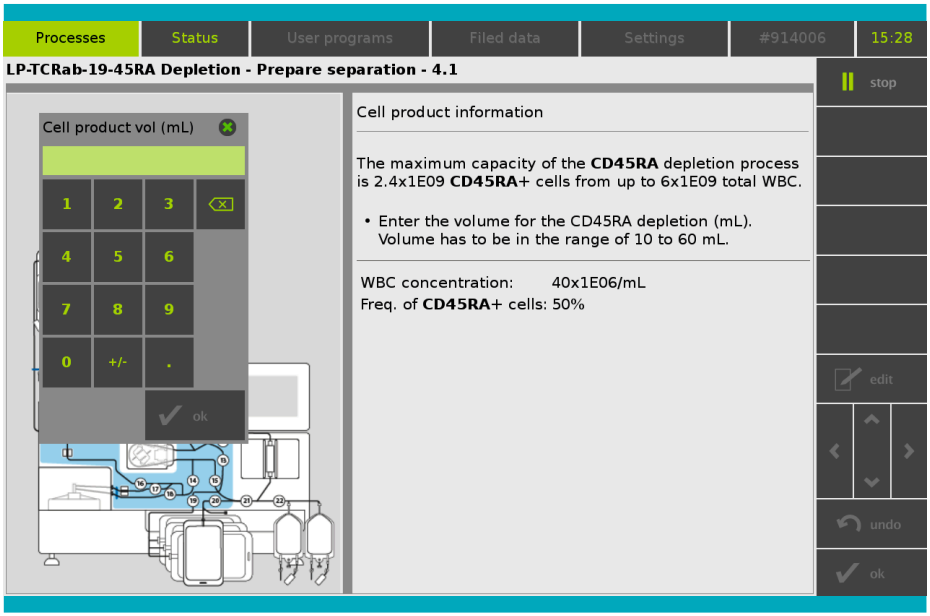
Screen 6.33: Entry of WBC concentration

After the WBC concentration of the cell product has been entered and confirmed, type in the frequency of labeled cells (all CD45RA-positive). The acceptable input range is shown on the screen.



Screen 6.34: Entry of frequency of labeled cells

After the frequency of CD45RA-positive cells has been entered and confirmed, type in the cell product volume to be used for the CD45RA depletion part. The acceptable range is shown on the screen.



Screen 6.35: Entry of cell product volume to be used for CD45RA depletion

IMPORTANT

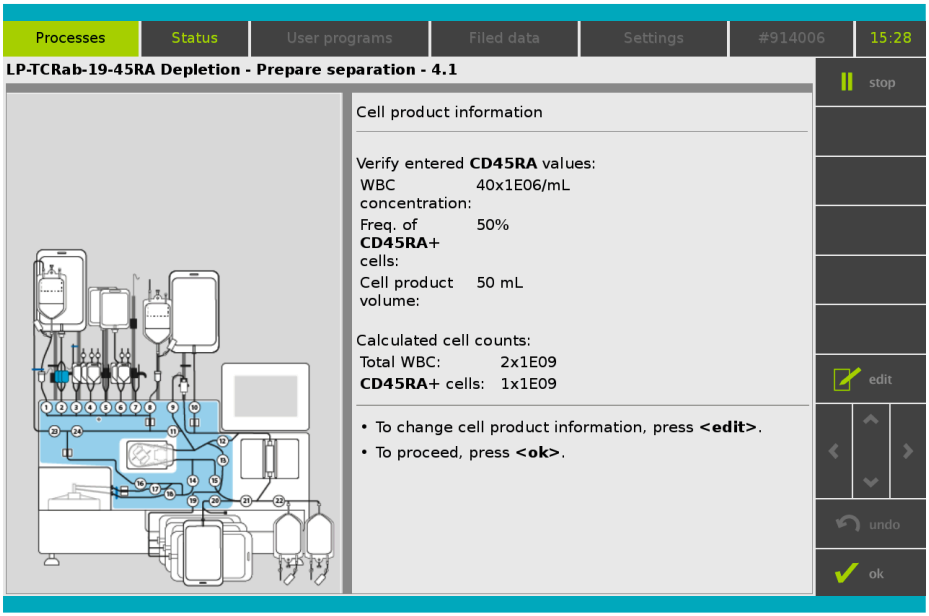
Acceptable starting cell product volume ranges depend on the use case selected. Table 6.2 lists all use case-specific starting volume ranges:

Use case	Process	Starting cell product volume
1	LP-45RA Depletion	10–350 mL
2	LP-TCRab Depletion	50–600 mL
3	LP-TCRab-19 Depletion	50–600 mL
4	LP-TCRab-45RA Depletion	10–60 mL (CD45RA) 50–600 mL (TCRα/β)
5	LP-TCRab-19-45RA Depletion	10–60 mL (CD45RA) 50–600 mL (TCRα/β(-CD19))

Table 6.2: Overview of starting cell product volume ranges for all use cases of the application

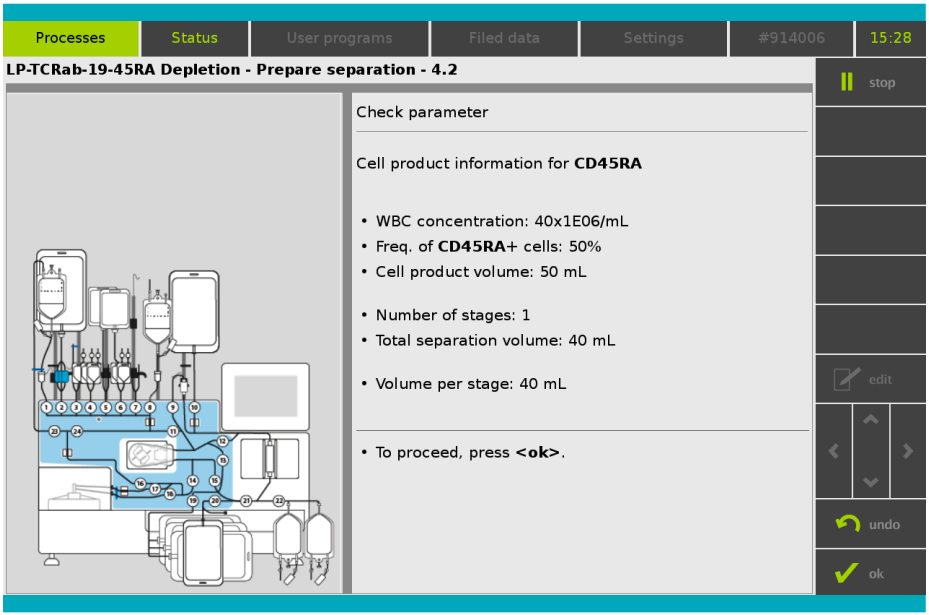
Based on the cell product information entered, total cell numbers are calculated automatically and compared to the given process specifications.

If required, tap <edit> to alter and re-calculate the cell product information.



Screen 6.36: Verification of entered values

Screen 6.37 shows an overview of the starting cell product information entered and characteristic calculated process parameters, like number of stages or total separation volume for the CD45RA depletion part.



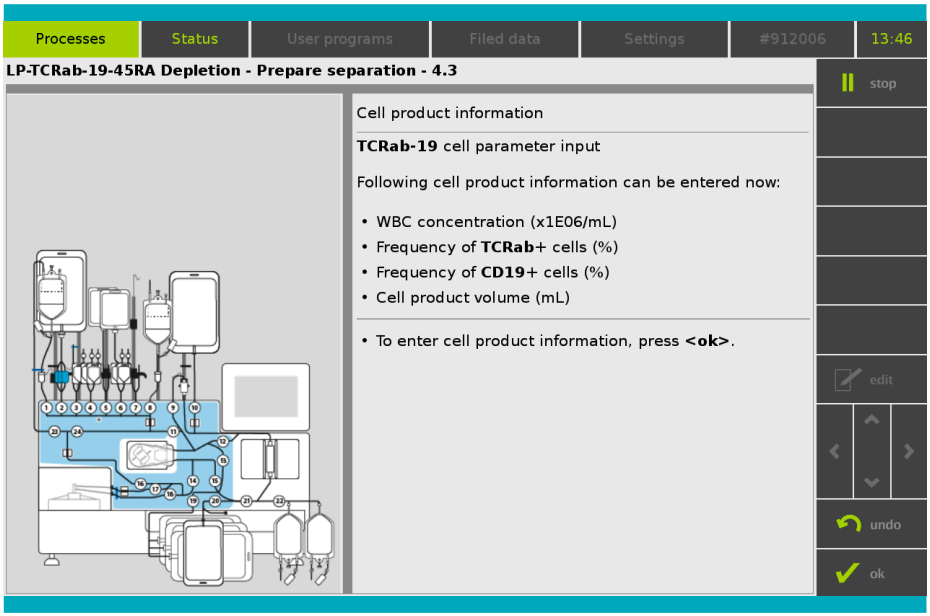
Screen 6.37: Overview cell product information and related process parameters (CD45RA depletion)

IMPORTANT

Tap <undo> to change cell product information if required.

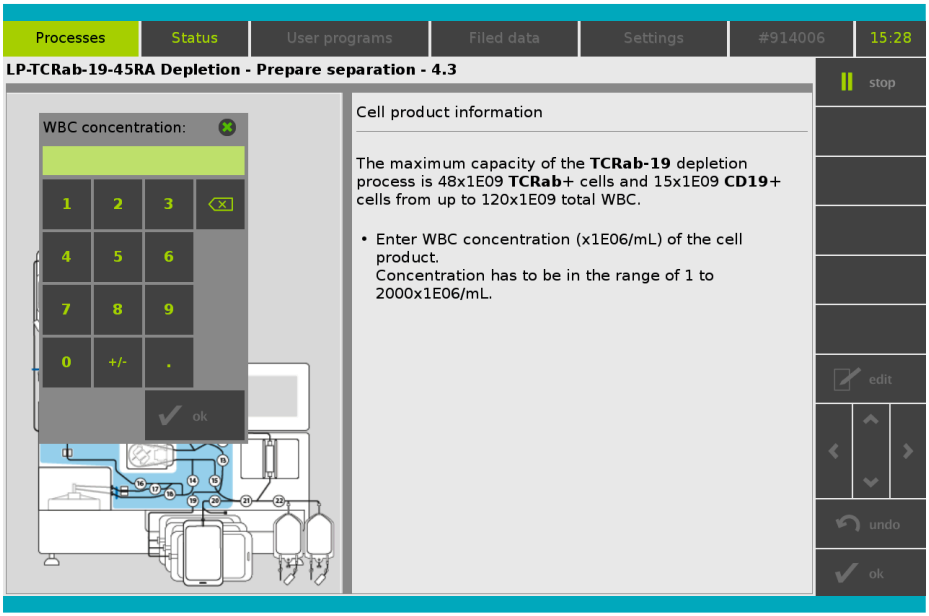
Cell parameter input for TCRα/β(-CD19) depletion

After cell parameter input for the CD45RA depletion part, cell product information entry related to the TCRα/β(-CD19) depletion part is required.



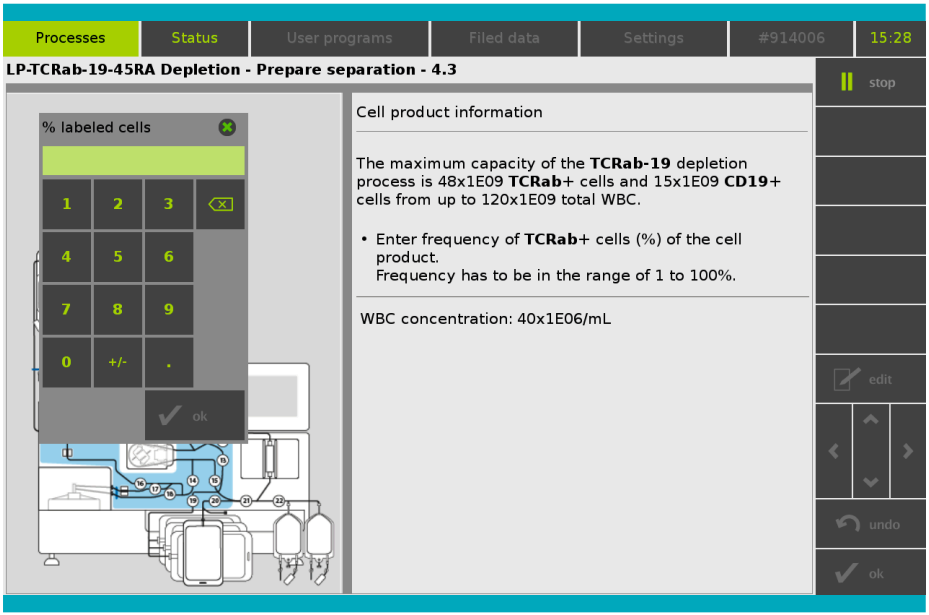
Screen 6.38: Cell product information input screen for the TCRα/β(-CD19) depletion part

Enter the WBC concentration of the starting cell product. The acceptable input range is shown on the screen.

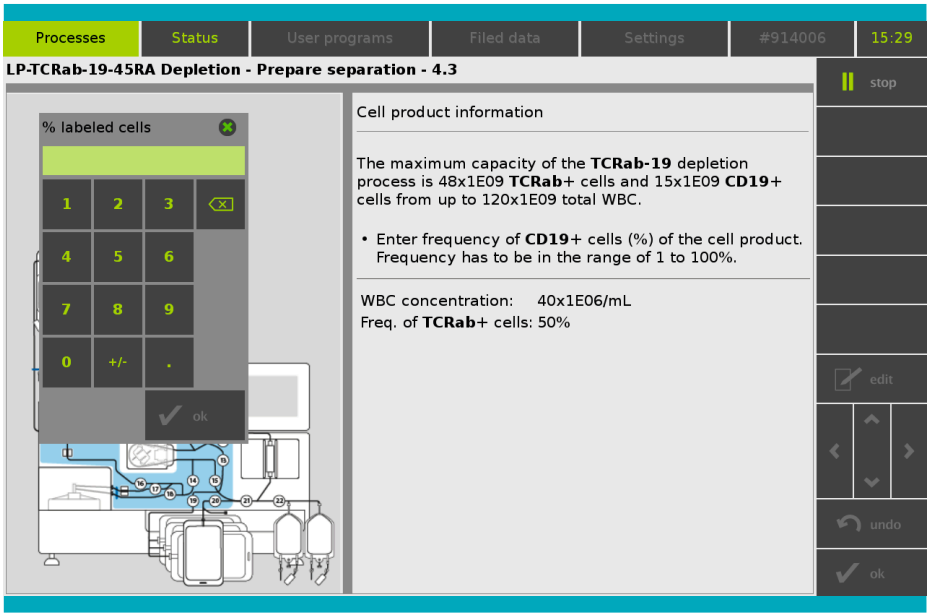


Screen 6.39: Entry of WBC concentration

After the WBC concentration has been entered and confirmed, type in the frequency of TCRab-positive cells followed by the frequency of CD19-positive cells. The acceptable input range is shown on the screen.



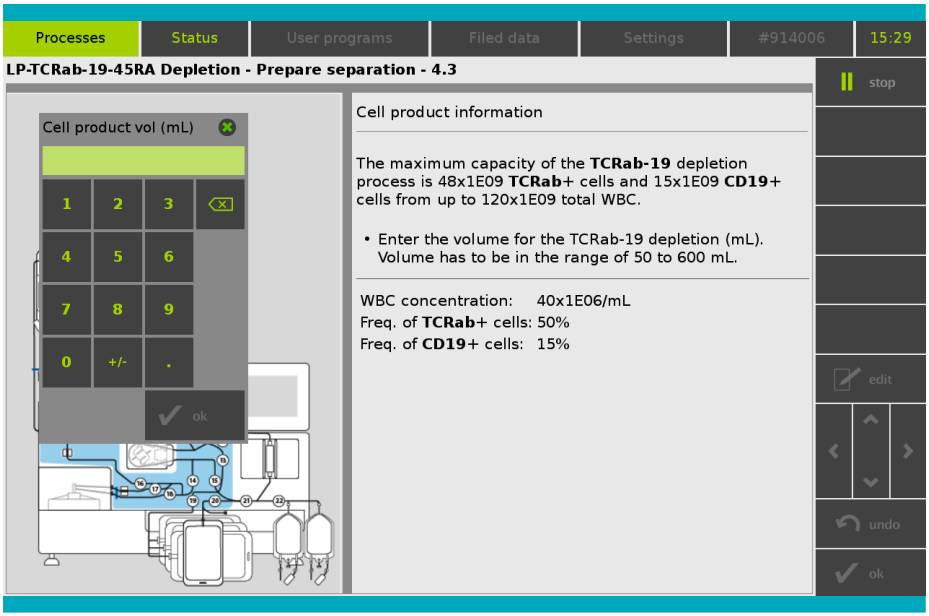
Screen 6.40: Entry of frequency of labeled cells (TCRα/β-positive cells)



Screen 6.41: Entry of frequency of labeled cells (CD19-positive cells)

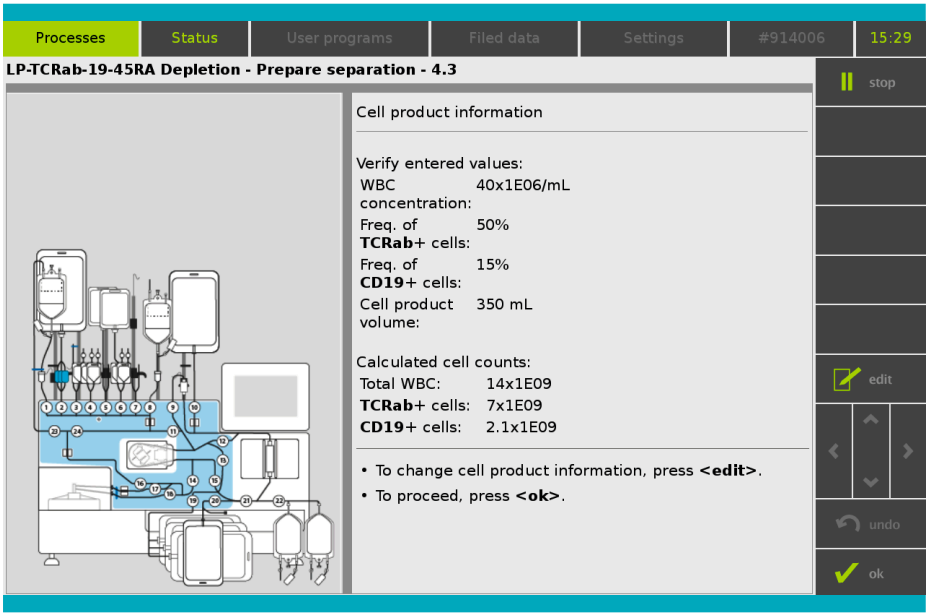
After frequencies of TCRα/β and CD19-positive cells have been entered and confirmed, type in the cell product volume used for the TCRα/β(-CD19) depletion part. The acceptable input range is shown on the screen.

Alternatively, see Table 6.2 in this section for an overview of use case-specific starting cell product volume ranges.



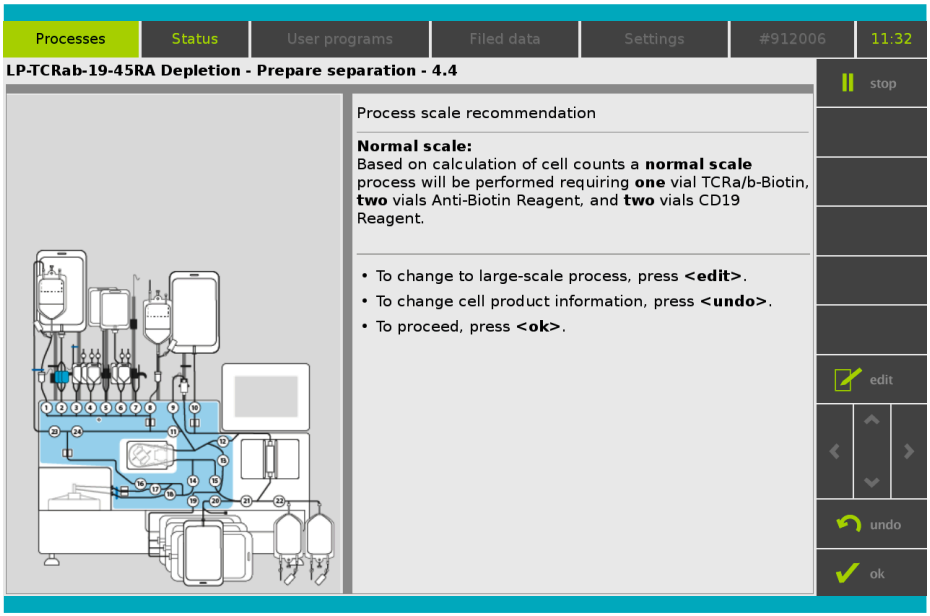
Screen 6.42: Entry of cell product volume for the TCRα/β(-CD19) depletion part

Based on the cell product information entered, total cell numbers are automatically calculated and compared to the given process specifications. If required, tap <edit> to alter and re-calculate the cell product information.



Screen 6.43: Verification of entered values

After confirmation of the cell product information entered, process scale and related reagent requirements are automatically calculated and shown on the screen. If required, tap <undo> to alter the cell product information. Subsequently, all process-related parameters are updated accordingly.

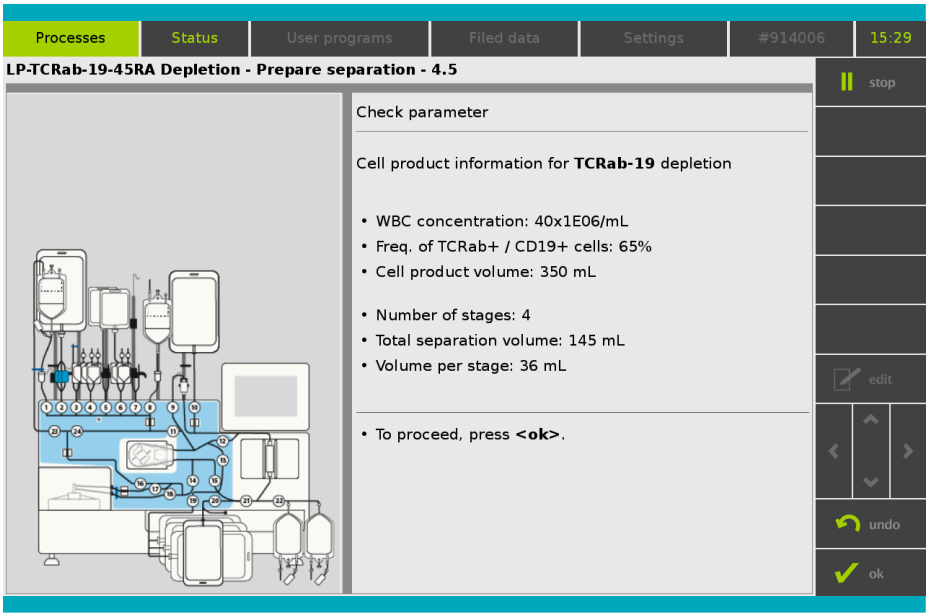


Screen 6.44: Process scale calculation and reagent requirements

IMPORTANT

- Strictly comply with the reagent requirements as outlined on Screen 6.44. Alternatively, see Table 3.4 in section 3.4 ´Summary of required materials´ for the use case-related overview of consumable quantities.
- It is possible to change from normal to large-scale processing for use case 2–5 of the LP-TCRab-19-45RA Depletion application, even if normal scale would be sufficient. If a large-scale process is recommended, it is not possible to change to a normal-scale process.
- Do not exceed the given process specifications. Adjust the volume of the starting cell product used for the process in order to keep the process specifications. Tap <undo> to change the cell product information accordingly and continue within the process specifications.

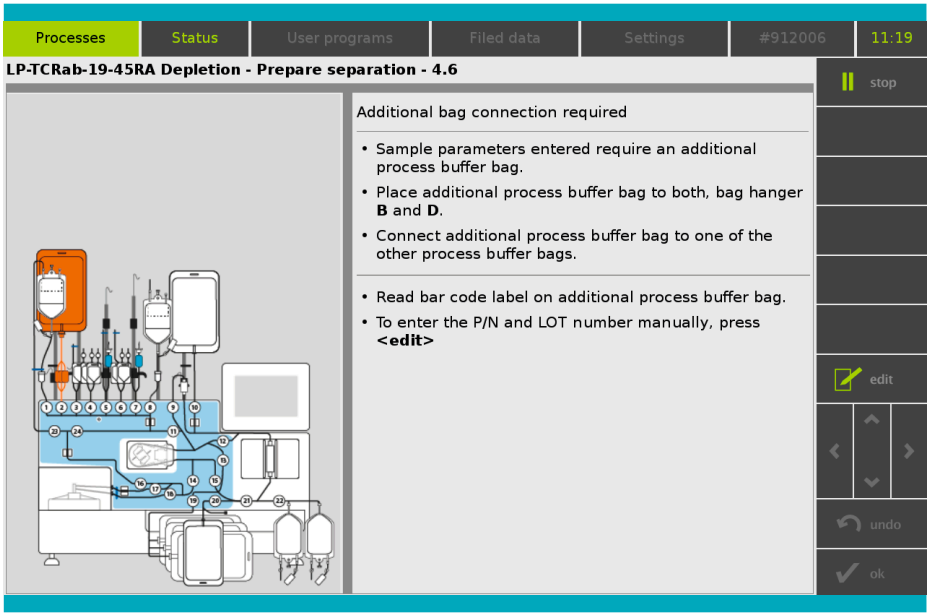
After proceeding within the process specifications, the next screen shows an overview of the TCRα/β and CD19-specific cell product information entered before. Based on these values, specific process-related parameters, e.g., number of loading stages or total separation volume, for the TCRα/β(-CD19) depletion part are shown.



Screen 6.45: Check parameters

Depending on the total numbers of TCRα/β and CD19-positive cells the connection of an additional process buffer bag (3 L) may be required. If a fourth process buffer bag is necessary for the process, this is automatically determined after confirmation of TCRα/β and CD19-related cell parameter input and shown on Screen 6.46.

If the use of a fourth process buffer bag is indicated, position the additional bag as indicated in Figure 6.11 and as instructed on Screen 6.46.



Screen 6.46: Connection of additional process buffer

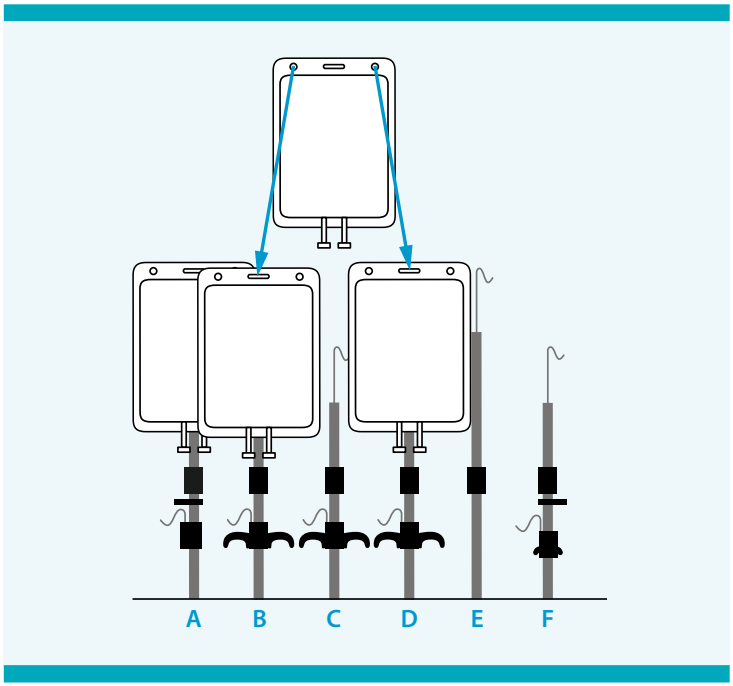
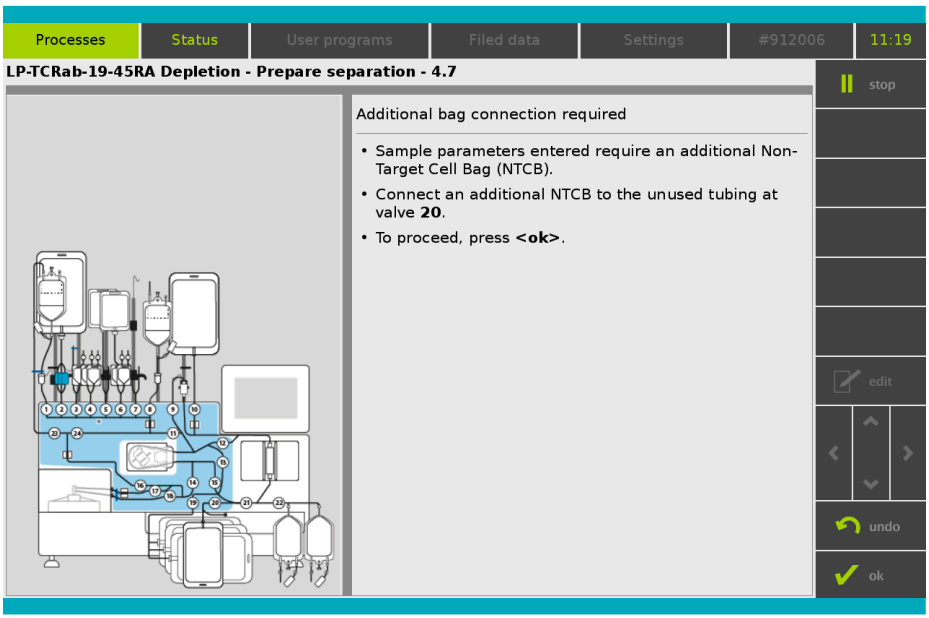


Figure 6.11: Positioning of the additional process buffer bag

Use a plasma transfer set for interconnection with one of the already installed process buffer bags. Clamp the plasma transfer set prior to connection. Follow the instructions given on the screen to enter product-specific data and place the bag to both, bag hangers B and D using the outer eyelets. Take care to open the clamp at the interconnection after the fourth buffer bag has been connected safely.

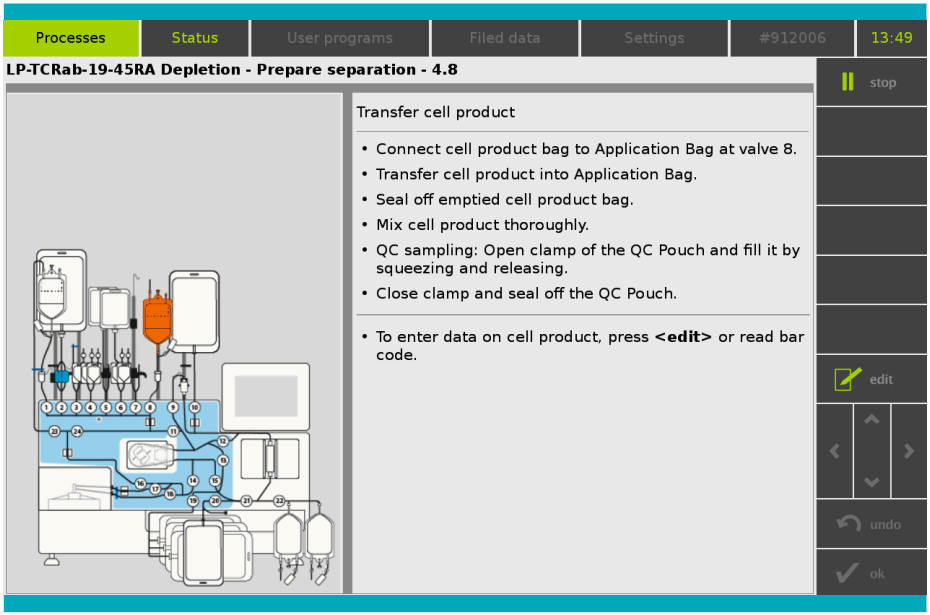
Depending on the total numbers of TCRα/β and CD19-positive cells the connection of an additional Non-Target Cell Bag (NTCB) may be required. If an additional NTCB is necessary for the process, this is automatically determined after TCRα/β and CD19-related cell parameter input and shown on Screen 6.47.

If applicable, weld a transfer bag of at least 1,000 mL volume to the unused tubing at the NTCB line below valve 20. Place the transfer bag together with the original NTCB outside of the bag compartment next to or below the instrument. Take care that the fluid pathway to the additional NTCB is open after connection.



Screen 6.47: Connection of an additional Non-Target Cell Bag (NTCB)

6.5.2 Transfer of the starting cell product



Screen 6.48: Transfer of the starting cell product into the Application Bag

For the transfer of the starting cell product into the Application Bag, follow the institution's standard operation procedures. For example, connect the starting cell product using either a sterile tubing connection, a Luer Lock connection, or a spike port connection. Transfer the starting cell product into the Application Bag. Seal off the emptied bag.

IMPORTANT

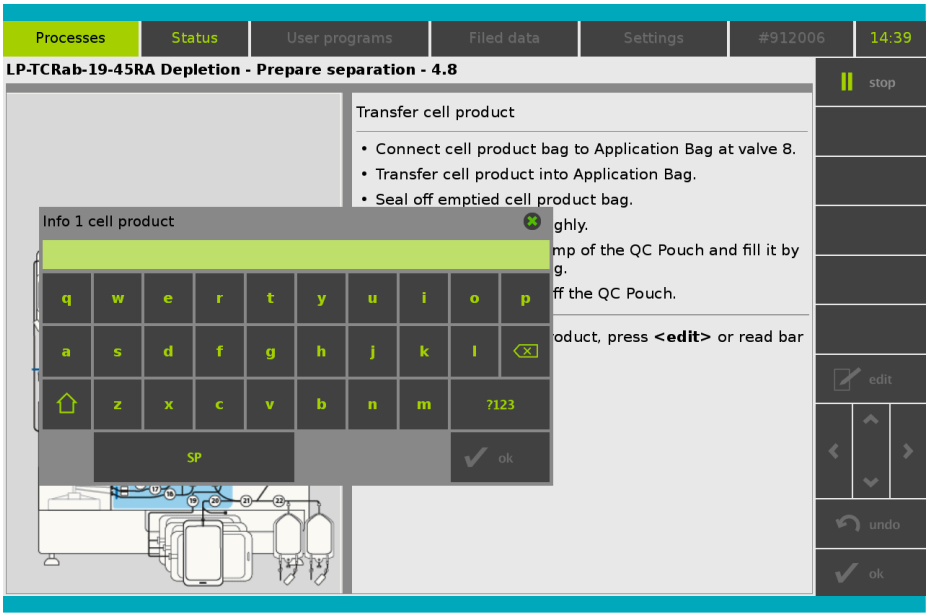
Gently but thoroughly mix the cells before QC sampling to ensure representative samples.

Use the QC Pouch to take a sample from the Application Bag. After the cell product has been transferred and mixed properly:

1. Open the QC Pouch clamp.
2. Squeeze and release the pouch once to transfer a representative sample into the pouch. Multiple squeezes and releases will increase the sample volume in the QC Pouch. The maximum volume of the QC Pouch is 4 mL.
3. Close the clamp and seal off the pouch.

The QC sample should be analyzed following the instituion's standard operating procedures.

Tap <edit> to enter cell product information.



Screen 6.49: Enter cell product identification

Different portions of the starting cell product are used for the CD45RA depletion part and the subsequent TCRα/β(-CD19) depletion part (depending on the use case selected). Separate cell information can be entered with the keyboard shown on the screen.

6.5.3 Connection of CliniMACS Reagents

⚠ WARNING

Risk of contamination. If reagent bags are not evacuated from air prior to reagent transfer, there is a risk of damaging the sterile filters during reagent transfer. Before transferring the reagent, remove remaining air from the reagent bag.

IMPORTANT

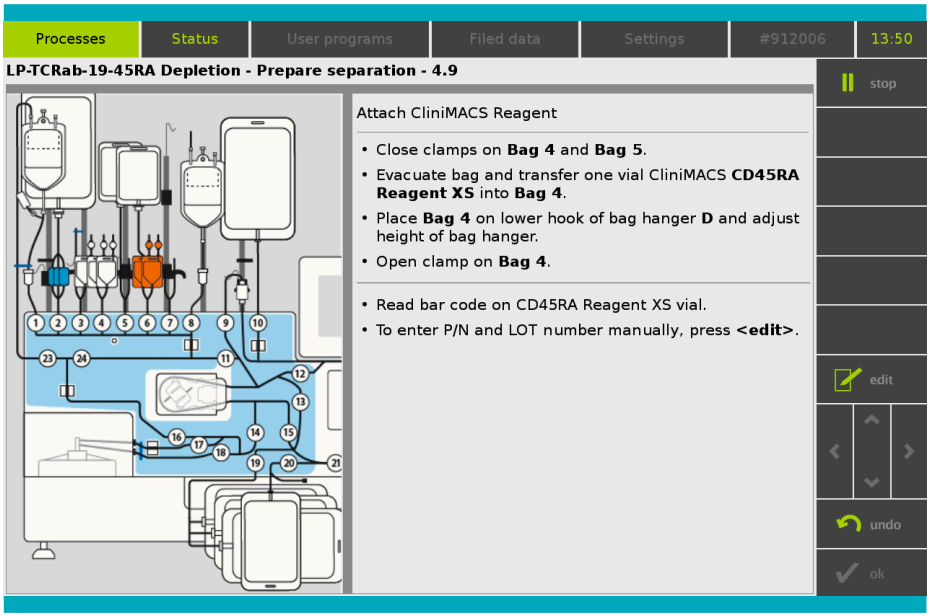
See Table 3.4 in section 3.4 ´Summary of required materials´ for an overview of use case-specific reagent requirements for normal and large-scale processing.

For appropriate reagent transfer and bag position, see Figure 6.12 and Table 6.3.

Connection of CliniMACS CD45RA Reagent XS

CliniMACS CD45RA Reagent XS connection is required for use cases 1, 4, and 5 of the LP-TCRab-19-45RA Depletion application.

Screen 6.50 illustrates the instructions for the transfer of CliniMACS CD45RA Reagent XS to the appropriate reagent bag.



Screen 6.50: Connection of CliniMACS CD45RA Reagent XS

Before reagent transfer, make sure that the clamps on reagent bag 4 and 5 are closed. Open the clamp on bag 4 after reagent transfer.

Read the barcode label on the reagent vial with the barcode reader. Alternatively, tap <edit> to enter part number (P/N) and lot number (LOT) manually with the keyboard shown on the screen.

CliniMACS Reagent	Bag no.	Bag hanger
CliniMACS TCRα/β-Biotin (or CliniMACS TCRα/β-Biotin XL)	1	B
CliniMACS Anti-Biotin (or CliniMACS Anti-Biotin XL)	2	C
CliniMACS CD19 Reagent (or CliniMACS CD19 Reagent XL)	3	C
CliniMACS CD45RA Reagent XS	4	D

Table 6.3: Overview reagent bag setup

Connection of CliniMACS TCRα/β-Biotin

Depending on the process scale CliniMACS TCRα/β-Biotin (normal scale) or CliniMACS TCRα/β-Biotin XL connection (large scale) is required for use cases 2–5 of the LP-TCRab-19-45RA Depletion application.

Normal-scale TCRα/β(-CD19) separations

Normal-scale separations always use the standard variant of the appropriate CliniMACS Reagent, i.e., CliniMACS TCRα/β-Biotin, CliniMACS Anti-Biotin Reagent, and CliniMACS CD19 Reagent, depending on the use case selected.

For transfer of the CliniMACS Reagents into the related reagent bags, one or two reagent vials of the same kind are required. For details on quantitative reagent requirements see Table 3.4 in section 3.4 ´Summary of required materials´.

IMPORTANT

To avoid blocking of the sterile filters pool two vials of the same reagent in a suitable syringe first before transfer to the corresponding reagent bag.

Large-scale TCRα/β(-CD19) separations

Large-scale separations always use one vial of the appropriate XL variant of CliniMACS Reagent, i.e., CliniMACS TCRα/β-Biotin XL, CliniMACS Anti-Biotin Reagent XL, and CliniMACS CD19 Reagent XL, depending on the use case selected. As only single XL reagent variants are used for large-scale processes, pooling is not applicable.

Note: Bag 5 is not used for any use case of the LP-TCRα/β-19-45RA Depletion application.

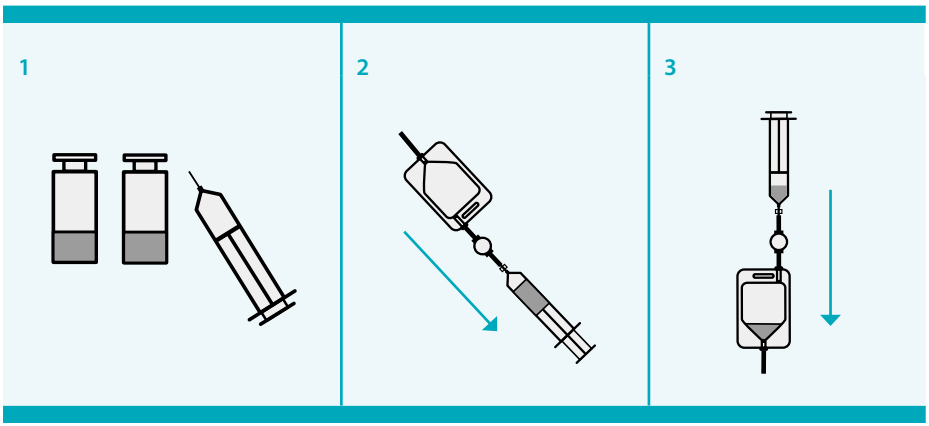
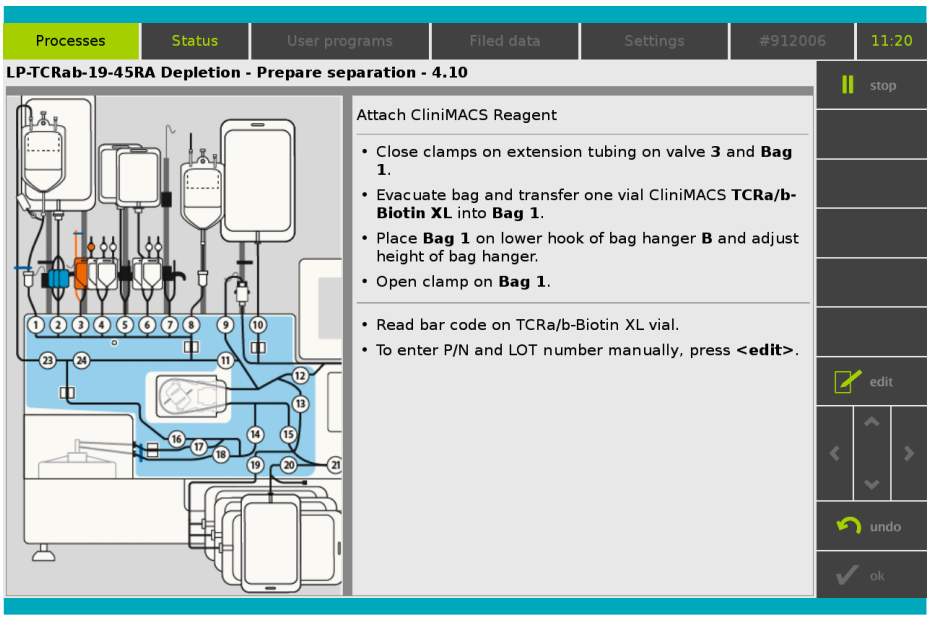


Figure 6.12: Transfer of reagent from vial(s) to reagent bag of the tubing set

Figure 6.12 illustrates how to transfer the contents from the reagent vial(s) into the related reagent bag:

1. Remove the plastic caps from the reagent vials. If two vials of the same reagent are required for the use case selected, first combine the contents of these vials in a suitable Luer Lock syringe equipped with a needle.
2. Remove the needle of the syringe carefully and connect the syringe via Luer Lock connection to the respective reagent bag. Initially, evacuate the air from the reagent bag as indicated.
3. Turn around bag and syringe and transfer the reagent (without air) in one go through the sterile filter into the bag for reagent transfer into corresponding reagent bag. Remove the syringe. Attach the reagent bag to the respective hook on the bag hanger. Make sure that the liquid can flow freely. See Table 6.3 for reagent transfer into corresponding bag and bag positioning.

Screen 6.51 illustrates the instructions for the transfer of CliniMACS TCRa/ß-Biotin XL to appropriate reagent bag for a large-scale LP-TCRab-19-45RA Depletion process.



Screen 6.51: Connection of CliniMACS TCRα/β-Biotin XL for a large-scale LP-TCRab-19-45RA Depletion process

IMPORTANT

Make sure that the appropriate reagent will be transferred, i.e., CliniMACS TCRα/β-Biotin for normal-scale or CliniMACS TCRα/β-Biotin XL for large-scale processing.

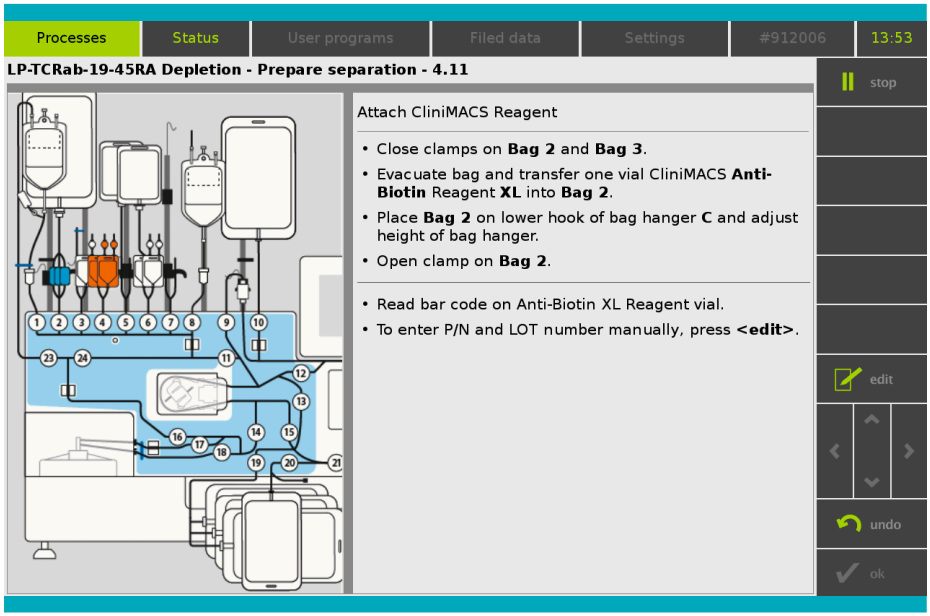
Before reagent transfer, make sure that the clamps on the reagent bag (Bag 1) and on the extension tubing at valve 3 are closed. After transfer of CliniMACS TCRα/β-Biotin (or CliniMACS TCRα/β-Biotin XL) to Bag 1, open the clamp to Bag 1.

Read the barcode label on the reagent vial with the barcode reader. Alternatively, tap <edit> to enter part number (P/N) and lot number (LOT) manually with the keyboard shown on the screen.

Connection of CliniMACS Anti-Biotin Reagent

CliniMACS Anti-Biotin Reagent (normal scale) or CliniMACS Anti-Biotin Reagent XL (large scale) connection is required for use cases 2–5 of the LP-TCRab-19-45RA Depletion application.

Screen 6.52 illustrates the instructions for the transfer of CliniMACS Anti-Biotin Reagent XL for a large-scale LP-TCRab-19-45RA Depletion process.



Screen 6.52: Connection of CliniMACS Anti-Biotin Reagent XL for a large-scale LP-TCRab-19-45RA Depletion process

IMPORTANT

Make sure that the appropriate reagent will be transferred, i.e., CliniMACS Anti-Biotin Reagent for normal-scale or CliniMACS Anti-Biotin Reagent XL for large-scale processing.

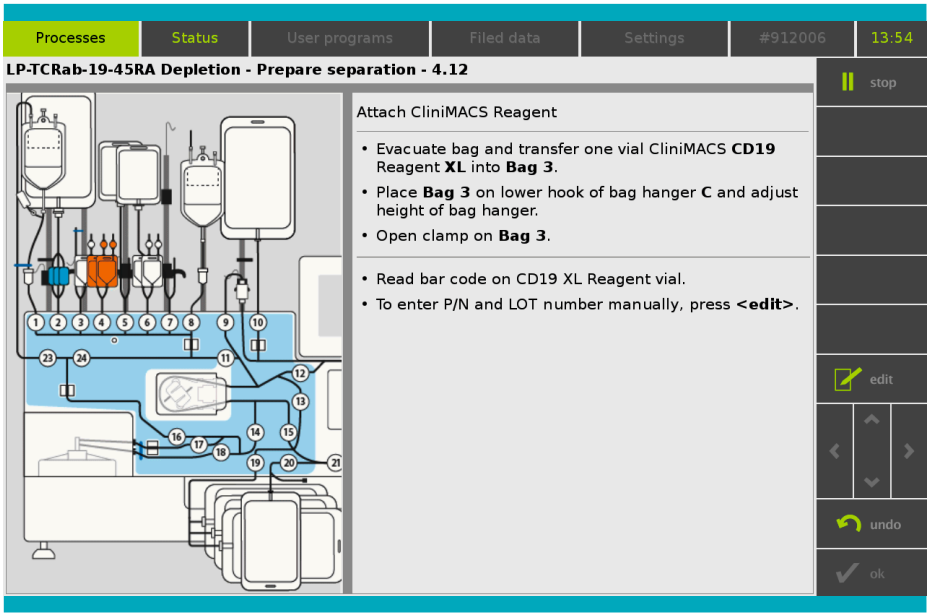
Before reagent transfer, make sure that the clamps on reagent bags (Bag 2 and 3) are closed. After transfer of CliniMACS Anti-Biotin Reagent (or Anti-Biotin Reagent XL) to Bag 2, open the clamp to Bag 2.

Read the barcode label(s) on the reagent vial(s) with the barcode reader. Alternatively, tap <edit> to enter part number (P/N) and lot number (LOT) manually with the keyboard shown on the screen.

Connection of CliniMACS CD19 Reagent

CliniMACS CD19 Reagent (normal scale) or CliniMACS CD19 Reagent XL (large scale) connection is required for use cases 3 and 5 of the LP-TCRab-19-45RA Depletion application.

Screen 6.53 below illustrates the instructions for the transfer of CliniMACS CD19 Reagent XL for a large-scale LP-TCRab-19-45RA Depletion process.



Screen 6.53: Connection of CliniMACS CD19 Reagent XL for a large-scale LP-TCRab-19-45RA Depletion process

IMPORTANT

Make sure that the appropriate reagent will be transferred to Bag 3, i.e., CliniMACS CD19 Reagent for normal-scale or CliniMACS CD19 Reagent XL for large-scale processing.

Before CD19 Reagent transfer, make sure that the clamp on reagent bag (Bag 3) is closed. After transfer of CliniMACS CD19 Reagent (or CliniMACS CD19 Reagent XL) to Bag 3, open the clamp to Bag 3.

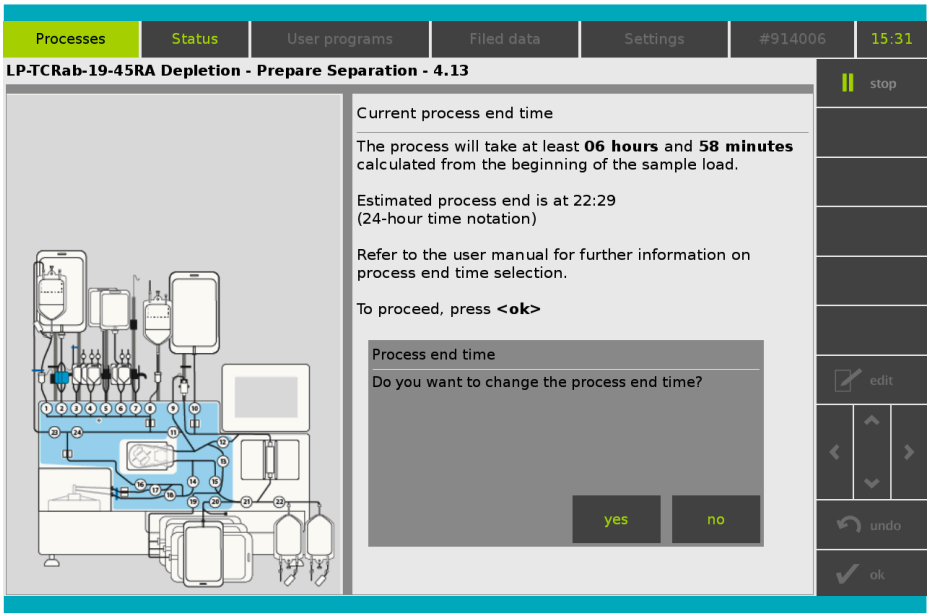
Read the barcode label on the reagent vial with the barcode reader. Alternatively, tap **<edit>** to enter part number (P/N) and lot number (LOT) manually with the keyboard shown on the screen.

6.5.4 Process end time (storage option)

Use cases 2–5 of the LP-TCRab-19-45RA Depletion application (use cases that include TCRα/β(-CD19) depletion) enable the user to decide on a particular time the final target cells (depleted of TCRα/β(-CD19)-positive cells) should be available.

In order to provide the TCRα/β(-CD19)-depleted target cells at an end time defined by the user, the process is paused after TCRα/β(-CD19) labeling, while the cells are transiently stored in the chamber adjusted to a WBC concentration of 2.5×10^8/mL and to a temperature of +6 °C (+43 °F). The process is paused for as long as it takes to provide the final cell product at the desired end time.

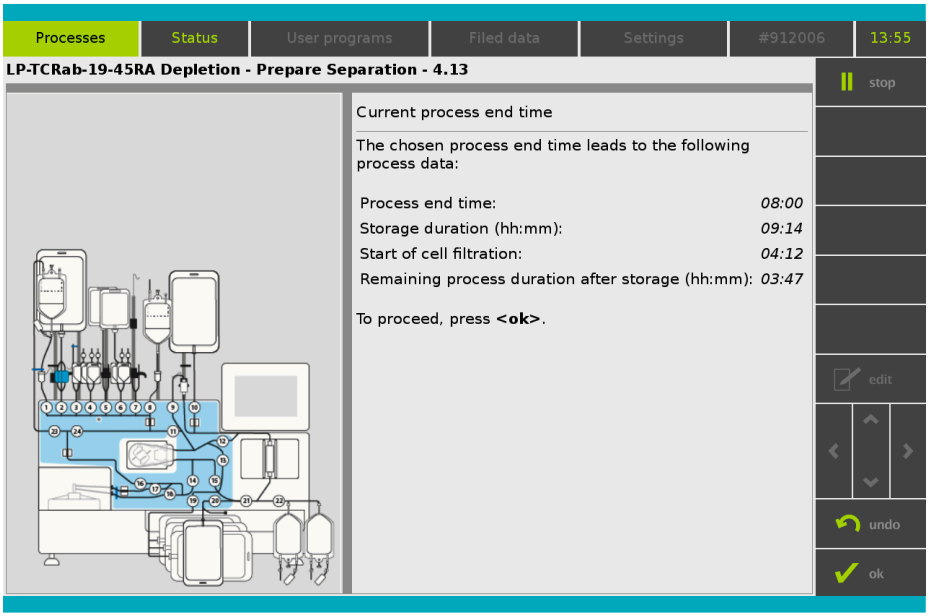
If a delay of the automatic process end time is desired, tap <yes> on Screen 6.54. Enter the desired end time for the TCRab(-CD19) depletion part with the keyboard shown on the screen.



Screen 6.54: Process end time entry option

If a change of the automatic process duration is not desired, tap <no> on Screen 6.54.

After entering a desired process end time, the following screen with important input-related process information is shown.



Screen 6.55: Important information after process end time input

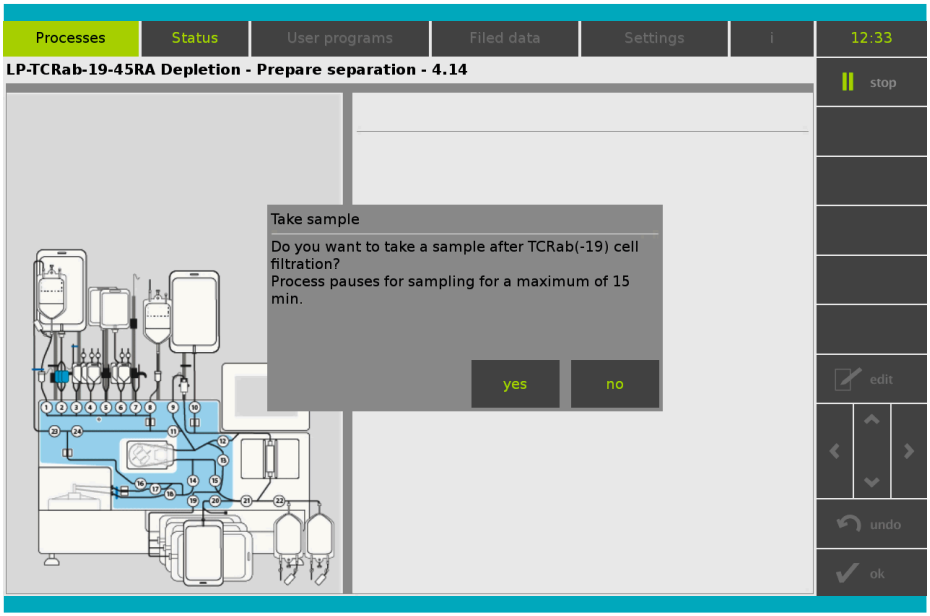
If a modification of the entered process end time is desired, tap <undo> and modify the desired end time with the keyboard shown on the screen.

IMPORTANT

- The maximum storage duration is 16 hours. If an individual end time input exceeds 16 hours of storage duration, adjust the desired end time accordingly. The entire process duration including a potential pause must not exceed 36 hours. If an individual process end time entered falls below the minimum required time for the process, the process end time entered will be automatically postponed by 24 hours.
- Use case 1 does not allow individual process end time input. Use case 2 and 3 enable to set an individual process end time for TCRα/β(-CD19) depletion.
- Use cases 4 and 5 enable to set an individual process end time prior to the CD45RA depletion part. The entered end time can later be adapted prior to the TCRα/β(-CD19) depletion part.

In-process QC sampling

Use cases 2–5 provide an in-process QC sampling option prior to TCRα/β(-CD19) depletion.



Screen 6.56: In-process QC sampling option

IMPORTANT

If requested, the in-process QC sampling must be executed after cell filtration and prior to to the start of the TCRα/β(-CD19) depletion part. The respective user interaction request appears automatically and is supported by a blinking blue light which turns to yellow after 3 minutes without confirmation of the pop-up shown in Screen 6.56. The QC sample must be taken within 15 minutes after the request appears. If QC sampling is not confirmed to be completed, the process will be continued automatically after 15 minutes. Mix the cells gently but thoroughly to ensure representative samples.

Use the QC Pouch to take a sample from the Filtration Bag. After the cell product has been transferred and mixed gently but thoroughly:

1. Open the QC Pouch clamp.
2. Squeeze and release the pouch once to transfer a representative sample into the pouch. Multiple squeezes and releases will increase the volume in the QC Pouch, if required. The maximum volume of the QC Pouch is 4 mL.
3. Close the clamp and seal off the pouch.

The QC sample should be analyzed following the institution's standard operating procedures.

6.5.5 Final process preparation

Figure 6.13 shows the tubing set with all connections required for the LP-TCRab-19-45RA Depletion process.

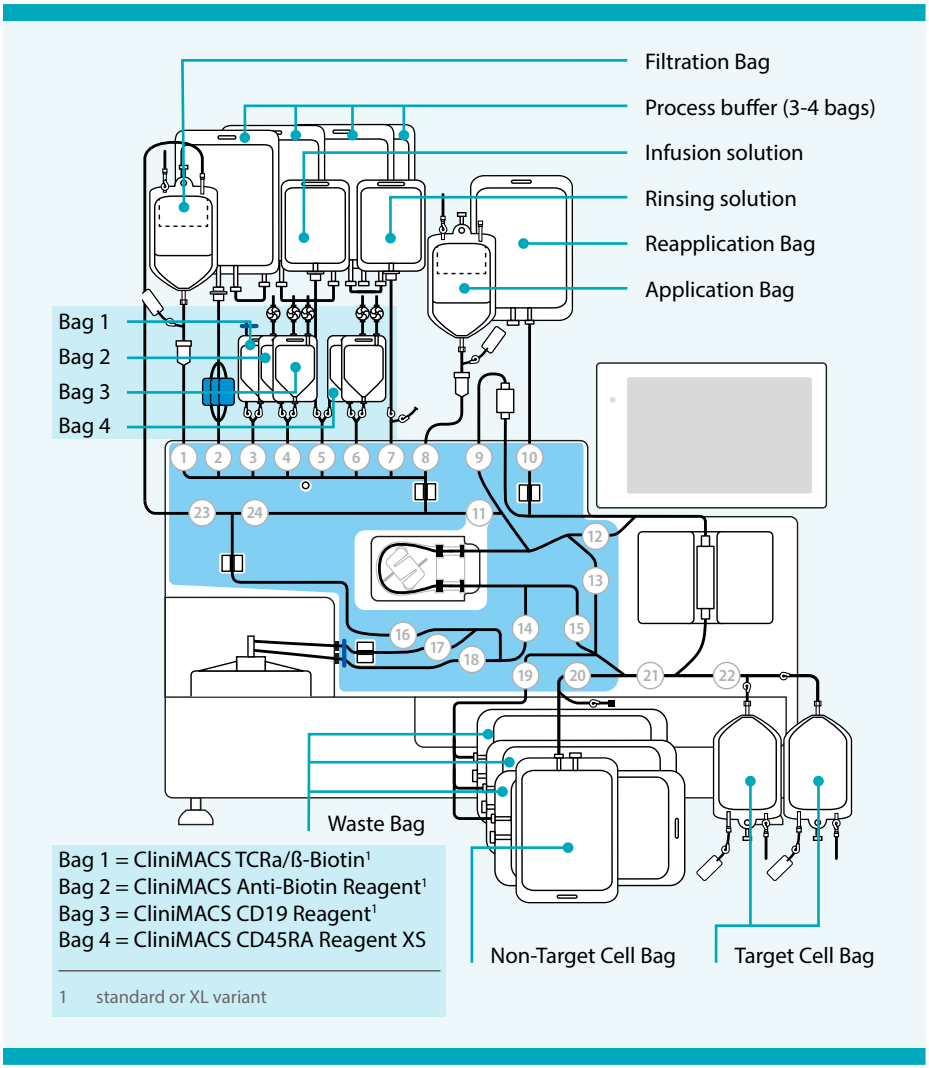
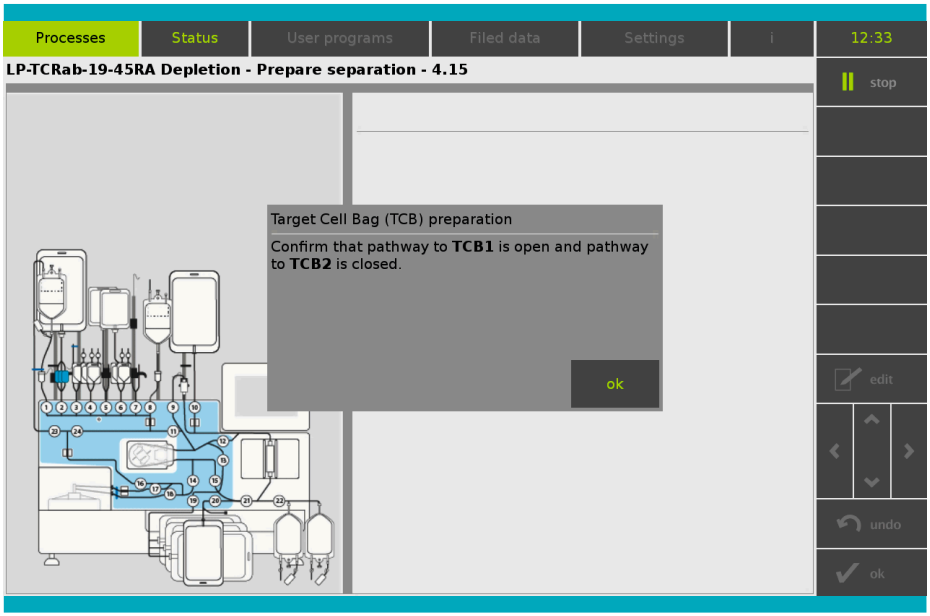


Figure 6.13: CliniMACS Prodigy TS 320 with all connections required for a LP-TCRab-19-45RA Depletion process, use case 5.

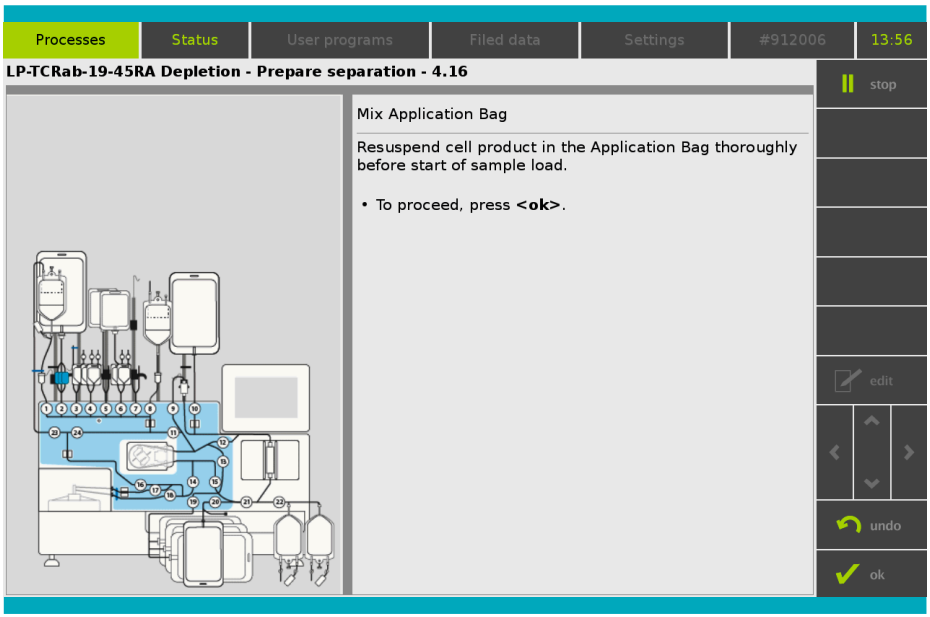
Make sure that the pathways to the Target Cell Bags (TCB1 and TCB2) are opened or closed according to the use case selected and as instructed by the software.



Screen 6.57: Target Cell Bag (TCB) preparation

IMPORTANT

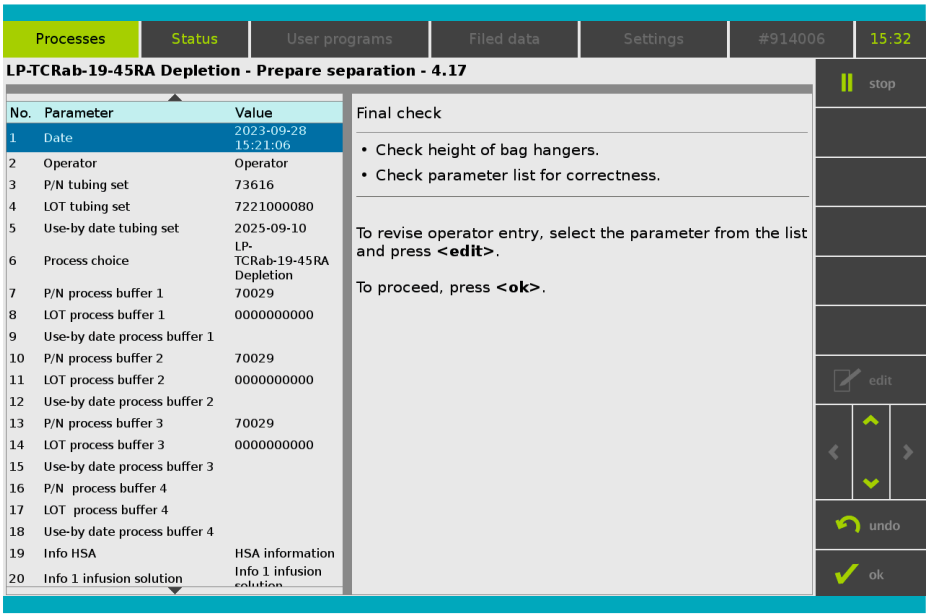
- For all use cases including a CD45RA depletion (i.e., use cases 1, 4, and 5), confirm that the pathway to TCB1 is open and the pathway to TCB2 is closed.
- For all use cases without CD45RA depletion (i.e., use cases 2 and 3), confirm that the pathway to TCB1 is closed and the pathway to TCB2 is open.



Screen 6.58: Mix Application Bag

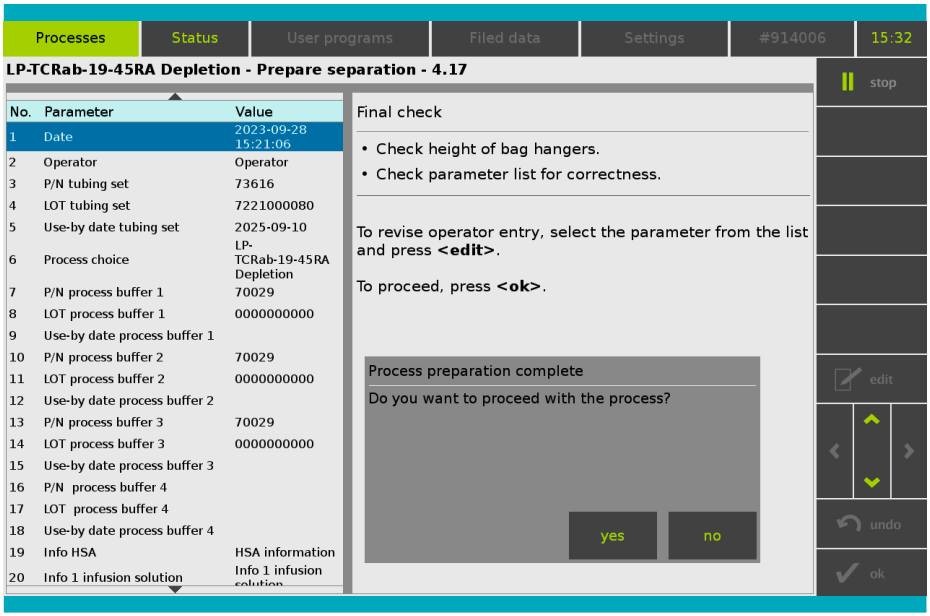
Mix the Application Bag gently but thoroughly.

A list of all entered parameters is shown on the screen.



Screen 6.59: Final process setup check

After confirming the shown parameter list tap <ok> to proceed with the process.



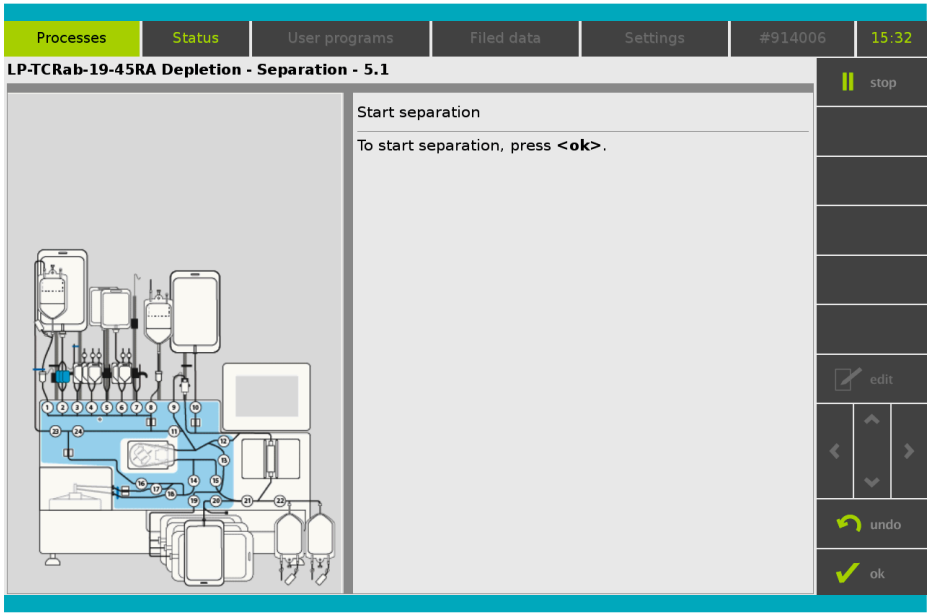
Screen 6.60: Completion of process preparation

Note: At this time, all preparative steps are completed, and the automated depletion process can be started.

6.6 Automated depletion process

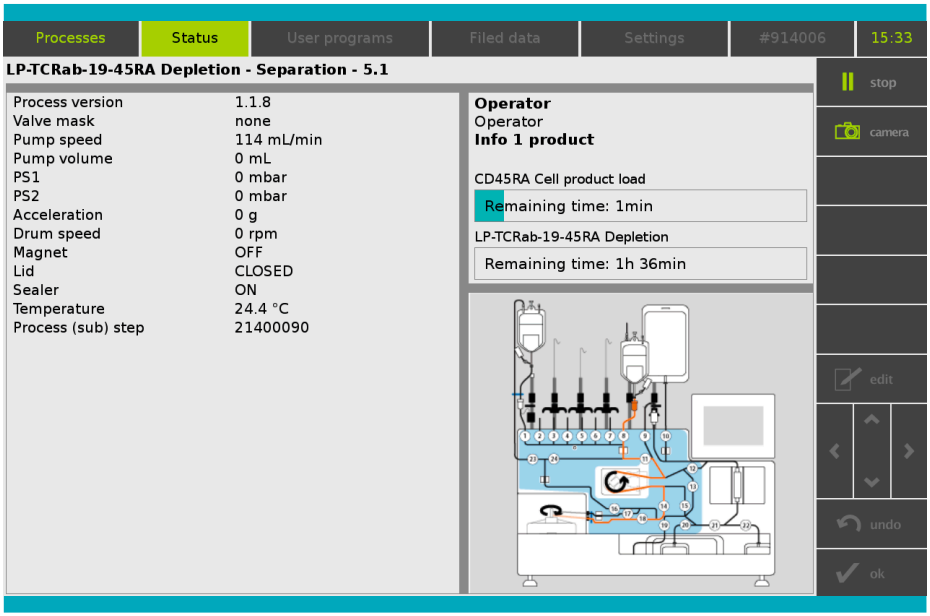
6.6.1 Start of the LP-TCRab-19-45RA Depletion process

To start the process for the use case selected, tap <ok> as shown in Screen 6.61.



Screen 6.61: Start of the automated depletion process

To monitor the different phases of the automated process tap <Status> on the menu bar. For every important process phase a specific screen is shown. Based on the screens shown, each process phase can be followed during the entire process.



Screen 6.62: Status screen – process information screen after start of the CD45RA depletion part

For a detailed description of the status screen, also see the CliniMACS Prodigy user manual (Instrument).

Depending on the use case selected, important process phases may include:

- CD45RA cell product load
- CD45RA labeling
- CD45RA reagent wash
- CD45RA depletion
- Tubing set cleaning
- TCRα/β(-CD19) cell product load
- TCRα/β(-CD19) cell wash
- TCRα/β(-CD19) labeling
- TCRα/β(-CD19) reagent wash
- TCRα/β(-CD19) storage phase (optional)
- TCRα/β(-CD19) cell filtration
- TCRα/β(-CD19) bulk depletion
- TCRα/β(-CD19) concentrate bulk product
- TCRα/β(-CD19) sensitive depletion

In detail: For CD45RA depletion a direct labeling is performed and a one-step depletion strategy is applied. At the end of the CD45RA depletion part (use cases 1, 4, and 5) the first target cell product, depleted of CD45RA-positive cells, is provided in a particular Target Cell Bag (TCB1) ready for further use. For use cases 2–5 CliniMACS Prodigy is subsequently prepared for the TCRα/β(-CD19) depletion part.

In the TCRα/β(-CD19) depletion part the intended volume of the leukapheresis product is depleted of TCRα/β(-CD19)-positive cells. Using an indirect labeling strategy, the starting cell product is first incubated with a primary antibody (CliniMACS TCRα/β-Biotin). After removal of the excess of the biotinylated primary antibody, the cell product is simultaneously labeled with CliniMACS Anti-Biotin Reagent (and CliniMACS CD19 Reagent) in a subsequent immunomagnetic labeling step. Afterwards, the excess of reagent is removed and the TCRα/β(-CD19) depletion is carried out in a continuous flow separation sequence using a two-step depletion strategy (bulk and sensitive depletion).

At the end of the second depletion part the (second) target cell product, depleted of TCRα/β(-CD19)-positive cells, is provided in a second Target Cell Bag (TCB2), ready for further use.

6.6.2 Intermediate handling after CD45RA depletion

IMPORTANT

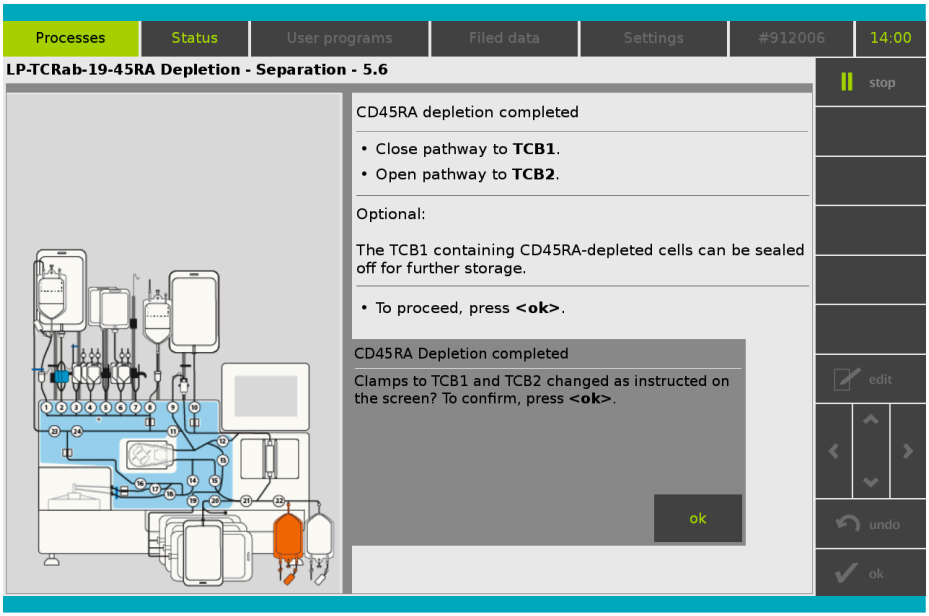
Use case 1 comprises a CD45RA depletion only. If use case 1 has been selected, continue with the instructions given on Screen 6.67. If use case 2 or 3 has been selected, section 6.6.2 ´Intermediate handling after CD45RA depletion´ is not applicable as these use cases do not contain a CD45RA depletion.

Use cases 4 and 5 combine a CD45RA depletion with a subsequent TCRα/β depletion with (use case 5) or without (use case 4) CD19 depletion.

This section is applicable for use cases 4 and 5 only. It describes the required user interactions after completion of the CD45RA depletion and to prepare the instrument for subsequent TCRα/β(-CD19) depletion.

For completion of the CD45RA depletion part, the following facts must be considered:

The CD45RA depletion part roughly takes two hours. After CD45RA depletion is completed, the CD45RA-depleted cells are provided in Target Cell Bag 1. The process pauses automatically. User attention is supported by a respective pop-up and a blinking blue light.



Screen 6.63: Fluid pathway changes after CD45RA depletion (intermediate process)

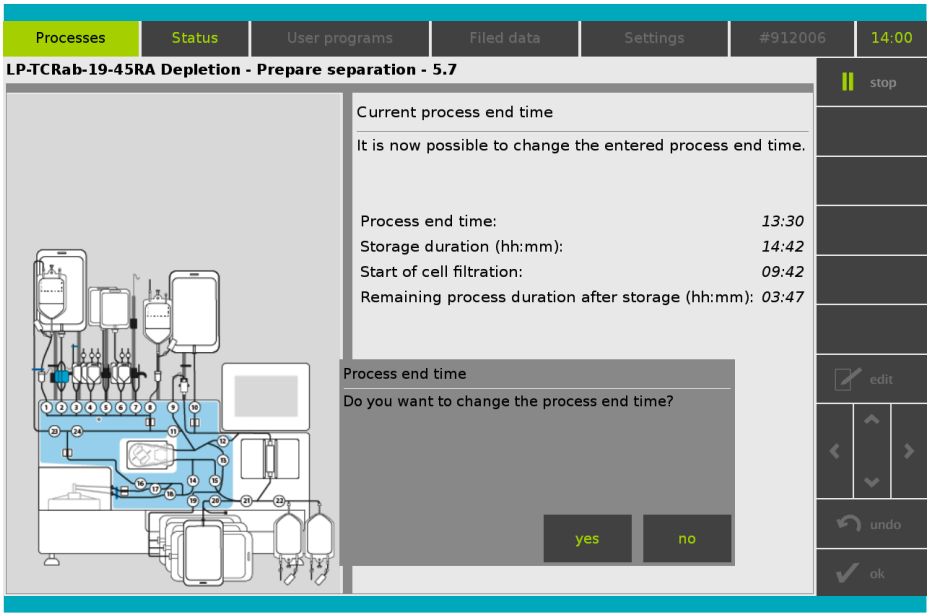
Screen 6.63 informs that the CD45RA depletion part is completed and the pathways to the Target Cell Bags must be changed.

Confirm the instructions given on the screen. Tap <ok> to continue the process.

IMPORTANT

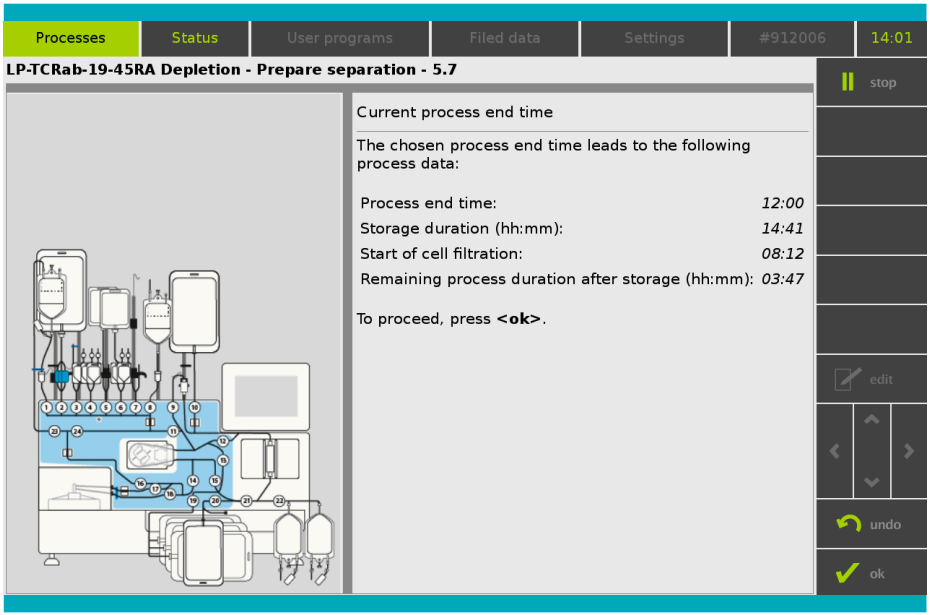
- Seal off and store the CD45RA-depleted cells in Target Cell Bag 1 (TCB1) according to the institutional guidelines.
- If the process is not continued within 30 minutes, an optical and acoustic warning appears. Note that the process would not be continued without confirmation of the Target Cell Bag pathway switch.

After CD45RA depletion is completed, the entered process end time can either be confirmed or adapted.



Screen 6.64: Changing the process end time after CD45RA depletion

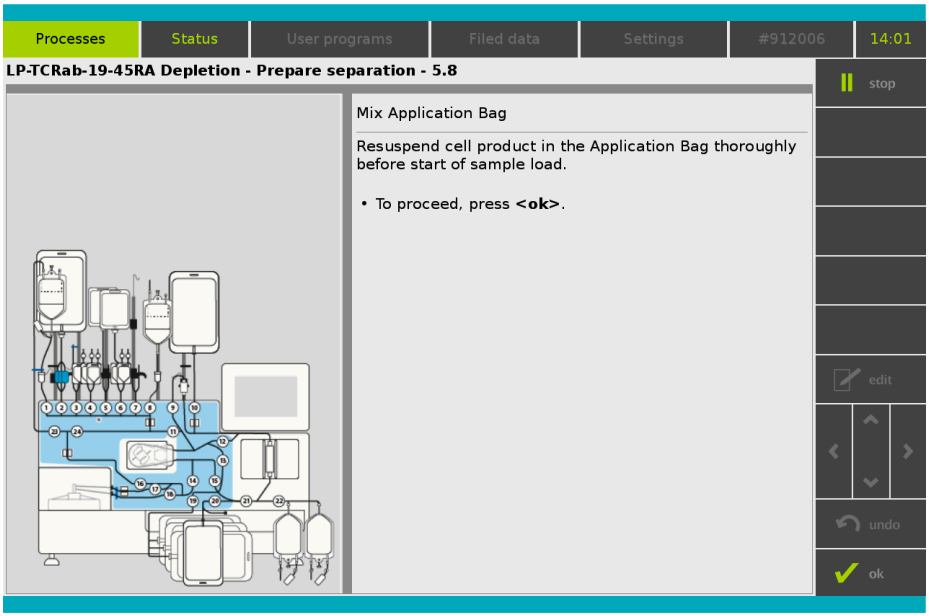
If adaption of the process end time is desired, tap <yes> and enter the new process end time with the keyboard shown on the screen. Once a new end time is entered, Screen 6.65 shows the new end time and related conditions.



Screen 6.65: After process end time has been adapted

Before starting the TCRα/β(-CD19) depletion part for the remaining cell product, mix the Application Bag gently but thoroughly.

Tap <ok> to continue the process with the TCRα/β(-CD19) depletion part.



Screen 6.66: Mix Application Bag

6.6.3 Completion of the depletion process

⚠ CAUTION

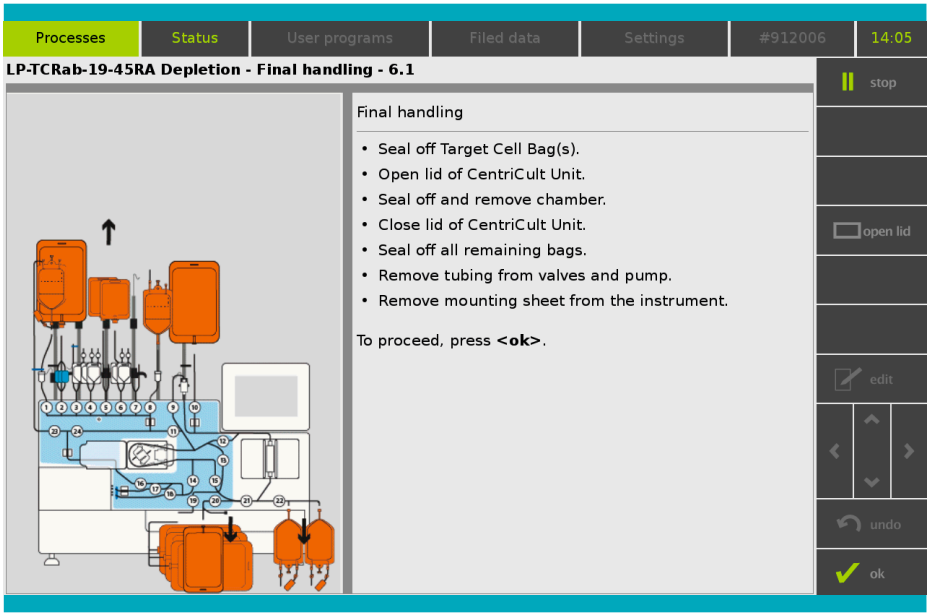
Risk of reduced quality of target cells. Other solutions for the resuspension of the final cell product than those specified in chapter ´Materials required´, can lead to reduced quality of the target cells. Make sure that the target cells are resuspended in a suitable infusion solution. For additional safety, check the remaining level in the infusion solution bag after process completion to see if the required volume has been used up.

After completion of the entire process, seal off the Target Cell Bag(s) and Non-Target Cell Bag(s). Use the QC Pouch(es) to take samples from Target Cell Bag(s) for QC analysis. Optionally take sample(s) from the Non-Target Cell Bag(s) as well.

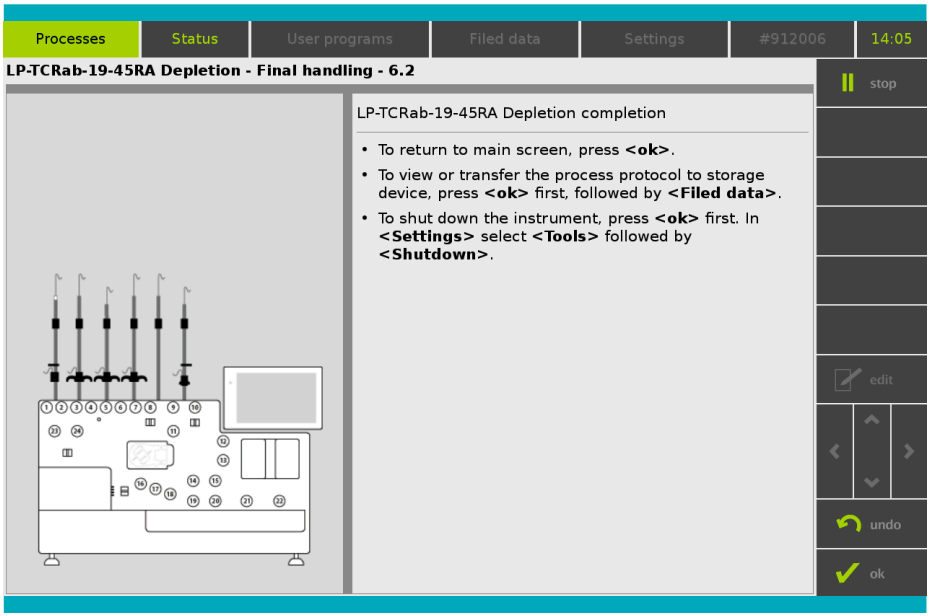
Store the samples according to institutional guidelines prior to analysis.

The CentriCult Unit lid unlocks automatically during this process step. If the lid does not unlock automatically, tap <open lid> on the menu bar. In case the lid could not be opened, contact Miltenyi Biotec Technical Support.

Note: Discard the tubing set as biohazardous material according to the institutional guidelines.



Screen 6.67: Final handling after depletion process is completed



Screen 6.68: Completion of the process

6.6.4 Download of process log files

Every CliniMACS Prodigy process generates a specific process log file including a process protocol. The process log file is saved and stored in the <Filed data> menu and can be transferred to other media with a USB storage device.

Select a process from the list shown in the <Filed data> menu. Tap <ok> to open the respective protocol. Tap <save> to save the entire process log file which contains amongst others the process protocol as a pdf on a USB storage device. To do so, the USB storage device should be inserted into the USB port at the right side of the touchscreen. See the CliniMACS Prodigy user manual (Instrument) for illustrated instructions.

6.6.5 Shutdown and switch off the instrument

Always perform a shutdown procedure before switching off the instrument. In the <Settings> menu, select the program <Shutdown> in the <Tools> group and tap <run>.

Once the shutdown is completed, the instrument can be switched off safely.

6.6.6 Cleaning and disinfection

⚠ CAUTION

Risk of cell damage by UV radiation. Exposure to UV radiation can lead to damage of cells. Do not use UV radiation for disinfection during the process.

The instrument should be cleaned and disinfected after each procedure. Refer to the CliniMACS Prodigy user manual (Instrument) regarding the cleaning and disinfection of the instrument. It is recommended to follow the standard operating procedures for the treatment of potentially infectious materials.

6.6.7 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.

Do not store samples in the QC Pouches. QC samples should be stored in suitable containers at +4 to +8 °C (+39 to +46 °F) or according to institutional guidelines. All QC samples should be analyzed according to standard operating procedures. Contact Miltenyi Biotec Technical Support for assistance, if required.

Note: Determine the volume(s) in the Target Cell Bag(s) for calculation of total WBC and target cell numbers. If required, determine the volume in the other fractions (e.g., Non-Target Cell Bag(s)) as well.

IMPORTANT

The Target Cell Bags included in the tubing set are not intended for storage or transport of cells for more than 24 hours. If the target cells are not used for direct application, special attention should be paid to preserve stability and functionality of the target cells in infusion solution and the storage container used.

7 Troubleshooting

In any case of instrument malfunction or process failure, contact the Miltenyi Biotec Technical Support at:

+49 2204 8306-3803
technicalsupport@miltenyi.com

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

8 Legal Notes

8.1 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
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Miltenyi Biotec

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

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

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

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

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

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

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

India
Miltenyi Biotec India Pvt. Ltd.
Vatika Business Centre,
Floor No. 6
Divya Sree Omega
Kondapur, Serilingampally
K.V. Rangareddy
Telangana 500084, India
+91 040 45175910
macsin@miltenyi.com

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

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

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

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

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

Spain
Miltenyi Biotec S.L.
C/Virgilio 2, Edificio II, Planta –1
28223 Pozuelo de Alarcón
Madrid, Spain
+34 91 512 12 90
+34 91 512 12 91
macses@miltenyi.com

Switzerland
Miltenyi Biotec Swiss AG
Soodstrasse 52
8134 Adliswil
Switzerland
+41 32 623 08 47
+49 2204 85197
macsCH@miltenyi.com

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

www.miltenyibiotec.com

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