Miltenyi Biotec

CliniMACS Prodigy®
T Cell Transduction – Large Scale System

User Manual

CAUTION:
Clinical Research System 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 LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE) or FDA approval. CliniMACS GMP MicroBeads are for *ex vivo* cell processing only, and are not intended for human *in vivo* applications.

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CliniMACS Prodigy®
T Cell Transduction – Large Scale System

User Manual

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Essential information

This user manual provides information for the use of the CliniMACS Prodigy T Cell Transduction – Large Scale 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 cell product are described in the CliniMACS Prodigy User Manual (Instrument). For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Prodigy T Cell Transduction – Large Scale 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 shown 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 could result in instrument malfunction, property damage, personal injury, and/or death.

Retain the user manuals for future reference. They should be kept accessible and readily available together with all other safety and operating documentation during the entire life cycle of the instrument for all personnel responsible for installation, operation, and maintenance.

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1 Introduction

1.1 General information

T cells can be stably modified by introduction of genetic material using viral vectors. For example, T cells can be genetically modified to express T cell receptors (TCRs), or chimeric antigen receptors (CARs) which redirect them to specific targeting and killing of tumor cells for improved clinical outcome of adoptive T cell therapy approaches.

The generation of genetically modified T cells requires sequential steps:

- cell separation
- T cell activation
- transduction
- expansion

The CliniMACS Prodigy T Cell Transduction – Large Scale System allows the entire process starting from fresh or frozen leukapheresis, including harvest of the cell product to be performed in an automated and closed system (see Figure 1.1). Samples for quality control can also be obtained during manufacturing using sterile technique. The T Cell Transduction – Large Scale System was especially developed for the manufacturing of high T cell numbers in a large cultivation chamber. The system provides four different harvest types to enable flexibility for downstream applications.

The components which are required to perform the T Cell Transduction with the CliniMACS Prodigy T Cell Transduction – Large Scale System are described in chapter 3 on page 17 of this user manual.

This user manual provides guidance for the use of the CliniMACS Prodigy T Cell Transduction – Large Scale System in order to manufacture genetically modified T cells for clinical application.

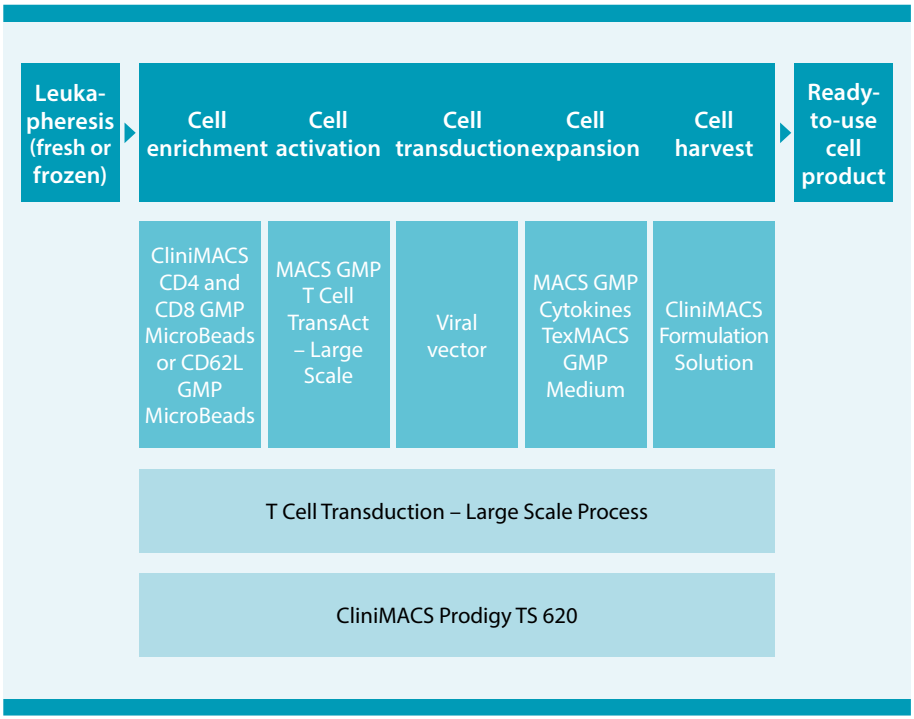


Figure 1.1: Generic manufacturing scheme using the CliniMACS Prodigy T Cell Transduction – Large Scale System

IMPORTANT

This user manual describes the full process and includes a recommended protocol. In order to ensure a high-quality cell product, Miltenyi Biotec recommends using the cytokines and media stated in this user manual.

1.2 Process

The T Cell Transduction – Large Scale System includes the following steps:

- Install the CliniMACS Prodigy TS 620 on the CliniMACS Prodigy.
- Supplement the CliniMACS PBS/EDTA Buffer with human serum albumin (HSA).
- Supplement the TexMACS™ GMP Medium with MACS® GMP Cytokines and human AB serum.
- Connect the supplemented medium to the tubing set.
- The CliniMACS Prodigy TS 620 is then primed.
- After priming of the tubing set, connect the fresh or frozen leukapheresis product to the tubing set.
- Connect the CliniMACS® GMP MicroBeads to the tubing set.
- Cell enrichment is performed.
- Initial washing, cell labeling and magnetic separation are performed automatically.
- Enriched cells will be directed to a dedicated bag from which a sample can be taken for analysis.
- After providing the required input, the required number of enriched cells are seeded in the chamber where all subsequent phases of cell cultivation take place.
- The T cells are then activated by MACS GMP Cytokines.
- The user is then prompted to plan the cultivation using an activity matrix which includes activation (e.g., with MACS GMP T Cell TransAct™ – Large Scale), transduction (viral vector), culture wash, feeding, medium exchange, chamber shaking, and rebuffering in formulation solution during harvest.
- The cell product is then ready for clinical application or further processing (e.g., formulation of a drug product with the correct cell concentration, another cultivation period, enrichment, cryopreservation, or functional testing).

Activities will be executed according to the user-defined activity matrix, which can also be edited during the cultivation period.

Perform culture monitoring and quality control with the available microscope and sampling functions.

The status of aforementioned processes can be monitored on the screen of the CliniMACS Prodigy.

The few manual handling steps are guided by the graphic user interface and this user manual.

In order to permit a more flexible use of the T Cell Transduction – Large Scale System, several options (named cases) are available to the user (see Figure 1.2). One of four "cases" must be chosen at the beginning of a manufacturing run:

Case 1: Full process

Choose this option to run the complete process. This includes tubing set installation, tubing set priming, T cell enrichment, setup of T cell culture, stimulation, transduction, expansion and harvest of cells as described above.

Case 2: T cell cultivation

Choose this option when only the setup of T cell culture, stimulation, transduction, and expansion should be performed. No T cell enrichment is performed. A tubing set must already be installed. Use this case for example if the tubing set was installed the day prior to the manufacturing run and a different cell source is used (e.g., use of thawed enriched cells or cells enriched by different means).

Case 3: T cell cultivation with tubing set installation

Use this option to perform a manufacturing run without T cell enrichment and when the tubing set has not yet been installed. (**Note:** The tubing set is primed with medium in this case).

Case 4: Resume cases

Use this option if the T cell cultivation phase was interrupted during cultivation or harvest and needs to be resumed. No culture preparation is performed. Depending on the situation, different resume subcases can be chosen.

⚠ CAUTION

Risk of low performance or loss of the cell product. If the process is interrupted there is the risk of low performance or loss of the cell product. Choose "Resume cases" to continue the cell cultivation process.

Case 4.1 Resume T Cell cultivation

This resume function starts directly with the culture. The activity matrix must be set up and the CO_2 concentration as well as the current culture volume must be entered.

Note: Be aware that entries in the activity matrix and logfile protocol entries are not saved on the device after an unexpected shutdown. When case 4.1 is executed the user has to start with a new activity matrix and the current day refers to day 0.

Case 4.2 Resume harvest

This resume function starts directly with the selection of one of the four harvest types.

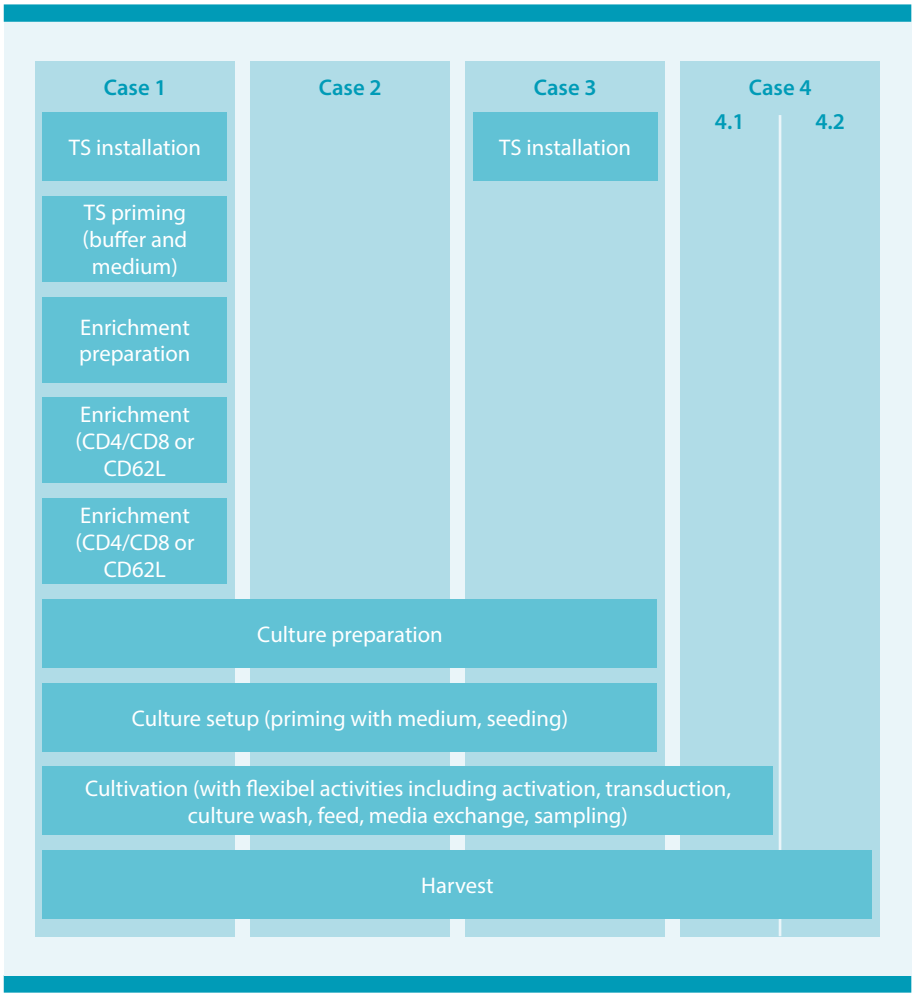


Figure 1.2: Versatility of the T Cell Transduction – Large Scale System

IMPORTANT

The CliniMACS Prodigy T Cell Transduction – Large Scale System has a maximum capacity with respect to cell enrichment and cell transduction. Details are described in section 5.1 on page 23 and section 7.2 on page 83.

2
Glossary

2.1 Graphical depiction

The following chart depicts the symbols used in this user manual to inform the user about potential risks if the outlined warnings and precautions are not followed. Icons on the left side specify the risk. 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.

⚠ WARNING

Indicates a hazardous situation which, if not avoided, could result in death or serious injury.

⚠ CAUTION

Indicates a hazardous situation which, 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

Is used to advise 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 information in the user manuals and labeling of the CliniMACS Products.

3
Materials required

The following section outlines the materials and consumables required for the generation of genetically modified T cells with the CliniMACS Prodigy T Cell Transduction – Large Scale System.

3.1 CliniMACS Prodigy T Cell Transduction – Large Scale System components

The following components are required for the CliniMACS Prodigy T Cell Transduction – Large Scale System (see Table 3.1):

Material	Quantity	Required for case(s)	Comment
CliniMACS Materials			
CliniMACS Prodigy	1	All[2]	
CliniMACS CD62L GMP MicroBeads[1]	1 vial	1	For CD62L enrichment only
CliniMACS CD4 GMP MicroBeads[1]	1 vial	1	In combination with CD8 enrichment
CliniMACS CD8 GMP MicroBeads[1]	1 vial	1	In combination with CD4 enrichment
CliniMACS Prodigy TS 620	1 set	All[2]	
CliniMACS PBS/EDTA Buffer	3 liters	1	Use 1×3,000 mL bag

Material	Quantity	Required for case(s)	Comment
MACS GMP Materials			
TexMACS GMP Medium	10 liters	All[2]	5 bags
MACS GMP Recombinant Human IL-7	5 vials 25 µg/vial	All[2]	In combination with IL-15
MACS GMP Recombinant Human IL-15	5 vials 25 µg/vial	All[2]	In combination with IL-7
MACS GMP Recombinant Human IL-2	5 vials 25 µg/vial	All[2]	If IL-2 is used for T cell expansion
MACS GMP T Cell TransAct – Large Scale	1 vial	1, 2, 3, optional in case 4.1[2]	
MACS GMP Vectofusin®-1	1 vial	Optional, for transduction e.g., with retroviral vectors	Will be pre-mixed with viral vector
CliniMACS Formulation Solution	1 bag	All[2]	Not needed for harvest type 2 or 4
Software			
T Cell Transduction – Large Scale (process version 2.0)			

1 The CliniMACS Reagents might be available in different countries as CliniMACS Reagents and/or CliniMACS GMP MicroBeads. In the following sections both will be generally referred to as CliniMACS GMP MicroBeads. This term will also include the CliniMACS Reagent version.

2 **Note:** For all the resume cases (case 4 with potential subcases), be aware that the required materials have been prepared/connected during the original run and can be further used for the resume cases. Depending on the time for the process to be resumed, material not yet provided will then be required for the resume cases.

Table 3.1: Materials required for the CliniMACS Prodigy T Cell Transduction – Large Scale System

IMPORTANT

The materials referenced above are recommended for the use in combination with the T Cell Transduction – Large Scale System. The use of CliniMACS GMP MicroBeads is recommended for cell selection. Amounts will differ if the protocol is modified. The user is responsible for the validation of the process.

3.2 Additional materials required

Besides the CliniMACS Prodigy T Cell Transduction – Large Scale System components, additional materials are required (see Table 3.2):

Material	Comment
Biological materials	
Heterogeneous hematologic cell sources • Fresh leukapheresis • Frozen leukapheresis	• Not older than 24 hours (fresh) • $<1\times10^{10}$ white blood cells (WBCs) • 50–600 mL for fresh leukapheresis • 50–300 mL for frozen leukapheresis
Human serum albumin (HSA)	Use only pharmaceutical grade HSA approved in your country.
Material	**Comment**
Human AB serum	Use only GMP grade product.
Sterile water	Pharmaceutical grade
Viral vector	Lentiviral or gamma-retroviral vector in a bag
Formulation solution	1 liter e.g., CliniMACS Formulation Solution
Consumables	
Syringes	• 5 mL, several • 10 mL, several • 50 mL, several
Hypodermic needles	20 gauge, several
Transfer bag, 150 mL	1 bag
Luer/Spike Interconnector	1 piece
Triple sampling adapter	On demand (in case more than 3 in-process controls are required during cultivation)
3-way Tube Adapter	On demand (in case 3 bags of TexMACS GMP Medium will be connected at once to valve 3)
Equipment	
Uninterruptable power supply	
CO_2 and compressed air supply	
Sterile tubing welder, e.g., Terumo Sterile Connecting Device	

Table 3.2: Additional materials required for the CliniMACS Prodigy T Cell Transduction – Large Scale System

IMPORTANT

This protocol requires the use of components and materials which are not part of the CliniMACS Prodigy T Cell Transduction – Large Scale System, e.g., human serum albumin (HSA) or human AB serum. Only use pharmaceutical grade HSA approved in your country and GMP grade human AB serum. 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 to humans.

4

Warnings and precautions

⚠ CAUTION

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

This section explains the potential risks associated with the operation of the CliniMACS Prodigy T Cell Transduction – Large Scale System.

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

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 cellular starting product are described in the CliniMACS Prodigy User Manual (Instrument). For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Prodigy T Cell Transduction – Large Scale System components, refer to the instructions for use provided for the respective component.

5 Preparative steps

⚠ WARNING

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

The process using the CliniMACS Prodigy T Cell Transduction – Large Scale System requires a few preparative steps.

5.1 Testing of cell product

The T Cell Transduction – Large Scale System contains an enrichment process, which is a multi-stage loading program that can process more cells than the single column capacity would allow. This means that the cell concentration of WBCs and the frequency of labelled cells must be determined before the enrichment in order to calculate the appropriate number of stages. A maximum of two stages can be processed with the T Cell Transduction – Large Scale System.

Therefore, analyze the cell product prior to connecting it to the tubing set. Determine the WBC concentration and the frequency of cells to be labeled (either CD4 and CD8 positive among viable CD45 positive cells or CD62L positive among viable CD45 positive cells depending on the selected separation reagent).

Note: If the cells are at a considerable low viability, it is recommended to consider the dead cells in the cell product to avoid an exceedance of the column capacity.

In order not to exceed the column capacity, start with a cell product volume containing a maximum of $1x10^{10}$ total WBCs or 3×10^{9} cells to be labeled (i.e., CD4 and CD8 positive cells or CD62L positive cells, respectively). If using a frozen leukapheresis, parameters referring to the frozen cell product bag can be used.

The minimum volume of the cell product is 50 mL and the maximum volume is 600 mL for a fresh cell product or 300 mL for a frozen cell product, respectively. For a frozen cell product an automatic dilution with medium can be performed with a maximum total volume (including the volume of the cell product) of 600 mL. If the volume of the fresh cell product is below 50 mL, dilute the cell product with process buffer manually (see section 5.2 on page 24).

The cell product parameters can be entered either at the beginning of the process or at the last possible time point directly before separation (e.g., if the analysis is performed while the cell labeling is running). It is recommended to determine the WBC concentration/volume/frequency before starting the process.

For further information see subsection 6.5.2 on page 70.

⚠ 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. Ensure that no leukocyte population is excluded from the analysis.

5.2 Preparation of process buffer

Supplement 3 liters 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 supplemented buffer is henceforth referred to as “process buffer”.

IMPORTANT

HSA is not a component of the CliniMACS Prodigy T Cell Transduction – Large Scale 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. The user must evaluate all risks arising from this material.

5.3 Preparation of medium

For optimal T cell proliferation, supplement 2 bags (= 4 L) of TexMACS GMP Medium with cytokines. For optimal results use IL-7 (MACS GMP Recombinant Human IL-7) in combination with IL-15 (MACS GMP Recombinant Human IL-15). Alternatively, IL-2 only (MACS GMP Recombinant Human IL-2) can be used.

Depending on the cell numbers used, varying cytokine concentrations and feeding schedule may be required. Titrate the cytokine concentration as needed.

The recommended dose ranges are:

- IL-7: 150–1,500 U/mL (5–25ng/mL)
- IL-15: 85–450 U/mL (5–25ng/mL)
- IL-2: 10–50 IU/mL

Note: Optimal results were obtained when using 1 vial (25 µg) of MACS GMP Recombinant Human IL-7 and 1 vial (25 µg) of MACS GMP Recombinant Human IL-15 per 2,000 mL bag of TexMACS GMP Medium. Make sure the lot-specific activities comply with the recommended dose ranges.

For agitated T cell culture in the CliniMACS Prodigy, it is highly recommended to supplement the TexMACS GMP Medium with 3% human AB serum. The TexMACS GMP Medium (bag) is henceforth referred to as "medium bag".

IMPORTANT

Human AB serum is not a component of the CliniMACS Prodigy T Cell Transduction – Large Scale System. Use only GMP grade human AB serum.

Note: It is possible to reduce the use of human AB serum if further medium bags are not supplemented with human AB serum (e.g., on cultivation day 5 or 6 during medium bag exchange).

To prepare the medium bag:

- Reconstitute the lyophilized MACS GMP Cytokines using 1 mL of sterile water.
- Add the appropriate amount of reconstituted cytokines to the medium bag using the Luer Lock port of the medium bag.
- Supplement both TexMACS GMP Medium bags with 60 mL (3%) human AB serum using the Luer Lock port of the medium bag.

If necessary, remove approximately 50 mL from the medium bag and store at +4 °C to +8 °C (+39 °F to +46 °F) to use for dilution of the activation reagent and viral vector.

- In case three TexMACS GMP Medium bags shall be connected at the same time, use a 3-way Tube Adapter and sterile connect it to valve 3.

IMPORTANT

UV light could lead to adverse effects on the TexMACS GMP Medium. Protect the TexMACS GMP Medium from UV light using the provided protection bag (yellow).

6

Separation process

6.1 Initialization of the CliniMACS Prodigy

Switch on the CliniMACS Prodigy with the ON/OFF switch located on the rear panel of the instrument.

Note: Parts of the text presented on the screens in the user manual may differ slightly from the screens displayed on the instrument.

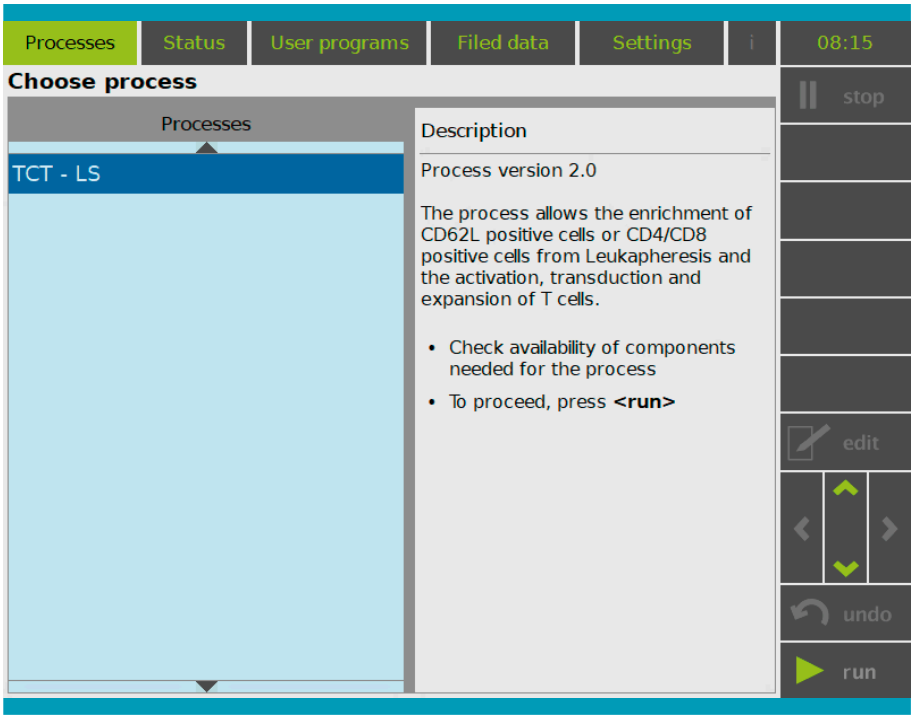
Use the touchscreen functionality and buttons on the menu and tool bar to operate the instrument. For details, refer to the CliniMACS Prodigy User Manual (Instrument).

After the instrument initialization phase the main screen is shown. Select the process “TCT-LS”. Press **run** to confirm the selection.

Note: A user interface showing screens with instructions and pictures will guide through the entire procedure.

⚠ 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. Ensure that the operator recognizes optical and acoustical alarm signals at any time during the process.



Screen 6.1: Main screen: Choice of process TCT-LS

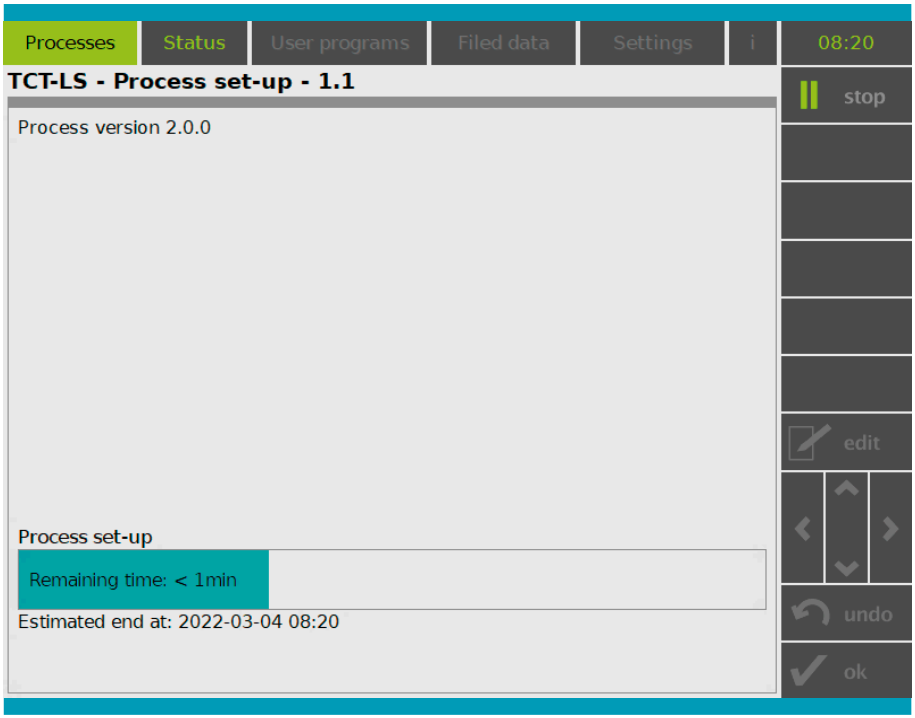
⚠ CAUTION

Risk of process failure. If the incorrect drive shaft is installed on the instrument, the T Cell Transduction - Large Scale System is not executed. The T Cell Transduction - Large Scale System is designed for the use with the CliniMACS Prodigy TS 620 only. The CliniMACS Prodigy TS 620 contains a large CentriCult™ Chamber requiring a specific drive shaft. Ensure the drive shaft is installed by Miltenyi Biotec Instrument Service before using the tubing set.

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

Contact Miltenyi Biotec Technical Support, in case a drive shaft exchange is required.

The instrument performs a short self-test, shown in Screen 6.2, and indicates the remaining time for this process step on the process bar before the next screen is shown.

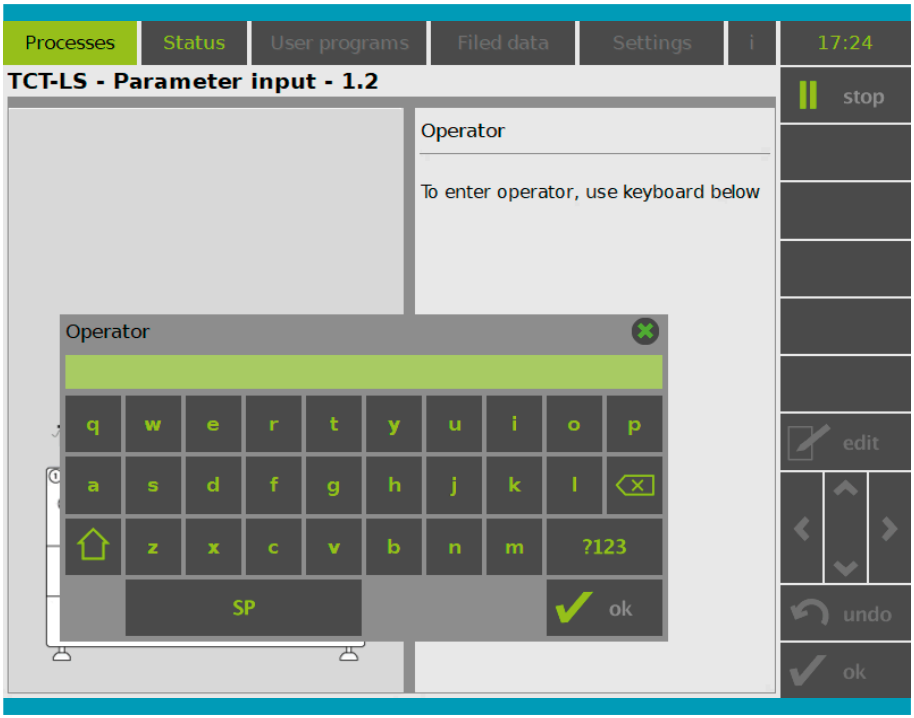


Screen 6.2: Main screen: Self-test

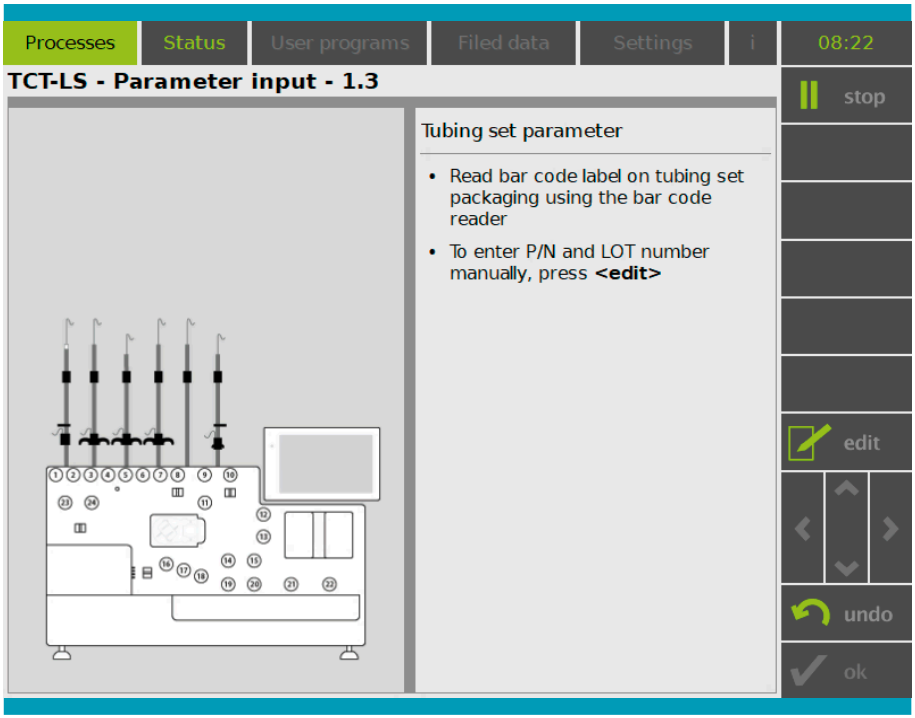
6.2 Parameter input and process setup choice

Multiple parameters must be entered for the T Cell Transduction – Large Scale System using either the bar code reader attached to the CliniMACS Prodigy or the keyboard shown on the screen. Press **ok** to confirm. All entered parameters are saved in a process-related protocol.

Within the next steps of the process enter information related to the operator (see Screen 6.3) and CliniMACS Prodigy TS 620 (see Figure 6.4 on page 43). For the manual entry of capital letters, press ⇧, for space press **SP**, for numbers press **?123**, and for backspace press ⌫.

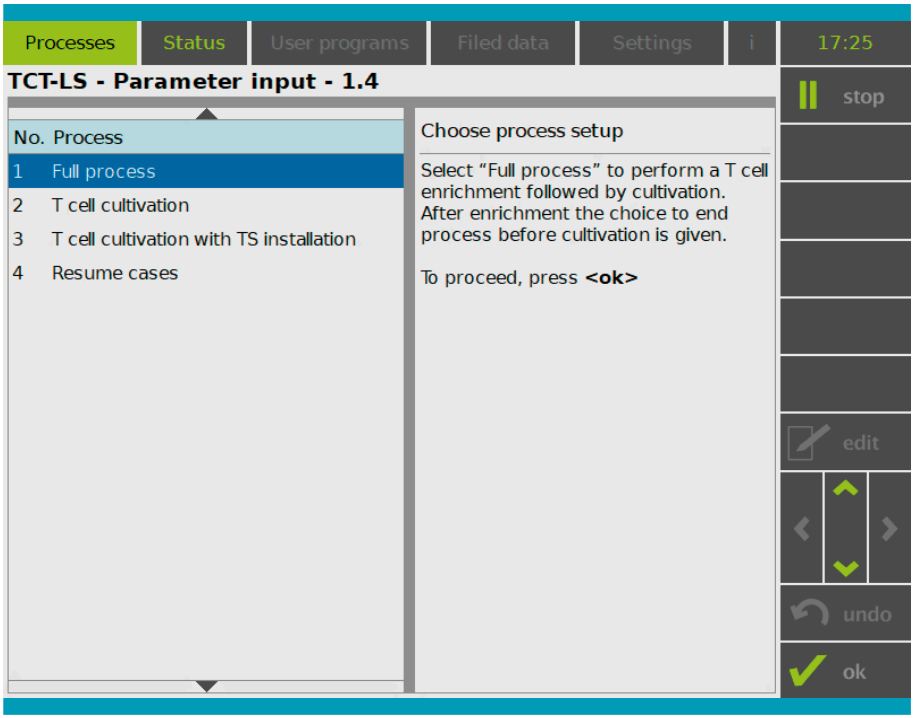


Screen 6.3: Entry of operator-related information



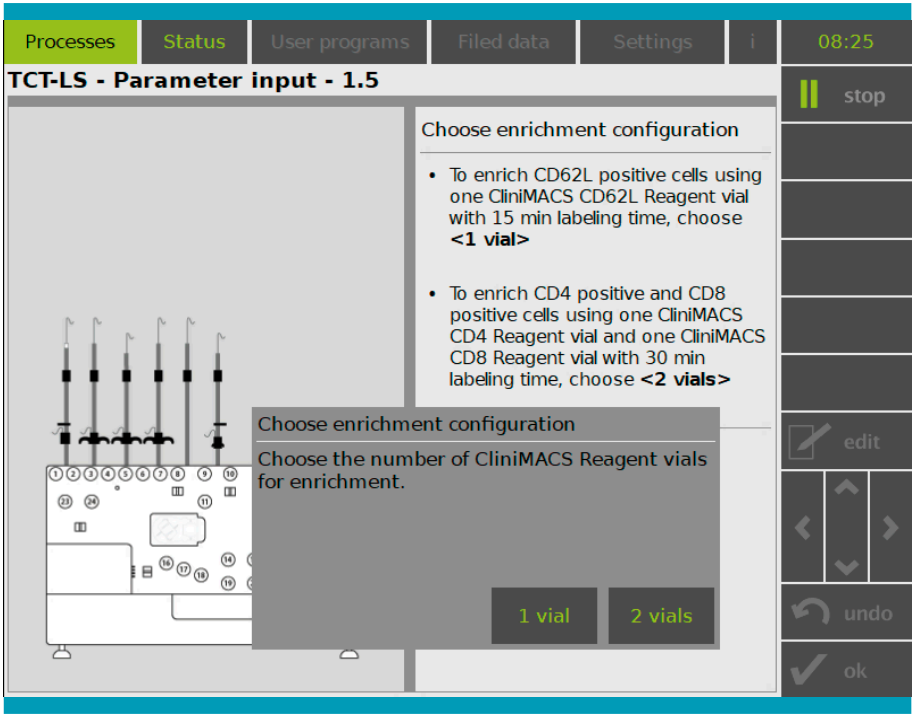
Screen 6.4: Entry of CliniMACS Prodigy TS 620 part number (P/N) and batch code (LOT)

Read the bar code label on the tubing set label. Alternatively, press **edit** to enter part number (P/N) and batch code (LOT) manually with the keyboard shown on the screen.



Screen 6.5: Process setup no. 1 "Full process"

After parameter input, choose between four different cases (see section 1.2 on page 11). Case 1, 2 and 3 have no subcases. Case 4 has two subcases (4.1 Resume T cell cultivation and 4.2 Resume harvest). This user manual describes the complete process using case 1 (Full process) as the default process (see Screen 6.5).



Screen 6.6: Selection of enrichment configuration (1 or 2 vials)

In the next step, choose the number of vials of CliniMACS GMP MicroBeads used for target cell enrichment. For CD62L enrichment, choose **1 vial**, for a combined CD4 and CD8 enrichment, choose **2 vials** (see Screen 6.6).

IMPORTANT

Use one vial of CliniMACS CD62L GMP MicroBeads to enrich CD62L cells. The use of CliniMACS CD62L GMP MicroBeads for frozen leukapheresis is not suitable due to the downregulation of the CD62L receptor[1]. Use one vial of CliniMACS CD4 GMP MicroBeads and one vial of CliniMACS CD8 GMP MicroBeads to enrich CD4 and CD8 cells.

1 Costantini A, Mancini S, Giuliodoro S, Butini L, Regnery CM, Silvestri G, Montroni M. Effects of cryopreservation on lymphocyte immunophenotype and function. J Immunol Methods. 2003 Jul;278(1-2):145-55. doi: 10.1016/s0022-1759(03)00202-3. PMID: 12957403.

6.3 Tubing set installation

6.3.1 Components of the CliniMACS Prodigy TS 620

The components of the CliniMACS Prodigy TS 620 are pre-assembled and provided in a sealed and sterilized package. All components of the tubing set are summarized in Figure 6.1.

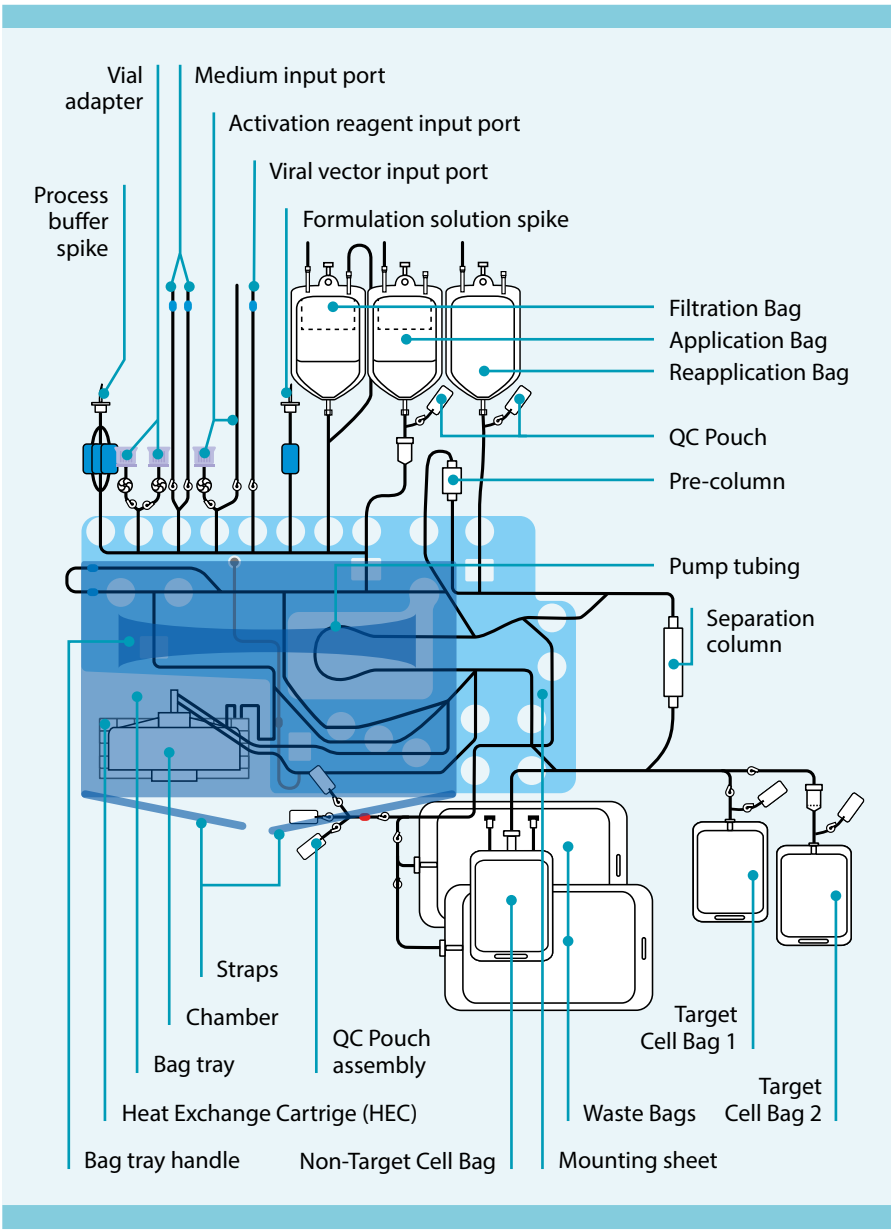


Figure 6.1: Components of the CliniMACS Prodigy TS 620

6.3.2 Installation steps

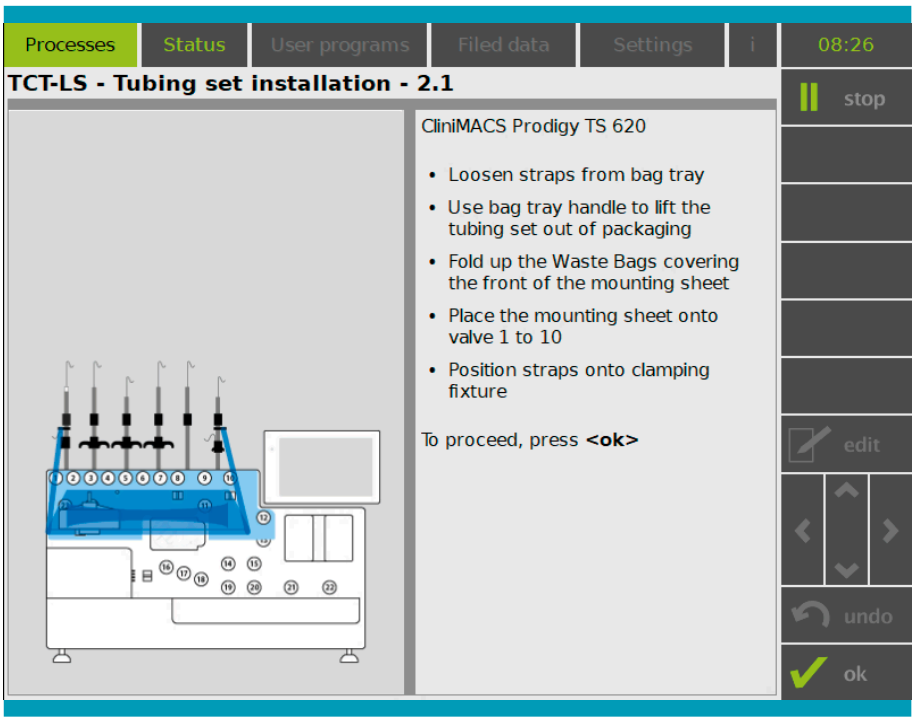
This section guides the user step by step through the installation of all tubing set components (see Screen 6.7 to Screen 6.28).

Each screen shows the written instructions supported by an informative image. Blinking and orange features in an image indicate areas of attention.

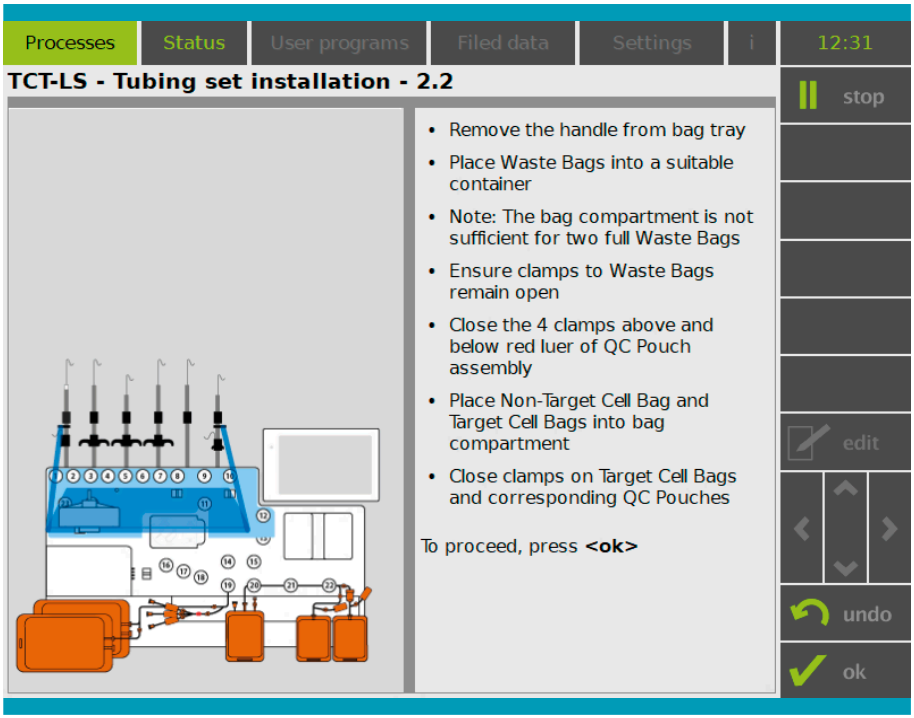
At any step during the installation press **undo** to return to a previous step.

⚠ 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 6.7: Installation of bag tray and mounting sheet onto the instrument



Screen 6.8: Placement of bags into bag compartment and closure of clamps

IMPORTANT

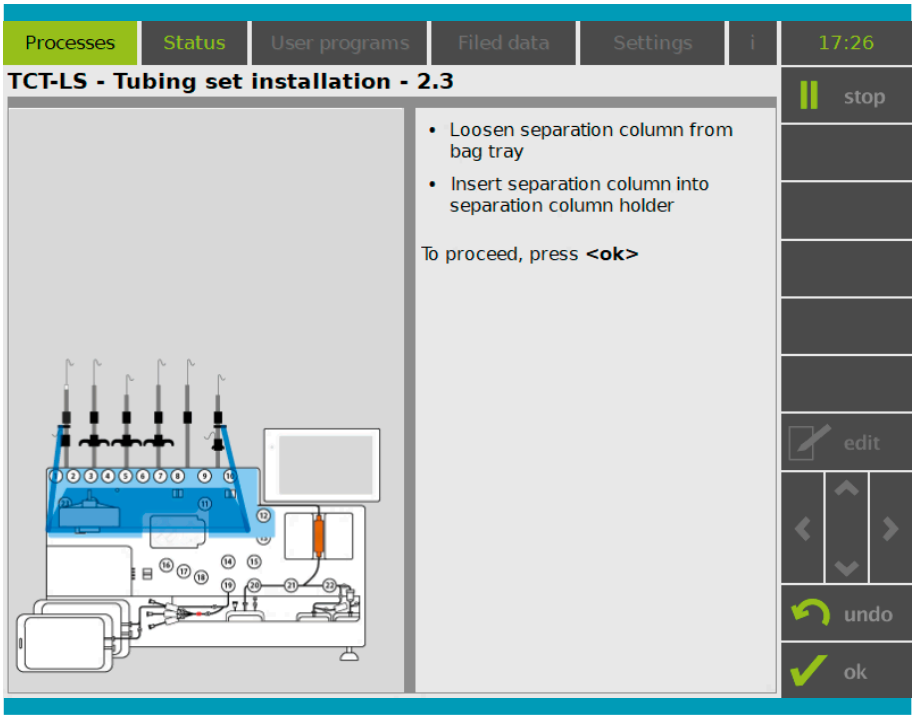
The Waste Bags must be safely placed outside of the bag compartment on a plain surface next to or below the CliniMACS Prodigy.

The CliniMACS Prodigy TS 620 contains two Waste Bags, which do not fit both into the bag compartment when completely filled. Therefore, use one of the following options:

- Store one bag in the bag compartment and keep the clamp on the other bag closed until replacement is required.
- Put both bags in a separate fitting bag compartment on the left-hand side of the CliniMACS Prodigy to be filled simultaneously.

IMPORTANT

Ensure that the clamps on the QC Pouches attached to Target Cell Bags are closed.



Screen 6.9: Insertion of separation column into magnet unit

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

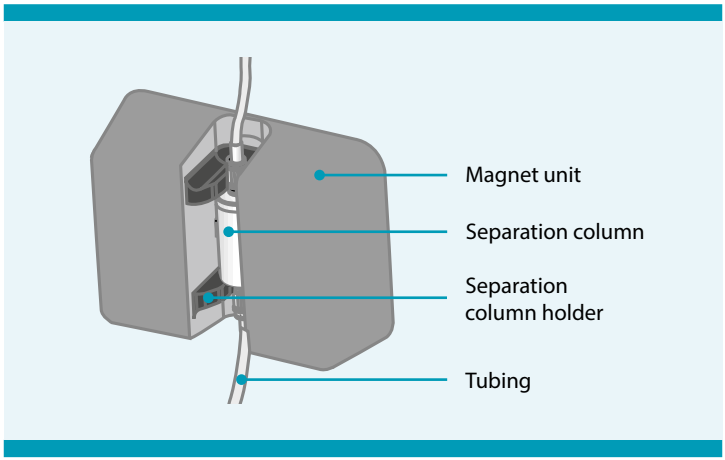
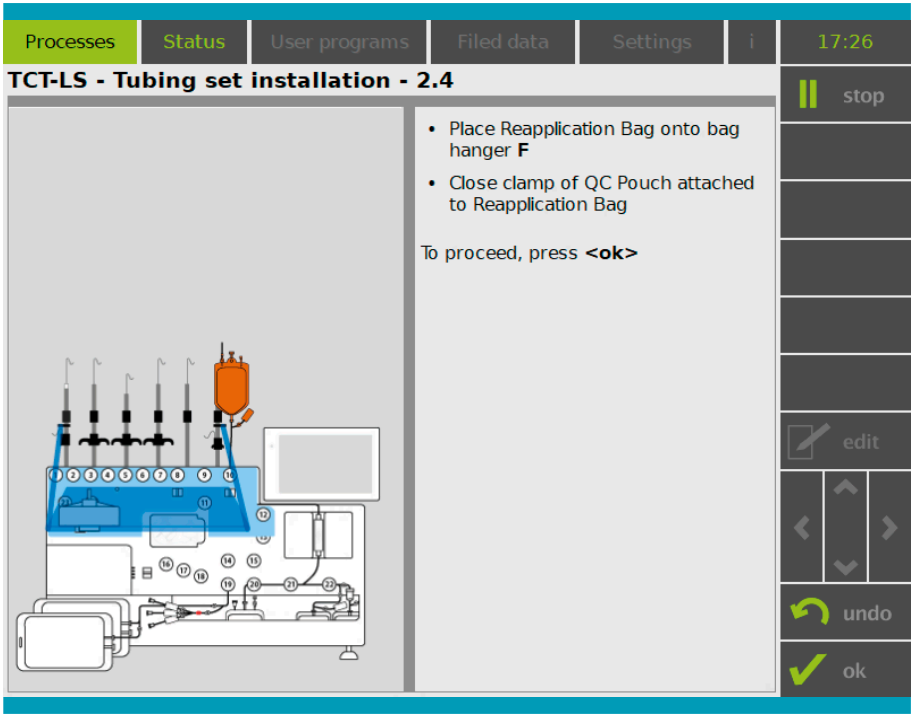


Figure 6.2: Separation column inserted into magnet unit

⚠ WARNING

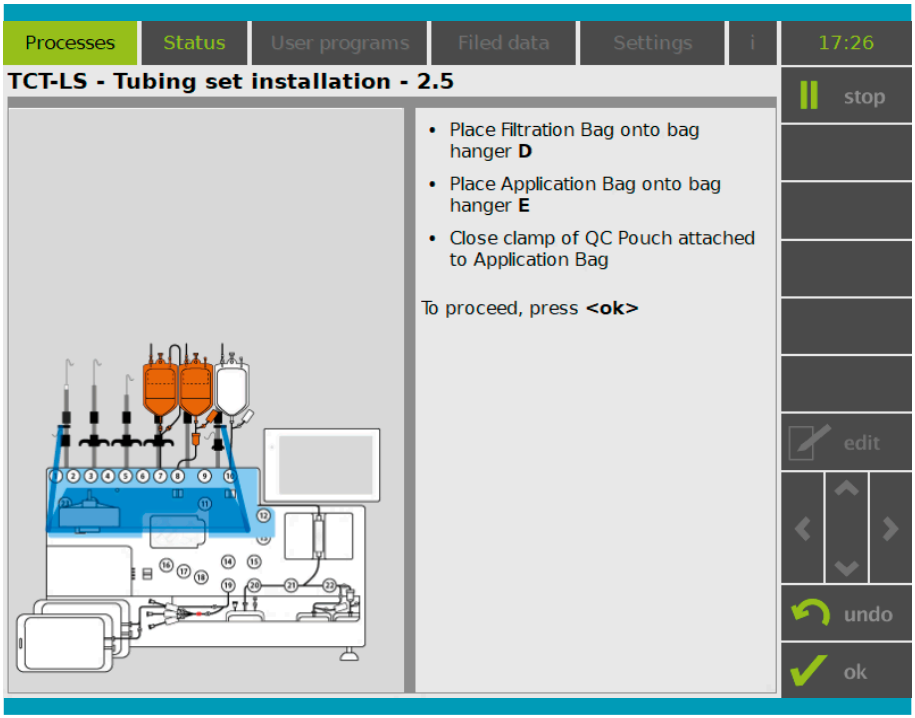
Risk of injury. If the separation column is inserted or removed while the magnet unit is switched on, then there is the risk of personal injury. Only insert or remove the separation column when the magnet unit is switched off.



Screen 6.10: Installation of Reapplication Bag

IMPORTANT

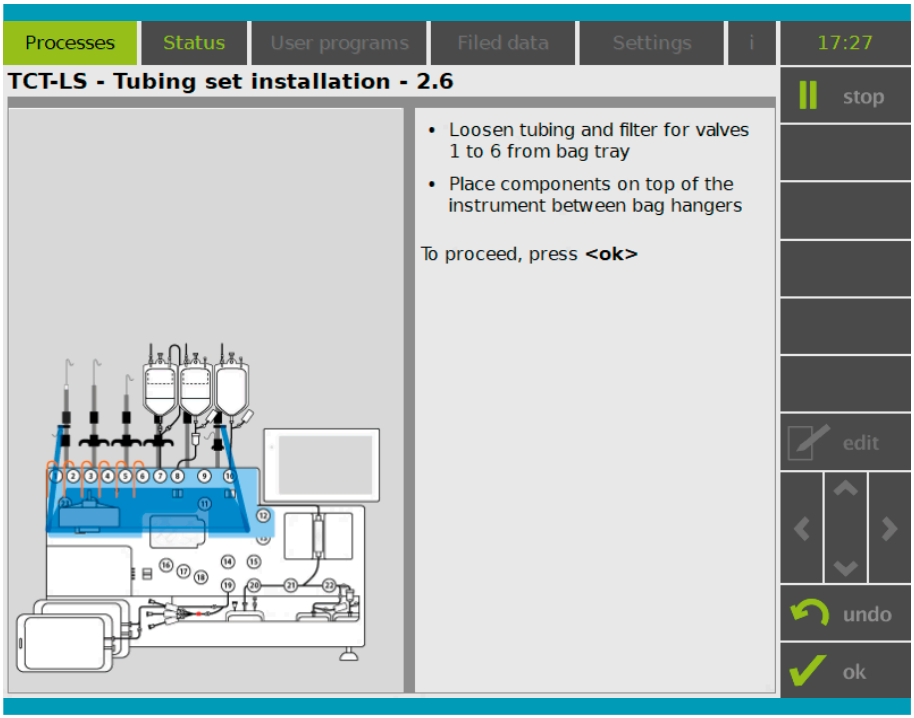
Ensure that the clamp on the QC Pouch attached to the Reapplication Bag is closed.



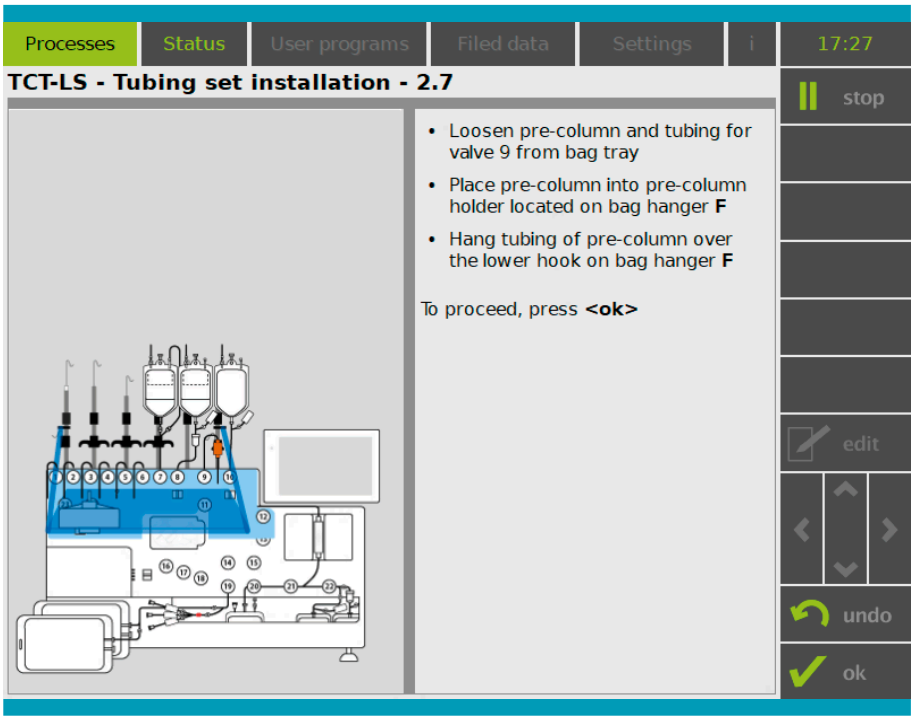
Screen 6.11: Installation of Filtration Bag and Application Bag

IMPORTANT

Ensure that the clamp on the QC Pouch attached to the Application Bag is closed.



Screen 6.12: Placement of tubing set components between bag hangers



Screen 6.13: Installation of pre-column into pre-column holder on bag hanger (F)

Figure 6.3 shows how to place the tubing over the lower hook on bag hanger (F).

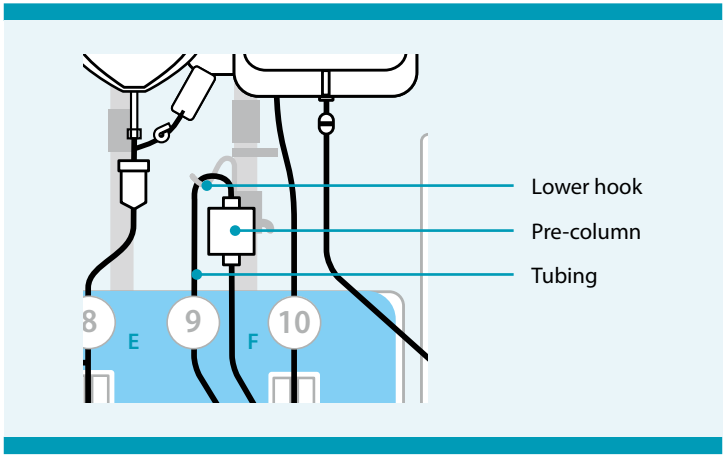
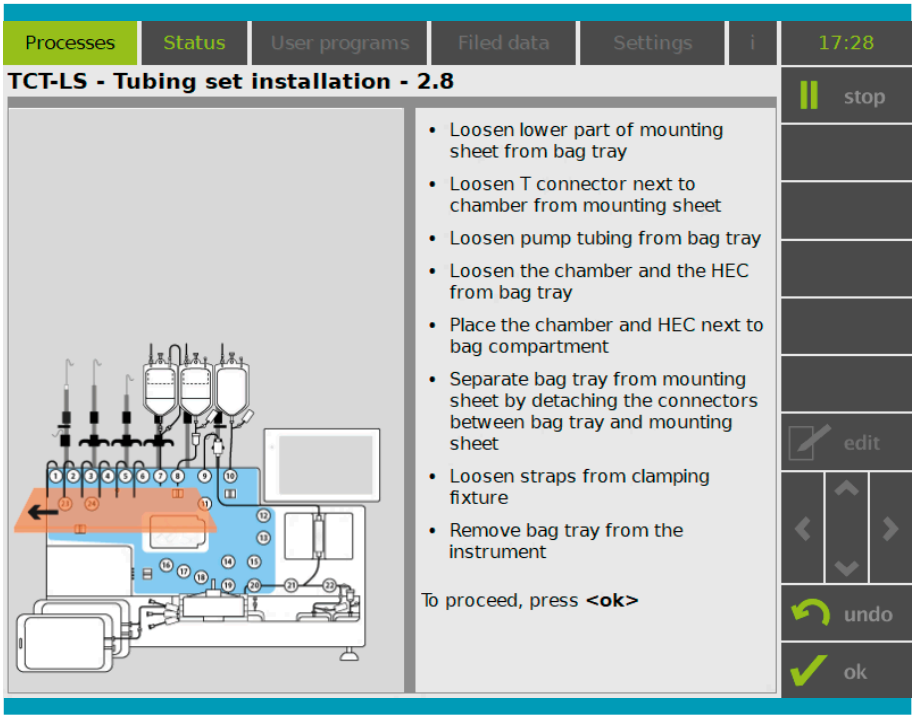
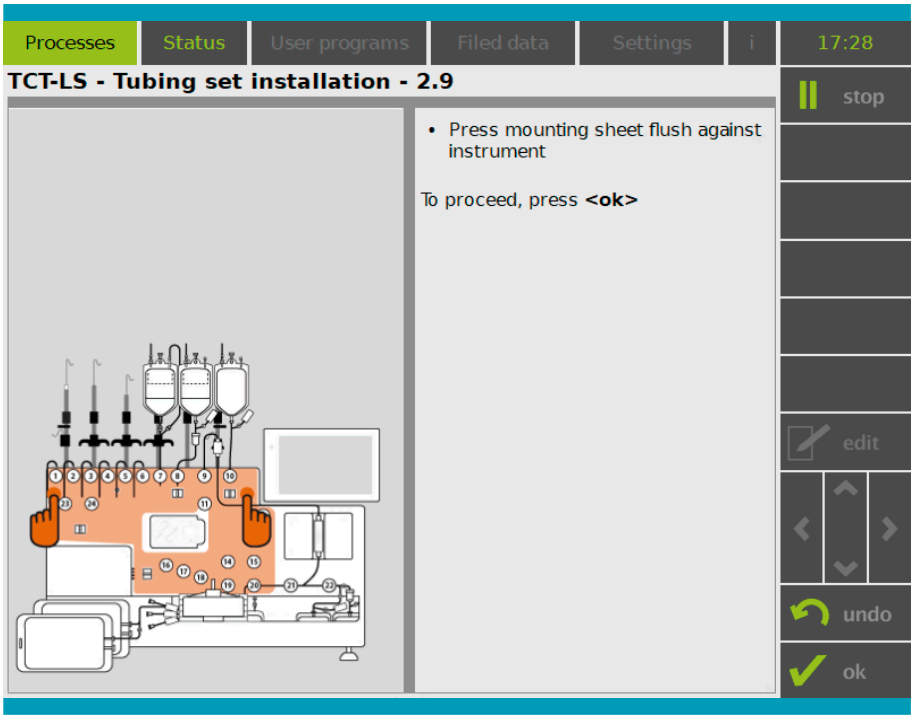


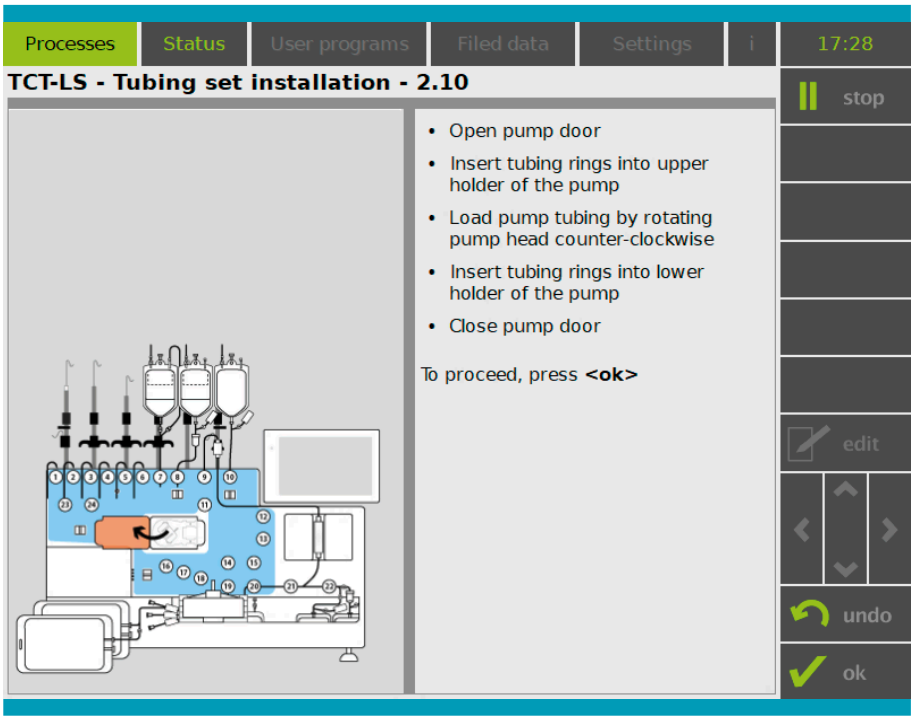
Figure 6.3: Guidance of pre-column-associated tubing



Screen 6.14: Removal of bag tray from instrument



Screen 6.15: Press mounting sheet flush against the instrument



Screen 6.16: 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 of the pump.
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. Ensure that the pump tubing is not pinched between the guide pins.
5. Turn the pump clockwise and counterclockwise to ensure the pump head moves freely.

If necessary, the tubing can be unloaded by lifting the lower end of the tubing and turning the pump head clockwise until the tubing is released.

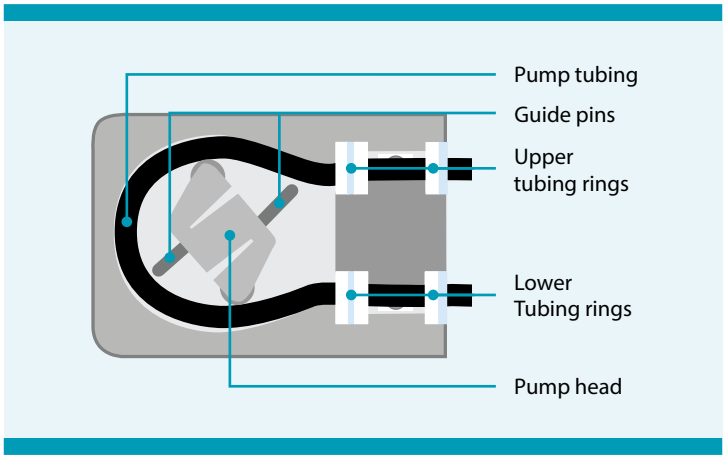
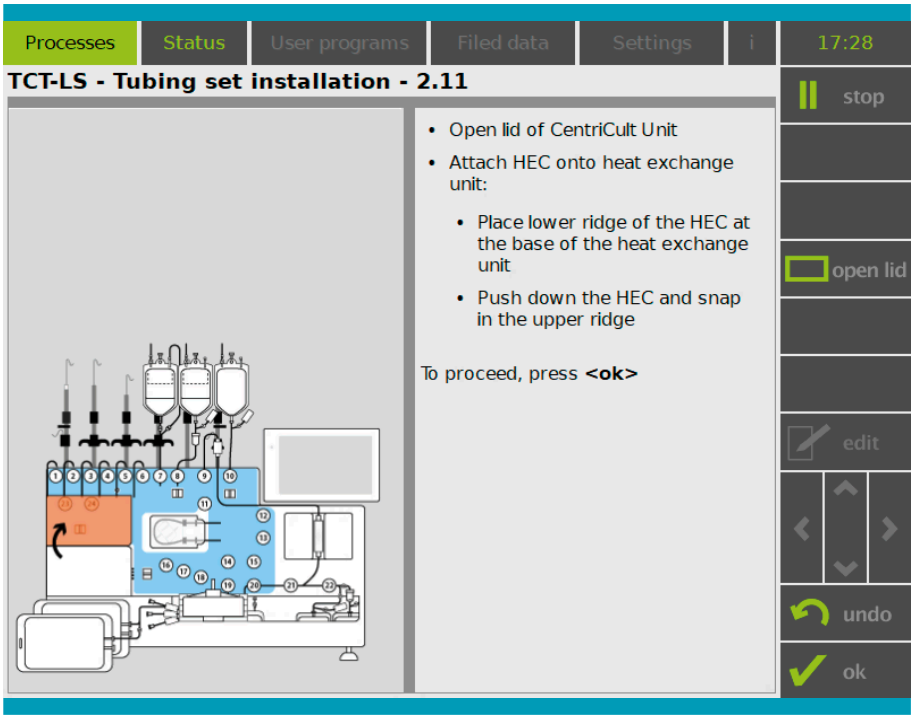


Figure 6.4: Pump tubing inserted into pump

The CentriCult Unit lid will unlock automatically for the installation of the Heat Exchange Cartridge (HEC) and chamber (see Screen 6.17 on page 44). If the lid does not unlock automatically, press **open lid** on the menu bar.



Screen 6.17: Installation of HEC onto heat exchange unit

Figure 6.5 illustrates the installation of the HEC onto the heat exchange unit. Confirm the HEC attaches tightly to the heat exchange unit to allow heating and cooling of liquid inside the HEC.

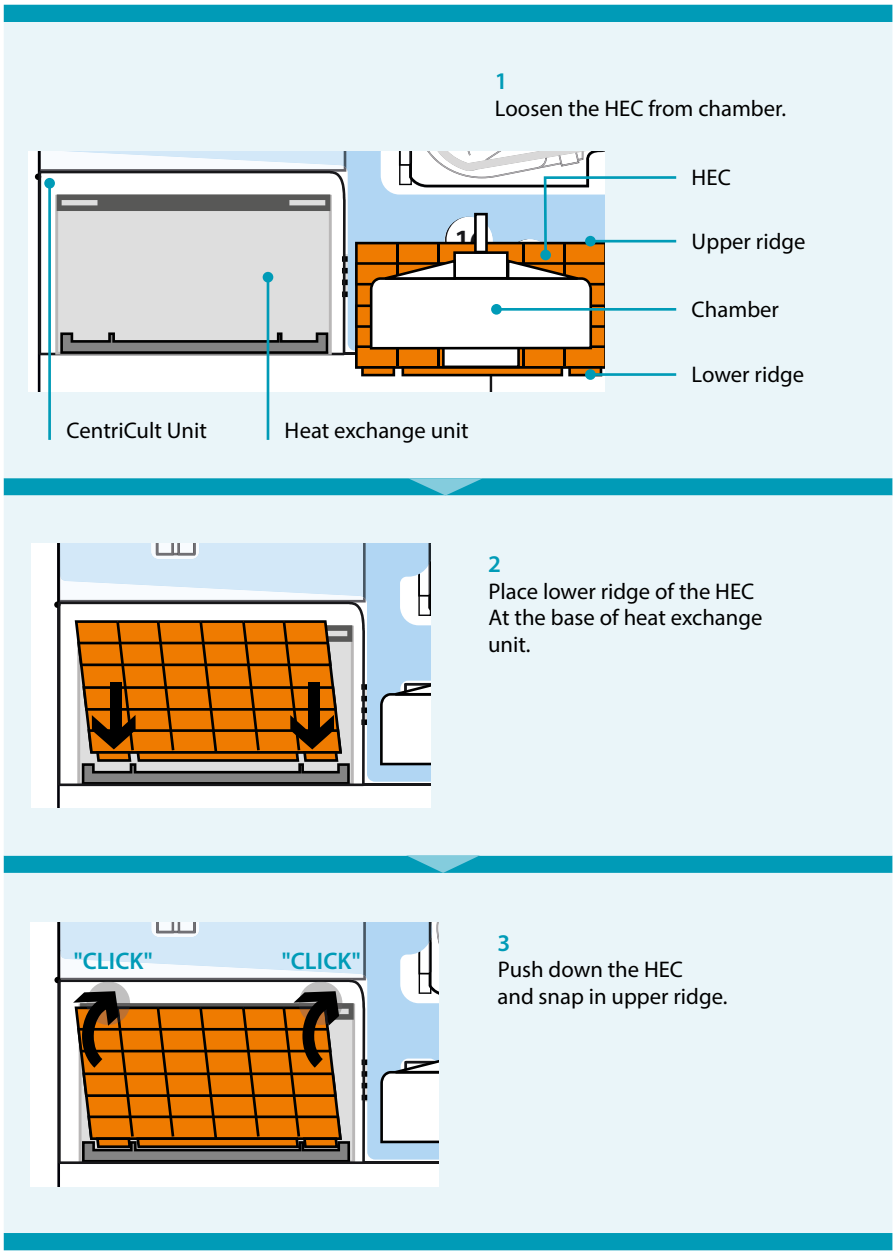
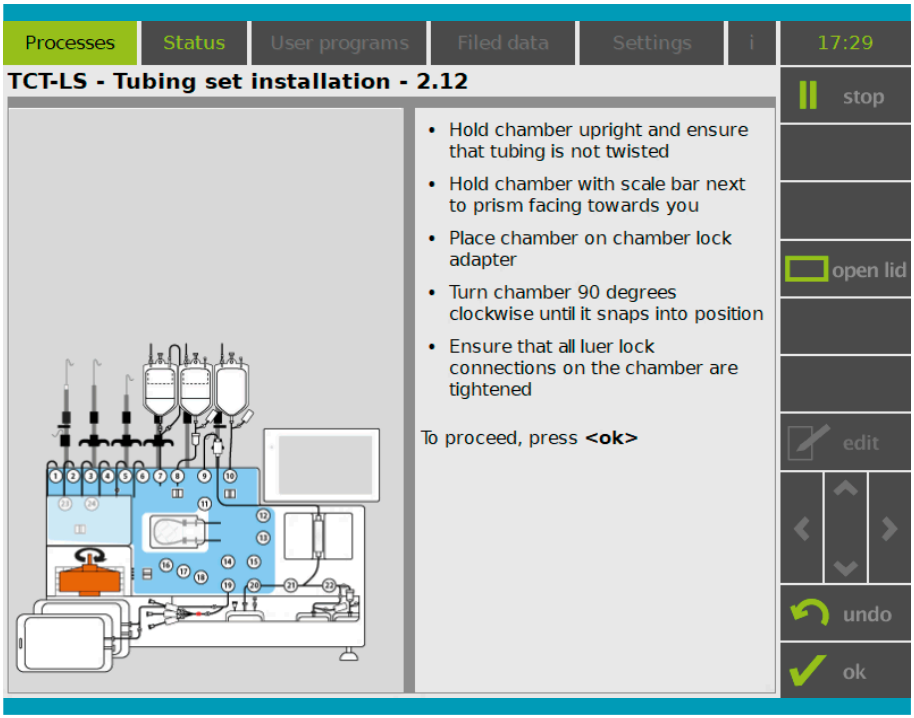


Figure 6.5: Installation of HEC onto heat exchange unit



Screen 6.18: Installation of chamber into CentriCult Unit

After inserting the HEC, install the chamber according to instructions on the screen (see Screen 6.18).

Figure 6.6 shows the components of the CentriCult Unit.

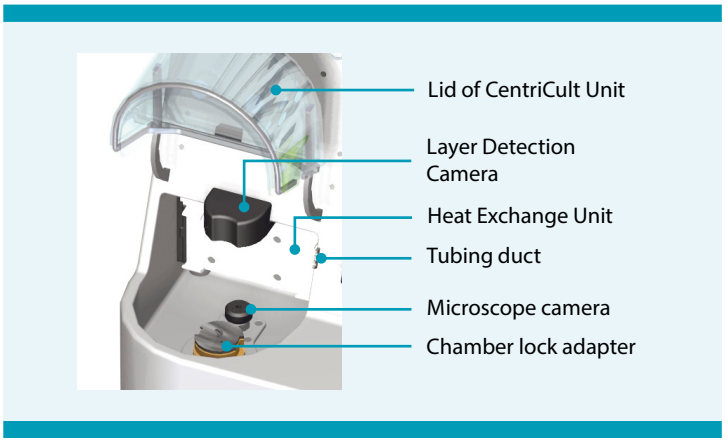


Figure 6.6: Components of the CentriCult Unit

IMPORTANT

The chamber has a scale bar on its lid. This scale bar has to point towards the user when placed on the chamber lock adapter.

Before closing the lid, ensure 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 correctly into position.

⚠ CAUTION

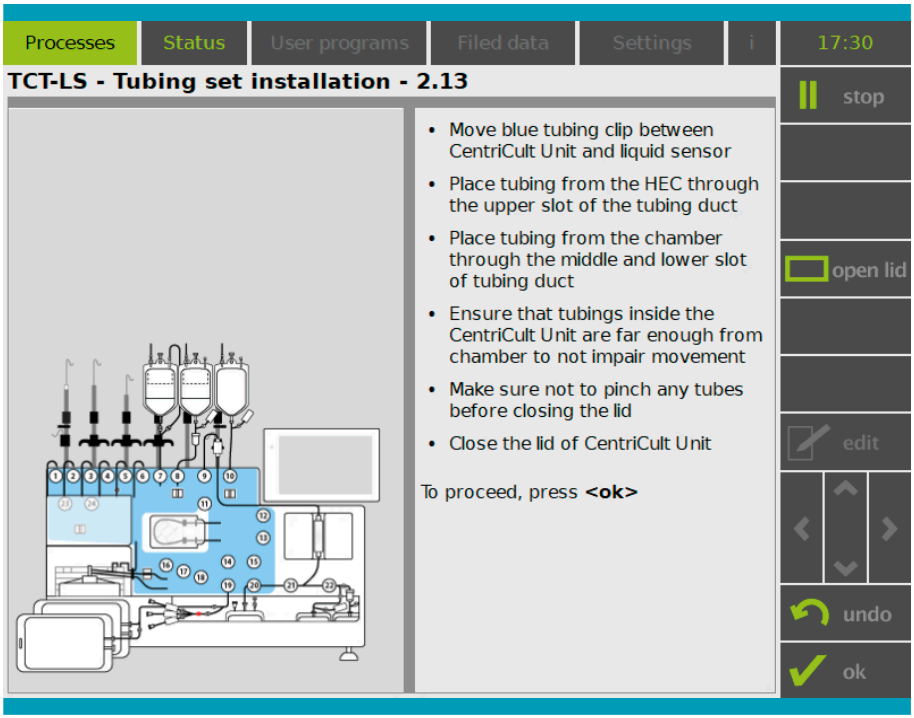
Risk of process failure. An incorrectly installed chamber can cause a process failure. Ensure that the chamber is tightly locked in position. Do not use the tubing set if the chamber cannot be installed properly.

⚠ CAUTION

Risk of process failure. Ensure that chamber does not touch tubes leading to HEC to avoid tube destruction during chamber rotation.

Also refer to important drive shaft requirements specified in section 6.1 on page 27.

If a twist or bending of the chamber tubing is recognized after the chamber is already locked, follow the instructions in the **IMPORTANT** section connected to Screen 6.27 on page 55 and correct the tubing position during that step.



Screen 6.19: Guidance of tubings from HEC and chamber through slots of tubing ducts

Figure 6.7 illustrates how to insert the two chamber tubing lines through the tubing duct. Ensure that the tubing is not twisted or pinched by the lid of the CentriCult Unit.

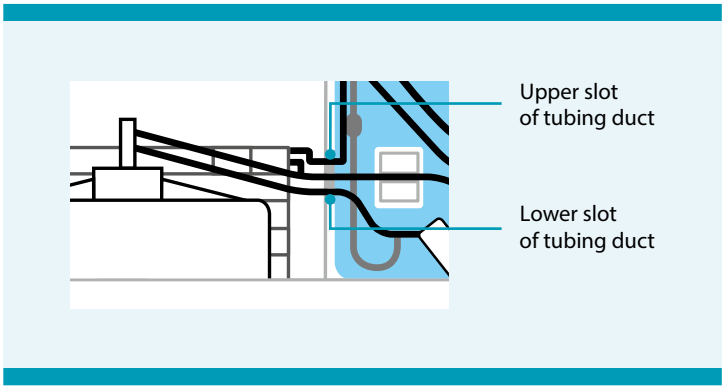
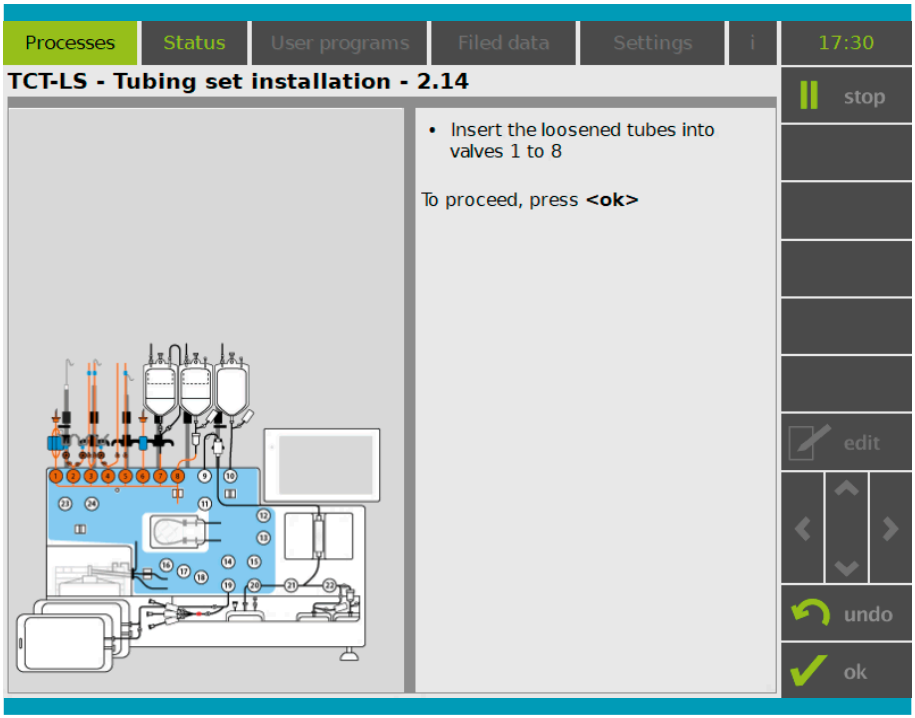


Figure 6.7: Guidance of tubing through tubing duct



Screen 6.20: Insertion of tubing into valves 1 to 8

Figure 6.8 shows how the tubing should be inserted correctly into the respective valve.

Insert the tubing only into open valves (when the valve button is pressed down). Otherwise, the tubing will not fit correctly.

If a tubing has to be adjusted, press the valve button to open the valve before adjustment.

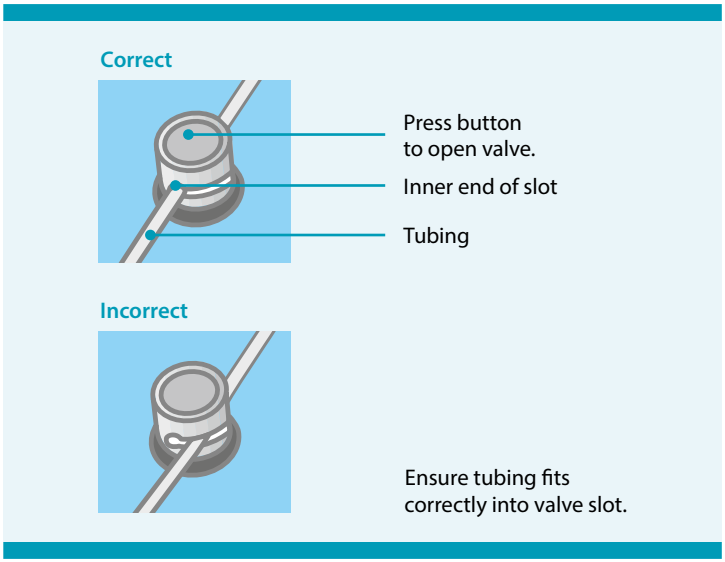
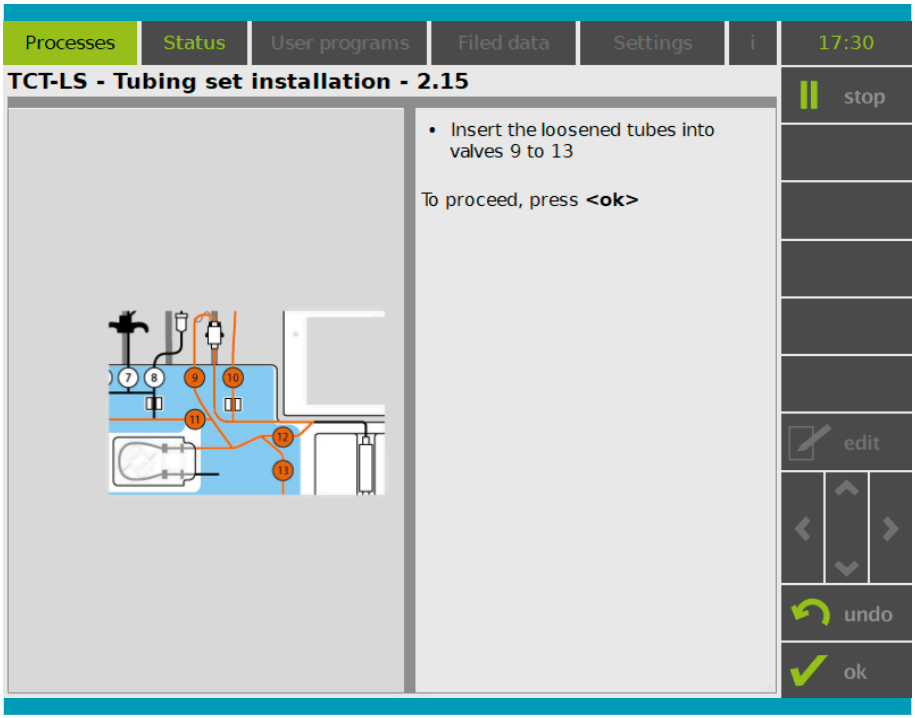


Figure 6.8: Insertion of tubing into valves



Screen 6.21: Insertion of tubing into valves 9 to 13

Figure 6.9 shows how to insert the tubing correctly into valve 12.

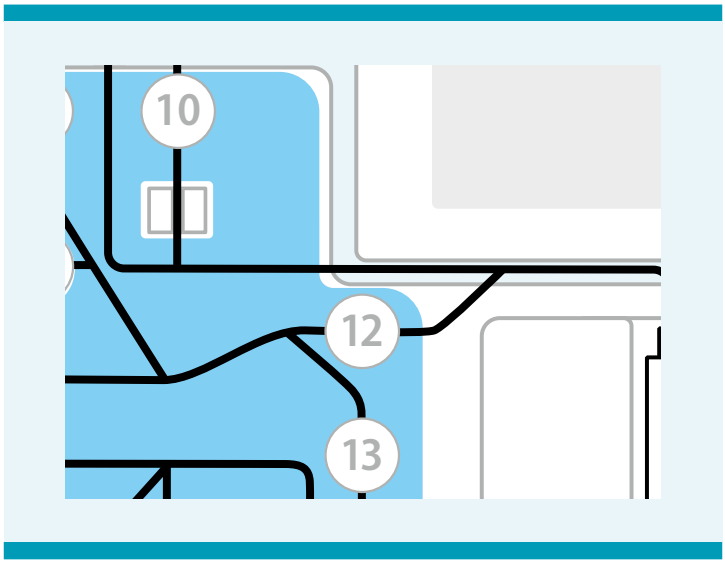
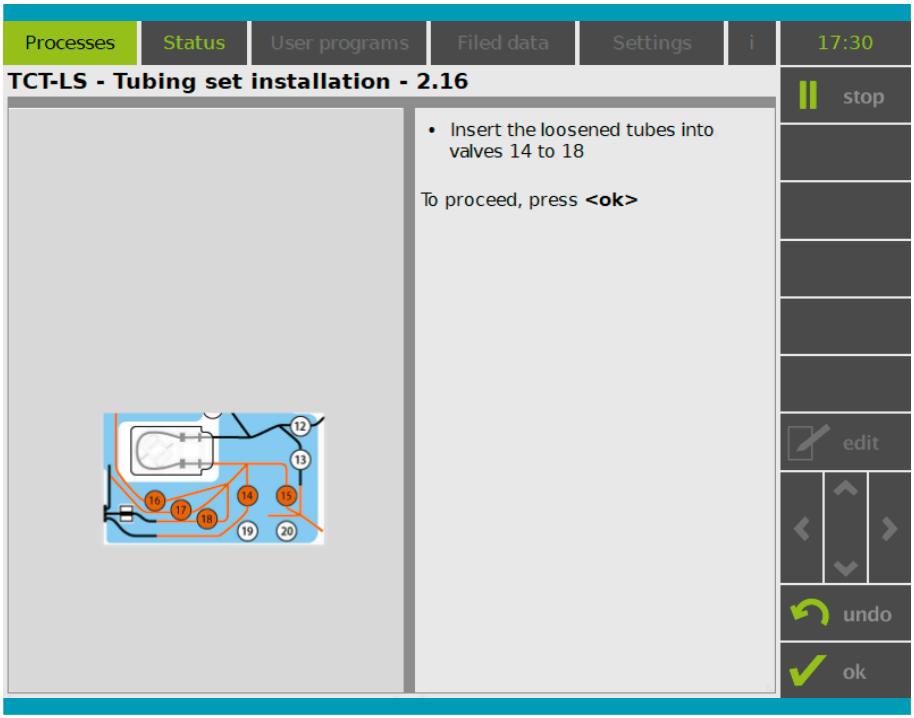
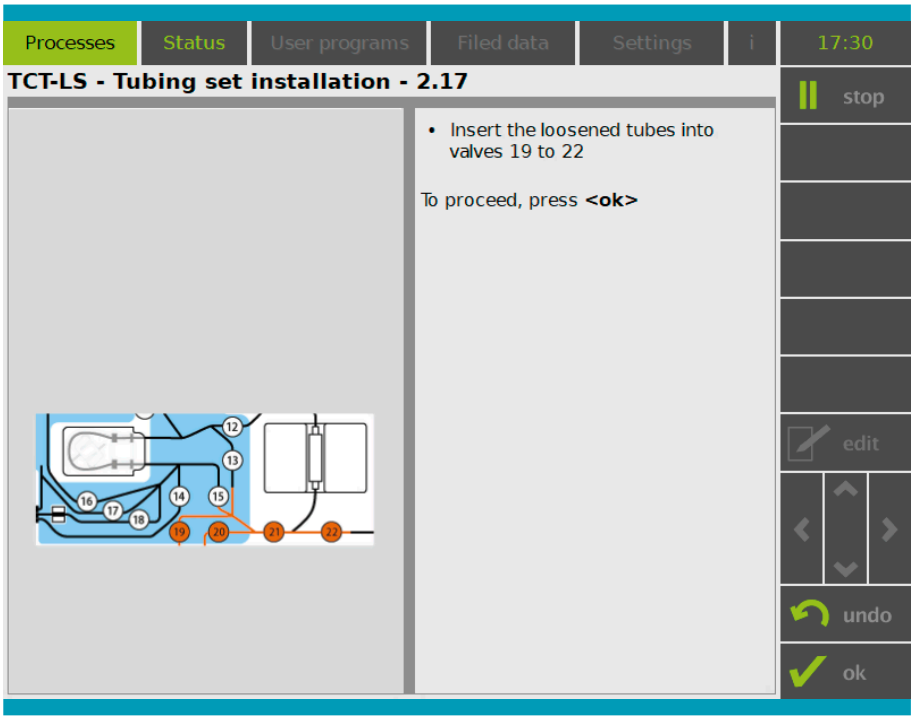


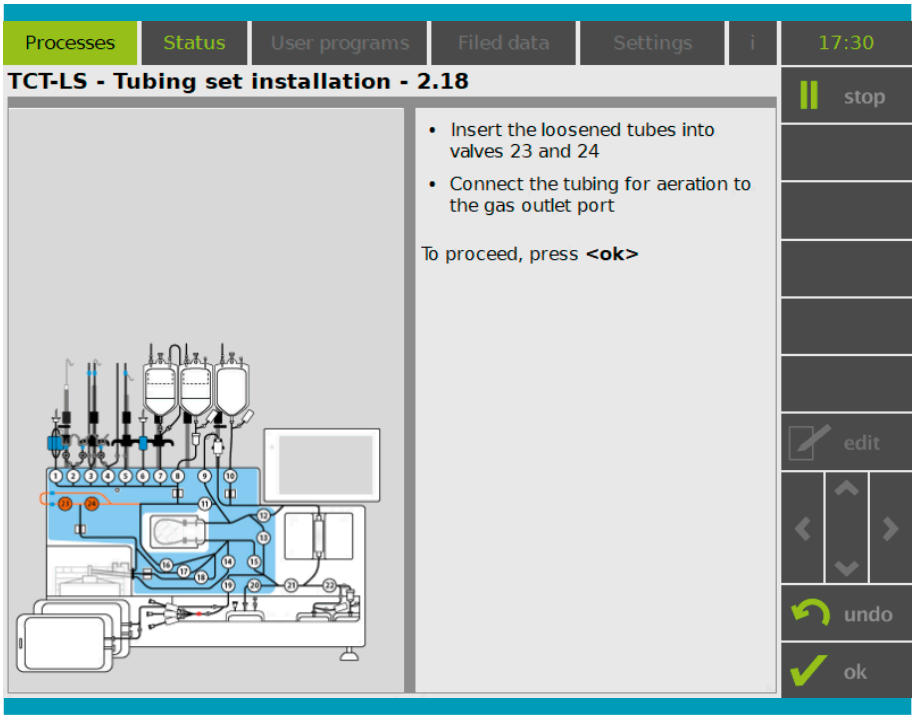
Figure 6.9: Guidance of tubing for separation column and valves 12



Screen 6.22: Insertion of tubing into valves 14 to 18



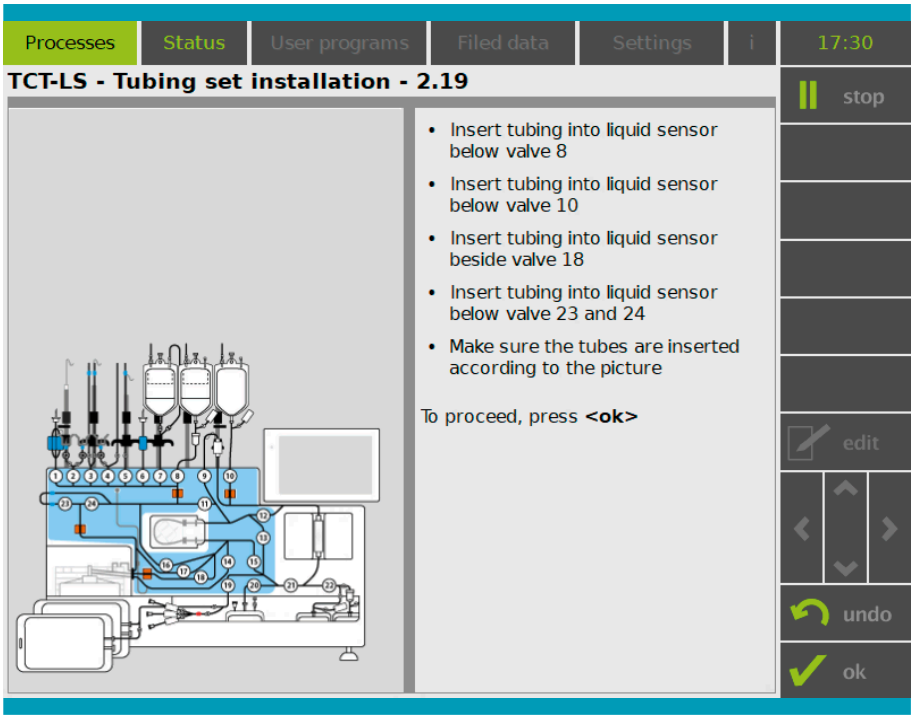
Screen 6.23: Insertion of tubing into valves 19 to 22



Screen 6.24: Insertion of tubing into valves 23 and 24 and connection of tubing for aeration to the gas outlet port

⚠ CAUTION

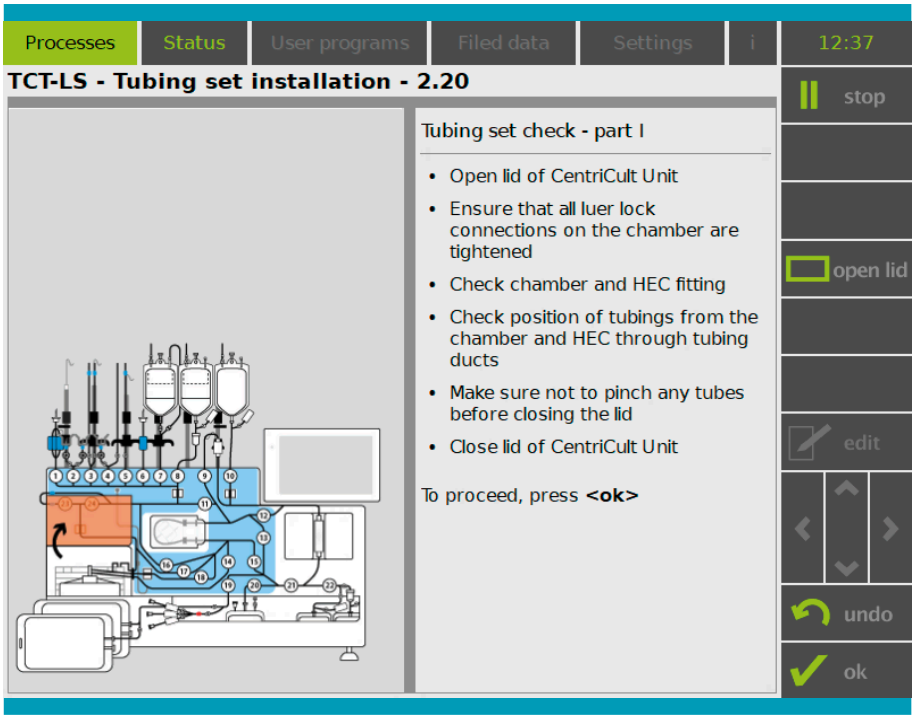
Risk of process failure. Improper gas flow can cause process failure. The tubing for aeration should be tightly connected to the gas outlet port to ensure a proper gas flow into the cultivation chamber.



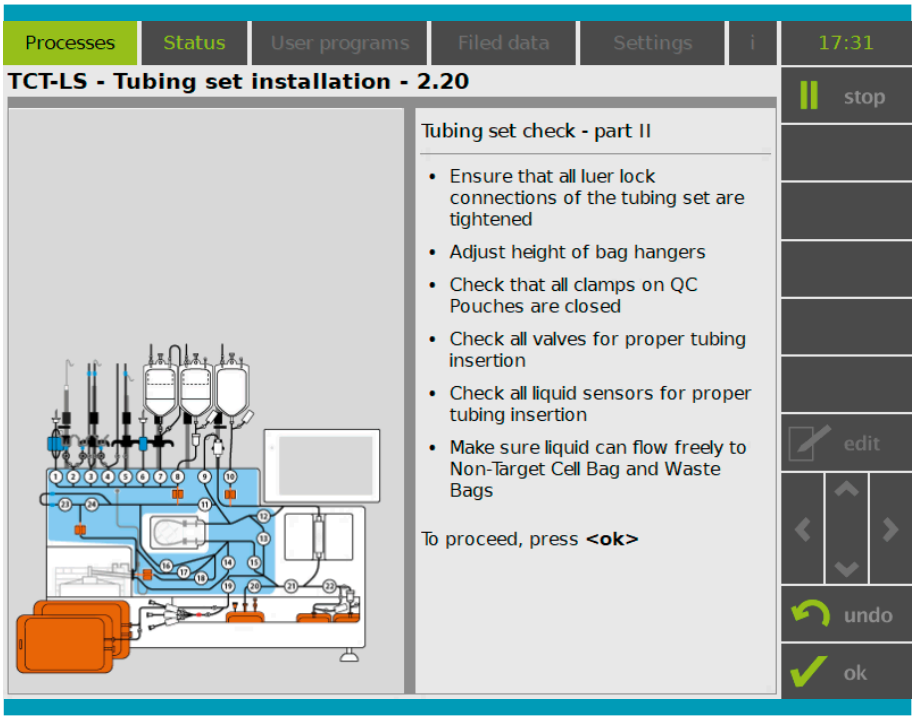
Screen 6.25: Insertion of tubing into liquid sensors

IMPORTANT

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



Screen 6.26: Tubing set check – part I



Screen 6.27: Tubing set check – part II

IMPORTANT

Review all tubing for kinks or severe bending that could restrict the flow of liquid through the tubing set. If a tubing position has to be adjusted, press the valve button to open the respective valve beforehand (see Figure 6.8 on page 50).

If necessary, raise or lower the bag hanger to adjust the height of the bag. Ensure that the bags are positioned high enough to prevent severe bending of the tubing and low enough to avoid the tubing or connections being stretched.

In addition, verify the tightness of all Luer Lock connections at the tubing set including both Luer Locks of the chamber.

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

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). In case the chamber needs to be removed, press **open lid** *on the tool bar. Wait until the lid opens. Turn the chamber 90 degrees counterclockwise to remove it from the drive shaft.*

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

When the tubing set installation is complete, all valves will open and close twice to verify proper functionality. In addition, the forward and backward movement of the magnet inside the magnet unit is tested. If a valve or the magnet does not operate correctly, then refer to chapter 7 on page 83 of this user manual.

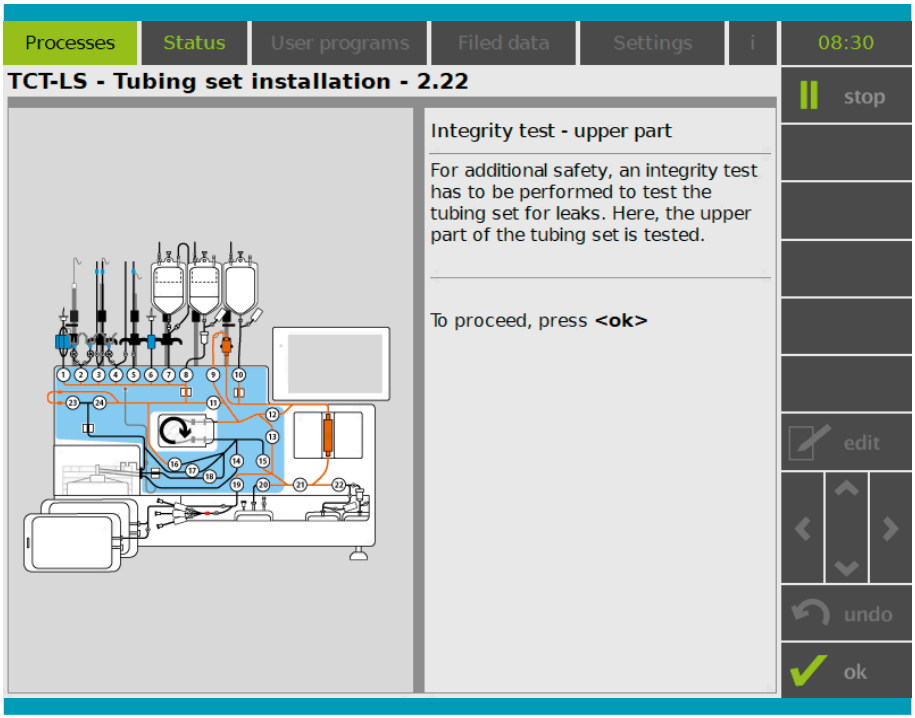
IMPORTANT

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

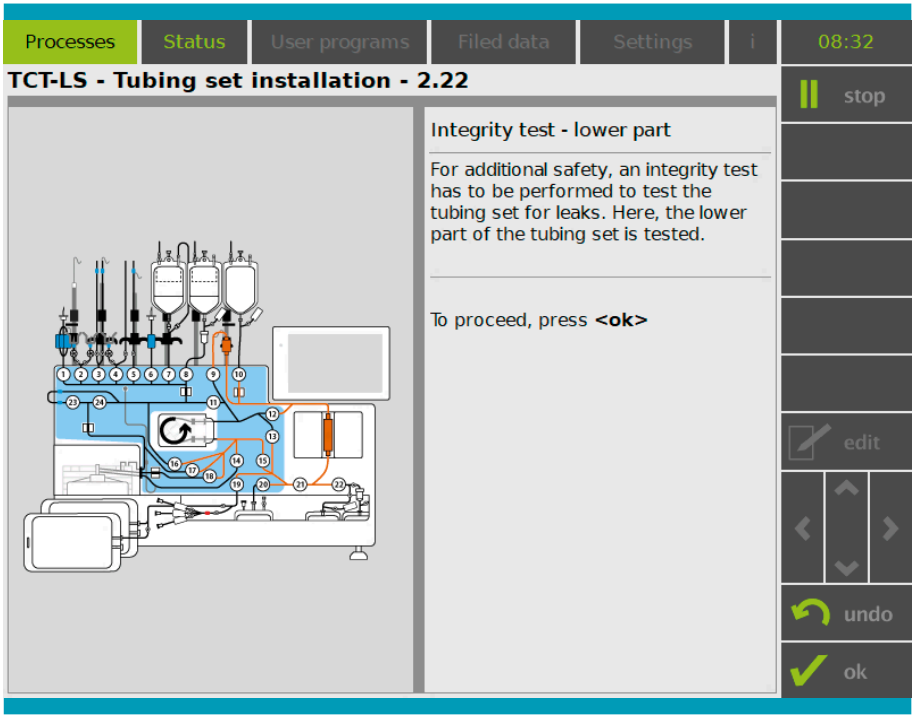
Follow the instructions on the screen to start the integrity test for the upper and lower part of the CliniMACS Prodigy TS 620.

It is not recommended to press **no** and skip the integrity test in the following pop-up.

Perform separate integrity tests for both upper and lower part of the tubing set.



Screen 6.28: Integrity test for upper part



Screen 6.29: Integrity test for lower part

⚠ 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.

6.4 Tubing set priming

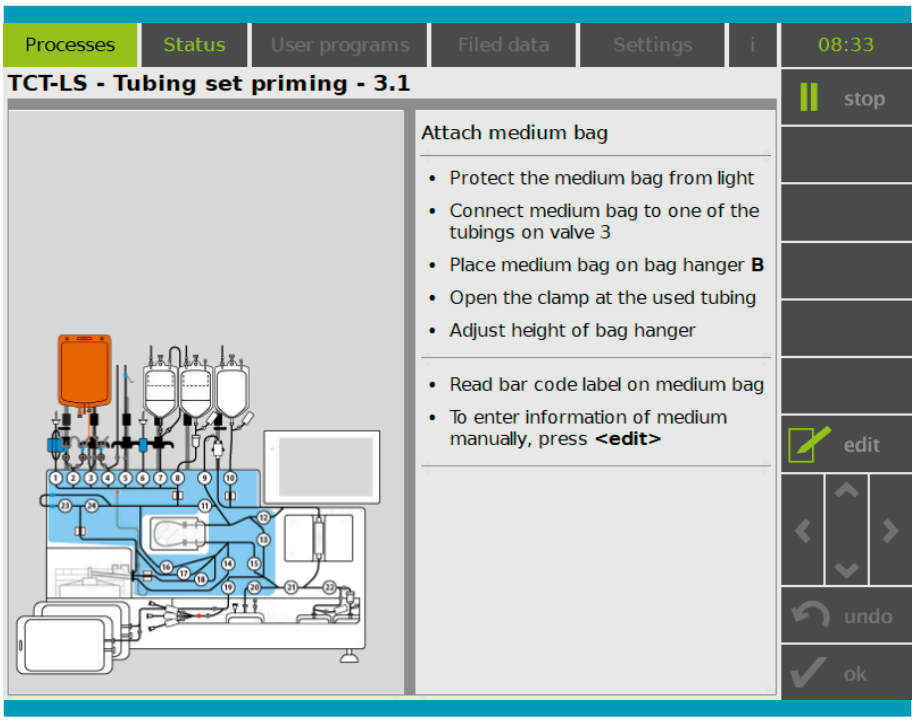
6.4.1 Connection of medium and buffer

Refer to chapter 5 on page 23 of this user manual for the preparation of the process buffer and medium.

NOTICE

Per bag hanger a maximal weight of 5 kg is allowed. If several bags weighing more than 5 kg in total are to be connected, they must be divided among several bag hangers.

Screen 6.30 to Screen 6.36 will guide the user through the priming phase.



Screen 6.30: Connection of medium bag

To protect the TexMACS GMP Medium bag from UV light use the provided protection bag (yellow) before connection to the tubing set.

IMPORTANT

UV light could lead to adverse effects on the TexMACS GMP Medium. Protect the TexMACS GMP Medium from UV light using the provided protection bag (yellow).

To connect the medium bag to the tubing set on valve 3 by sterile welding, use a sterile tubing welder placed next to the instrument.

Place the medium bag on bag hanger B.

Read the bar code label on the medium bag with the bar code reader. Alternatively, press **edit** to enter part number (P/N) and batch code (LOT) manually with the keyboard.

IMPORTANT

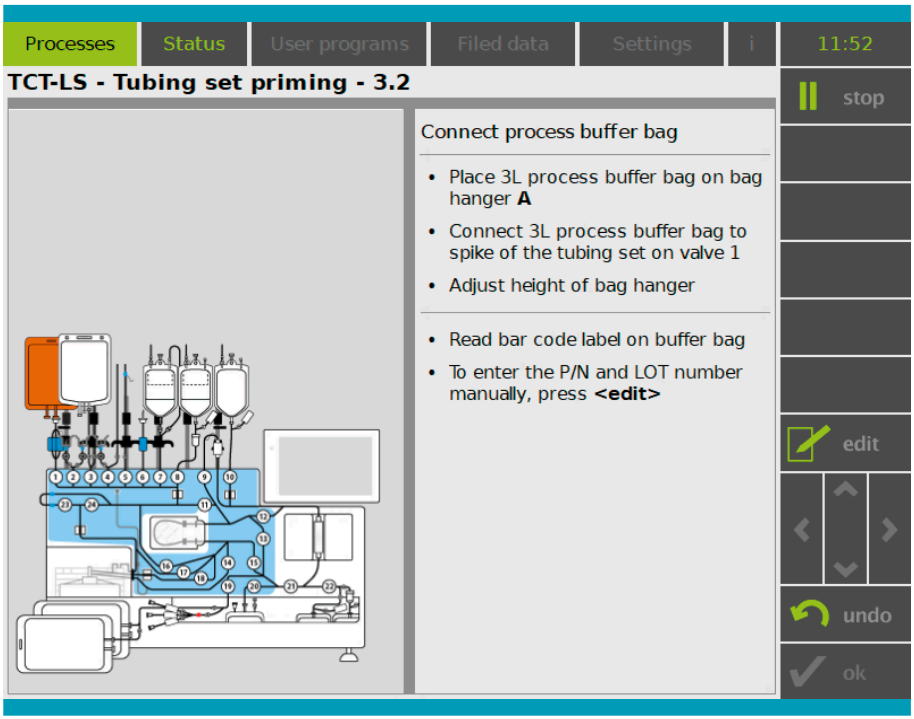
Confirm that the clamp of the medium bag is open at this point.

Press **edit** to enter information on supplements.

If necessary, raise or lower the hanger to adjust the height of the bag hanger. Ensure that the bag is positioned at a suitable height to prevent severe bending or overstretching of the tubing.

IMPORTANT

It is recommended to use TexMACS GMP Medium for the T Cell Transduction – Large Scale System. Using other medium could result in a lower performance.



Screen 6.31: Connection of process buffer bag

To connect the process buffer bag to the tubing set on valve 1:

- Uncap the spike of the tube connected to valve 1.
- Spike it into the process buffer bag.
- Place the process buffer bag on bag hanger A.

Alternatively, the process buffer bag can be connected to the CliniMACS Prodigy TS 620 by sterile welding.

Read the bar code label on the process buffer bag. Alternatively, press **edit** to manually enter the part number (P/N) and batch code (LOT).

Press **edit** to enter information on the HSA.

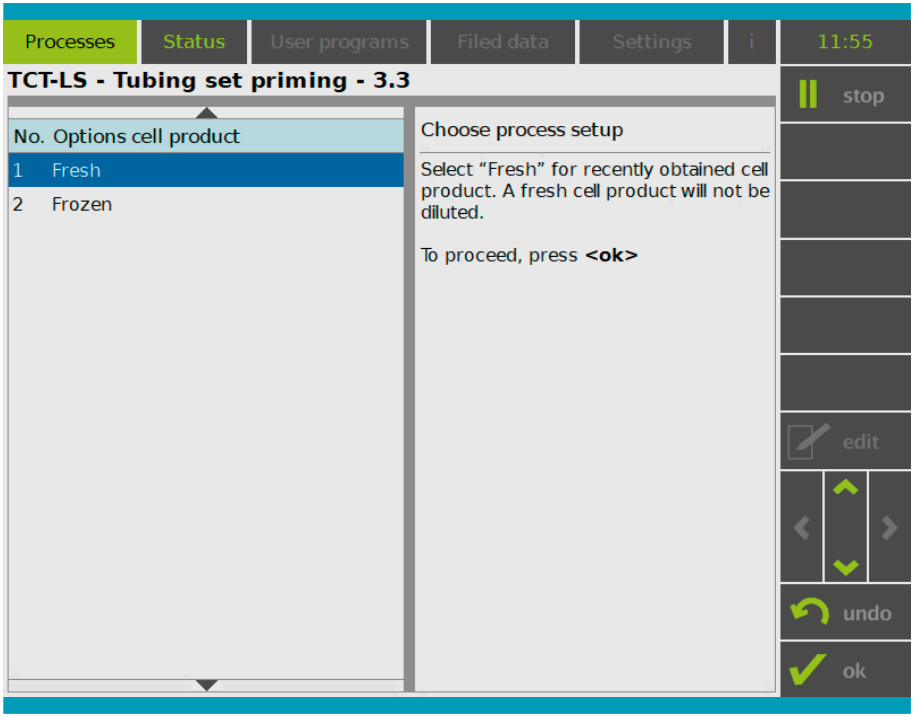
If necessary, raise or lower the bag hanger to adjust the height of the hanger. Ensure that the bag is positioned at a suitable height to prevent severe bending or overstretching of the tubing.

An overview of the positioning of the different bags on the bag hangers is provided below in Table 6.1.

Bag hanger ID	Bag
A	3,000 mL process buffer bag
B	2,000 mL medium bag
C	-
D	Filtration Bag
E	Application Bag
F	Reapplication Bag

Table 6.1: Positions of bags on bag hangers during priming and enrichment

6.4.2 Selection of fresh cell product



Screen 6.32: Selection of fresh or frozen starting cell product

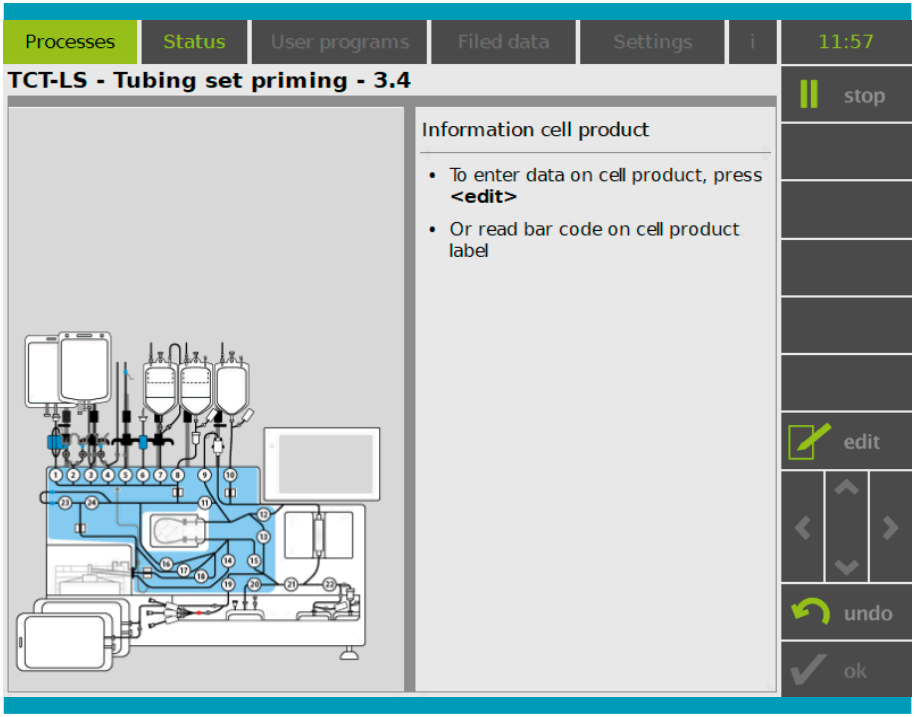
Select a fresh or a frozen leukapheresis as starting cell product and confirm with **ok**.

In case a frozen cell product is used refer to subsection 6.4.3 on page 63.

⚠ CAUTION

Risk of low separation performance. The T Cell Transduction – Large Scale System only allows the use of fresh or frozen leukapheresis. Do not use whole blood or buffy coats as cell products for this process.

6.4.3 Selection of frozen cell product



Screen 6.33: Information of cell product

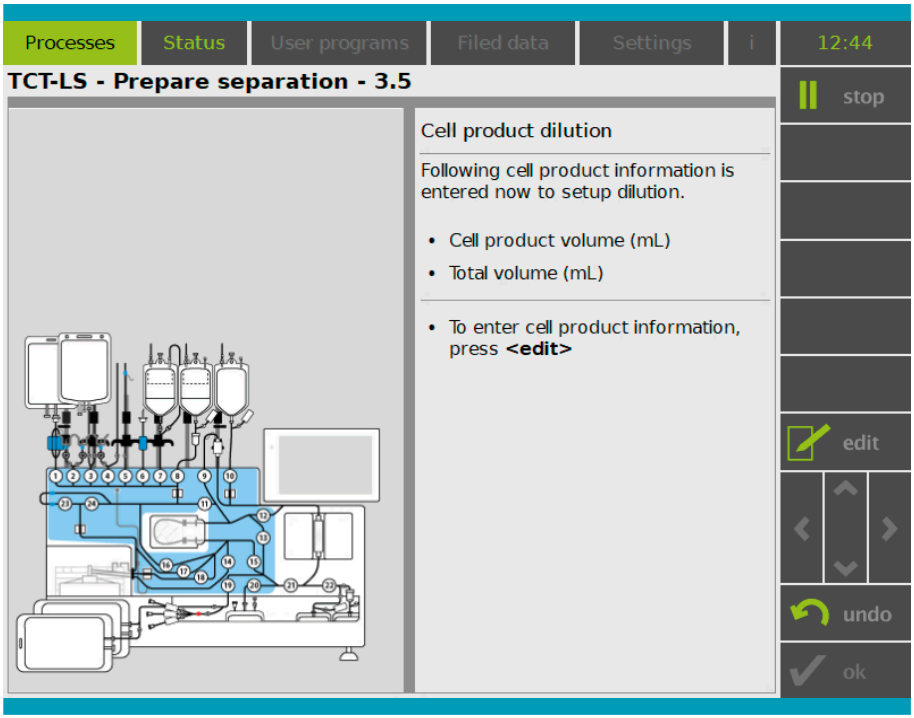
Press **edit** to enter the information of the cell product or read the bar code label (see Screen 6.33). The transfer of the cell product will happen later during the process (refer to subsection 6.5.2 on page 70).

6.4.4 Frozen cell product

⚠ CAUTION

Do not thaw the frozen leukapheresis until instructed to do so! Long holding time of the thawed cellular product might result in low cell viabilities and low separation performance.

To enter the information of the cell product or read the bar code label press **edit** in the first screen (see Screen 6.33).



Screen 6.34: Cell product dilution for frozen leukapheresis

An automatic dilution of the cellular product with medium can be performed when the entered total volume is higher than the cell product volume.

Enter the frozen cell product volume (e.g., 100 mL) and the total volume (e.g., 300 mL) that shall be achieved after dilution (see Screen 6.34). The corresponding dilution volume (e.g., 200 mL) is calculated automatically.

This volume of medium will be pumped into the Application Bag directly after the priming sequence.

Note: Be aware that the maximal capacity of the Application Bag is 600 mL. Make sure that the cell product volume does not exceed 300 mL.

Note: In case the cell product volume is the same as the total volume, no dilution will be performed. An orange dialog box appears to inform the operator.

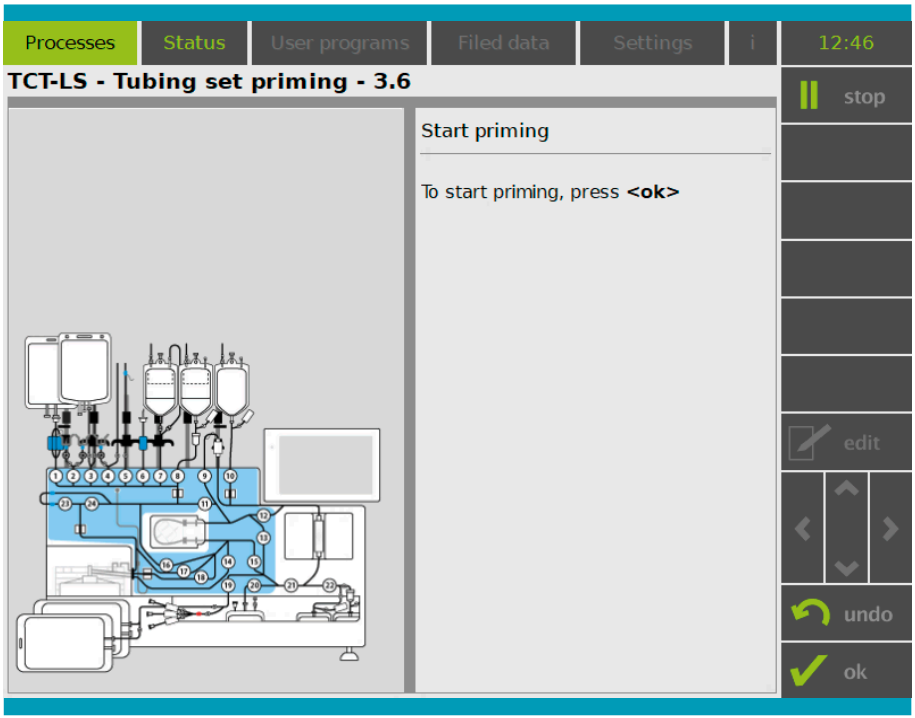
To confirm the entered values, press **ok**. To change the entered values, press **edit**. Otherwise press **ok** to proceed.

Be aware that after values were entered and confirmed the process does not allow a change of previously entered values. The dilution volume that will be pumped into the Application Bag cannot be changed and might result in a wrong dilution of the thawed cell product.

IMPORTANT

The combination of a frozen leukapheresis and an enrichment of CD62L positive cells is biologically not possible, because CD62L is downregulated after thawing.

6.4.5 Priming phase

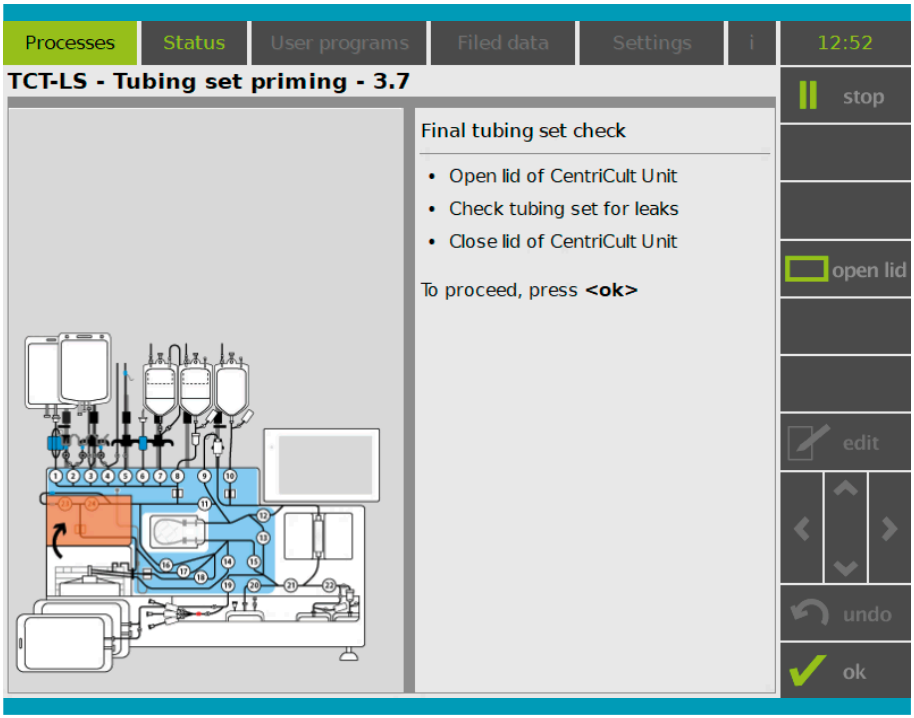


Screen 6.35: Start of priming phase

To start the priming of the tubing set press **ok**.

During the priming phase, the Reapplication Bag is filled with medium, and the tubing set is filled with process buffer.

The time for the priming phase will be displayed as a process bar on the screen.

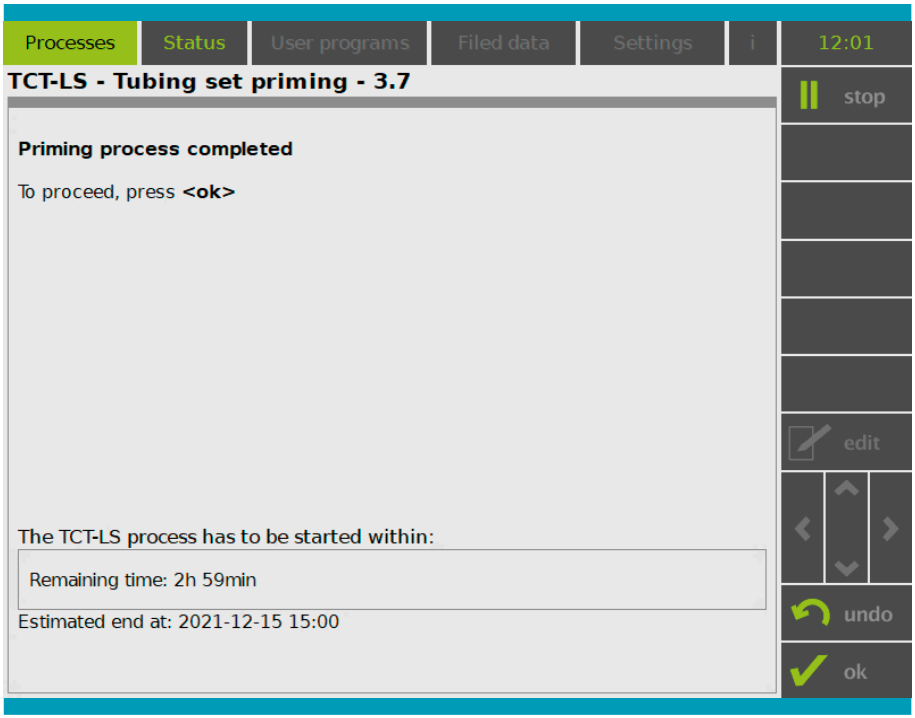


Screen 6.36: Final tubing set check after priming phase

When the priming phase is completed, check all tubing, fittings, valves, chamber, and columns for leaks or kinks that may block the fluids (see Screen 6.36). In case there is any leakage, restart the process with a new tubing set, medium, and buffer.

The CentriCult Unit lid unlocks automatically during this process step. If the lid does not unlock automatically, press the **open lid** button on the tool bar (see Screen 6.36). In the event the lid cannot be opened, contact Miltenyi Biotec Technical Support.

Note: If an automated dilution for frozen cell product was performed, confirm that medium is provided in the Application Bag before transferring the cell product.



Screen 6.37: End of priming phase

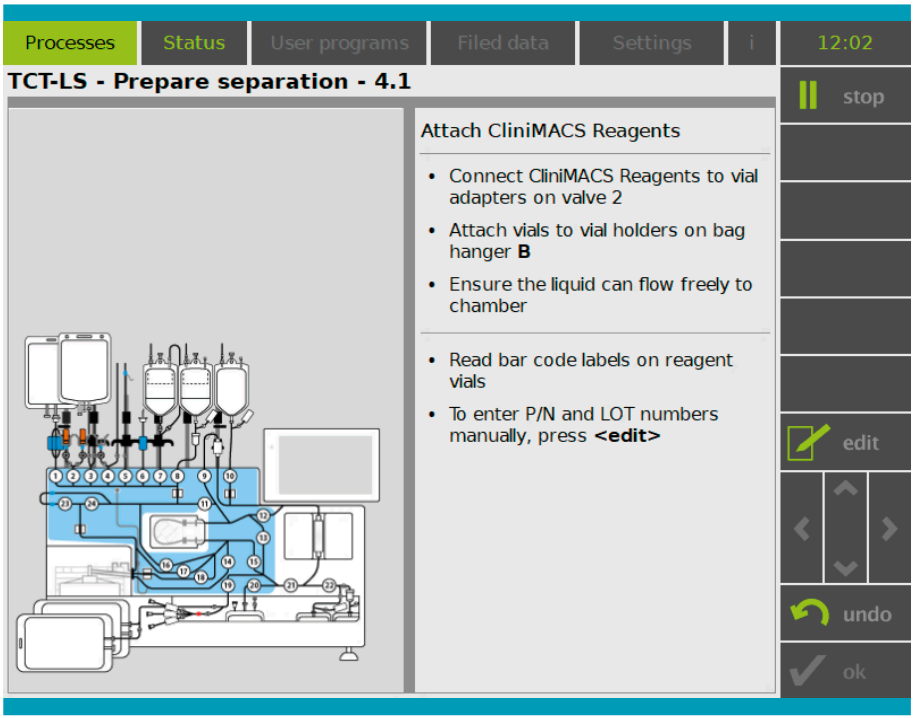
IMPORTANT

It is recommended to perform the tubing set priming directly before the enrichment process and not more than 3 hours in advance. Check that the separation reagent(s) and the cellular starting product are available for the separation phase.

6.5 Preparation for the automated enrichment process

6.5.1 Connection of CliniMACS GMP MicroBeads

According to prior process selection, connect either one vial of the CliniMACS CD62L GMP MicroBeads or one vial of each CliniMACS CD4 GMP MicroBeads and CliniMACS CD8 GMP MicroBeads to the vial adapters on valve 2.



Screen 6.38: Connection of CliniMACS GMP MicroBeads

If connecting one vial of CliniMACS CD62L GMP MicroBeads:

- Close one of the clamps on the vial adapters first and place this vial adapter on top of the instrument between the bag hangers.
- Turn the other vial adapter upside down and place it over the CliniMACS CD62L GMP MicroBeads vial.
- Carefully push the vial into the adapter while slightly turning until the spike penetrates the elastomer stopper and the adapter plastic ring snaps into place.
- Ensure that the septum is punctured, allowing free flow of liquid.

- In one smooth motion, turn the vial and vial adapter back again and insert the CliniMACS CD62L GMP MicroBeads vial into the vial holder on bag hanger B. For correct connection, refer to Figure 6.9 on page 51.
- Adjust height of vial holder.

If connecting one vial of CliniMACS CD4 GMP MicroBeads and one vial of CliniMACS CD8 GMP MicroBeads:

- Turn both vial adapters upside down and place them over the vials.
- Carefully push both vials into the adapters, while slightly turning until the spike penetrates the elastomer stopper and the adapter plastic ring snaps into place.
- Ensure that the septum of each vial is punctured, allowing free flow of liquid.
- In one smooth motion, turn the vials upside down and insert both CliniMACS GMP MicroBeads vials into the vial holder on bag hanger B. For correct connection, see Figure 6.9 on page 51.
- Adjust height of vial holder.

Read the bar code label on the vial(s) or press **edit** and enter part number(s) (P/N) and batch code(s) (LOT) manually with the keyboard displayed on the screen (see Screen 6.38).

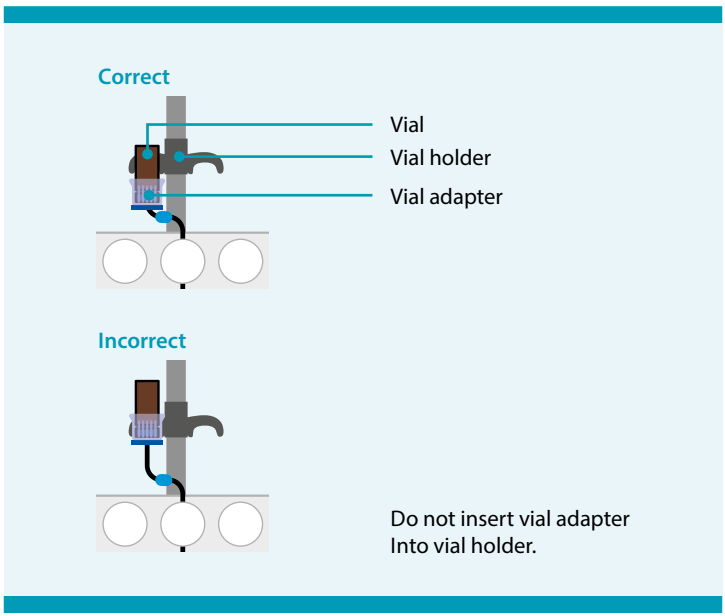


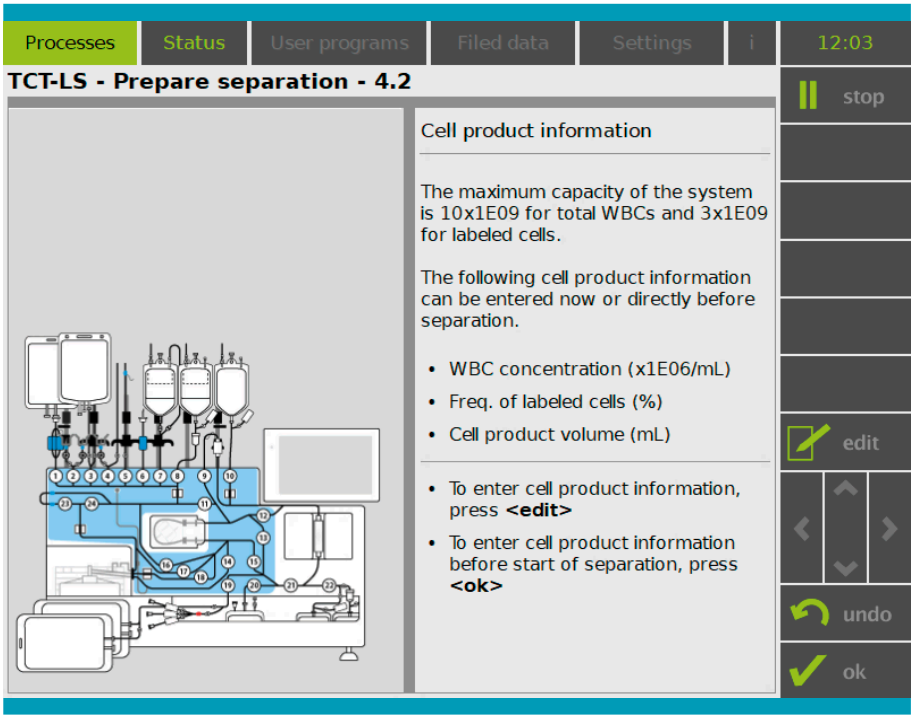
Figure 6.10: Connection of vials to vial holder

6.5.2 Transfer of cell product

Transfer of fresh cell product

Before connecting the cell product bag to the tubing set, determine the volume, the WBC concentration and the frequency of cells to be labelled.

Note: If the total number of cells to be labelled is higher than 3×10^9 cells, the process capacity is exceeded, and target cells will be lost in the negative fraction and will end up in the Non-Target Cell Bag. For details regarding how to calculate the number of target cells refer to section 5.1 on page 23.



Screen 6.39: Entry of fresh cell product information

To enter the information of the cell product press **edit**.

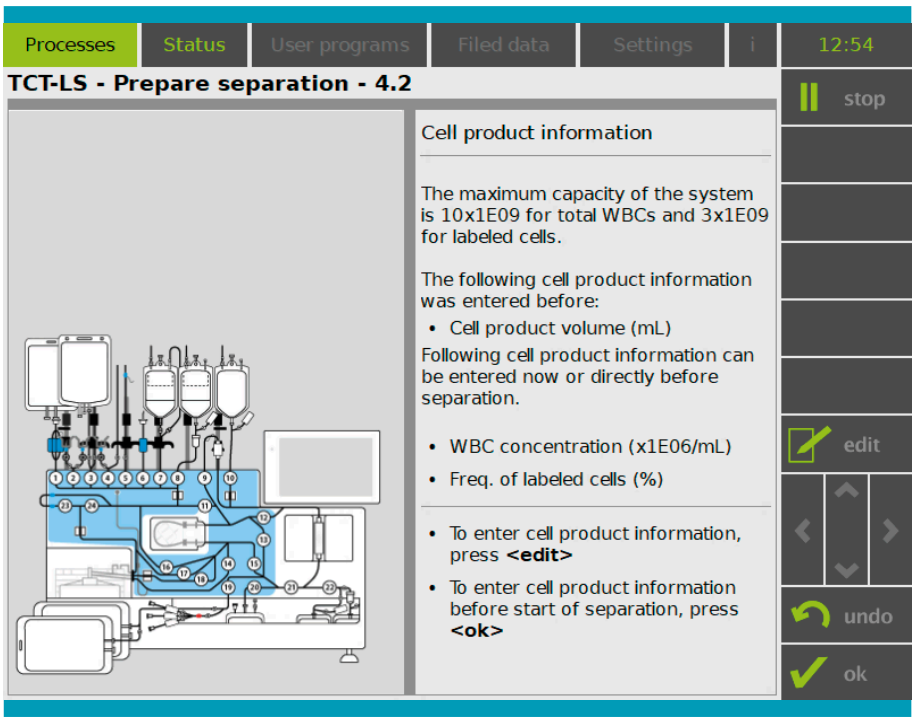
Enter the WBC concentration ($\times10^6$/mL), the frequency of labeled cells (i.e., percentage (%) of CD4/CD8 positive cells or percentage (%) of CD62L positive cells) and the volume of the cell product that will be transferred to the Application Bag (see section 5.1 on page 23).

Optionally, press **ok** to enter the parameters later during the process. If choosing to continue without parameter input at this time, the same input will have to be done in the middle of the automatic enrichment process, specifically right before the cells are loaded onto the column.

Transfer of frozen cell product

⚠ CAUTION

Risk of low performance or loss of cell product. Do not thaw the frozen leukapheresis until instructed to do so! Long holding time of the thawed cellular product might result in low cell viabilities and low separation performance.



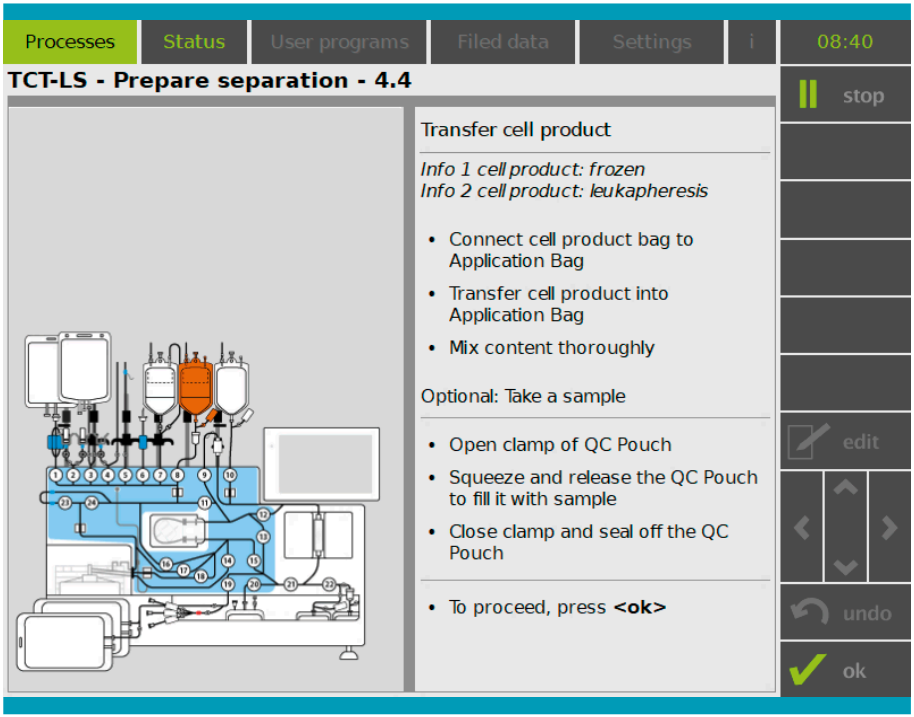
Screen 6.40: Entry of frozen cell product information

To enter the information of the cell product press **edit**.

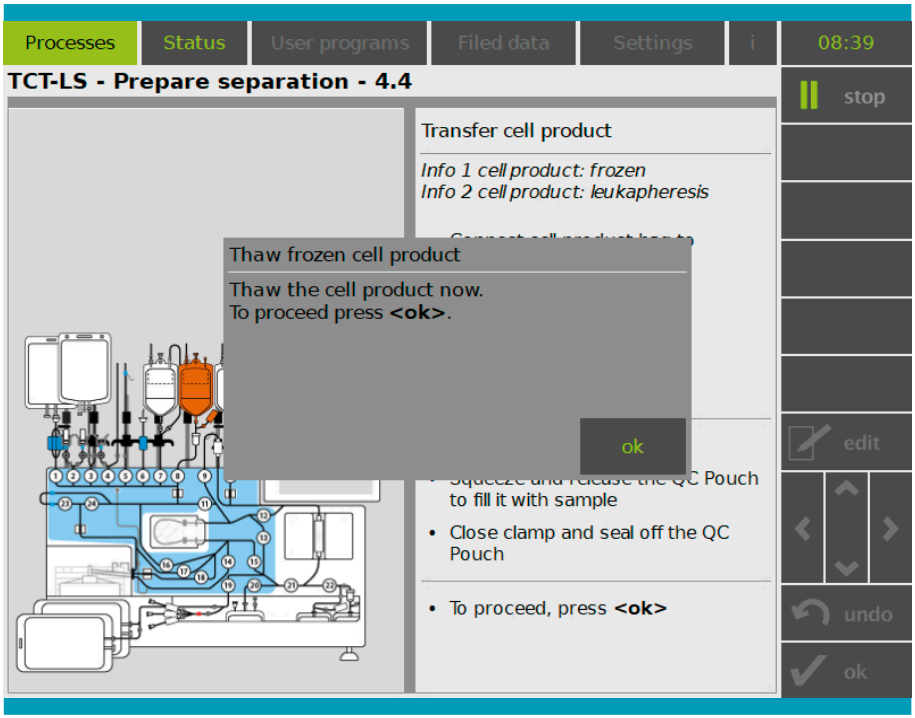
Enter the WBC concentration ($\times 10^6$/mL), the frequency of labeled cells (i.e., percentage (%) CD4/CD8 positive cells or percentage (%) CD62L positive cells) (see Screen 6.40). Be aware to enter the parameters referring to the frozen cell product.

Optionally, press **ok** to enter the parameters later during the process. If choosing to continue without parameter input at this time, the same input will have to be done in the middle of the automatic enrichment process, specifically right before the cells are loaded onto the column.

Note: For WBC concentration values that are obtained from the thawed and diluted cell product drawn from the Application Bag, the dilution factor must be considered.



Screen 6.41: Connection of frozen cell product



Screen 6.42: Thawing of frozen cell product

The frozen cell product bag should be thawed, as soon as the pop-up with the instruction appears (see Screen 6.42).

⚠ CAUTION

Risk of tubing set damage. Dimethyl sulfoxide (DMSO) concentrations higher than 20 % (v/v) (22% (w/v)) with longer exposure times than 24 h and temperatures above room temperature are not recommended for the CliniMACS Prodigy Tubing Set. If solutions with higher DMSO concentration should be connected to the tubing set it is recommended to dilute the solution in advance.

To transfer the cell product to the Application Bag, follow the institution's standard operating procedures. For example, connect a 1m tube extension with a luer to the spike connector and spike it into the spike port of the cell product bag. Then connect the cell product bag to the Application Bag via sterile welding. Transfer the cell product into the Application Bag and seal off the empty bag using a heat sealer. Make sure to gently mix the Application Bag after transfer to ensure homogeneous dilution of the cell product.

To take a sample from the blood product:

- Open the clamp of the QC Pouch
- Squeeze and release the pouch once to fill the QC Pouch with cellular starting product.
- Multiple squeezes and releases will increase the sample volume in the QC Pouch. The maximum volume capacity of the QC Pouch is 4 mL.
- Close the clamp and seal off the QC Pouch.

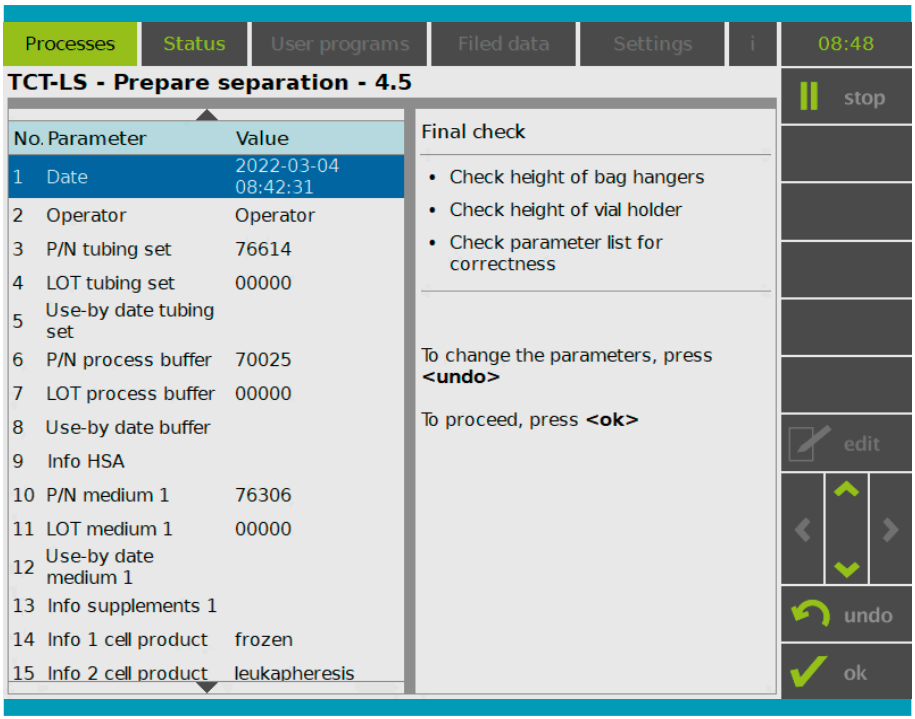
The QC sample should be analyzed following the institution's standard operating procedures.

Press **edit** to enter information regarding the cell product.

⚠ CAUTION

Risk of low separation performance. The application software calculates the stages with the original cell product volume. In case the sample is taken from the diluted cell product in the Application Bag, the dilution factor must be considered during parameter entry.

6.5.3 Final check



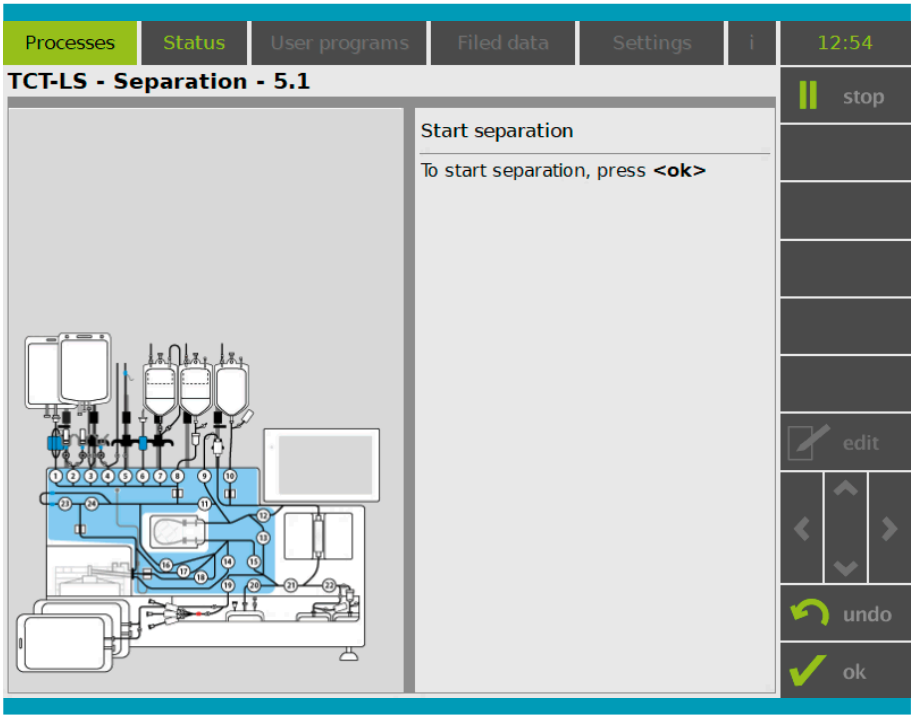
Screen 6.43: Final set-up check

Parameters can only be revised but not edited at this point. To return to the previous screen, press **undo** (see Screen 6.43).

Note: At this time all pre-handling steps for the enrichment part of the T Cell Transduction – Large Scale System are complete, and the automated enrichment process can begin.

6.6 Automated enrichment process

6.6.1 Start of enrichment process



Screen 6.44: Start of automated enrichment process

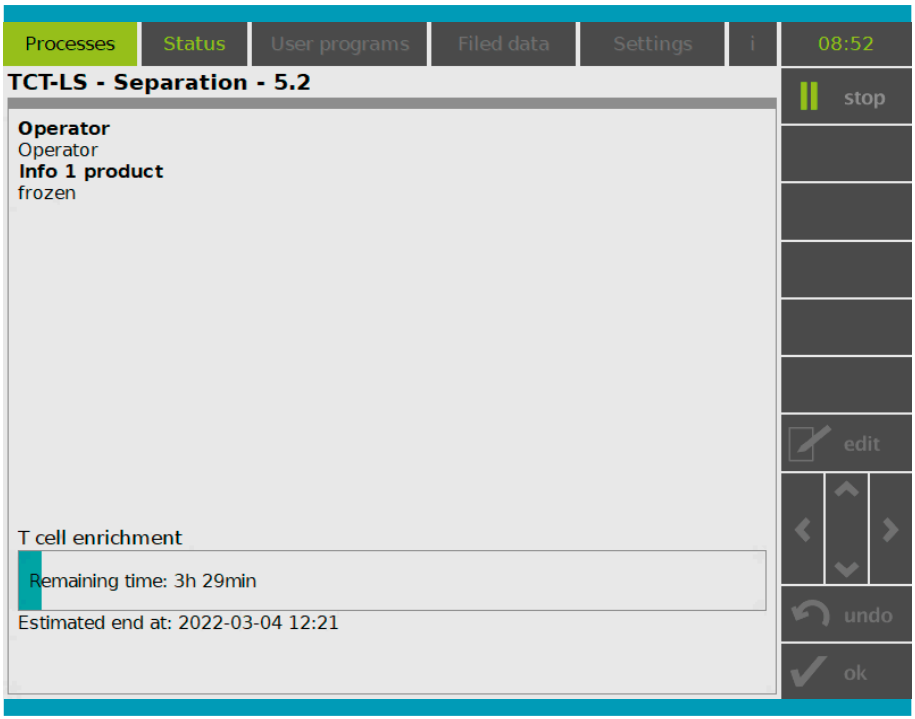
To start the enrichment press **ok** (see Screen 6.44). A pop-up window will appear.

Press **yes** to confirm the start of the enrichment process.

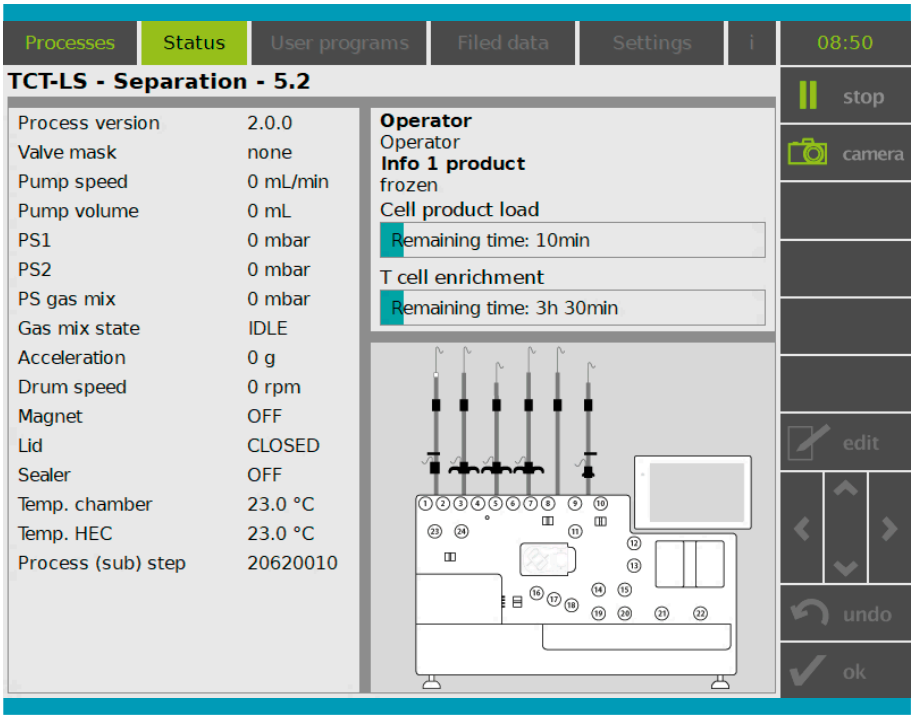
The fully automated enrichment process is divided into several phases:

1. cell product load
2. washing/volume adjustment
3. cell labeling
4. removal of unbound beads
5. cell filtration
6. magnetic separation
7. elution

Press **Status** on the menu bar to monitor the different phases during the process. For details, refer to CliniMACS Prodigy User Manual (Instrument).



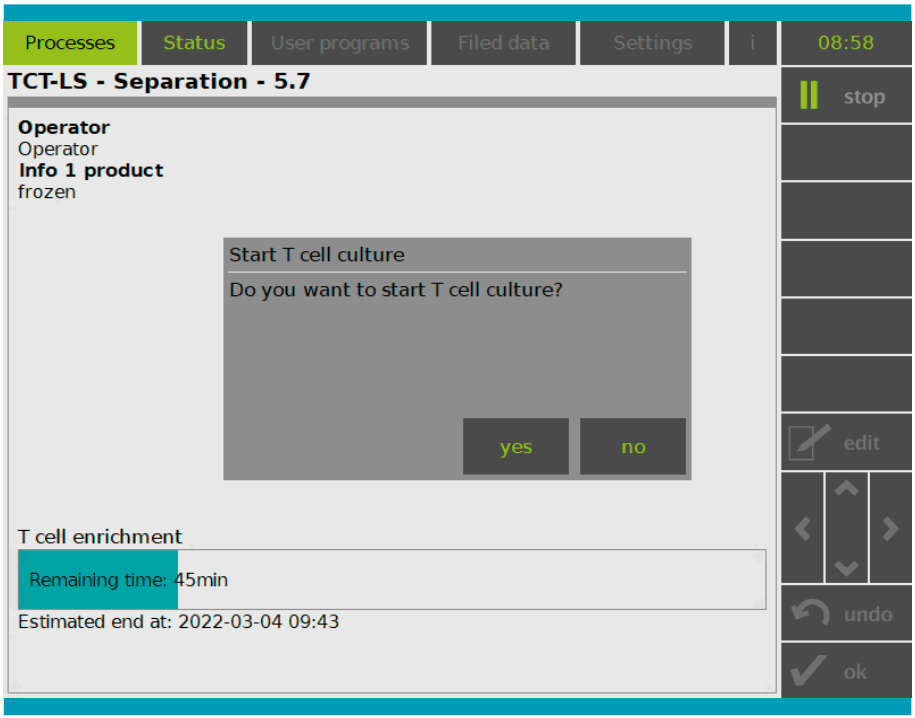
Screen 6.45: (Processes): Generic information screen



Screen 6.46: (Status): Generic status screen displaying real time process info

6.6.2 Completion of enrichment process

After the enrichment process, a pop-up window will ask if the process should be continued with cultivation.



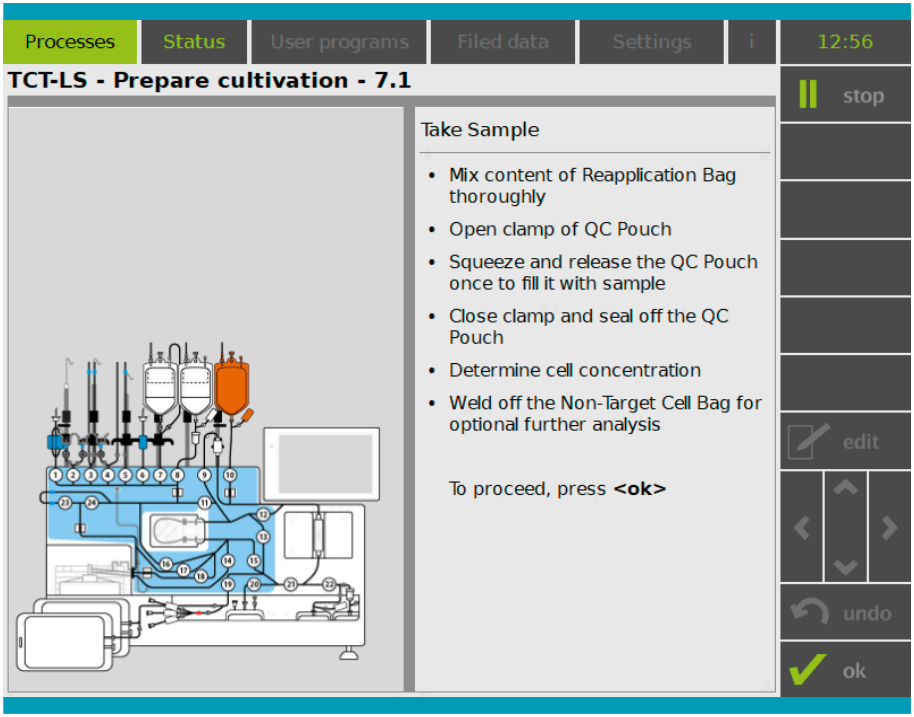
Screen 6.47: Pop-up with start of T cell culture

If no cultivation is required press **no** and proceed to final handling (see section 7.5 on page 128).

The Reapplication Bag above valve 10 contains the enriched target cell fraction. Seal off the Reapplication Bag to retrieve the enriched cells.

To perform cultivation, press **yes** and proceed with the analysis of the target cell fraction (see subsection 6.6.3 on page 80).

6.6.3 Analysis of enriched target cell fraction



Screen 6.48: Removal of sample from target cell fraction

To take a sample of the enriched target cell fraction from the Reapplication Bag:

- Mix the content of the Reapplication Bag thoroughly.
- Open the clamp of the QC Pouch.
- Squeeze and release the pouch once to fill the QC Pouch with cellular starting product.
- Multiple squeezes and releases will increase the sample volume in the QC Pouch. The maximum volume capacity of the QC Pouch is 4 mL.
- Close the clamp at the top end of the tubing and seal off the tubing.

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

Store all QC samples at +4 °C to +8 °C (+39 °F to +46 °F).

Analyze QC samples according to the institution's standard operating procedure.

⚠ 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.

To analyze the non-target cell fraction, seal off the Non-Target Cell Bag below valve 20.

7

Cultivation of T cells

7.1 General remarks

IMPORTANT
During the entire cultivation process the following must be considered:

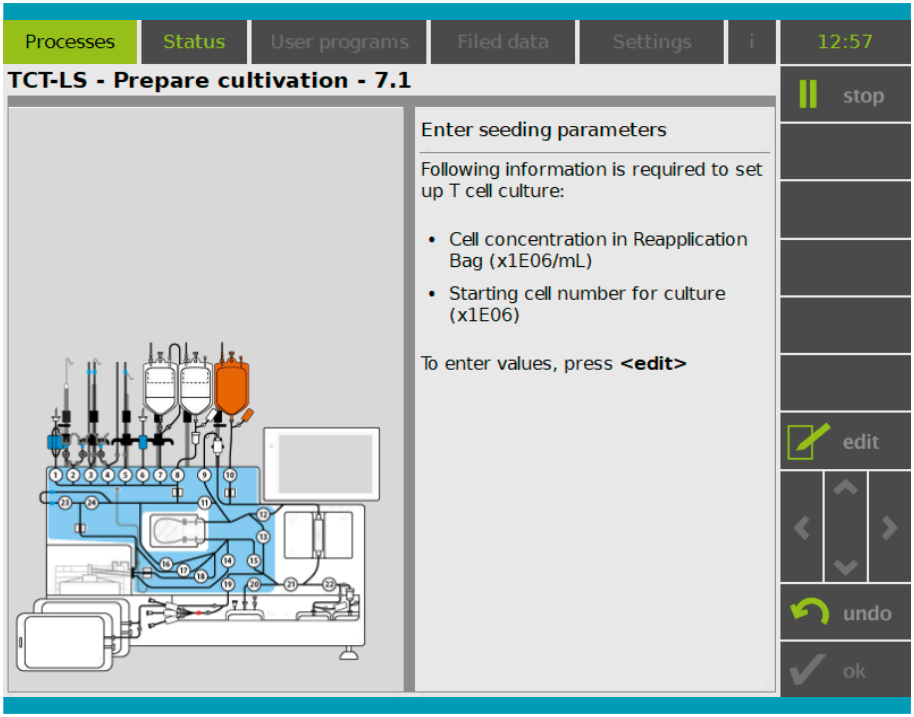
Every 10 hours, the tubing set is rinsed with 10 mL of medium connected to valve 3. Therefore, sufficient medium should always be connected to the tubing on valve 3 (e.g., if the device is running for 72 hours, a minimum of 80 mL medium must be connected).

Each Waste Bag connected to the CliniMACS Prodigy TS 620 has a capacity of five liters. If a long process with many feeding and/or washing steps is to be performed, regularly check that the Waste Bag is not overfilled.

7.2 Preparation for the cultivation

This section guides the user step by step through the setup of the cultivation part of the T Cell Transduction – Large Scale. Each screen displays the instructions supported by a picture. At any step during the setup press **undo** in case you want to return to a previous step.

7.2.1 Input of cell seeding parameters



Screen 7.1: Input of seeding parameters

Enter the cell concentration of the enriched cell fraction in the Reapplication Bag determined in subsection 6.6.3 on page 80 and the total number of cells to be seeded.

It is recommended to seed a total cell number of 400×10^6. This corresponds to an approximate cell seeding density of 2.72×10^6 cells per cm^2.

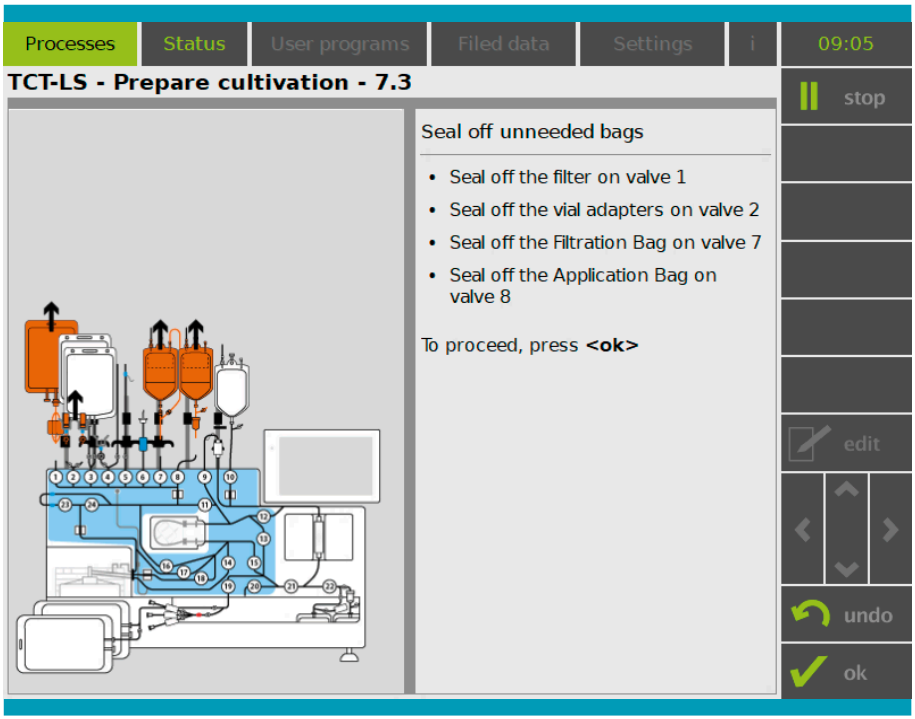
The volume to be transferred is automatically calculated.

Note: To retrieve the remaining enriched target cells (e.g., to be frozen as back-up) seal off the Reapplication Bag after the automatic setup of cultivation is completed (see subsection 7.2.6).

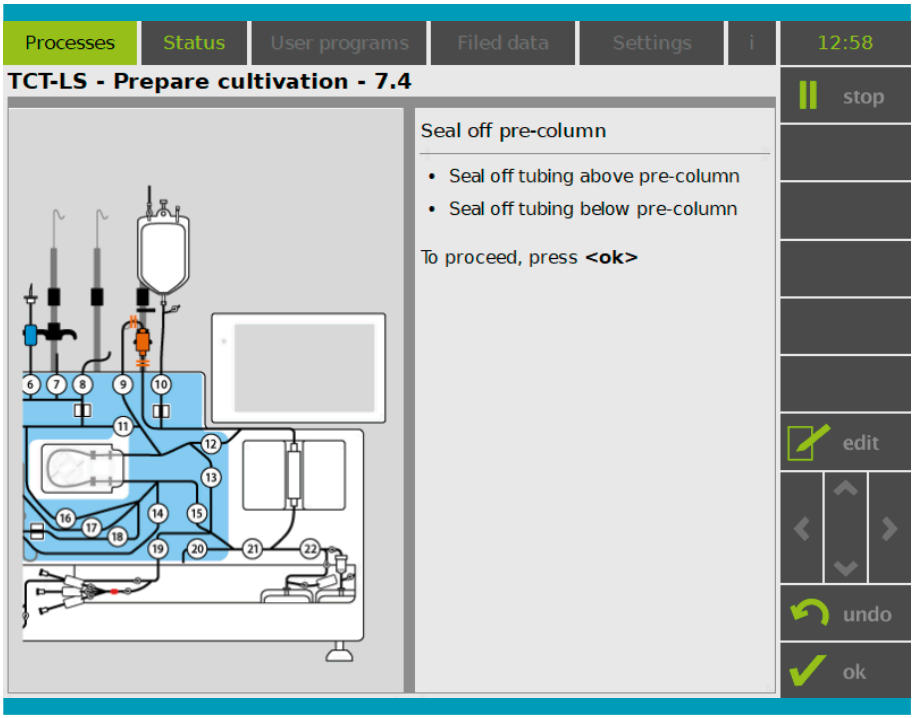
In the following screen the user is instructed to connect a second medium bag. For further instructions refer to subsection 6.4.1 on page 59.

7.2.2 Sealing off non-needed equipment

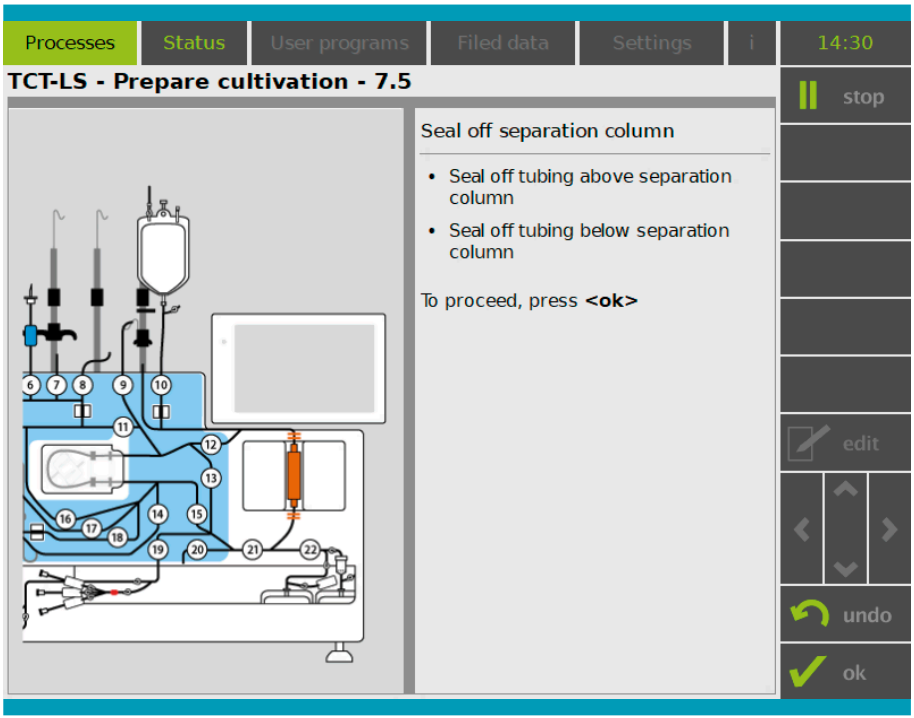
In order to avoid diffusion of solutions or cells from unneeded equipment during the cultivation step, bags on valve 1, valve 7 and valve 8, vial adapters on valve 2, the pre-column and the separation column of the tubing set should be sealed off or clamped off as shown on Screen 7.2, Screen 7.3 and Screen 7.4).



Screen 7.2: Sealing off non- needed bags and vial adapters on valve 1, 2, 7, 8



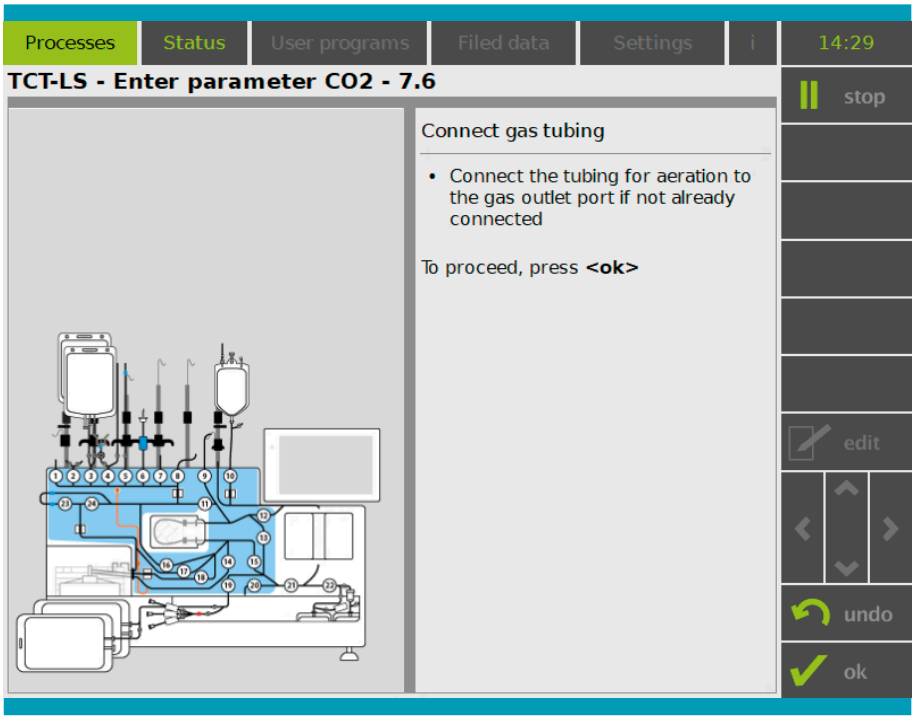
Screen 7.3: Sealing off the pre-column



Screen 7.4: Sealing off the separation column

7.2.3 Input of CO_2 and temperature parameters

If running the full process (case 1), the gas tubing has already been connected (see subsection 6.3.2 on page 35), press **ok** to proceed.

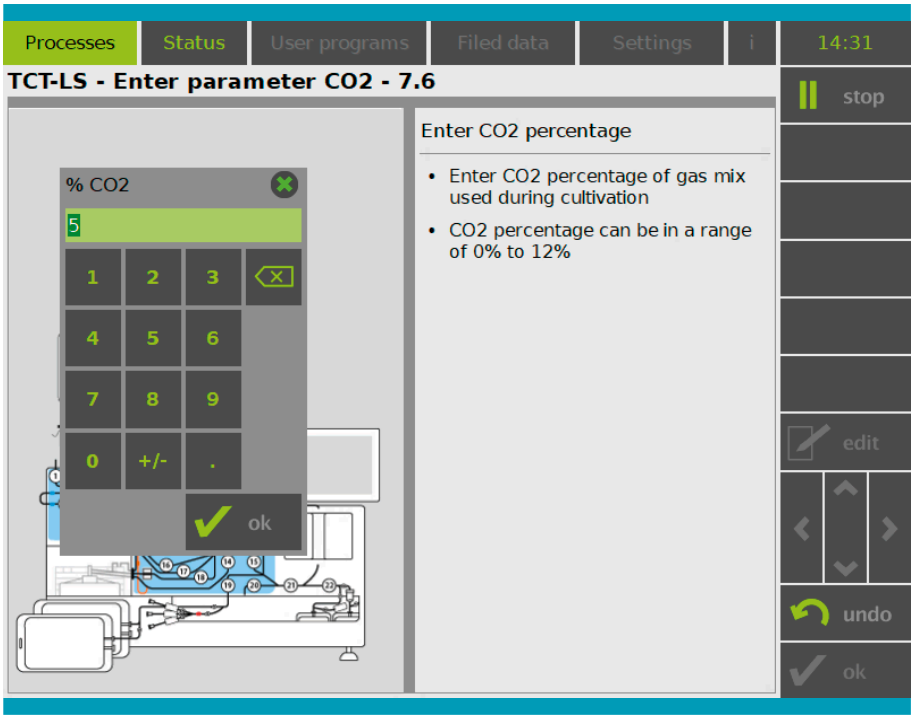


Screen 7.5: Connection of gas tubing

For the cultivation of the cells, the values for CO_2 concentration must be entered. For TexMACS GMP Medium enter **5% CO_2** (see Screen 7.6).

IMPORTANT

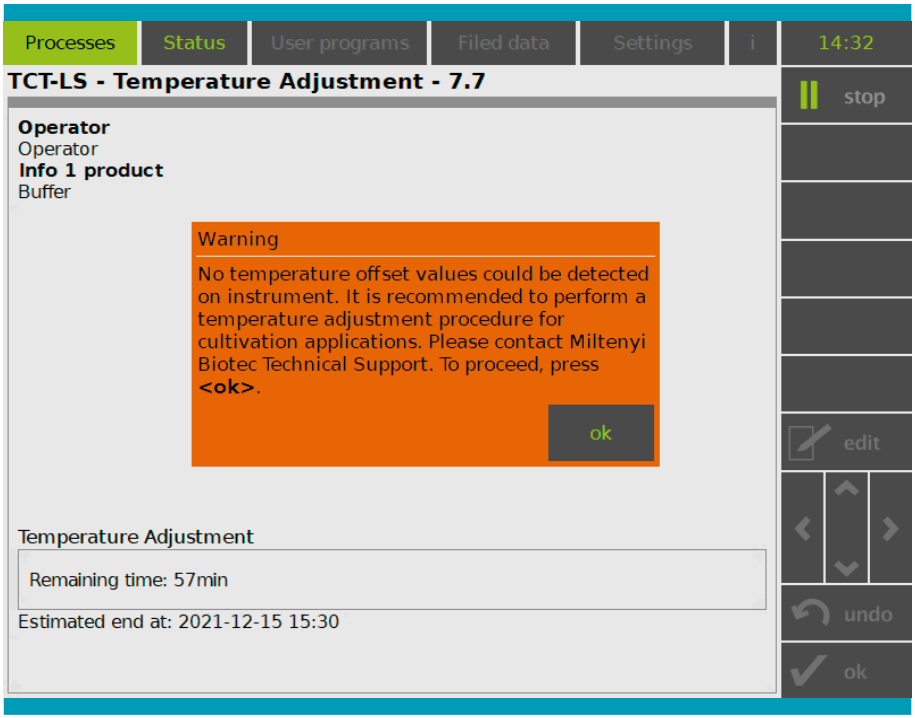
Connect the gas tube tightly to the gas outlet port to ensure proper aeration of the cells.



Screen 7.6: Input of CO_2 concentration

The temperature parameter will be set automatically to +37 °C (+99 °F), if temperature offset values are detected on the device.

If the instrument has no temperature offset values, a warning will appear stating that no temperature offset values could be detected on the instrument (see Screen 7.7). In this case the temperature parameter will be set automatically to +39 °C (+102 °F), which equals a cultivation temperature of +37 °C (+99 °F).

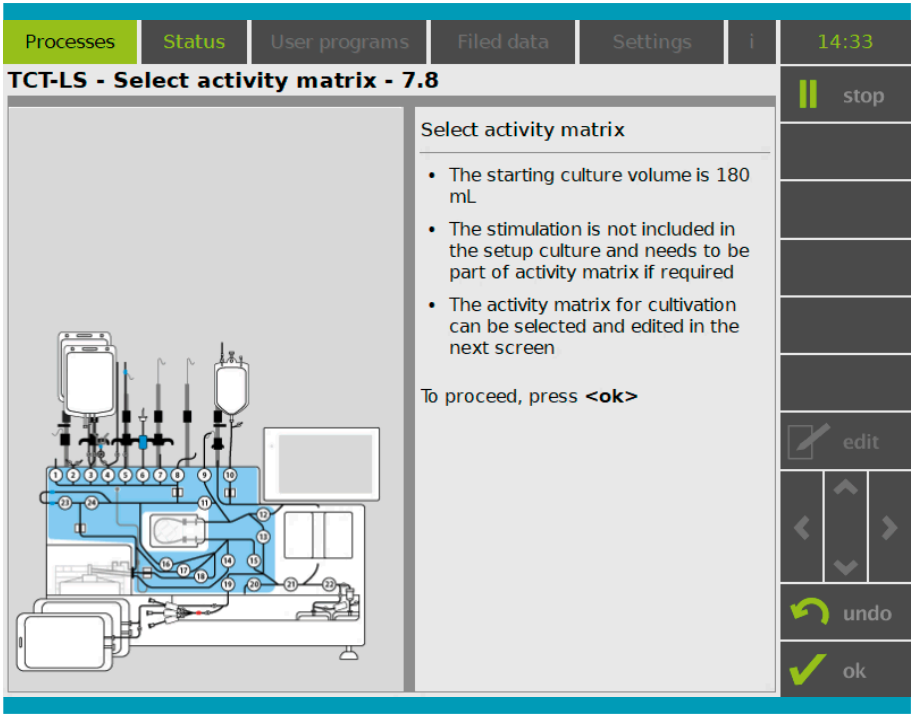


Screen 7.7: Warning which states that no offset values are detected on the instrument

IMPORTANT

It is highly recommended to receive a temperature adjustment and validation procedure for the instrument. For further information contact Miltenyi Biotec Technical Support.

7.2.4 Setup of activity matrix



Screen 7.8: Set activity matrix

The T Cell Transduction – Large Scale System allows customized programming of T cell cultivation steps. All T cell cultivation steps are programmed in an activity matrix. This can either be done by choosing a default matrix (see "Set default matrix" on page 91) or by creating an individual and permanently saved activity matrix on the CliniMACS Prodigy. Individually designed activity matrices can be imported and exported across CliniMACS Prodigy devices (see "Creation of individual protocols" on page 94). Each activity matrix can be modified during the cultivation phase, but those changes cannot be saved for further processes.

IMPORTANT

All steps programmed in the activity matrix are performed after the automated culture setup. The culture setup is performed automatically in one step (see subsection 7.2.6 on page 102) and is not part of the activity matrix. It cannot be modified.

The starting culture volume after the culture setup is always 180 mL and cannot be changed.

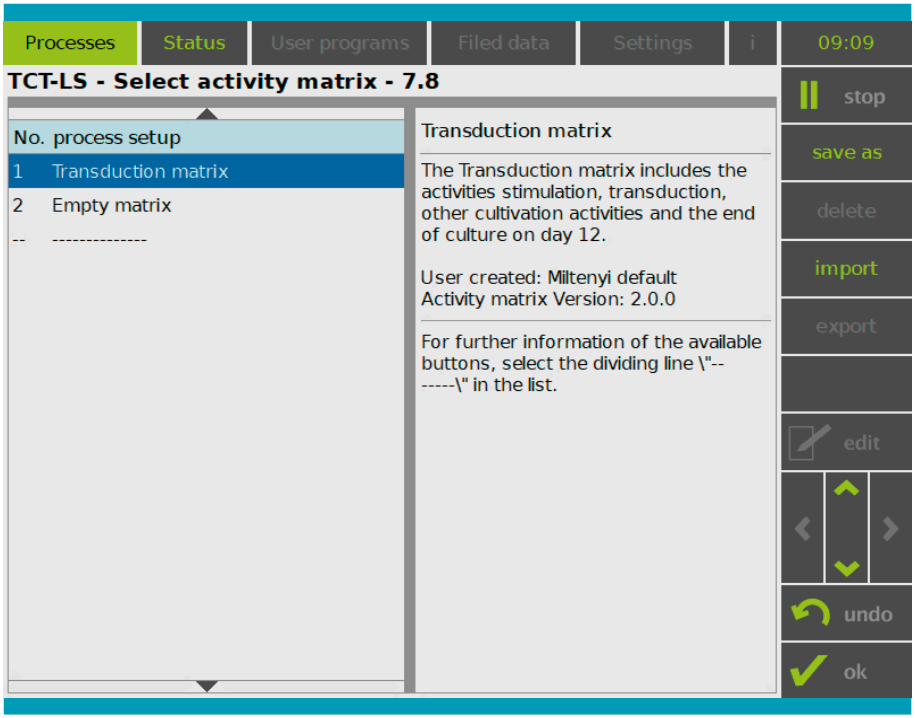
The following activities (listed in subsection “Modification of activities” on page 97) have an influence on the volume in the chamber. This volume is theoretically calculated in the activity matrix in the column “Volume” (see Screen 7.10).

The total volume in the chamber must be maintained between 40 mL and 600 mL. If these values are exceeded during the setup of the activity matrix a warning will be displayed at the final confirmation step.

Note: In the T Cell Transduction – Large Scale System the stimulation of T cells is decoupled from the culture setup and must be programmed into the activity matrix if required.

Note: The T Cell Transduction – Large Scale System is only verified for a cultivation time of up to 12 days.

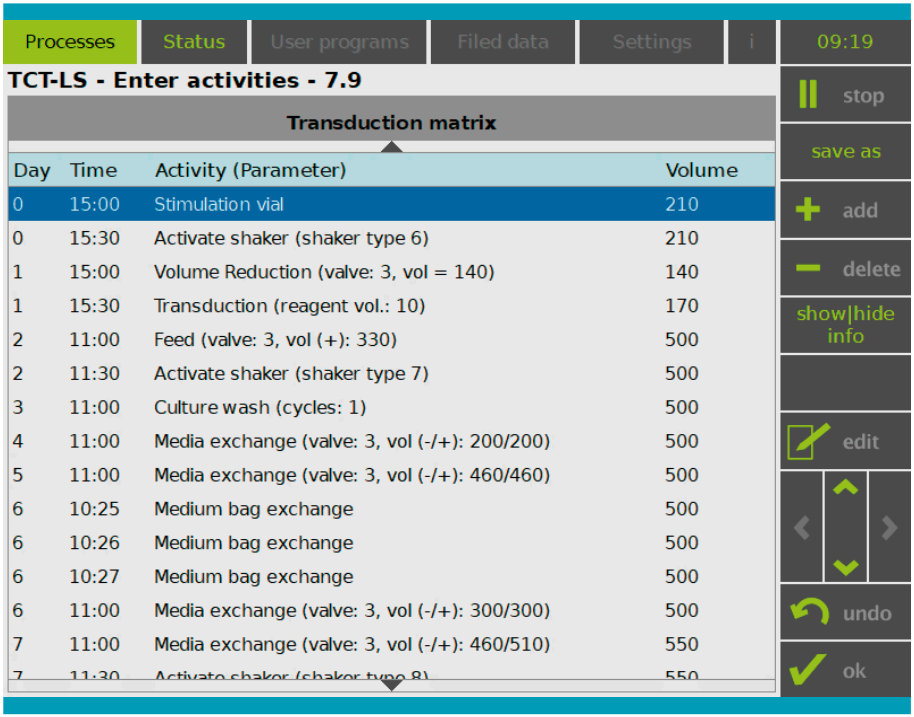
Set default matrix



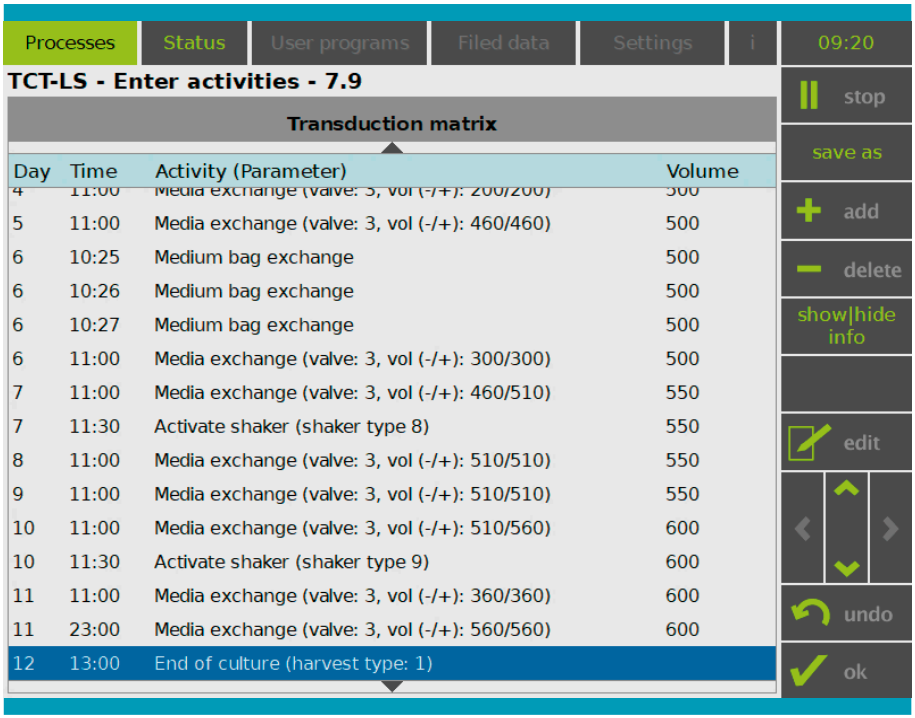
Screen 7.9: Set default matrix

The default setting is displayed showing at which day and time an activity will be performed.

Depending on the usage strategy of the Waste Bags and medium bags the schedules for “Waste Bag exchange” and “Medium bag exchange” may be different from the schedules in the default protocols. Note the schedule of the respective protocol and ensure that the bags are exchanged accordingly.



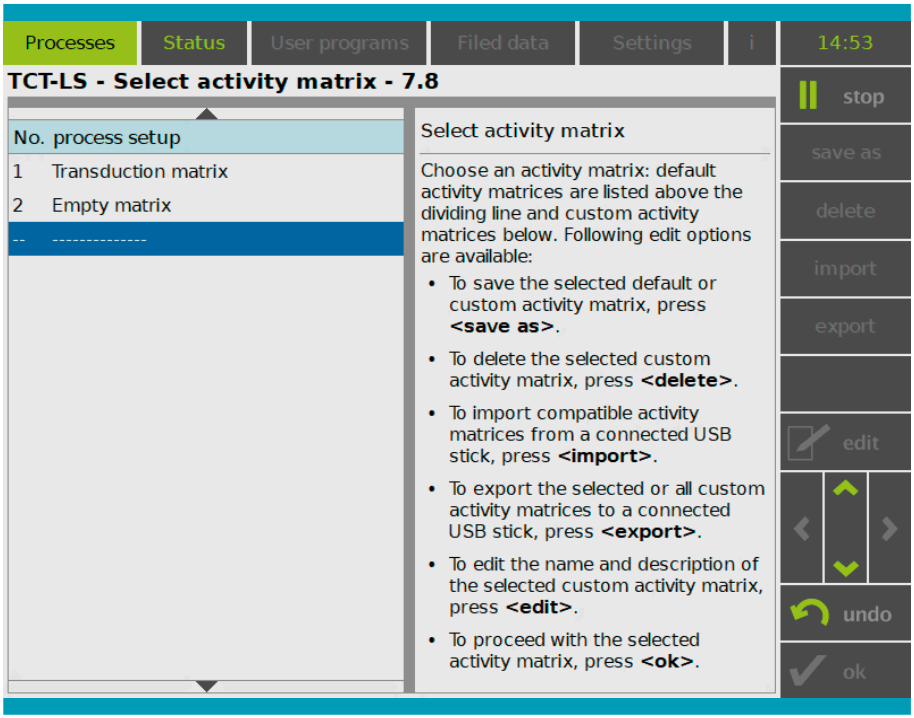
Screen 7.10: Default activity matrix – Standard protocol



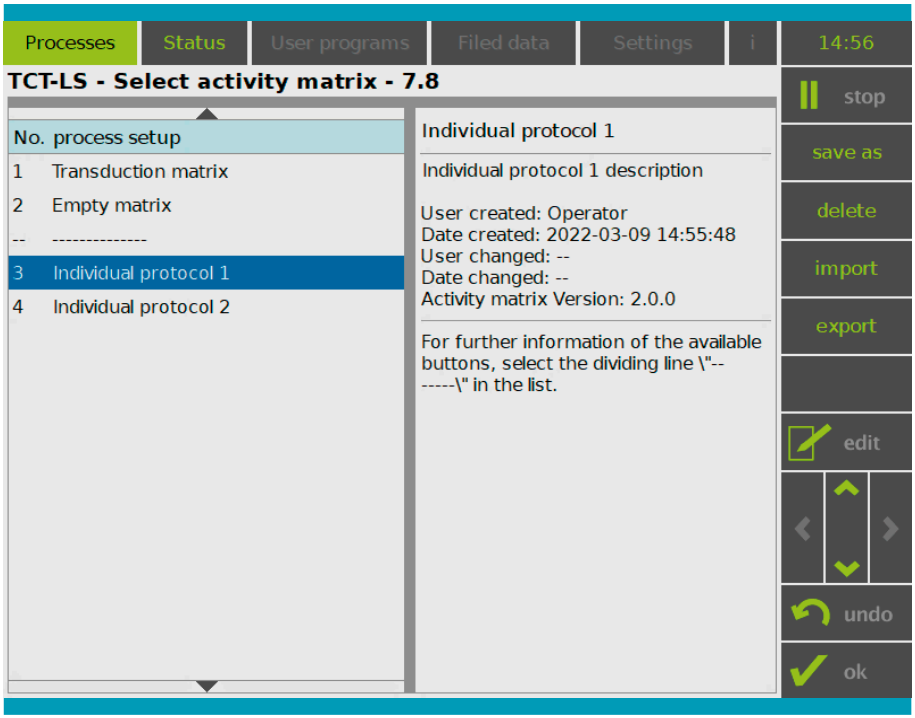
Screen 7.11: Default activity matrix – Standard protocol

Press **show/hide** info to show extra information on the entered parameters and instructions to edit the activity matrix.

Creation of individual protocols



Screen 7.12: Creation of individual designed activity matrices



Screen 7.13: Creation of individual designed activity matrices

The user can create individual activity matrices which remain permanently on the CliniMACS Prodigy. For detailed instructions press the dividing line ("-- ----------") in the list on the left-hand side of the screen (see Screen 7.12).

To create an individual activity matrix, select a default matrix as template ("Transduction matrix" or "Empty matrix") and press **save as**. Rename the new activity matrix with maximal 30 digits (e.g., "Individual protocol 1") and give it an individual description with maximal 300 digits. Special characters are not allowed.

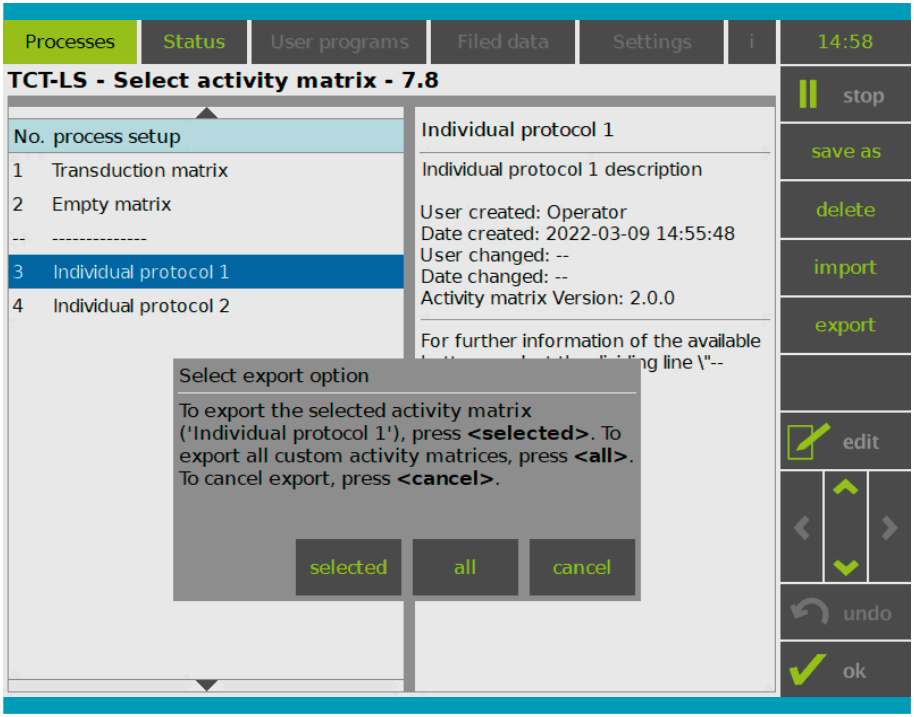
To edit an existing activity matrix, select it (typo) from the list and press **edit** (see Screen 7.13).

Including the two default matrices up to 50 activity matrices can be saved on the CliniMACS Prodigy concurrently. The protocols are serially numbered in the list.

Note: Do not use the numbering of the matrix as an identifier as the numbering can change if matrices are deleted out of the list.

The username and the current date will be logged under the description on the right side. New created activity matrices can also be edited and deleted by pressing **edit** or **delete**.

Note: Changes to the activity matrix which are made during culture will only be effective for the current run.



Screen 7.14: Export of individual designed activity matrices

Individual activity matrices can be exported and imported to further CliniMACS Prodigy devices. For this a USB stick is required. It is only possible to transfer the selected or all individual activity matrices.

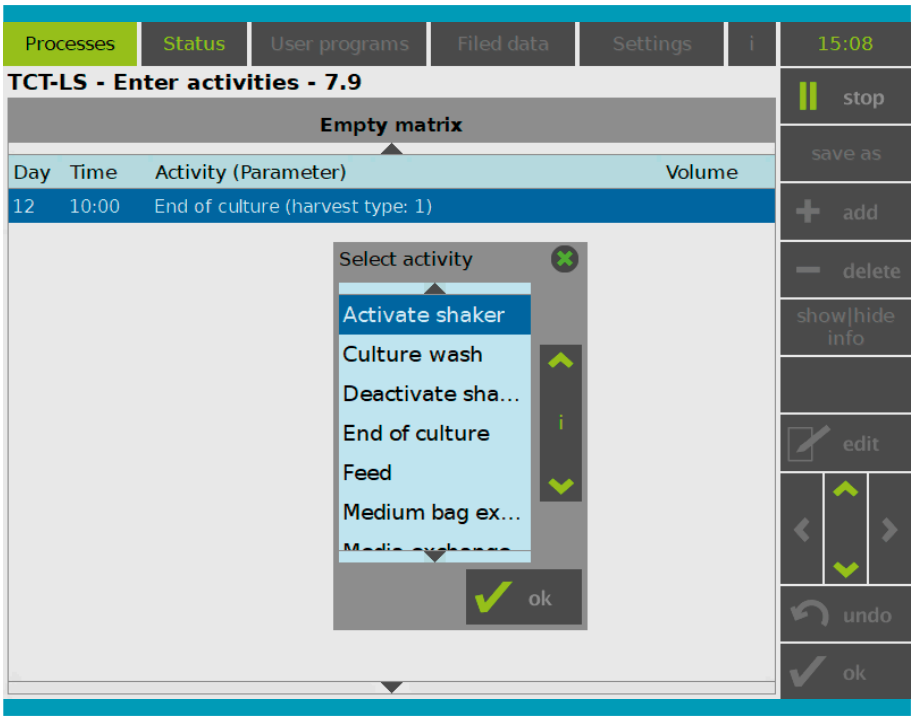
Note: To use the export and import function make sure the same application software versions are installed among devices.

Modification of activities

Activity	Description	Remark
Activate shaker	Start a continuous shaking of the chamber for suspension culture	Continuous in background
Culture wash	Wash of culture to remove the stimulation reagent and/or viral vector	Automatic execution
Deactivate shaker	Switch off a previously activated shaker	Automatic execution
End of culture	Harvest type 1: Cells will be rebuffered in formulation solution and transferred to the Target Cell Bag in a final volume of 100 mL Harvest type 2: Cells will be transferred to the Target Cell Bag with an optional fill-up with medium to max. 900 mL Harvest type 3: Cells will be rebuffered in formulation solution and stored in chamber in a final volume of 100 mL at 8°C with slow shaking Harvest type 4: Cells will be stored in chamber in the current culture volume at 8°C with slow shaking	User confirmation/ Interaction upon execution require
Feed	Add fresh medium	Automatic execution
Media exchange	Remove old medium and add fresh medium	Automatic execution
Medium bag exchange	Prompt and user guidance to exchange medium bag	User confirmation/ Interaction upon execution required
Spin	Centrifugation step to be used after addition of viral vector for spinoculation protocol	Automatic execution
Stimulation bag	Transfer of stimulation reagent to chamber	User confirmation/ interaction upon execution required
Stimulation vial	Transfer of MACS GMP T Cell TransAct – Large Scale to chamber	User confirmation/ interaction upon execution required
Temperature change	Change of cultivation temperature	Automatic execution

Activity	Description	Remark
Transduction	Transfer of viral vector to chamber	User confirmation/ interaction upon execution required
Volume reduction	Volume reduction of culture using centrifugation, e.g., to be used before transduction with large viral vector volumes	Automatic execution
Waste Bag exchange	Prompt and user guidance to exchange Waste Bag	User confirmation/ Interaction upon execution required

Table 7.1: Available activities for cultivation

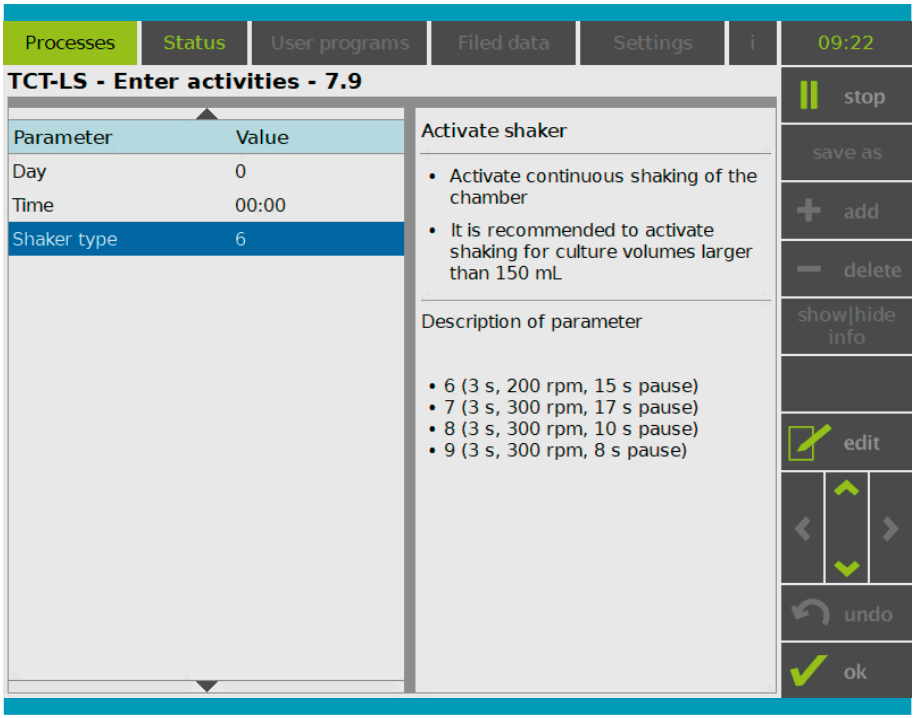


Screen 7.15: Addition of an activity

To add an activity, press **add** and select the activity from the pop-up list (see Screen 7.15). A screen specific for that activity opens.

Press **edit** to change the parameters of the activity.

To confirm and to return to the matrix press **ok**.

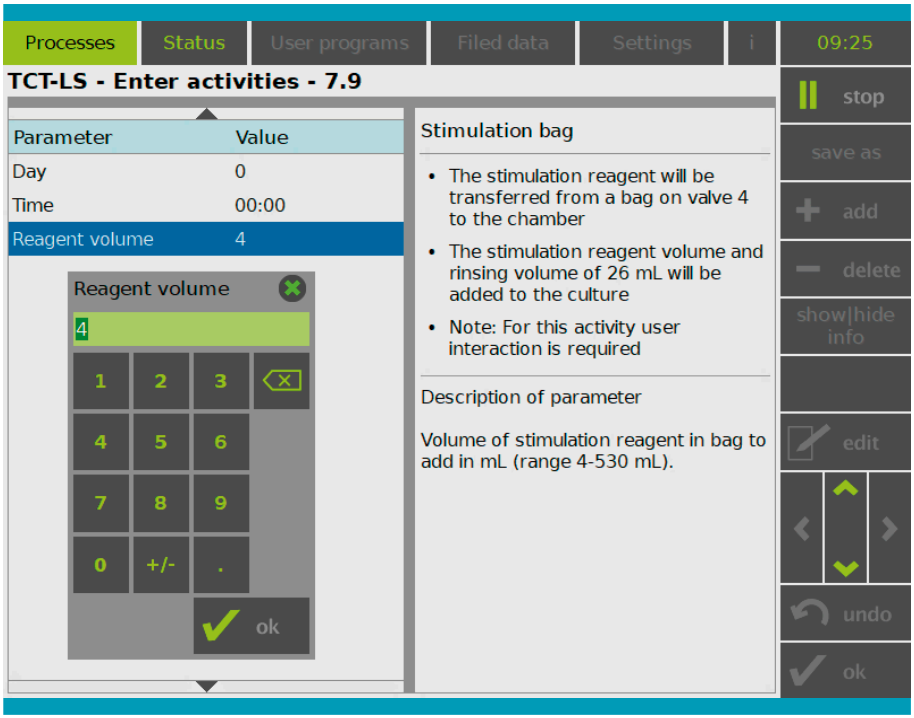


Screen 7.16: Editing of entered activity "Activate shaker"

Several shaker types are available for varying culture densities.

IMPORTANT

For optimal T cell expansion, it is recommended to shake cells from the very beginning of the culture.



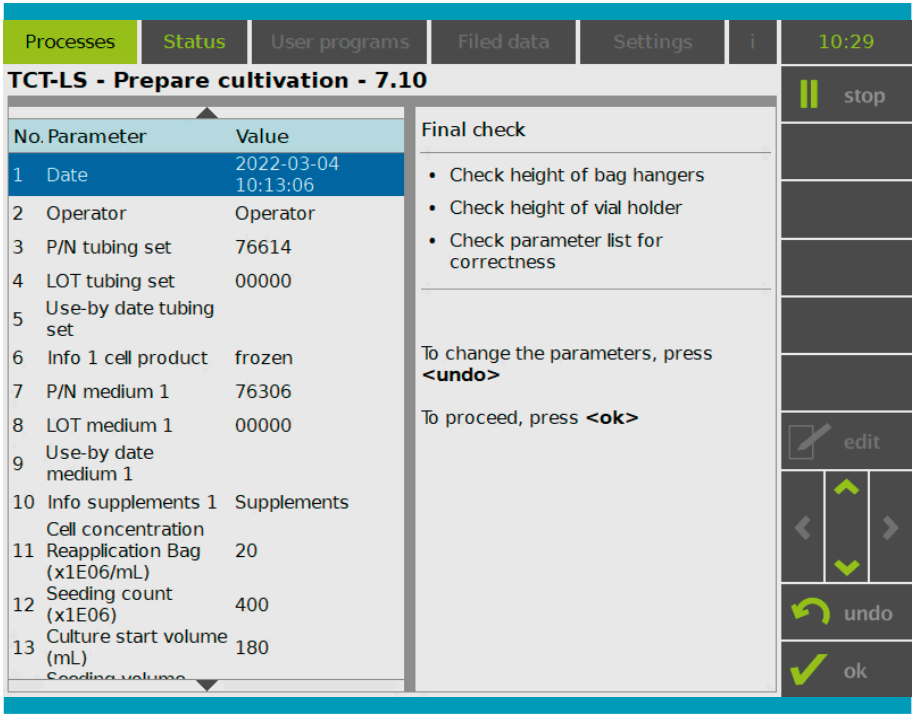
Screen 7.17: Editing of parameter "Stimulation bag"

Note: When planning the process, consider that user interaction is required prior to the execution of the activities "Stimulation vial", "Stimulation bag", "Transduction", "Medium bag exchange", "Waste Bag exchange", and "End of culture" (see Table 7.1 on page 98).

Note: The replacement of the medium bag should be planned according to the activity matrix and the corresponding usage of medium. The exchange of one Waste Bag should be planned depending on the activity matrix and the corresponding filling of the Waste Bags. If the two Waste Bags are used one after another, seal off the full Waste Bag and open the clamp on the empty one.

When the activity matrix is created and all editing is completed, press **ok** to proceed to the final setup check. Press **yes** to confirm.

7.2.5 Final setup check

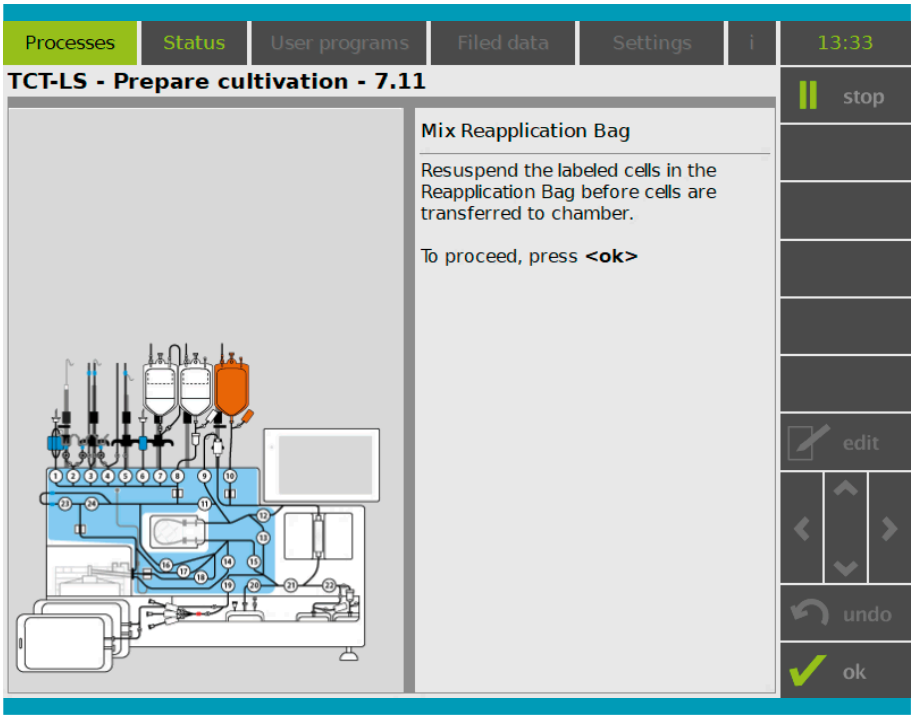


Screen 7.18: Final check

The user is asked to check the height of the bag hangers and the vial holders as well as the parameter list (see Screen 7.18).

Parameters can only be revised but not edited at this point. To return to the previous screen, press **undo**.

After confirming the parameter list, a further screen appears to remind the user to mix the labelled cells before they will be transferred to chamber (see Screen 7.19).



Screen 7.19: Mix Reapplication Bag

Note: At this point, all preparative steps are complete, and the automated cultivation process can begin.

Press **ok** to proceed. Press **yes** to confirm the set up. The automated culture set up is initialized (see Screen 7.20).

7.2.6 Automated culture setup

The culture setup is performed automatically and includes the following steps:

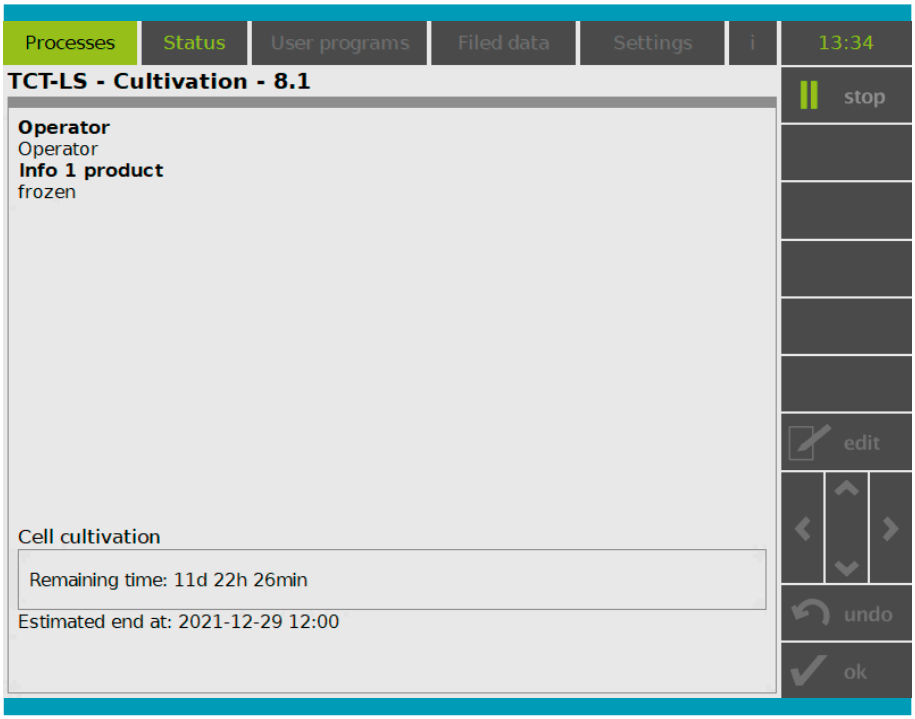
1. Rinsing and priming of the chamber and the tubing set with medium.
2. Transfer of the selected amount of cells to the chamber.
3. Washing of the cells with medium.
4. Fill-up to 180 mL in cultivation chamber.
5. Setup of aeration and temperature.

Mix the Reapplication Bag thoroughly before the automatic culture setup is initiated to ensure the correct cell seeding density.

Note: If case 4 “Resume T cell cultivation” has been selected in section 6.2 on page 30 (see Screen 6.5 on page 32), only step 5 is executed.

IMPORTANT

All steps programmed in the activity matrix are performed after the automated culture setup. The culture setup is performed automatically in one step and is not part of the activity matrix. The culture setup cannot be modified.



Screen 7.20: Cultivation (Processes)

7.3 Cultivation



Screen 7.21: Cultivation (Processes)

Press **edit** to change the activity matrix during cultivation.

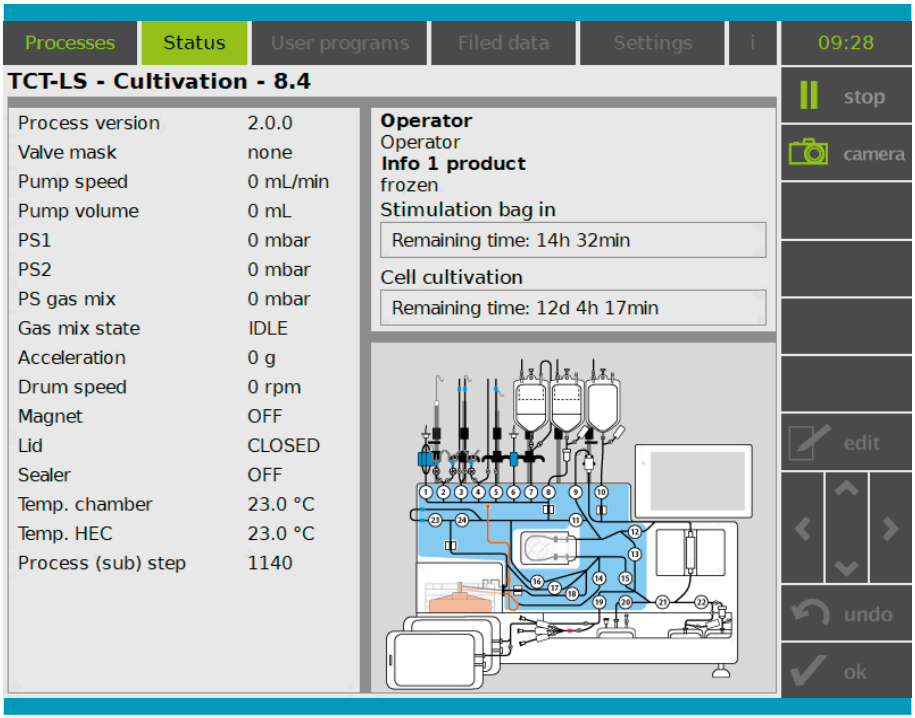
To show the current activity matrix, press **show/hide**.

Press **microscope** to make a photographic record of cells.

To take a sample during cultivation, press **sample**.

For details refer to the following sections.

When programming the cultivation matrix, be aware: If one activity is not finished yet and the next one is supposed to start, the first activity will be completed. The following activity will be delayed. No activity will be skipped.



Screen 7.22: Cell cultivation (Status)

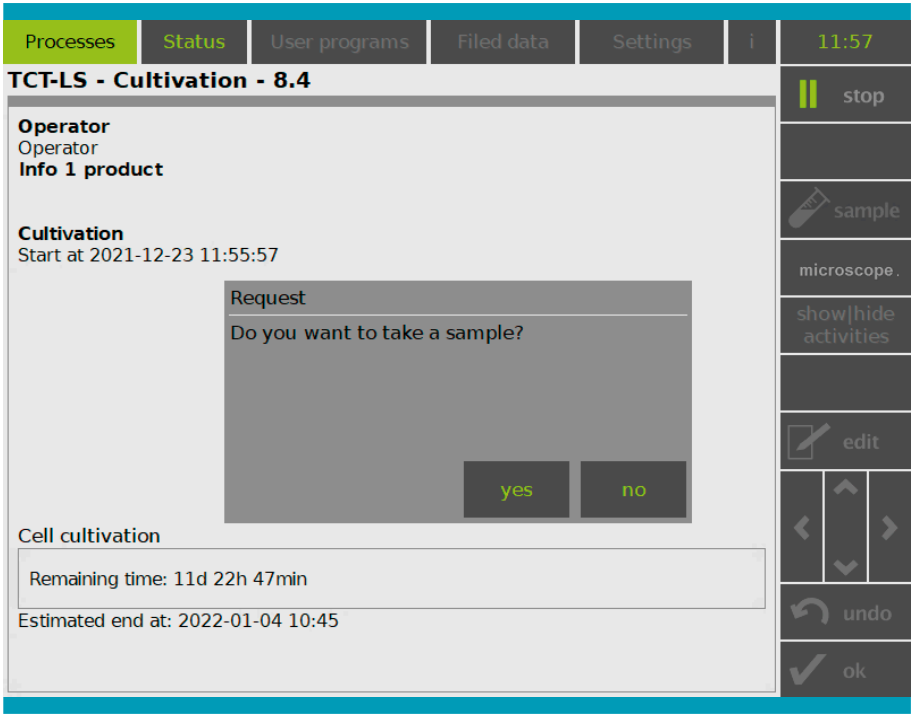
After initial setup of the cultivation, the monitor displays the current step and the remaining time of the cultivation process in the **Status** screen.

Note: The remaining enriched target cells can now be retrieved (e.g., to be frozen as back-up) by sealing off the Reapplication Bag.

7.3.1 Monitoring of cultivation via sensor values

During the cultivation, several sensor values can be monitored on the **Status** screen. The pressure sensor “PS gas mix” is inside the gas mix unit and should show values between 0 mbar and 800 mbar during the setup and between 200 mbar and 800 mbar during cultivation.

7.3.2 Sampling using the sampling pouch accessories



Screen 7.23: Taking a sample

To take a sample from the chamber during cultivation, change to the **Processes** screen and press **sample**. Follow the instructions in the pop-ups leading through the sampling procedure.

Note: When the instructions mention to open/close the clamp leading to the sampling pouches, be aware that actually two clamps need to be opened/closed. Hold the sampling pouch with the sterile filter facing up while the sampling procedure is performed to prevent filter clogging.

To remove the residual sample liquid from the tubing after the sample has been taken:

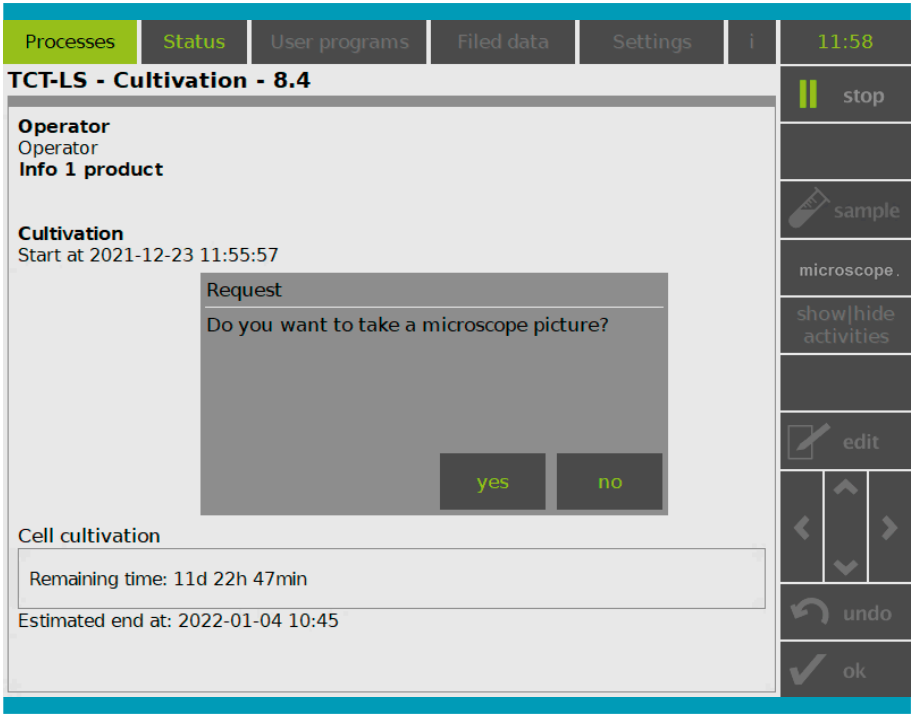
- Open all clamps from an empty sample pouch leading to the Waste Bag.
- Squeeze the sample pouch to press residual liquid to the Waste Bag.
- Close the sample pouch assembly and the clamp of the sample pouch.
- Remove the blue cap on the filter of the used sample pouch to release the vacuum.
- Reinstall the cap.
- After the sampling is completed, seal off the pouch first and then collect the sample by unscrewing the sterile filter.

If more than three samples are required weld on another Triple Sampling Adapter using a sterile tubing welder.

IMPORTANT

Do not remove the sterile filter during sampling as it could result in a contamination of the cell product.

7.3.3 Taking microscope pictures during cultivation



Screen 7.24: Taking a microscope picture

To take a microscope picture during cultivation, follow the procedure described below:

- Press **Processes**.
- Press **microscope**.
- Press **yes** to confirm taking a microscope picture.
- Press **edit** to enter the desired position (1–20) and brightness (e.g., 100) for the picture.
- Now the camera will autofocus and take a picture.
- Press **Status** and select the **camera** button to display the camera screen. The picture will be displayed seconds later. To leave the camera view, press **ok**.
- Press **yes** to take another picture. Otherwise, press **no** to return to the cultivation.

Note: At higher cultivation volumes and during agitated culture (with an active shaker) the quality of the picture might decrease. This is mainly caused by light dispersion and by the fact that the cells are in suspension and not settled on the bottom of the chamber. Allow the cells to settle before taking a picture to increase picture quality.

7.3.4 Stimulation

IMPORTANT

Once the activity has started by appearance of the pop-up, it is not possible to change any parameters (e.g., time or volume). Make sure to have entered all parameters correctly. Also be aware once the pop-up has appeared no gassing or temperature control is performed.

Note: The activity stimulation bag and stimulation vial require user interaction.

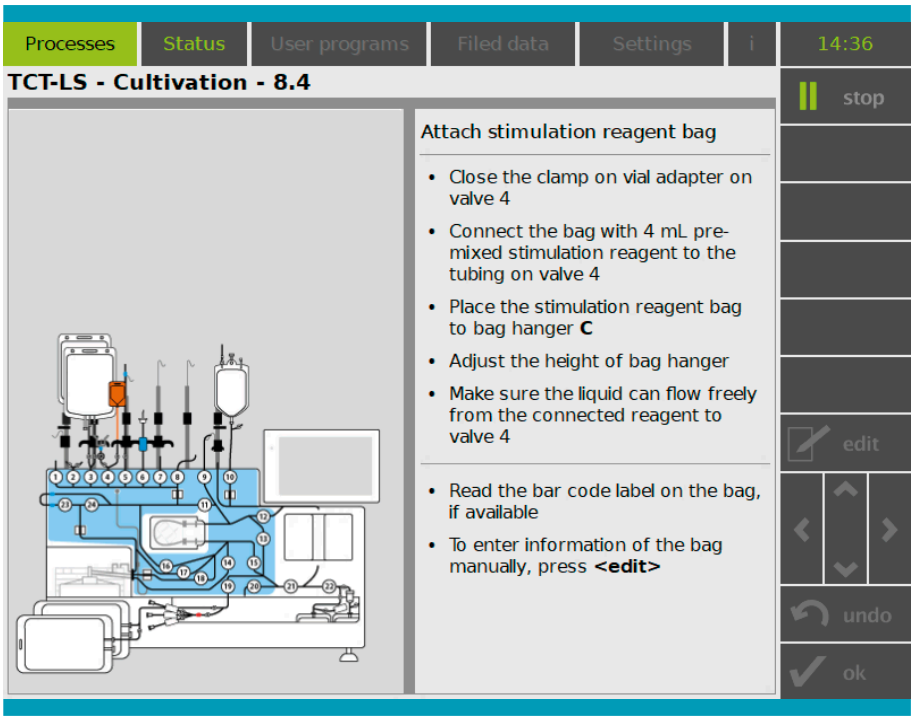
For stimulation of the selected T cells, the activity "Stimulation bag" or "Stimulation vial" must be programmed into the activity matrix (see subsection 7.2.4 on page 90).

⚠ CAUTION

Risk of cell loss. Incomplete loading of the stimulation reagent can lead to delayed cell preparation. Make sure that the stimulation reagent has been loaded completely after the activity to ensure optimal activation results.

Stimulation bag

Follow the specific instructions on the screen for the stimulation bag (see Screen 7.25).



Screen 7.25: Connection of the stimulation reagent (bag)

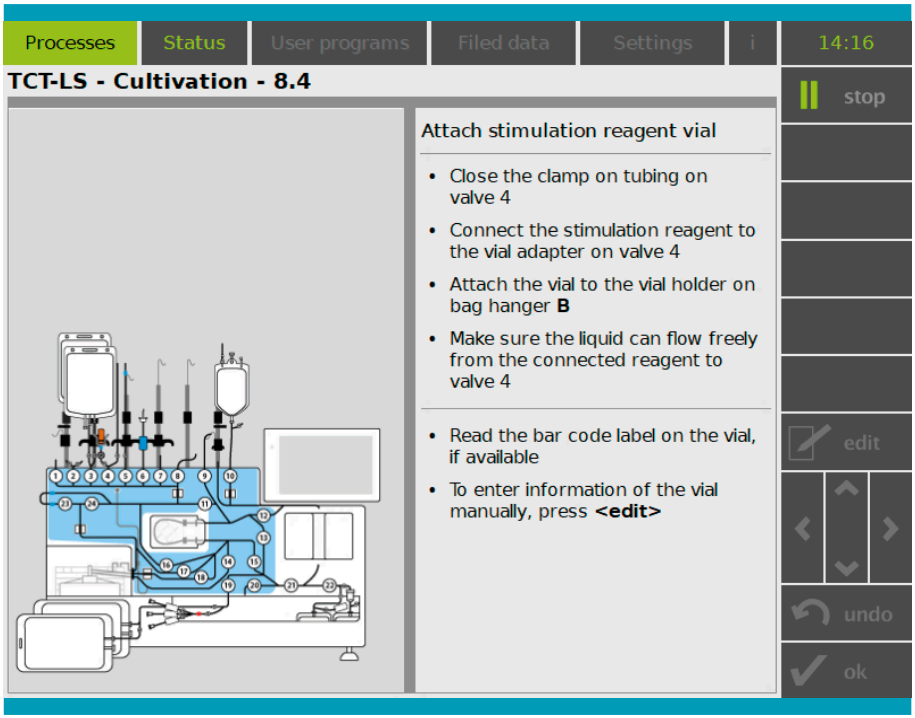
Note: The volume (4–530 mL) must be programmed into the activity of the matrix during set up. The user must ensure the bag contains the exact volume which is programmed.

Stimulation vial

Follow the specific instructions on the screen for the stimulation vial (see Screen 7.26).

IMPORTANT

It is mandatory to use MACS GMP T Cell TransAct– Large Scale as a stimulation vial. Connect the vial as described in the GUI screen. Ensure that the MACS GMP T Cell TransAct – Large Scale is used in the correct dilution. The culture volume in the chamber must be 180 mL to achieve the correct dilution.



Screen 7.26: Connection of the stimulation reagent (vial)

Read bar code label on the reagent vial or press **edit** and enter part number (P/N) and batch code (LOT) manually (see Screen 7.26).

IMPORTANT

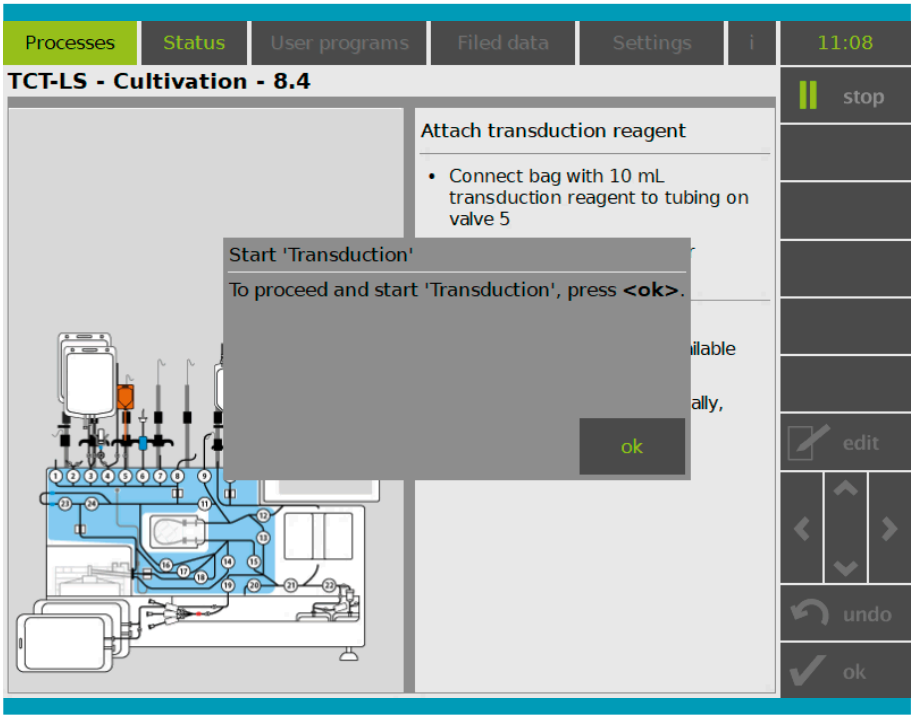
In case a vial is used for stimulation, ensure that the filter at the vial adapter on valve 4 is unused and dry. A used vial adapter must be replaced with a new "Single Vial Adapter".

After the activity the chamber volume will be 210 mL.

7.3.5 Transduction

IMPORTANT

Once the activity has started by appearance of the pop-up, it is not possible to change any parameters (e.g., time or volume). Make sure to have correctly entered all parameters for the connection of the transduction reagent.



Screen 7.27: Transduction

For transduction of the activated T cells, the activity "**Transduction**" has already been programmed into the activity matrix (see subsection 7.2.4 on page 90).

Note: The activity "**Transduction**" will not start without confirmation by the user (see Screen 7.27).

When the scheduled time point is reached, user interaction is required. Follow the instructions on the screen to connect the viral vector bag.

Note: It is recommended to perform the transduction approx. 24 h after stimulation.

The viral vector should be prepared under sterile conditions in a bag that can be connected to the tubing set via sterile welding.

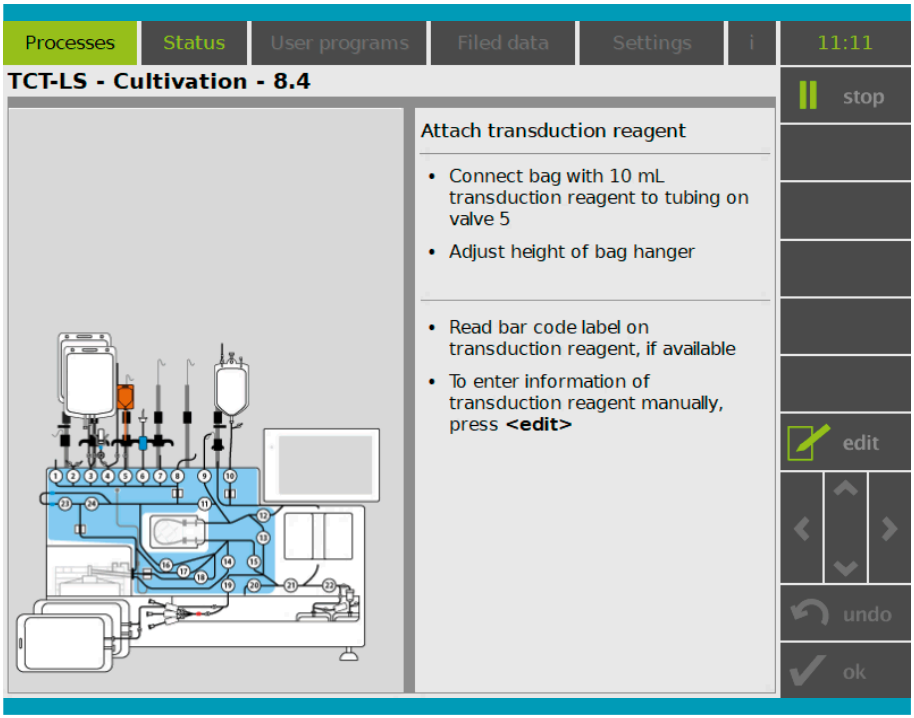
The viral vector must be in a volume between 5 mL and 250 mL.

It is recommended to use concentrated viral vector in a volume of 10 mL.

Note: Only connect the exact volume that shall be used for transduction. The CliniMACS Prodigy will stringently pump the volume that is programmed into the activity matrix.

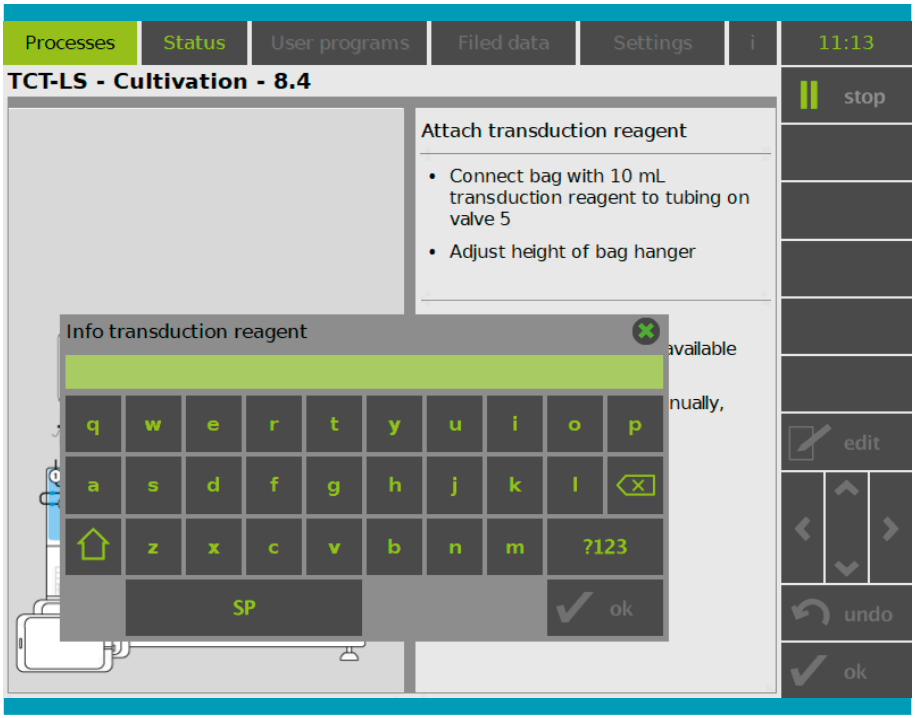
If the vector is in a much larger volume, consider reducing the volume of the culture prior to transduction using the activity “Volume reduction”.

By adding the viral vector and the following rinsing steps, the volume of the culture in the chamber increases by the volume of the vector plus 20 mL medium used for rinsing.



Screen 7.28: Attachment of viral vector

Use a sterile tubing welder to connect the viral vector to the tubing on valve 5.



Screen 7.29: Entering information on viral vector

Press **edit** and enter information on the viral vector with the keyboard displayed on the screen.

7.3.6 Culture wash

Note: The activity "**Culture wash**" will be executed **automatically**.
It is recommended to perform a culture wash on day 3 to remove excess activation reagent and viral vector particles.

Note: Confirm that there is enough medium connected prior to the activity. The amount of medium needed for the culture wash depends on the cycles programmed into the activity. For 1, 2, and 3 cycles a respective volume of 1,000 mL, 1,300 mL, and 1,600 mL must be available in the bag on valve 3.

After the culture wash activity, the total culture volume is always 500 mL.

Use the activity "Medium bag exchange" to attach a new medium bag.

7.3.7 Volume reduction and spin

Note: The activity “Volume reduction” and “Spin” will be executed automatically.

The activity “Volume reduction” will reduce culture volume to the desired volume by centrifugation and supernatant removal.

Confirm that there is medium connected to valve 3. The minimal target volume is 40 mL.

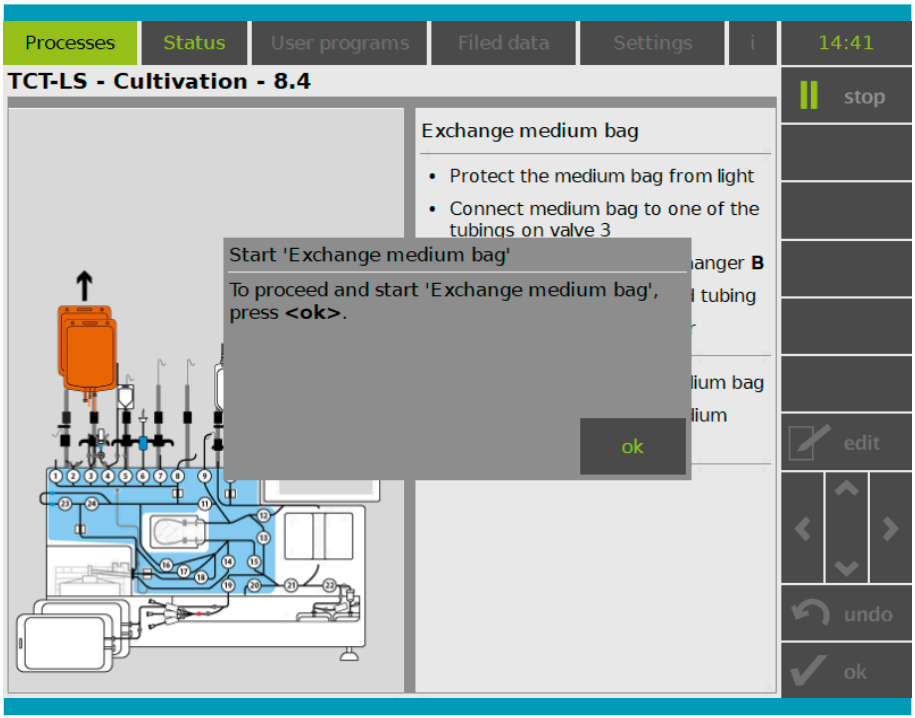
Spinoculation might be used to enhance transduction efficiency by spinning the cells and virus at 400 ×g for up to 8 h. During spinoculation the temperature can be adjusted between +18 °C to +39 °C (+64 °F to +102 °F).

IMPORTANT

In the Spin activity the temperature can be adjusted. For this purpose, it is highly recommended to receive a temperature adjustment and validation procedure for the instrument. If the instrument has no temperature offset values, a warning will appear stating that no temperature offset values could be detected on the instrument (see Screen 7.7 on page 89). In this case the temperature parameter cannot be guaranteed. For further information contact Miltenyi Biotec Technical Support.

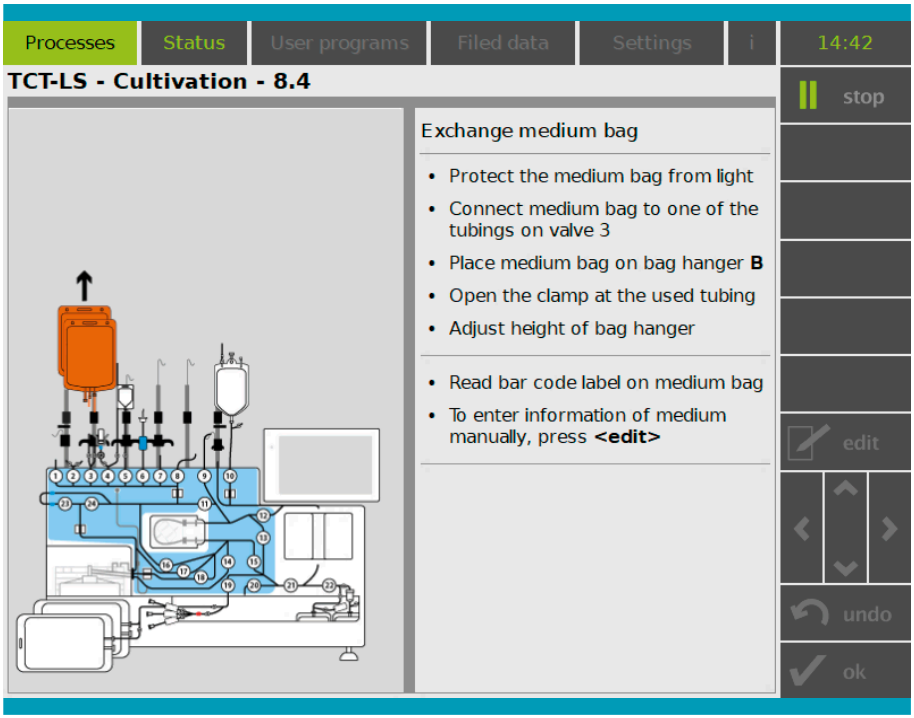
7.3.8 Medium and Waste Bag exchange

Note: The activities "**Medium bag exchange**" and "**Waste bag exchange**" will not start without confirmation by the user.



Screen 7.30: Start of activity medium bag exchange

Refer to chapter 5 on page 23 of this user manual for the preparation of the medium.



Screen 7.31: Connection of medium bag

When the programmed time point for the activity “Medium bag exchange” is reached, user interaction is required. Follow the instructions on the screen to connect a new medium bag (for details see subsection 6.4.1 on page 59).

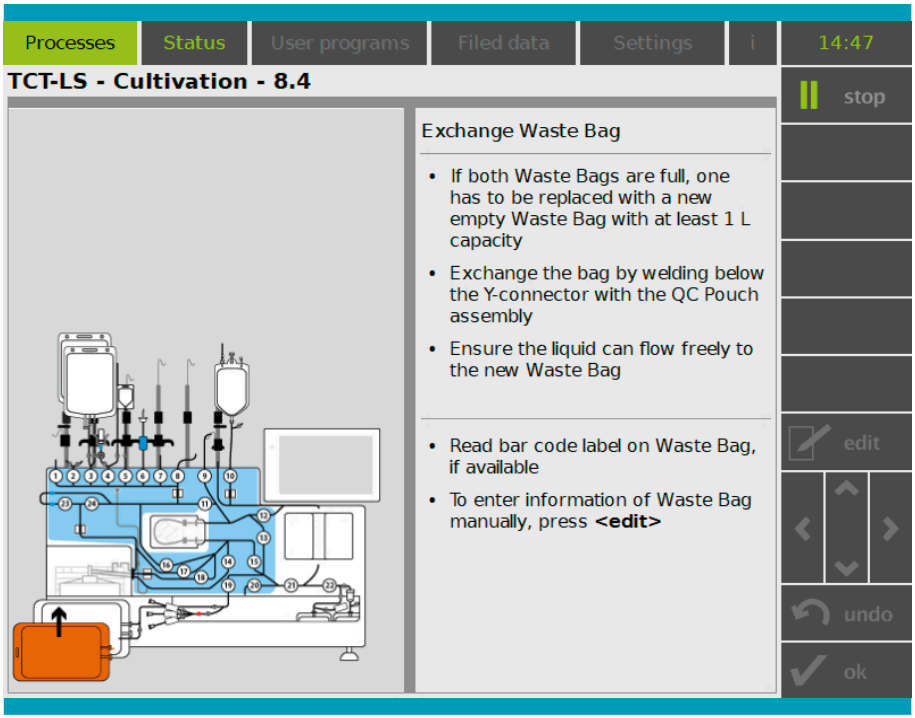
Read the bar code label on the medium bag. Alternatively, press **edit** to manually enter the information, then press **ok**.

Press **edit** to enter information regarding supplements. Confirm the entered values with **ok** to complete the activity and return to cultivation.

Note: In case three medium bags shall be connected make sure to use a 3-way Tube Adapter and ensure each bag is connected to valve 3.

IMPORTANT

It is strongly recommended that the user monitors the medium bag capacity to prevent any errors in the process.



Screen 7.32: Connection of new empty waste bag

When the programmed time point for the activity “Waste Bag exchange” is reached, user interaction is required. Follow the instructions on the screen to connect a new empty Waste Bag (see Screen 7.32).

Read the bar code label on the Waste Bag. Alternatively, press **edit** to enter the information manually, then press **ok**. Confirm the entered values with **ok** to complete the activity and return to cultivation.

Ensure that the waste liquid can flow freely to the available Waste Bag in use.

⚠ WARNING

Risk of biological hazard. The Waste Bag may comprise infectious biological material or residual viral vector. Standard procedures for handling biological materials have to be followed. Dispose of the used Waste Bag as biohazardous material according to the standard operating procedures of the institution. Consider the genetic engineering biosafety level of the used viral vector for waste disposal.

7.3.9 Feeding/Exchange of media

Note: The activity "**Feed**" and "**Media exchange**" will be executed automatically.

When the programmed time for activity "Feed" and "Media exchange" is reached (see subsection 7.2.4 on page 90), the activity will start automatically (no user interaction required).

Confirm there is sufficient medium in the medium bag connected to the selected valve (default is valve 3).

If a different valve than valve 3 is selected, ensure that there is always medium connected to valve 3 for the tubing set rinsing which is automatically performed every 10 hours.

Use the activity "Medium bag exchange" to attach a new medium bag.

7.3.10 Temperature change

IMPORTANT
It is highly recommended to receive a temperature adjustment and validation procedure for the instrument. If the instrument has no temperature offset values, a warning will appear stating that no temperature offset values could be detected on the instrument (see Screen 7.7 on page 89). In this case the temperature parameter cannot be guaranteed. For further information contact Miltenyi Biotec Technical Support.

The activity "Temperature change" (see subsection 7.2.4 on page 90) will be executed
automatically.

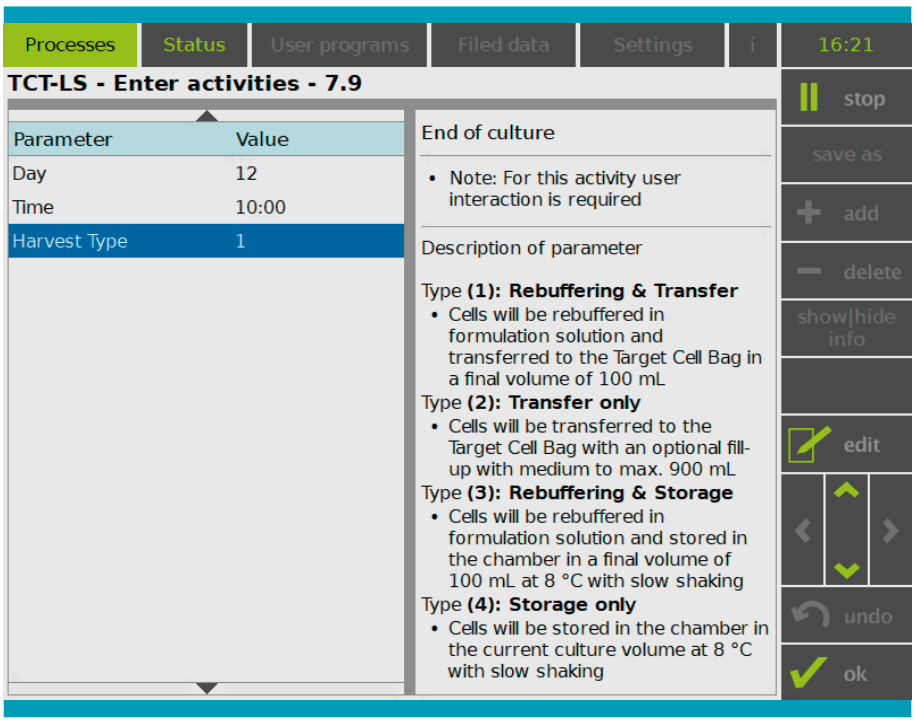
During cultivation the temperature can be adjusted by using this activity. If a temperature adjustment was performed, the desired temperature can be entered and the temperature for the chamber will be set to the defined value.

7.4 End of culture

The user can choose between 4 harvest types (see Screen 7.33). The harvest type must be programmed into the activity matrix during set up (see subsection 7.2.4 on page 90).

⚠ CAUTION

Risk of low performance. Be aware that cultivation times longer than 12 days can cause lower cell viability and might cause a loss of the cell product. It is recommended to perform the end of culture activity on day 12.



Screen 7.33: End of culture – Choice between four different harvest types

Harvest type 1: Rebuffering & Transfer

Cells will be rebuffered in formulation solution and transferred into the Target Cell Bag in a final volume of 100 mL.

Harvest type 2: Transfer only

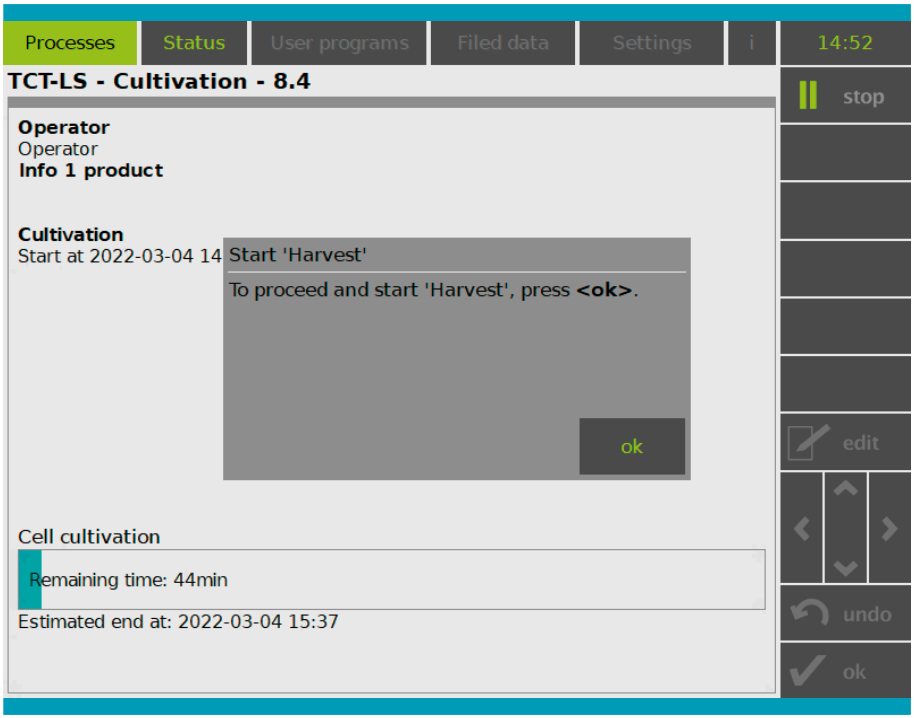
Cells will be directly transferred from the chamber to the Target Cell Bag. Optionally, the Target Cell Bag can be filled up with medium to an intended volume of up to 900 mL.

Harvest type 3: Rebuffering & Storage

Cells will be rebuffered in formulation solution and stored in the chamber in a final volume of 100 mL at 8 °C (46 °F) with slow shaking.

Harvest type 4: Storage only

Cells will be stored in the chamber in the current culture volume at 8 °C (46 °F) with slow shaking.



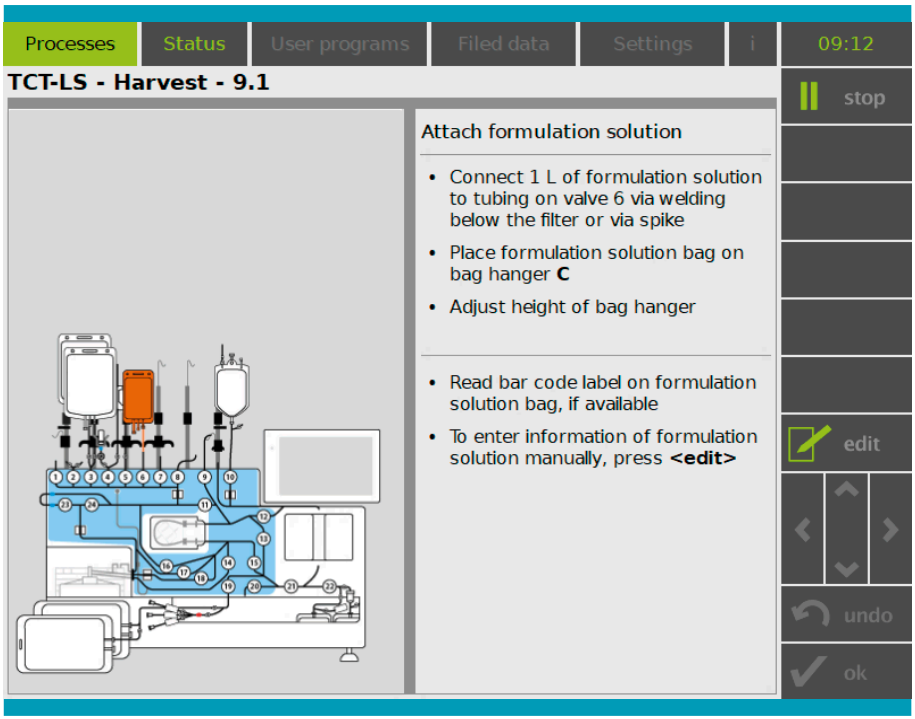
Screen 7.34: End of culture – Start Harvest

Note: The harvest will not start without confirmation by the user (see Screen 7.34). Immediately start the harvest when asked in the pop-up.

When the programmed time for “End of culture” is reached (see subsection 7.2.4 on page 90)
user interaction is required.

IMPORTANT
It is not possible to change any parameters of the end of culture activity at this point. Proceed immediately when the pop-up appears as temperature-monitoring and gassing is not maintained at this point.

7.4.1 Harvest type 1



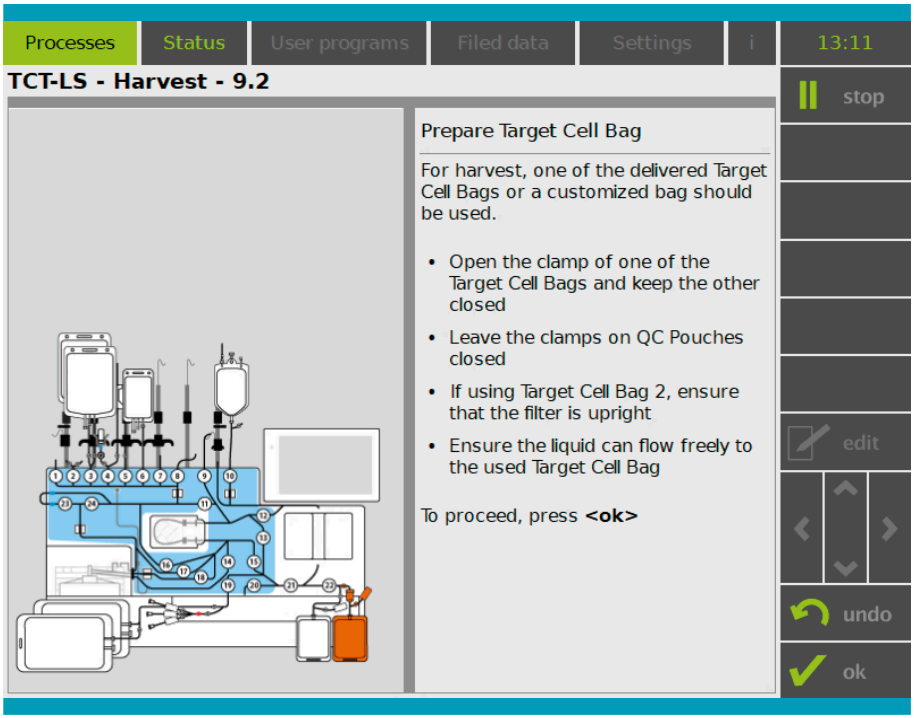
Screen 7.35: Attachment of formulation solution

Connect the formulation solution to the spike port on valve 6 or via sterile welding below the filter (e.g., for CliniMACS Formulation Solution).

Note: When a protein containing formulation solution is used (e.g., CliniMACS Formulation Solution), ensure to weld below the filter to remove it from the tubing set. Protein might cause clogging of the filter and impede sufficient fluid flow.

Read the bar code label on the formulation solution bag or press **edit** to enter the information manually (see Screen 7.35).

Press **ok** to continue.



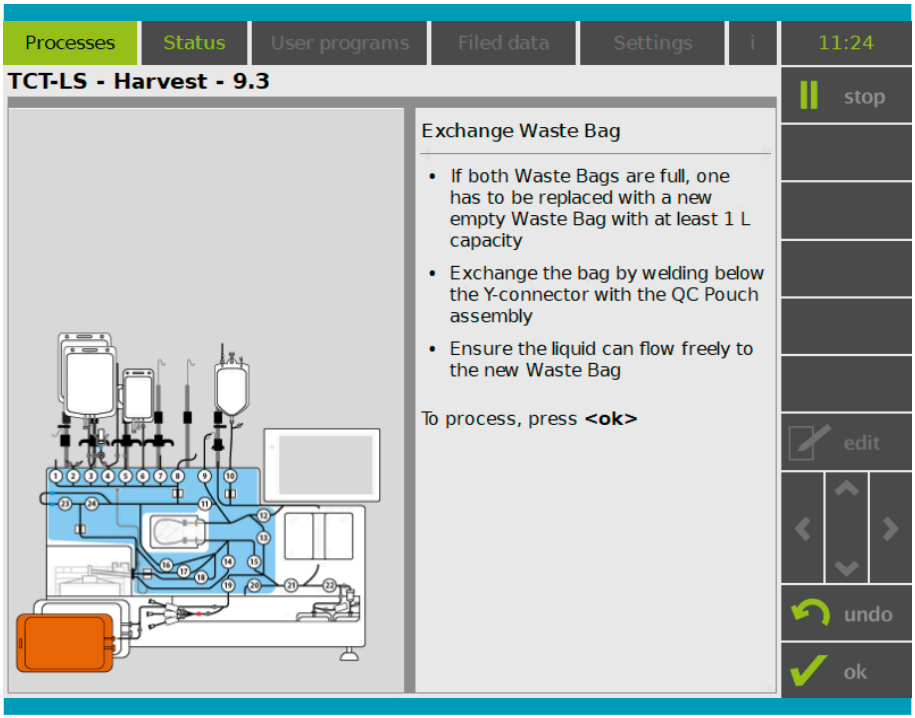
Screen 7.36: Preparation of Target Cell Bag

Cell filtration is an option when harvesting target cells. This is available with the pre-assembled in-line filter connected to one of the two designated Target Cell Bags. If using the in-line filter on the Target Cell Bag, make sure that the in-line filter is in an upright position.

This can be achieved by replacing the separation column that has been sealed-off and removed from the magnet unit with the in-line filter to assure that the filter is hanging in an upright position during the transfer of cells into the Target Cell Bag.

If filtration of the cells is not required, choose the alternative Target Cell Bag. If another specific Target Cell Bag is required, sterile connect this Target Cell Bag between valve 22 and the in-line filter.

Close the clamp of the Target Cell Bag that will not be used. Follow the instructions on the screen to prepare the Target Cell Bag (see Screen 7.36). The volume of the cell product will be 100 mL.



Screen 7.37: Exchange of waste bag

With the next screen the user is asked to exchange the Waste Bag. This is only necessary if the capacity of the current bag is nearly reached, and additional waste liquid capacity is required.

In this case, connect a new empty Waste Bag via sterile welding (see Screen 7.37).

Press **ok** to continue. If an exchange of the Waste Bag is not required, press **ok** directly.

The automated rebuffering and harvesting step will take about 45 minutes. The cells in the chamber are washed twice with the formulation solution and are then transferred to the chosen Target Cell Bag on valve 22.

7.4.2 Harvest type 2

In the first screen the user is asked to exchange the Waste Bag. This is only necessary if the capacity of the current bag is nearly reached and additional waste liquid capacity is required (see subsection 7.4.1 on page 123, Screen 7.37).

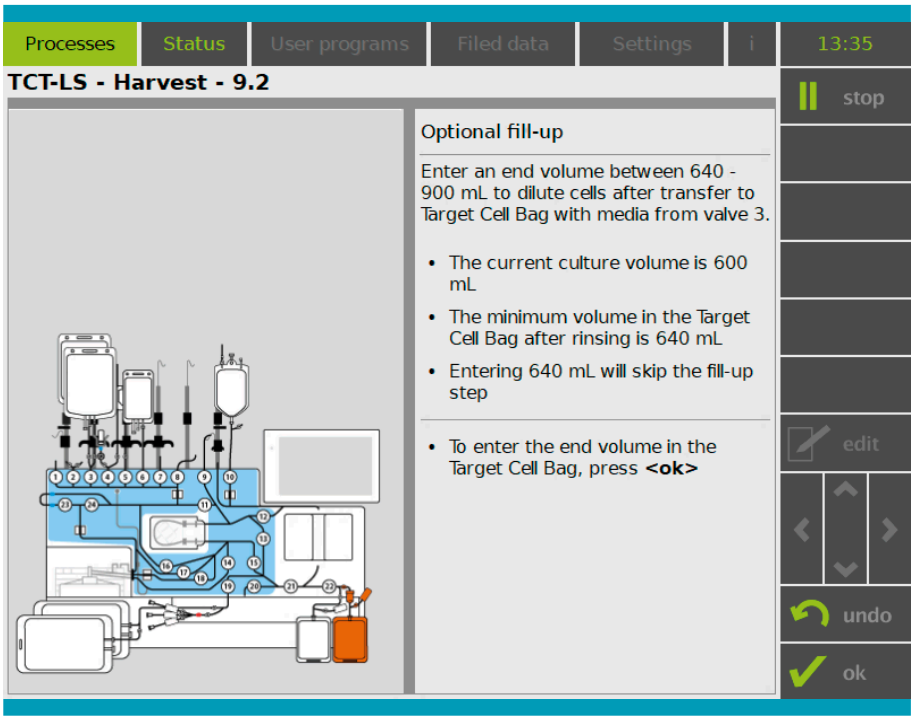
The Harvest Type 2 transfers the cell suspension to the Target Cell Bag. After the transfer, the chamber is rinsed twice with medium. The volume in the Target Cell Bag will be the current culture volume before harvest plus 40 mL.

Optionally, the cell suspension in the Target Cell Bag can be automatically filled with medium to an intended volume of up to 900 mL.

Note: For volumes larger than 300 mL, the Target Cell Bag must be exchanged by a customized bag with sufficient volume capacity by sterile welding.

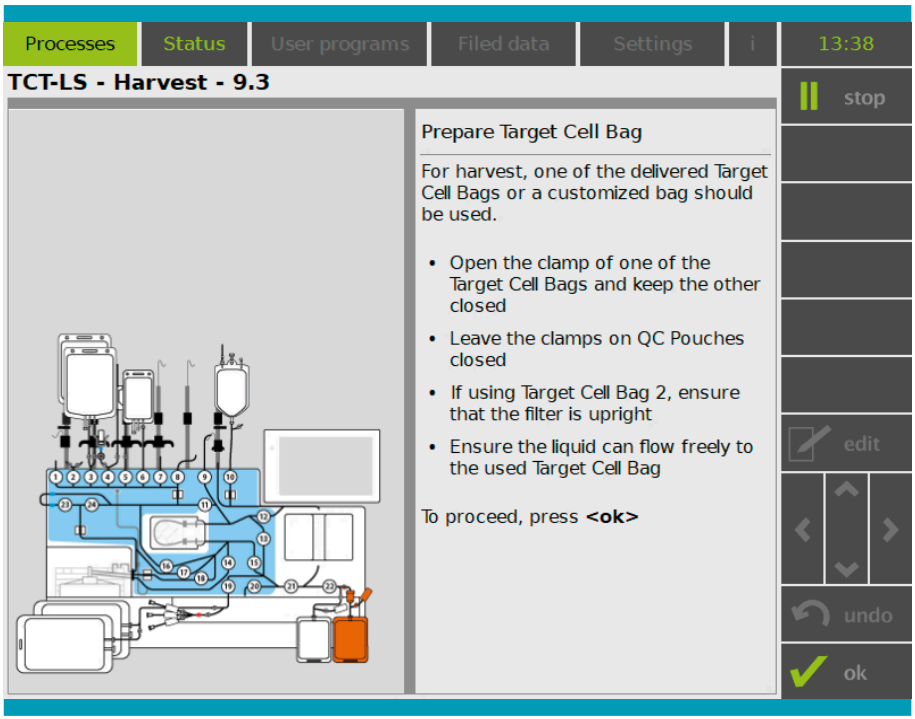
⚠ CAUTION

Risk of cell loss. The Target Cell Bag has a maximal capacity of 500 mL. It is recommended to fill the bag with maximal 300 mL of cell suspension to enable sufficient mixing and safe handling of the bag after harvest. Take this into account while programming the optional fill-up.



Screen 7.38: Optional medium fill-up during harvest type 2

Note: By setting the end volume to the current culture volume plus 40 mL (rinse volume), no additional fill-up will be performed (see Screen 7.38).



Screen 7.39: Preparation of Target Cell Bag or customized bag during harvest type 2

7.4.3 Harvest type 3

In the first screen the user is asked to attach the formulation solution (see subsection 7.4.1 on page 123., Screen 7.35 on page 123).

In the second screen the user is asked to exchange the Waste Bag. This is only necessary if the capacity of the current bag is nearly reached and additional waste liquid capacity is required (see subsection 7.4.1 on page 123, Screen 7.37).

Cells will be rebuffered in formulation solution and stored in chamber in a final volume of 100 mL at 8 °C (46 °F) with slow shaking.

⚠ CAUTION

Risk of low performance. There is no time limit to stop the process when the cells are stored in the chamber. Keep the cells under storage conditions as long as preparations for downstream processes are performed. The user should proceed soon to ensure cell viability.

7.4.4 Harvest type 4

In harvest type 4 cells are stored in the chamber in the current culture volume and medium at 8 °C (46 °F) with slow shaking.

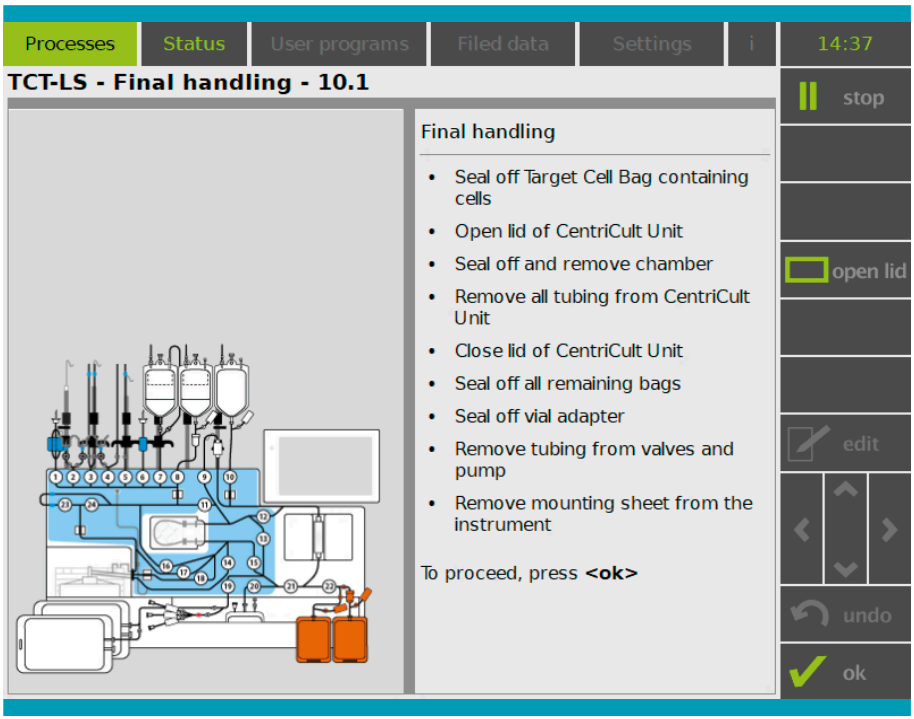
⚠ CAUTION

Risk of low performance. There is no time limit to stop the process when the cells are stored in the chamber. Keep the cells under storage conditions as long as preparations for downstream processes are performed. The user should proceed soon to ensure cell viability.

7.5 Final handling

7.5.1 Final handling after harvest type 1 and 2

After termination of the process, the program guides the user through the final handling steps (see Screen 7.40).



Screen 7.40: Final handling

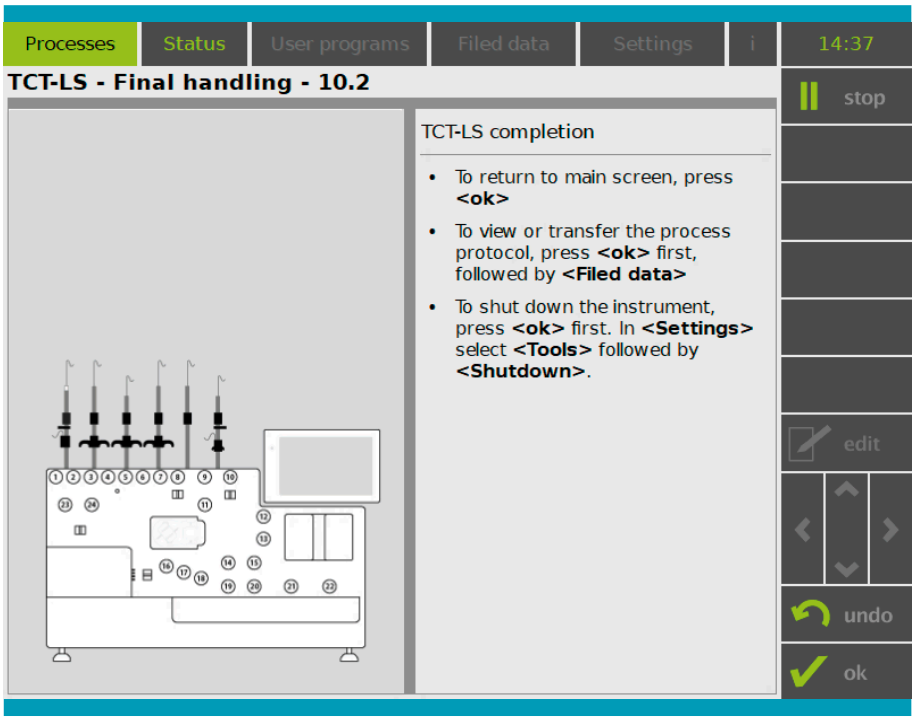
Seal off the Target Cell Bag containing the cell product. Take a sample from the Target Cell Bag for further analysis. Store samples at +4 °C to +8 °C (+39 °F to +46 °F).

Note: If no cultivation was performed and the final handling takes place directly after enrichment (coming from subsection 6.6.2 on page 79), seal off the Reapplication Bag containing the enriched target cells. If required, take a sample from the Reapplication Bag for further analysis.

Follow the instructions given on the screen to remove the tubing set and to finalize the process (see Screen 7.40). The lid of the CentriCult Unit unlocks automatically during this process step. If the lid does not unlock automatically, press the **open lid** button on the menu bar. In the event the lid cannot be opened, contact Miltenyi Biotec Technical Support.

Note: Dispose of the tubing set as biohazardous material according to the standard operating procedures of the institution. Consider the genetic engineering biosafety level of the used viral vector for waste disposal.

After removal of the tubing set the process is completed.



Screen 7.41: Process completion

The process protocol is stored in **Filed data** and can be transferred to other media with a standard USB storage device. To do so, plug an USB storage device into the USB port located at the side of the touch screen. Press **Filed data** on the menu bar and select the requested process. Then press **save** to transfer the process protocol to the USB storage device.

Clean and disinfect the CliniMACS Prodigy 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 institution's standard procedures for the treatment of infectious materials.

⚠ 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.

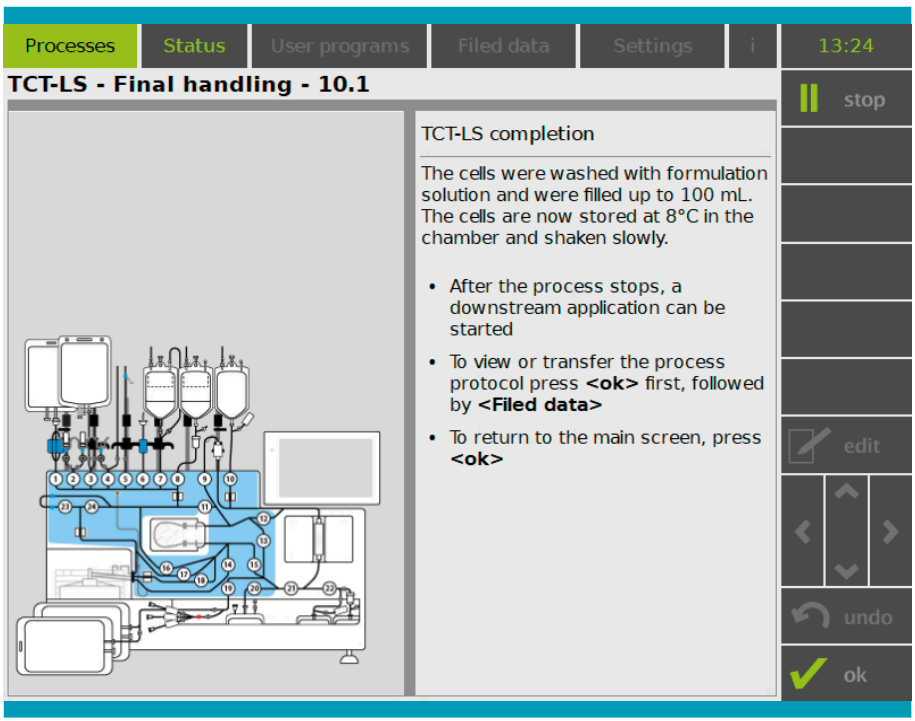
All QC samples should be analyzed according to standard operating procedures. For the manufacture and use of the engineered T cells, national and international legislation and regulations must be followed.

Miltenyi Biotec as the provider of the CliniMACS Prodigy T Cell Transduction - Large Scale System does not provide any recommendations regarding the use of the manufactured cells for therapeutic purposes and does not make any claim regarding a clinical benefit.

7.5.2 Final handling after harvest type 3 and 4

After harvest type 3 and 4 cells remain in chamber at 8°C (46 °F) with slow shaking.

During this time preparations for downstream processes should be performed.



Screen 7.42: Process completion after harvest type 3

Press **ok** to stop the temperature control and shaking (see Screen 7.42).

The CliniMACS Prodigy can then be used for a (customized) downstream process using the same CliniMACS TS 620.

The process protocol is stored in **Filed data** and can be transferred to other media with a standard USB storage device. To do so, plug an USB storage device into the USB port located at the side of the touch screen. Press **Filed data** on the menu bar and select the requested process. Then press **save** to transfer the process protocol to the USB storage device.

The CliniMACS Prodigy 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 institution's standard procedures for the treatment of infectious materials.

⚠ 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.

All QC samples should be analyzed according to standard operating procedures. For the manufacture and use of the engineered T cells, national and international legislation and regulations must be followed.

Miltenyi Biotec as the provider of the CliniMACS Prodigy T Cell Transduction – Large Scale System does not provide any recommendations regarding the use of the manufactured cells for therapeutic purposes and does not make any claim regarding a clinical benefit.

8 Troubleshooting

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

+49 2204 8306-3803
technicalsupport@miltenyi.com

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

9 Legal notices

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