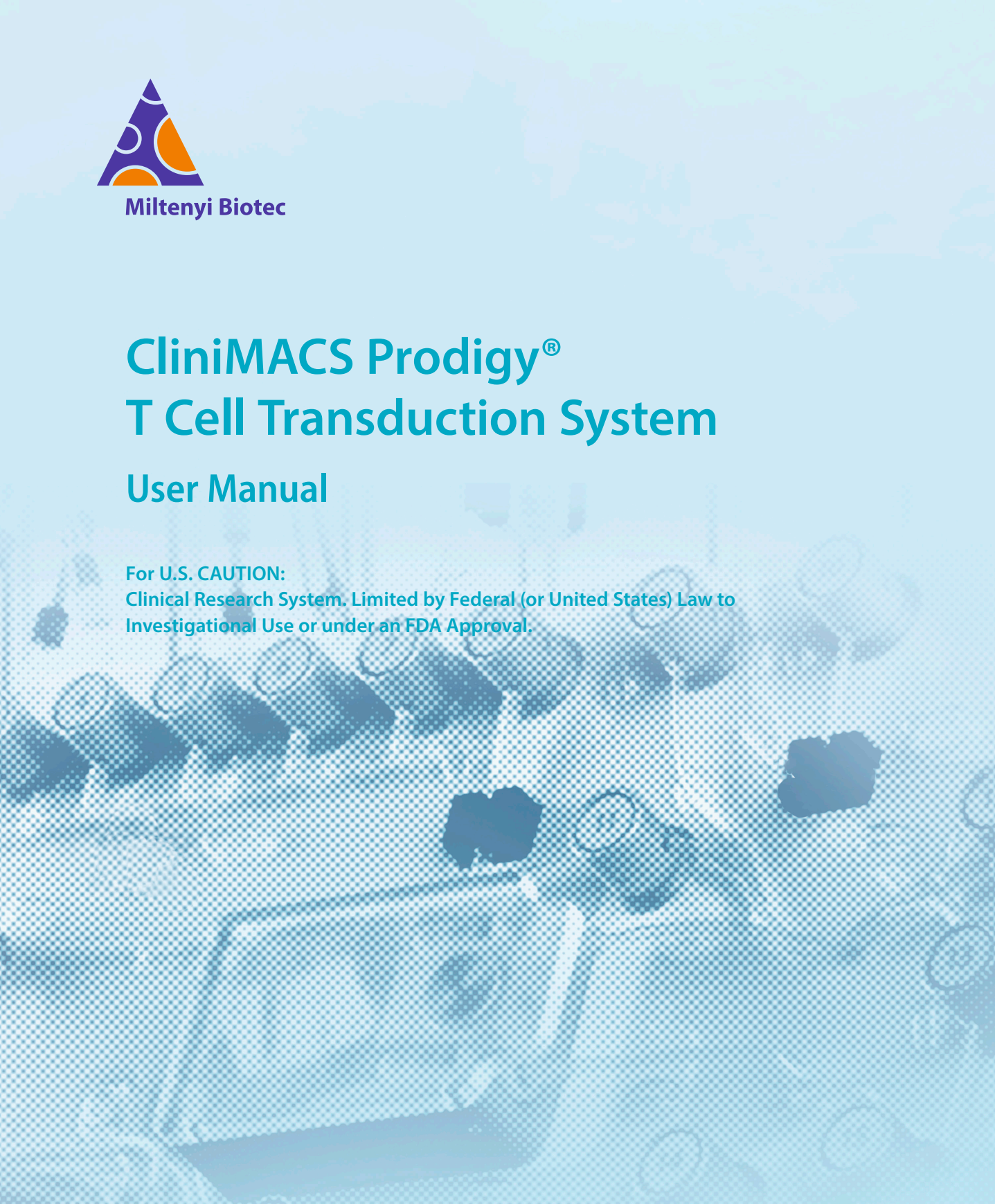
Miltenyi Biotec

CliniMACS Prodigy®
T Cell Transduction System

User Manual

For U.S. 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. In Canada, the CliniMACS T Cell Transduction System was licensed by Health Canada according to Section 36 of Canadian Medical Devices Regulations SOR/98-282. We ensure and declare that we fulfill the obligations imposed by Part 1 of Canadian Medical Devices Regulations SOR/98-282, and that the above mentioned medical device meets the Safety and Effectiveness Requirements of Canadian Medical Devices Regulations articles 10 – 20. The establishment and maintenance of a quality system according to ISO 13485 under MDSAP was certified by the certification body TÜV SÜD America Inc. MACS GMP Products 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 System

User Manual

Process version 2.x

Issued: 2023-04
38024/06

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Essential information

This user manual provides information for the use of the CliniMACS Prodigy T Cell Transduction System. For further details on other processes run 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 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 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 this user manual, chapter 3 "Important safety information") and in any safety-related recommendations issued by Miltenyi Biotec. Pay special attention to all warnings displayed on the instrument or provided with consumables and accessories. The operator must adhere to all instructions and procedures at all times during the operation of the instrument, confirming that all safety information, warnings, precautions, and instructions are observed. Failure to follow the safety information, warnings, precautions, and instructions contained in the instructions for use could result in instrument malfunction, property damage, personal injury, and/or death. Equipment safety may be compromised if the instrument is not used according to the manufacturer's instruction.

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

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1 Introduction

1.1 General information

T cells can be stably modified by introduction of genetic material using viral vectors. For example, such gene modified T cells can be made 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 gene modified T cells requires sequential steps consisting of cell separation, T cell activation, transduction, and expansion. The CliniMACS Prodigy T Cell Transduction System allows the entire process, including formulation of the final product to be performed in an automated and completely closed system (see Figure 1.1). Samples for quality control can also be obtained during manufacturing using sterile technique.

The components that are required to perform the T cell transduction with the CliniMACS Prodigy T Cell Transduction System are described in chapter 3 of this user manual.

This user manual provides guidance for the use of the CliniMACS Prodigy T Cell Transduction System in order to manufacture gene-modified T cell products for clinical application. Refer to the CliniMACS Prodigy T Cell Transduction publication Lock et al., (2017) Human Gene Therapy 10: 914 - 925[1], for information regarding performance results of this process.

1 Lock, D. & Mockel-Tenbrinck, N. et al. (2017) Automated manufacturing of potent CD20-directed chimeric antigenreceptor T cells for clinical use. Hum. Gene Ther. 28: 914–925. https://doi.org/10.1089/hum.2017.111

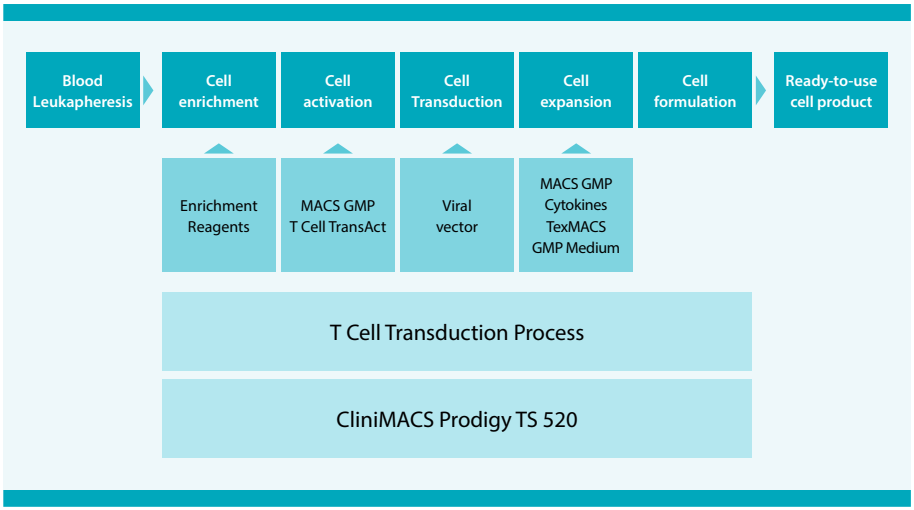


Figure 1.1: Generic manufacturing scheme using the CliniMACS Prodigy T Cell Transduction System

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 listed in this user manual. For Canada, only the CliniMACS CD4 Reagent and CliniMACS CD8 Reagent must be used for the cell enrichment step within the T Cell Transduction Process.

1.2 Process

The T Cell Transduction Process starts with the installation of the CliniMACS Prodigy TS 520 on the CliniMACS Prodigy. Once the tubing set is installed on the instrument, CliniMACS PBS/EDTA Buffer supplemented with human serum albumin (HSA) and TexMACS™ GMP Medium supplemented with MACS® GMP Cytokines and human AB serum are connected to the instrument. The CliniMACS Prodigy TS 520 is then primed before the blood product is connected to the tubing set.

Once the blood product is connected, CliniMACS GMP MicroBeads/CliniMACS Reagents are connected to the tubing set and the cell enrichment occurs. Initial washing, cell labeling and magnetic separation are all automatically performed. 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 is seeded in the chamber where all subsequent phases of cell cultivation take place. The T cells are then activated by MACS GMP T Cell TransAct™. The user is then prompted to plan the cultivation using an activity matrix that includes transduction (viral vector), culture wash, feeding, medium

exchange, chamber shaking, and formulation for harvest. This final product is then ready for clinical application or further processing (e.g. culture, 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.

Culture monitoring and quality control can be performed 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 Process, several options (named cases) are available to the user (see Figure 1.2). One of four "cases" has to be chosen at the beginning of a manufacturing run:

Case 1: Full process

This option is chosen to run the complete process, including tubing set installation, T cell enrichment, T cell activation, transduction, and expansion as described above.

Case 2: T cell cultivation

This option is chosen when only the T cell activation, transduction, and expansion is to be performed without T cell enrichment, and a tubing set is already installed. This, for example, is used in cases where 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

This option is used in order 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 T cell cultivation

This option is used if the T cell cultivation process was interrupted and needs to be resumed. This resume function starts directly with the activity matrix setup.

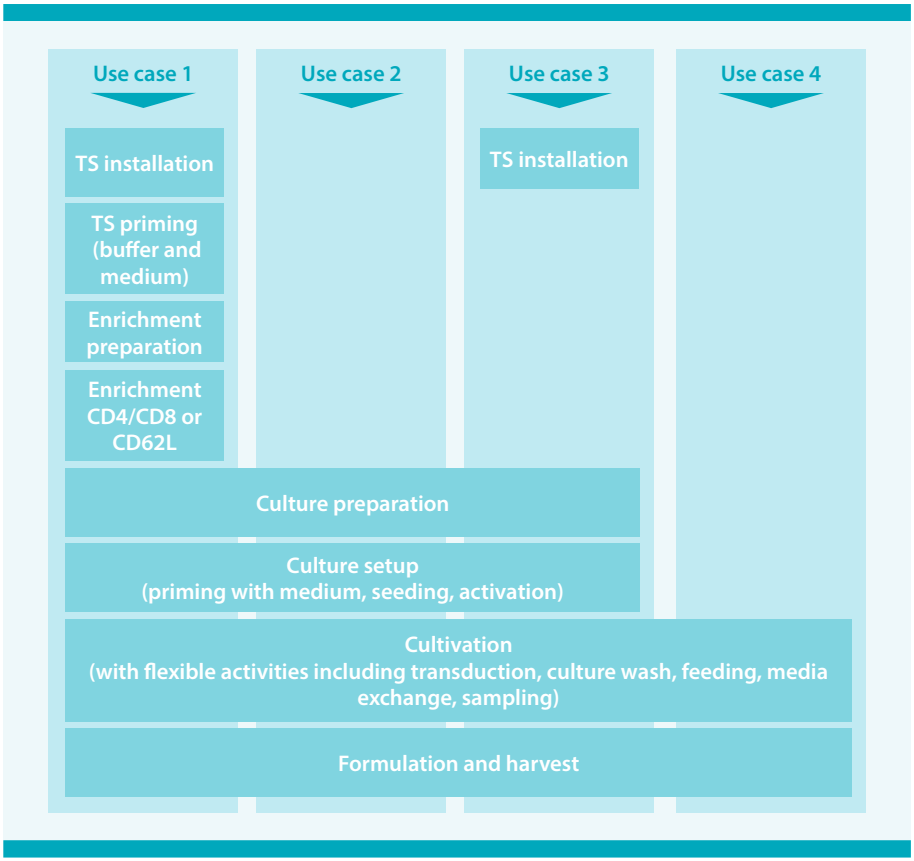


Figure 1.2: Versatility of the T Cell Transduction Process

IMPORTANT

The CliniMACS Prodigy T Cell Transduction System has a maximum capacity with respect to cell enrichment and cell transduction. Details are described in section 5.1 and section 7.2.

2
Glossary

2.1 Graphical depiction

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

⚠ WARNING

Indicates a hazardous situation 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

Is used to address practices or information not related to personal injury but may lead 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 labeling of the CliniMACS Products.

3
Materials required

The following chapter outlines the materials and consumables required for the CliniMACS Prodigy T Cell Transduction System.

3.1 CliniMACS Prodigy T Cell Transduction System components

The following CliniMACS and MACS GMP Materials (see Table 3.1) are recommended for the generation of gene modified T cells:

Material	Quantity	Comment
CliniMACS Materials		
CliniMACS Prodigy	1	
CliniMACS CD62L GMP MicroBeads/ CliniMACS CD62L Reagent[1]	1 vial	For CD62L enrichment only
CliniMACS CD4 GMP MicroBeads/ CliniMACS CD4 Reagent[2]	1 vial	In combination with CD8 enrichment
CliniMACS CD8 GMP MicroBeads/ CliniMACS CD8 Reagent[2]	1 vial	In combination with CD4 enrichment
CliniMACS Prodigy TS 520	1 set	
CliniMACS PBS/EDTA Buffer	3 liters	Use 1×3,000 mL bag

1 **Note:** CliniMACS CD62L Reagent/Microbeads are not approved for use in Canada.
2 **Note:** Only CliniMACS CD4 Reagent and CliniMACS CD8 Reagent are approved for use in Canada.

Materials	Quantity	Comment
MACS GMP Materials		
TexMACS GMP Medium	4 liters	
MACS GMP Recombinant Human IL-7	2 vials 25 µg/vial	In combination with IL-15
MACS GMP Recombinant Human IL-15	2 vials 25 µg/via	In combination with IL-7
MACS GMP Recombinant Human IL-2	4 vials 25 µg/vial	If IL-2 is used for T cell expansion
MACS GMP T Cell TransAct	1 vial	
MACS GMP Vectofusin®-1	1 vial	Will be pre-mixed with viral vector
Software		
T Cell Transduction Process (process version 2.x)		

Table 3.1: Materials recommended for the CliniMACS Prodigy T Cell Transduction System

IMPORTANT

The cytokines and the vectofusin-1 referenced above are recommended for use in combination with the T Cell Transduction Process. The user is responsible for the validation of the process.

3.2 Additional materials and equipment required

Besides the CliniMACS Prodigy T Cell Transduction System components, additional materials and equipment are required for the T Cell Transduction Process (see Table 3.2):

Material	Comment
Biological materials	
Heterogeneous hematologic cell sources	• Not older than 24 hours • <20×10^9 white blood cells (WBCs) • 50–280 mL
Human serum albumin	Use only pharmaceutical grade HSA approved in your country.
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., physiologic sodium chloride solution
Consumables	
Syringes	• 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)
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 System

IMPORTANT

This protocol requires the use of components and materials which are not part of the CliniMACS Prodigy T Cell Transduction System (e.g. human serum albumin (HSA) or human AB serum). Therefore, use only 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

Warning and precaution

⚠ 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.

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 System components, refer to the instructions for use provided for the respective component.

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

5
Preparative steps

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

⚠ WARNING

Use aseptic working conditions for all preparative steps.

5.1 Testing of starting material

The T Cell Transduction Process 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 numbers and target cell frequency must be determined before the enrichment in order to calculate the appropriate number of stages. A maximum of three stages can be processed with the T Cell Transduction Process.

Therefore, analyze the cellular starting product prior to connecting it to the tubing set. Determine the WBC concentration and the frequency of target cells (either CD4 and CD8 positive among viable CD45 positive cells or CD62L positive among viable CD45 positive cells depending on the selected separation reagent). In order not to exceed the process capacity, start with a cell product volume containing a maximum of 3×10^9 target cells (i.e. CD4 and CD8 positive cells or CD62L positive cells, respectively). The minimum volume of the cell product is 50 mL and the maximum volume is 280 mL. Dilute the cell product with process buffer if necessary. The cell product information 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 cell number/volume/frequency before starting the process.

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 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.

⚠ WARNING

Store the buffer for cell preparation at +19 °C to +25 °C (+66 °F to +77 °F). Lower or higher temperature may influence the quality of the target cells.

5.3 Preparation of medium

For optimal T cell proliferation, supplement 2 liters of TexMACS GMP Medium with cytokines. IL-7 (MACS GMP Recombinant Human IL-7) in combination with IL-15 (MACS GMP Recombinant Human IL-15). Alternatively, only IL-2 (MACS GMP Recombinant Human IL-2) can be used. Depending on the cell numbers used, varying cytokine concentrations may be required. Titrate the cytokine concentration as needed.

The recommended dose ranges are:

- IL-7: 150–1500 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 2000 mL bag of TexMACS GMP Medium which corresponds to 1,350 IU/mL and 450 IU/mL respectively.

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

Note: It is possible to adapt the T cell culture and reduce the use of human AB serum by using 3% supplementation only in the first 2,000 mL bag and using a second 2,000 mL bag of TexMACS GMP Medium without supplementation of AB serum starting from day 6 of culture.

Work under sterile conditions 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 the TexMACS GMP Medium with 60 mL (3%) human AB serum using the Luer Lock port of the medium bag.
- 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.

IMPORTANT

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

6
Separation process

6.1 Initialization of the CliniMACS Prodigy

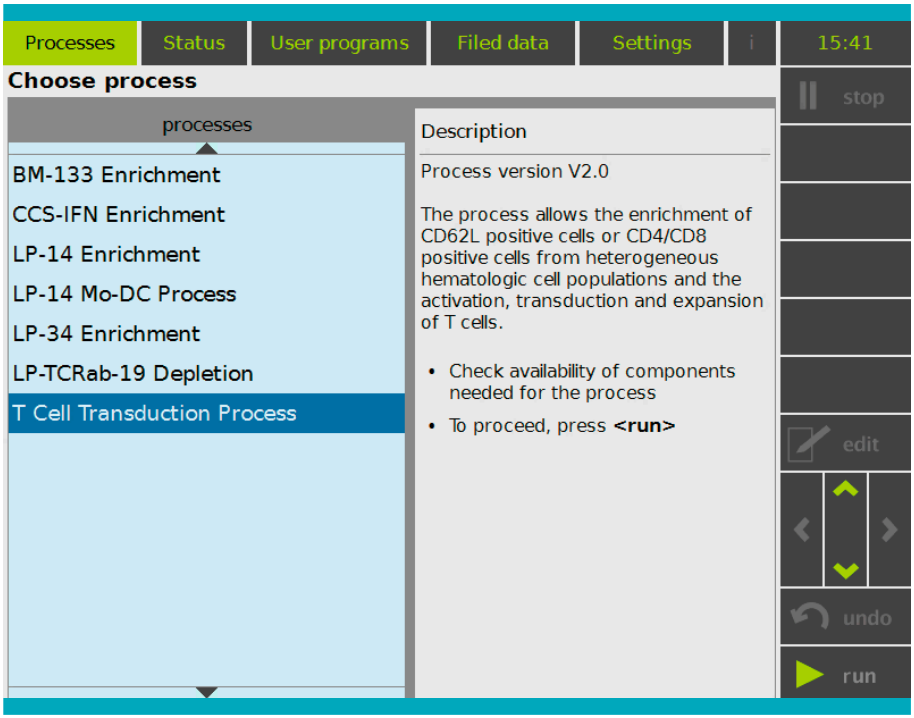
Switch on the CliniMACS Prodigy by using the ON/OFF switch located on the back panel of the instrument.

Note: A user interface showing screens with instructions and pictures will guide the user through the procedure. Parts of the text presented on the screens in the user manual may differ slightly from the screens displayed on the instrument. Touchscreen functionality and buttons on the menu and tool bar can be used. For details, refer to the CliniMACS Prodigy User Manual (Instrument).

After the instrument initialization phase the main screen is displayed. Select the process "T Cell Transduction Process" and confirm the selection by pressing **run**.

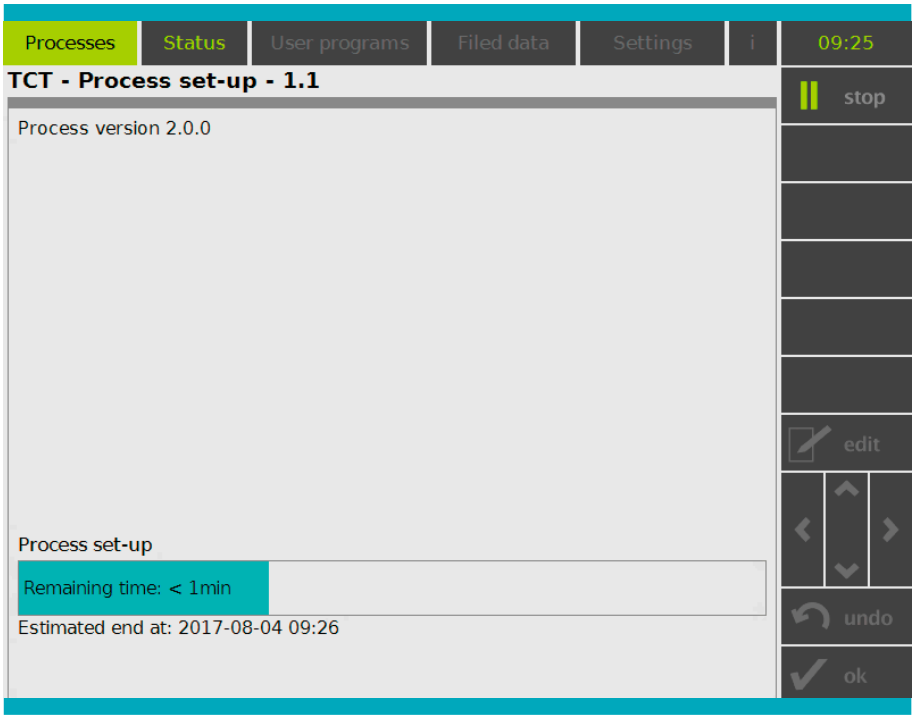
⚠ CAUTION

Risk of process failure. Ensure that optical and acoustical alarm signals can be recognized by the operator at any time during the process.



Screen 6.1: Choice of process "T Cell Transduction Process"

The instrument performs a short self-test, shown in Screen 1.1, and indicates the remaining time for this process step on the process bar before the next screen is displayed.

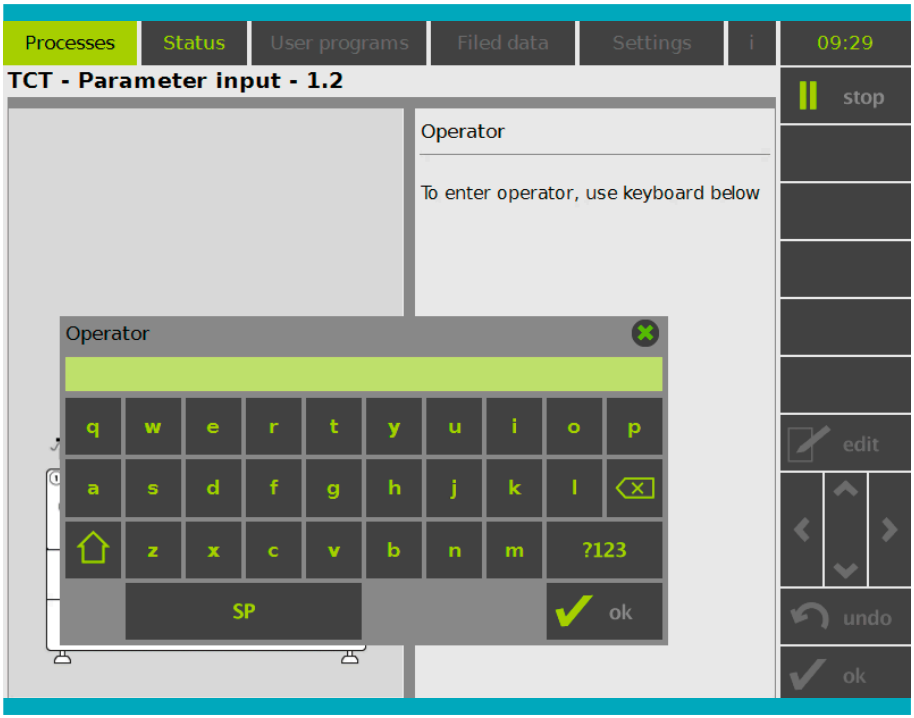


Screen 6.2: Self-test

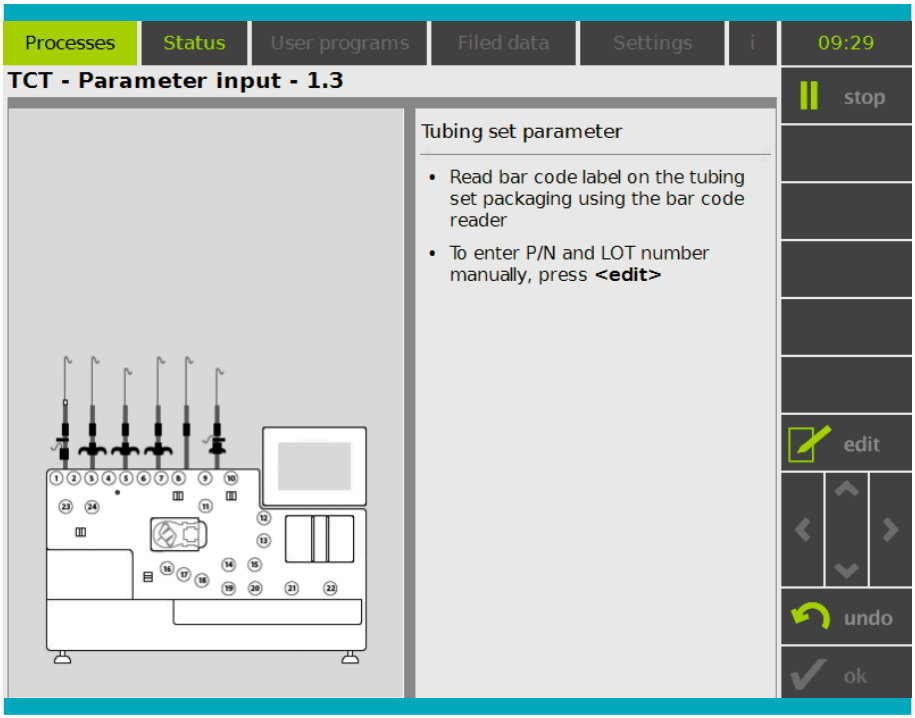
6.2 Parameter input and process setup choice

Multiple parameters can be entered for the T Cell Transduction Process using either the bar code reader attached to the CliniMACS Prodigy or the keyboard displayed on the screen. All entered parameters are confirmed by pressing **ok** and will then be saved in a process-related protocol.

Within the next steps of the process, information related to the operator (see Screen 1.2) and CliniMACS Prodigy TS 520 (see Screen 1.3) must be entered. For the manual entry of capital letters, press ⇧, for space press **SP**, for numbers press **?123**, and for backspace press ⌫.

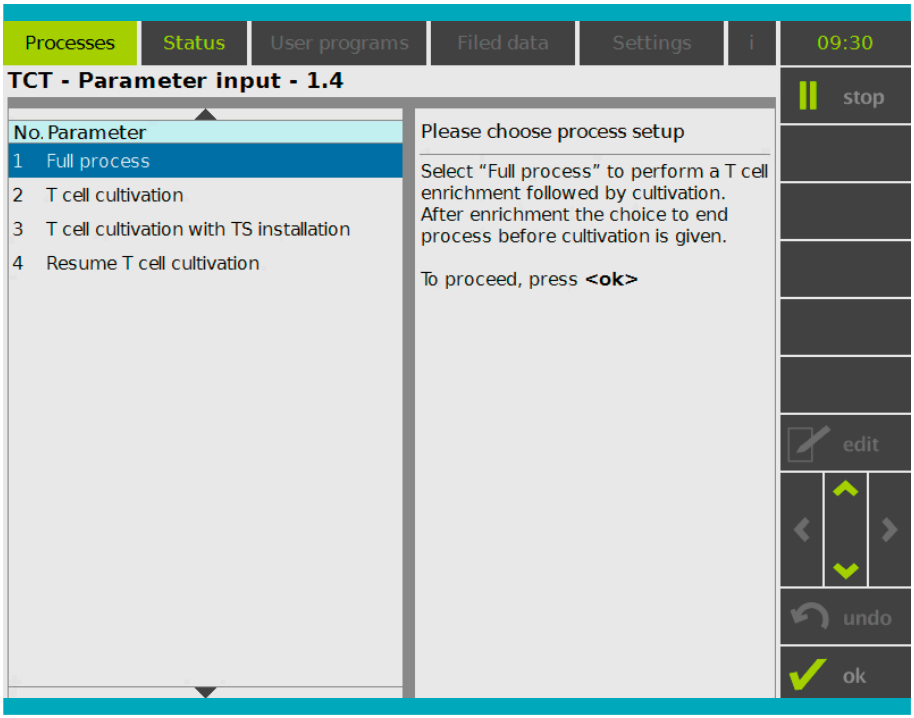


Screen 6.3: Entry of operator-related information



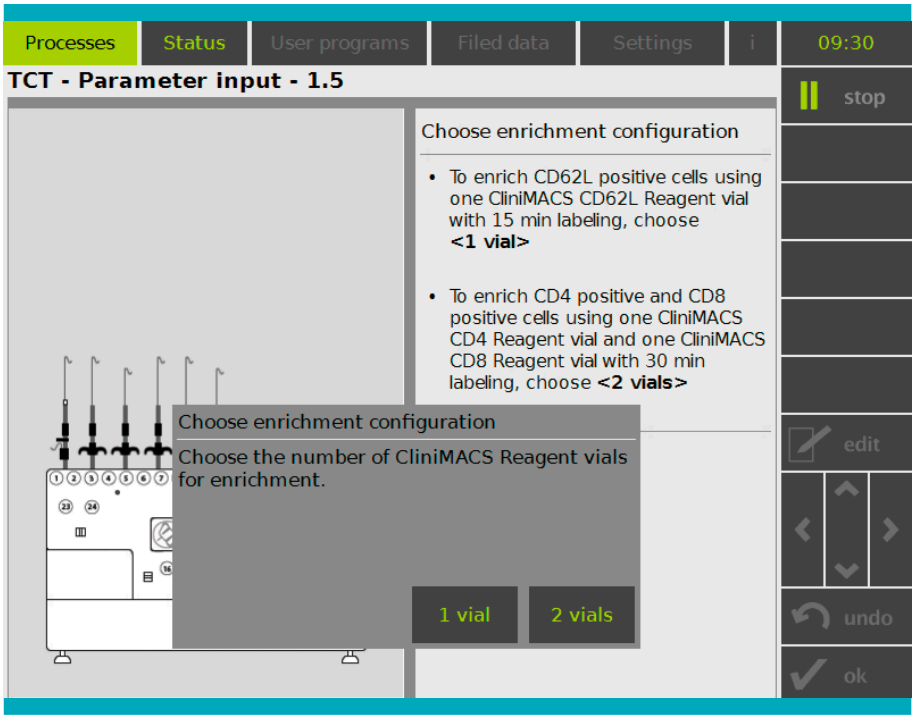
Screen 6.4: Entry of CliniMACS Prodigy TS 520 part number (P/N) and batch code (LOT)

Read the bar code label on the tubing set label or press **edit** to enter part number (P/N) and batch code (LOT) manually by using the keyboard displayed on the screen.



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

After parameter input, the user can choose between four different cases (see section 1.2). This user manual describes the complete process using case 1 (Full process) as the default process.



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

In the next step, the user chooses the number of CliniMACS GMP MicroBeads/ CliniMACS Reagents vials used for target cell enrichment. For CD62L enrichment, choose **1 vial**, for a combined CD4 and CD8 enrichment, choose **2 vials**.

IMPORTANT

Use one vial of CliniMACS CD62L GMP MicroBeads/CliniMACS CD62L Reagent[1] to enrich CD62L cells. Use one vial of CliniMACS CD4 GMP MicroBeads/CliniMACS CD4 Reagent and one vial of CliniMACS CD8 GMP MicroBeads/CliniMACS CD8 Reagent to enrich CD4 and CD8 cells[2].

1 **Note:** CliniMACS CD62L Reagent/Microbeads are not approved for use in Canada.
2 **Note:** Only CliniMACS CD4 Reagent and CliniMACS CD8 Reagent are approved for use in Canada.

6.3 Tubing set installation

6.3.1 Components of the CliniMACS Prodigy TS 520

The components of the CliniMACS Prodigy TS 520 are pre-assembled and provided in a sealed and sterilized package. All components of the tubing set are summarized in Figure 6.1.

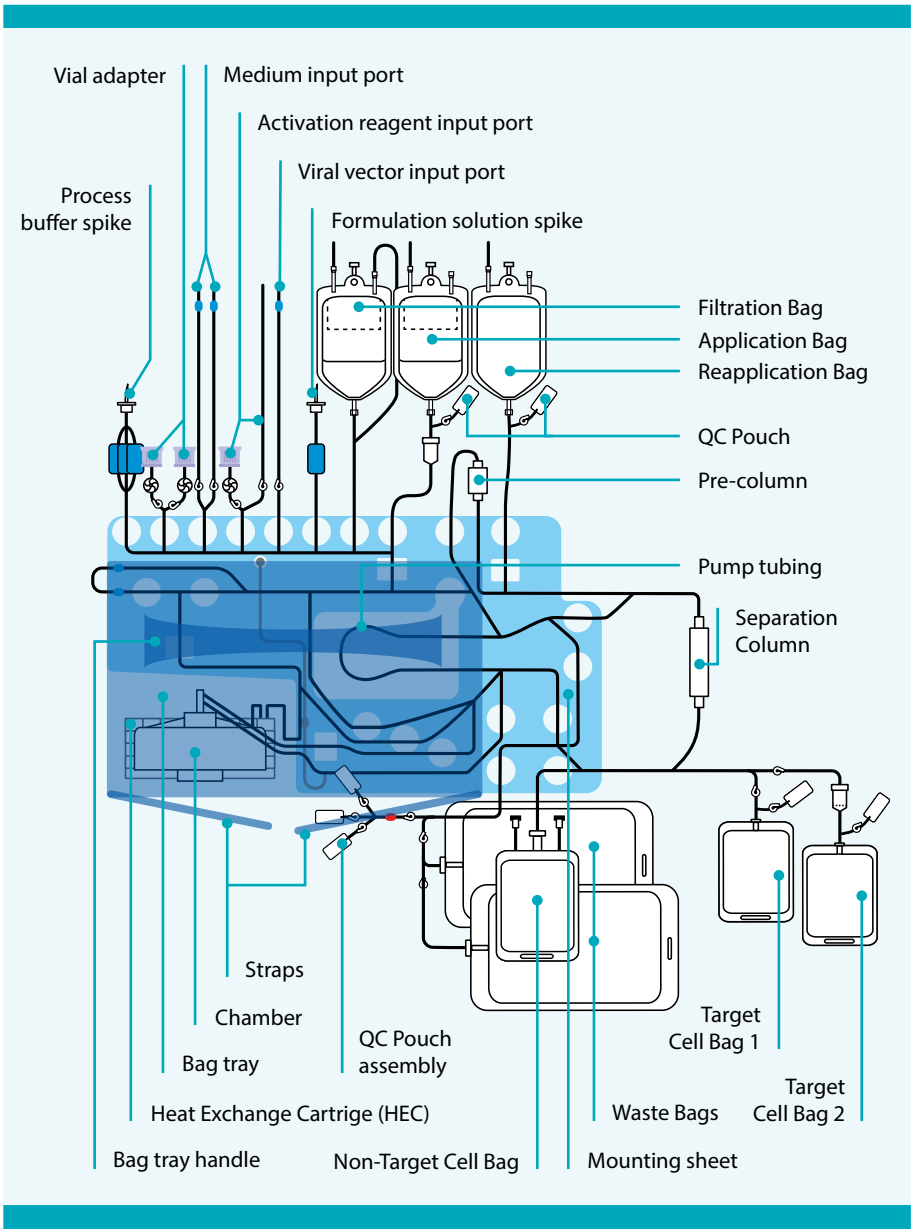


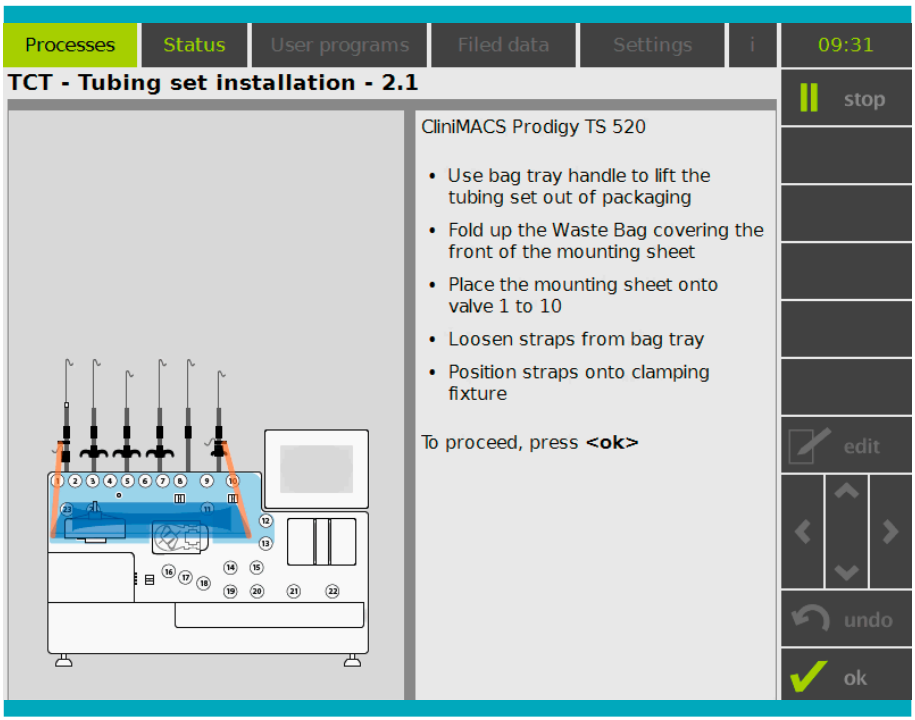
Figure 6.1: Components of the CliniMACS Prodigy TS 520

6.3.2 Installation steps

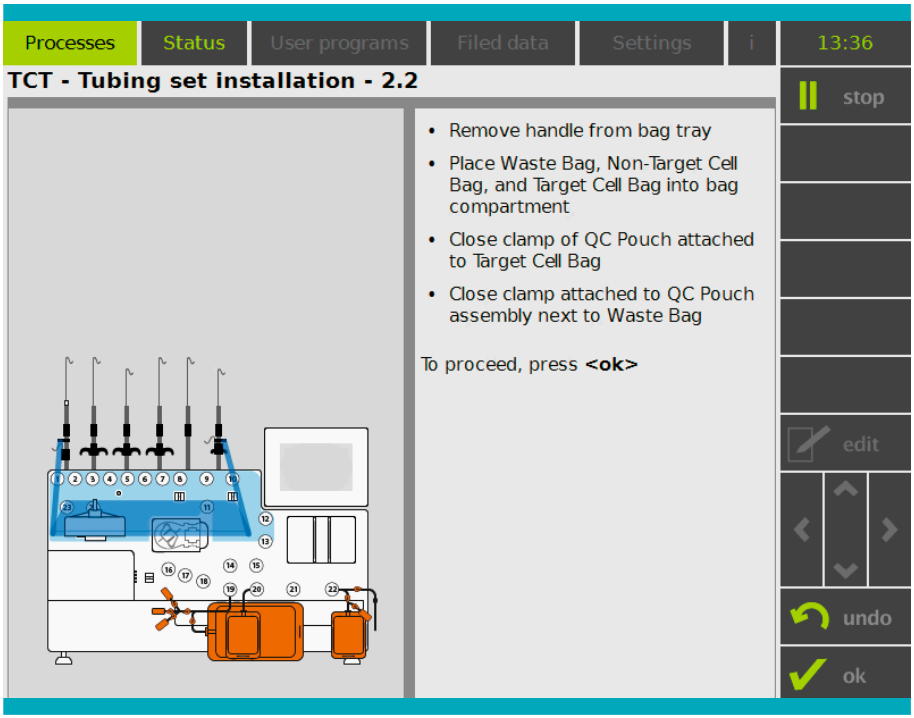
This section guides the user step by step through the installation of all tubing set components (see Screens 2.1 to 2.23). Each screen displays the instructions supported by a picture. Blinking and orange features on the screen indicate areas of attention. At any step during the installation, the **undo** key can be pressed to return to previous steps.

⚠ WARNING

Prior to opening the tubing set, inspect the packaging for damage, punctures, or tears. Use tubing set only if package is undamaged and sealed.

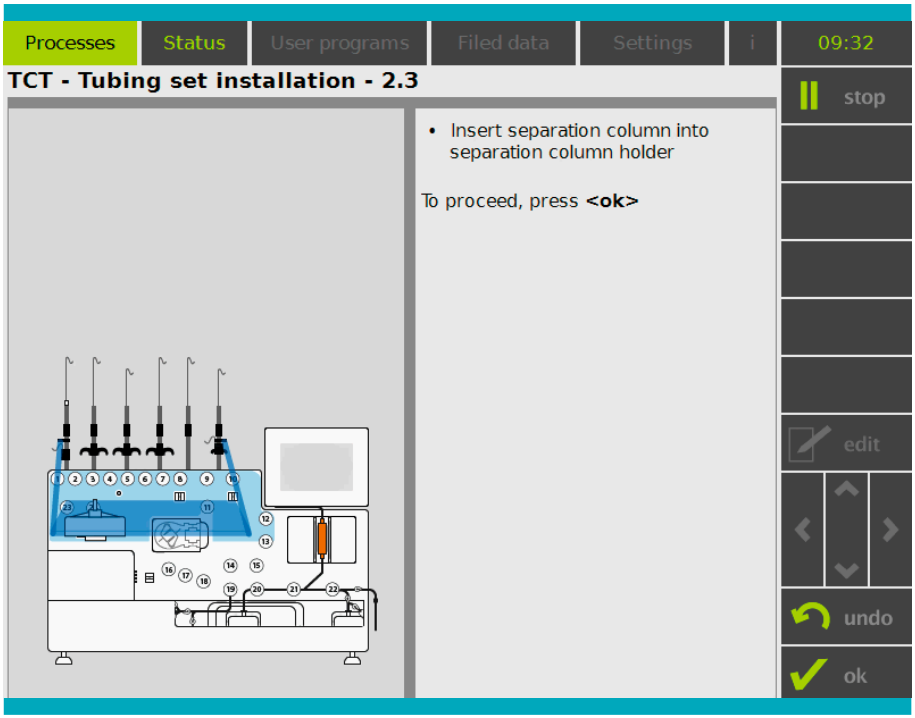


Screen 6.7: Installation of bag tray and mounting sheet onto the instrument



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

Waste Bags may be stored to the left hand side of the CliniMACS Prodigy in a suitable container if both waste bags are supposed to be used at the same time. If only one bag is used at a time, it can be stored in the bag compartment. Ensure clamps of both 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. To avoid possible pinch injuries, hold the top and the bottom part of the column between thumb and index finger. The column label should be facing the user. Place the column carefully into the column holder. Ensure that no tubing is pinched in between the column and the column holder.

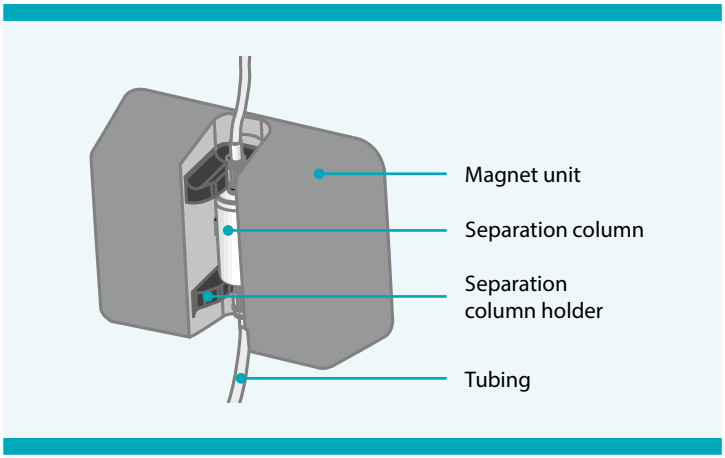
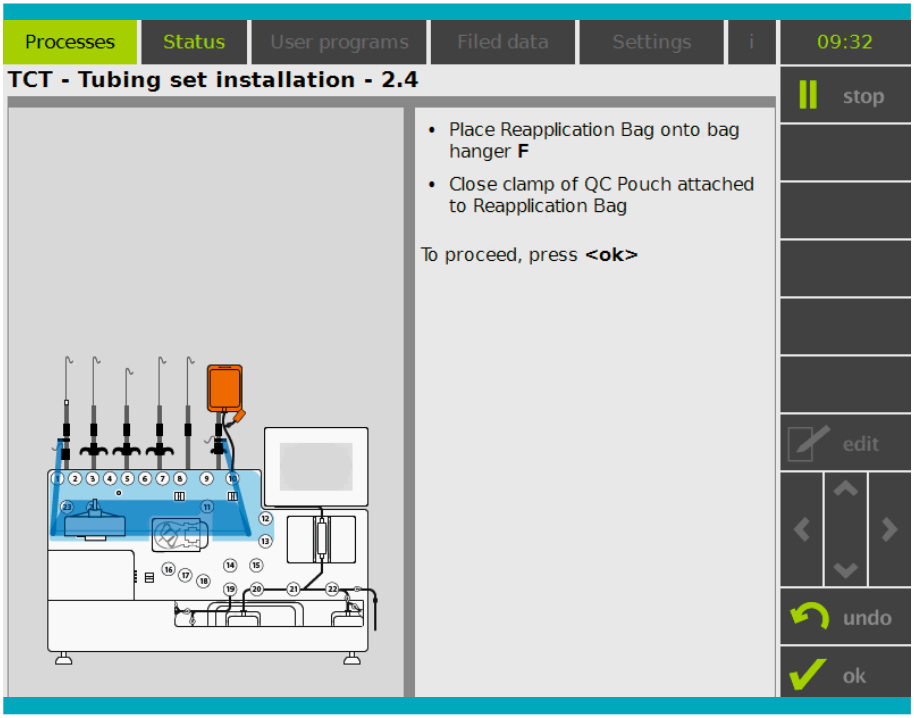
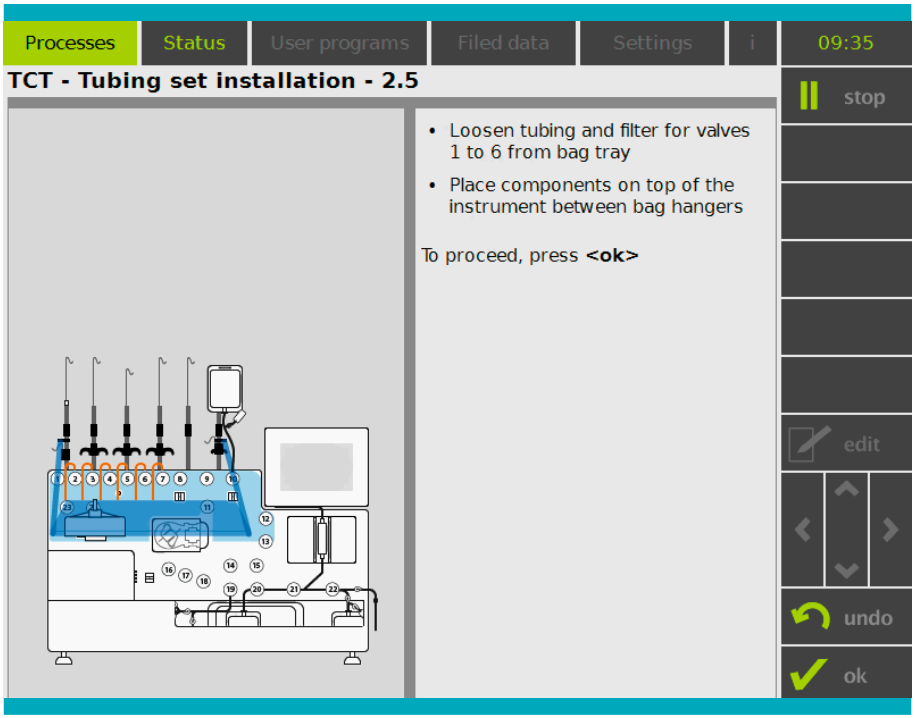


Figure 6.2: Separation column inserted into magnet unit

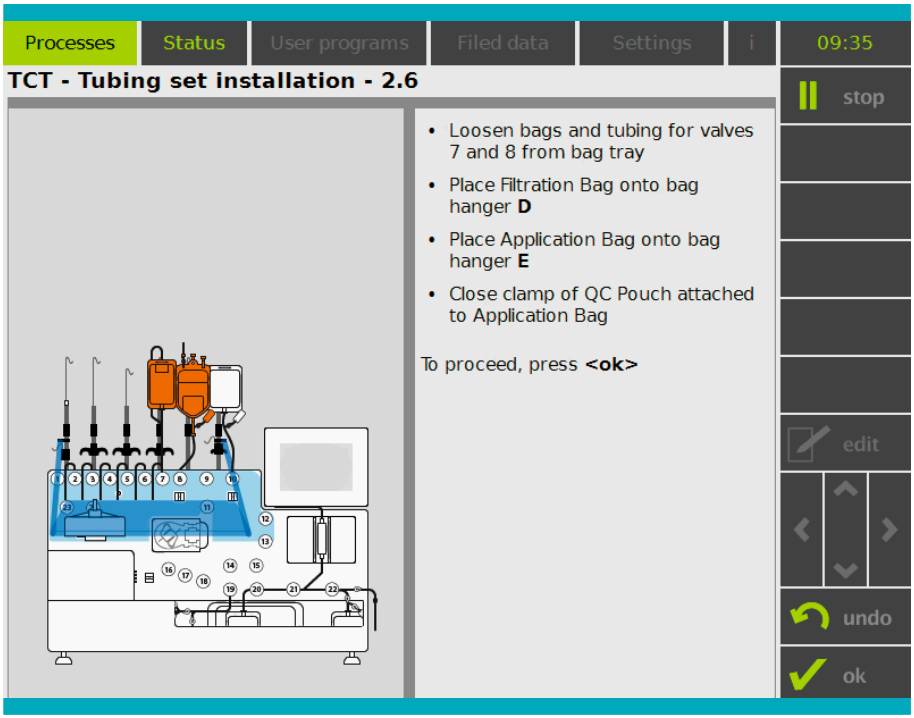


Screen 6.10: Installation of Reapplication Bag



Screen 6.11: Placement of tubing set components between bag hangers

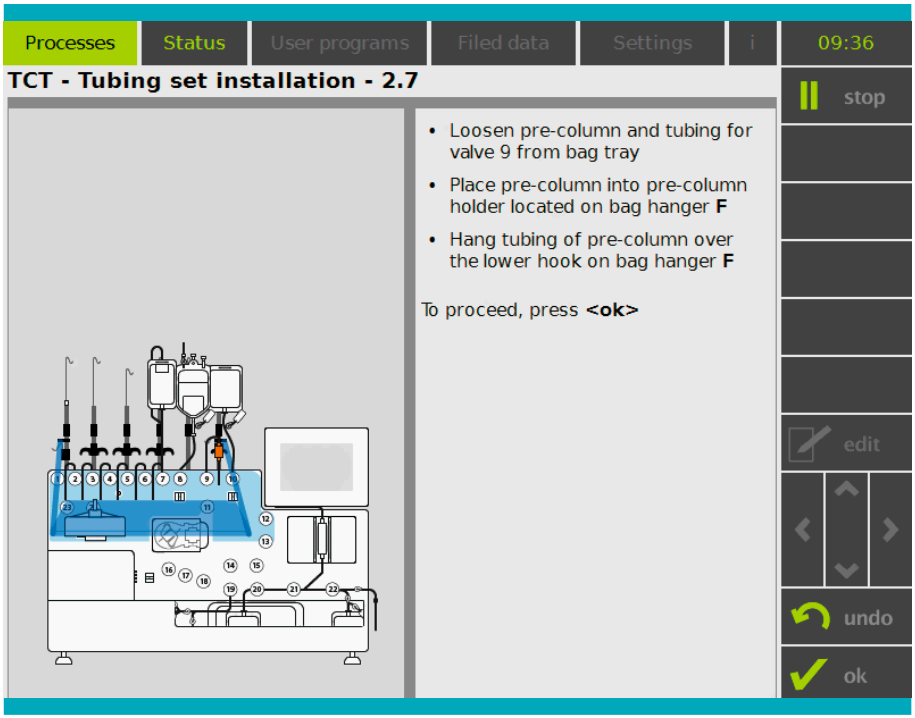
Note: Place each vial adapter in the vial holder temporarily. The final position of the vial adapters is described in Figure 6.9.



Screen 6.12: Installation of Filtration Bag and Application Bag

IMPORTANT

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



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).

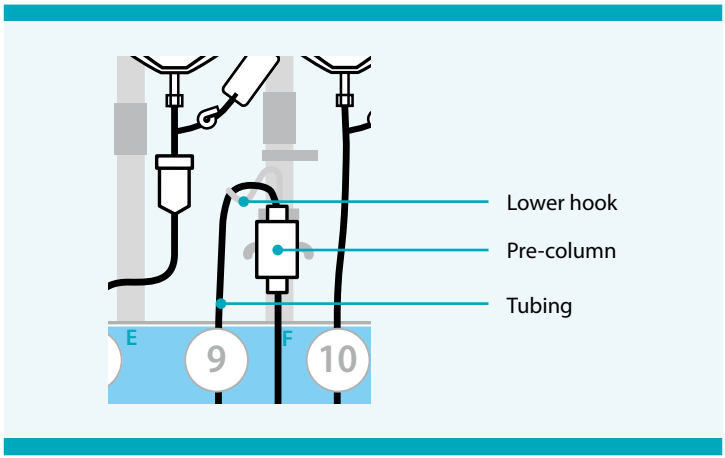
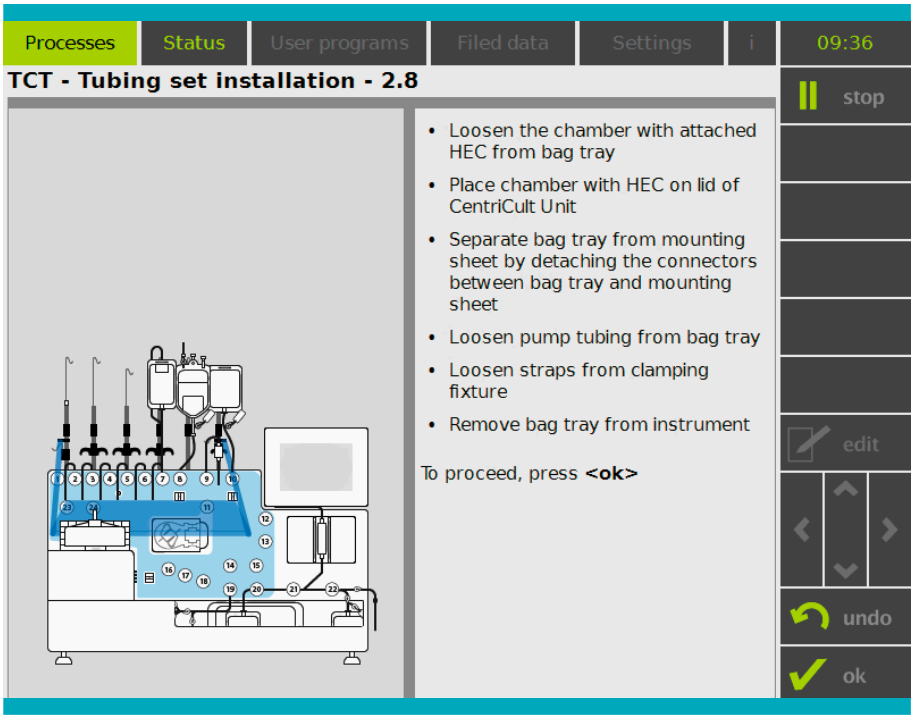
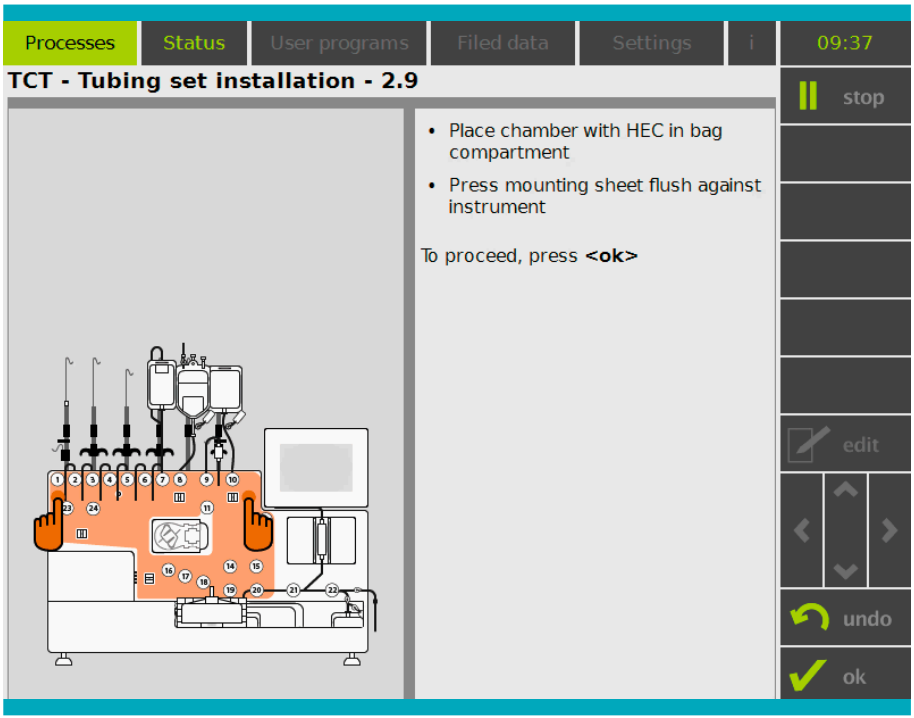


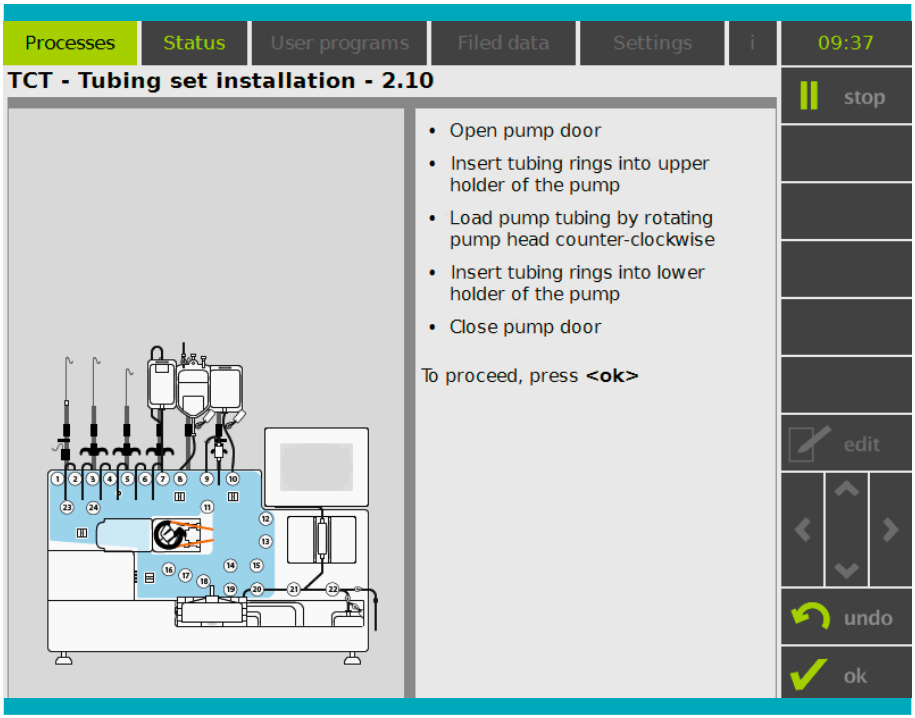
Figure 6.3: Guidance of tubing over the lower hook on bag hanger (F)



Screen 6.14: Removal of bag tray from instrument



Screen 6.15: Placement of chamber with Heat Exchange Cartridge (HEC) in bag compartment



Screen 6.16: Insertion of pump tubing into pump

Figure 6.4 shows how to insert the pump tubing into the pump. Insert the upper pump tubing ring next to the edge of the pump. Rotate the pump head counter-clockwise until the tubing is threaded between both sets of the tubing guide pins. Then insert the lower outer tubing ring next to the edge of the pump. Check that the pump tubing is not pinched between the guide pins. Repeat clockwise and counter-clockwise rotation of the pump head 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.

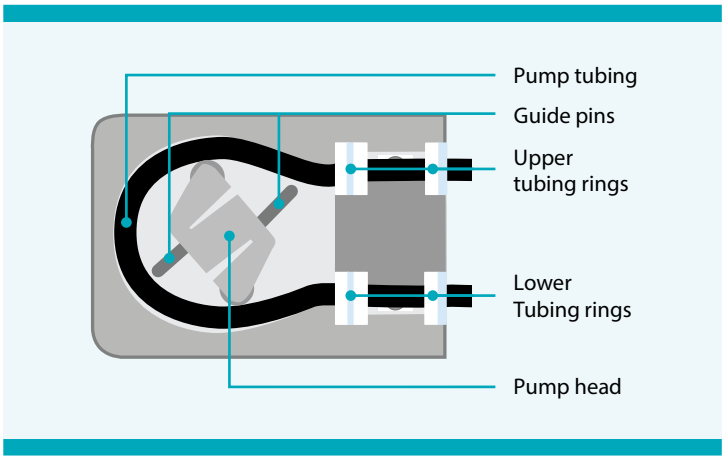
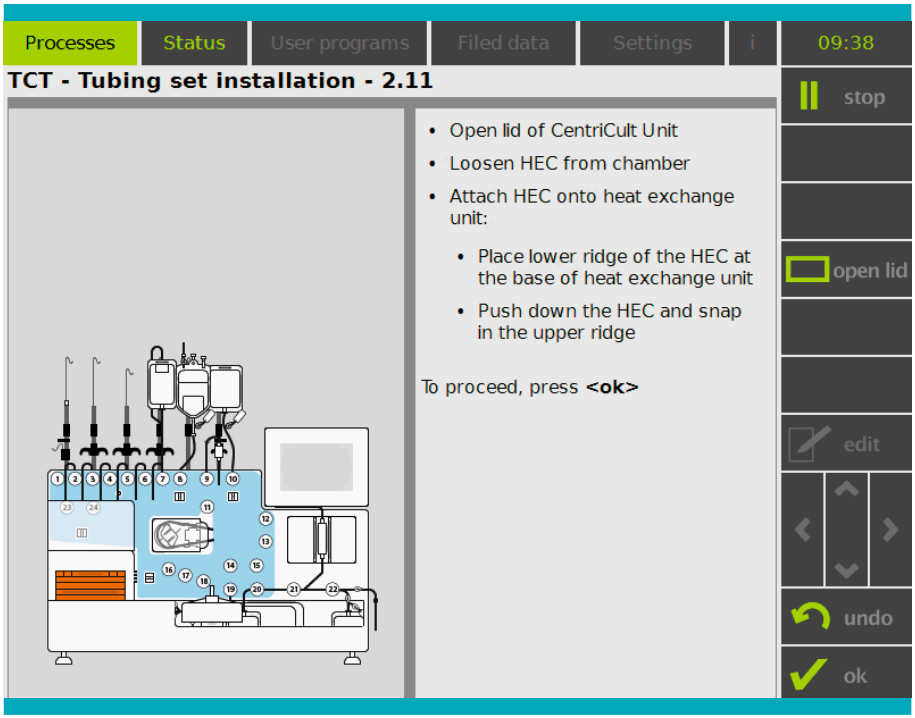


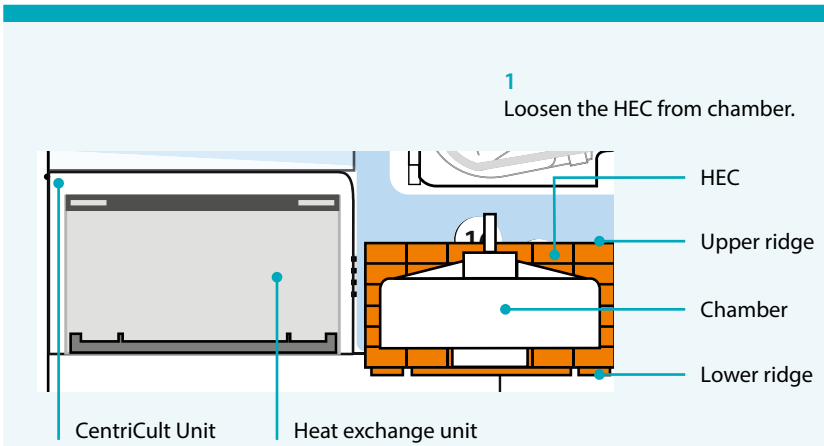
Figure 6.4: Pump tubing inserted into pump

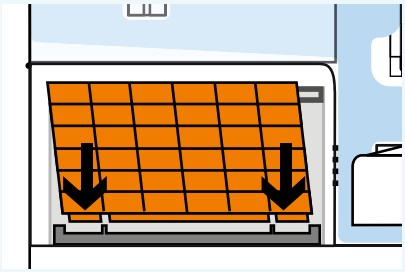


Screen 6.17: Installation of HEC onto heat exchange unit

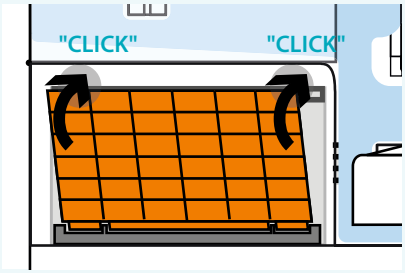
The CentriCult™ Unit lid unlocks automatically during this process step. If the lid does not unlock automatically, press the **open lid** button on the tool bar.

Figure 6.5 illustrates the installation of the Heat Exchange Cartridge (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.



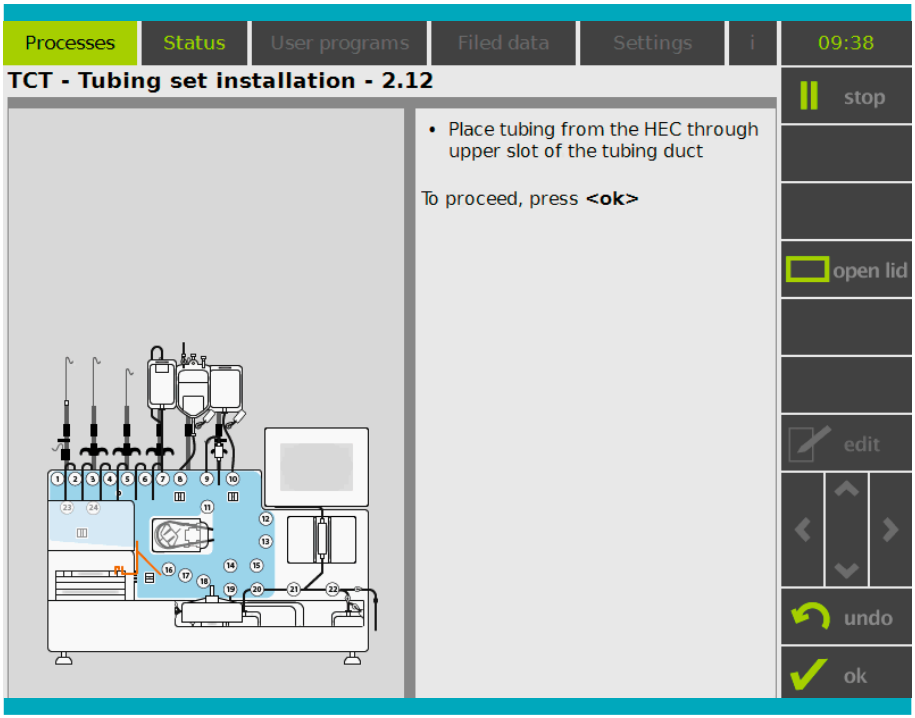


2
Place lower ridge of the HEC
At the base of heat exchange unit.

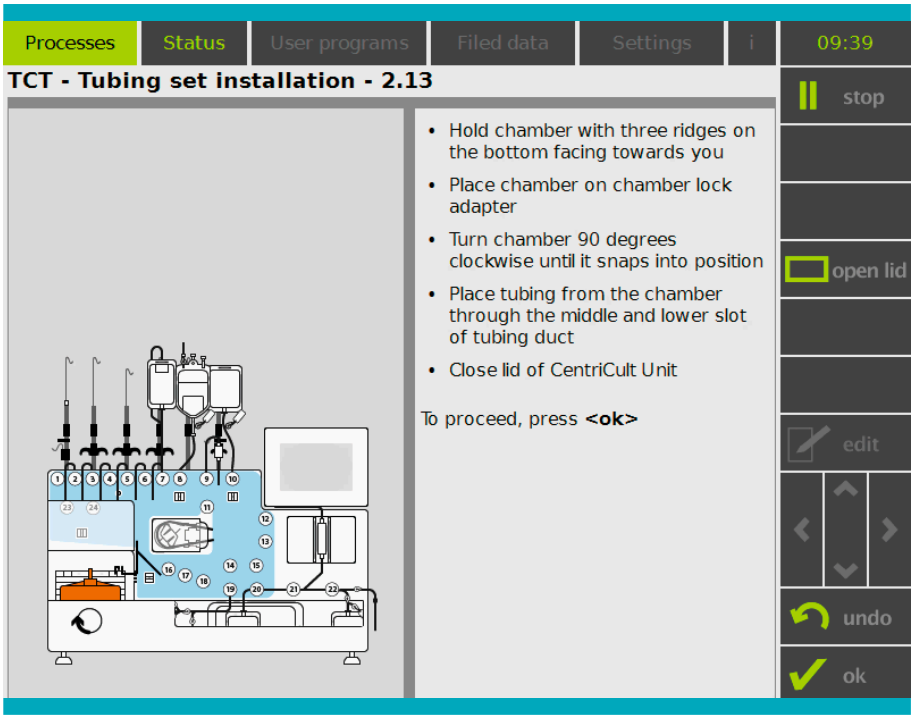


3
Push down the HEC
and snap in upper ridge.

Figure 6.5: Installation of HEC onto heat exchange unit



Screen 6.18: Guidance of tubing from HEC through upper slot of tubing duct



Screen 6.19: Installation of chamber into CentriCult Unit

Note: Before positioning the chamber onto the chamber lock adapter, ensure that the tubing attached to the chamber is in a parallel position and not twisted or bent. The chamber should not be detachable from the chamber lock adapter if it is snapped correctly into position.

⚠ WARNING

Chamber must be tightly locked into position. Do not use tubing set if chamber cannot be installed properly.

Figure 6.6 shows how to insert the three sections of the tubing through the tubing duct. Ensure that the tubing is not crossed or pinched by the lid of the CentriCult Unit.

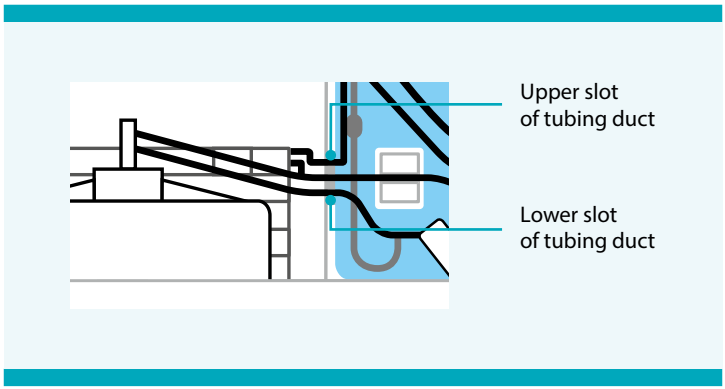
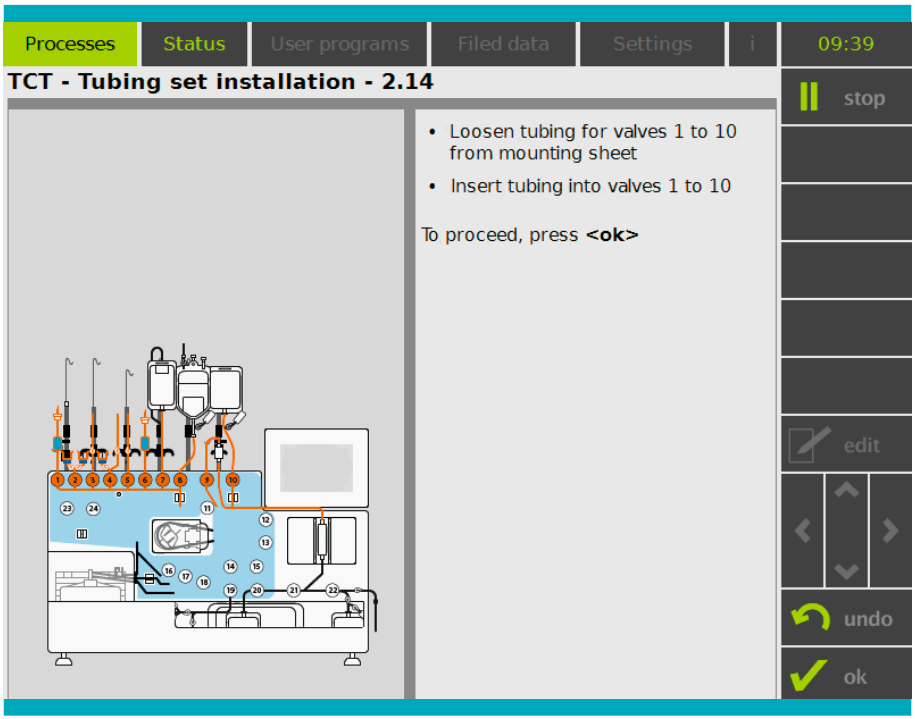


Figure 6.6: Guidance of tubing through tubing duct



Screen 6.20: Insertion of tubing into valves 1 to 10

Figure 6.7 shows how the tubing should be inserted correctly into the respective valves. Insert tubing only into open valves (when button is pressed down). Otherwise, the tubing will not fit correctly. If the tubing has to be adjusted, press the valve button to open the valve beforehand.

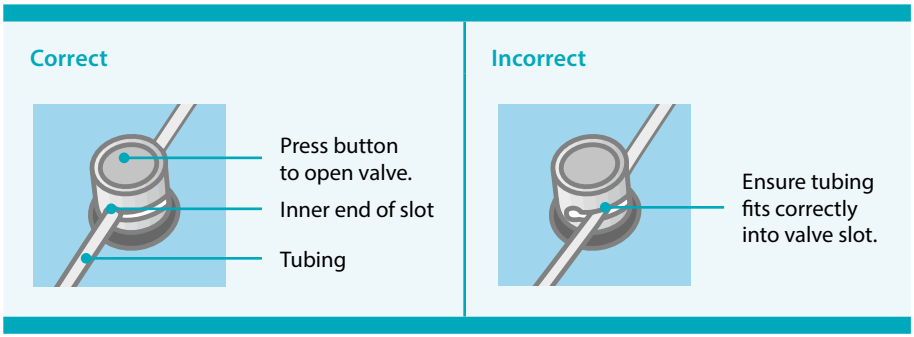
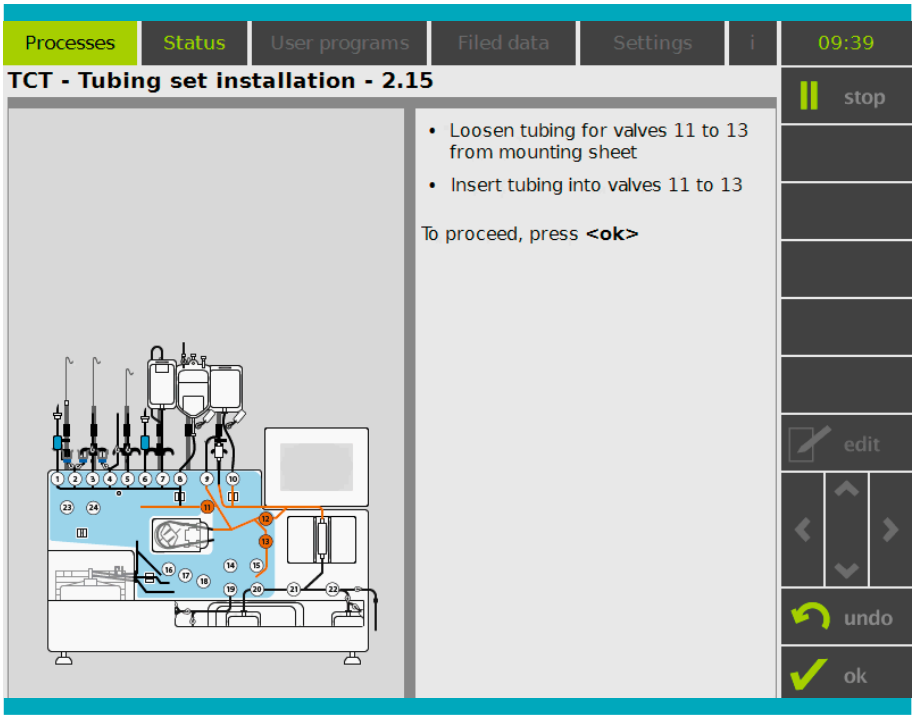


Figure 6.7: Insertion of tubing into valves

Fix the tubing with double vial adapters on valve 2 to the vial holders on bag hanger B.



Screen 6.21: Insertion of tubing into valves 11 to 13

Figure 6.8 shows how to insert the tubing correctly into valves 10, 12, and 13.

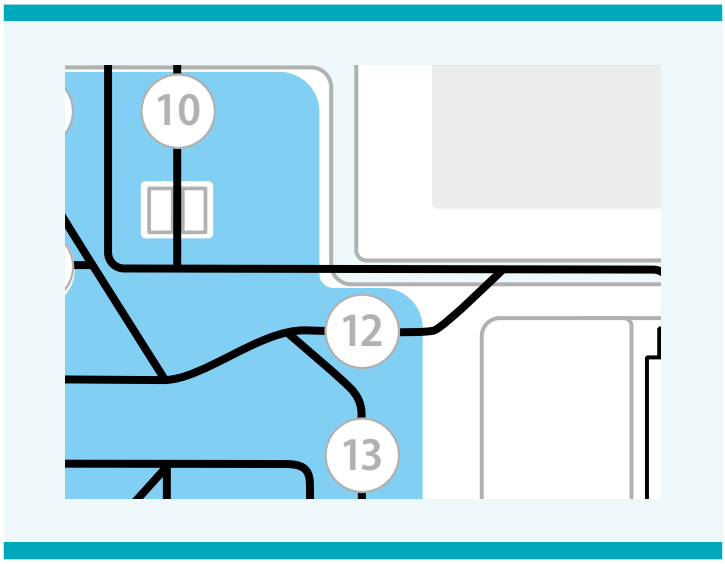
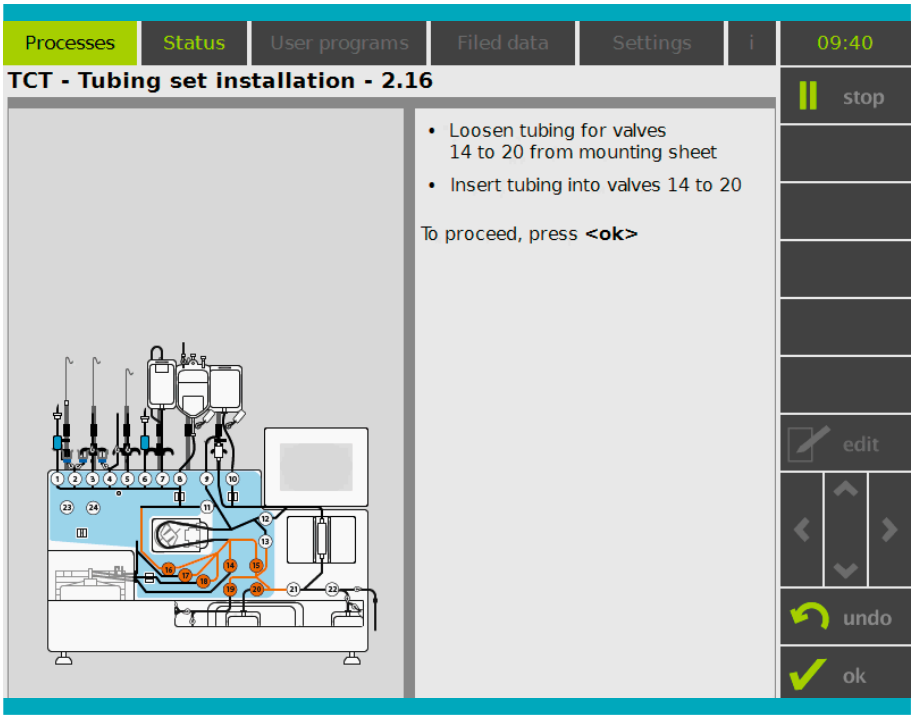
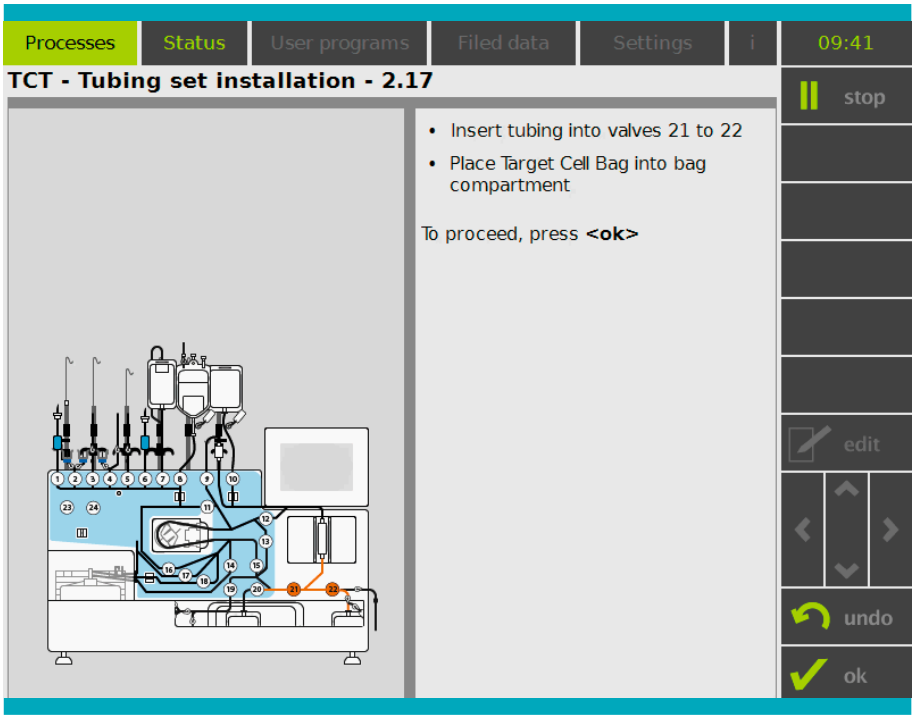


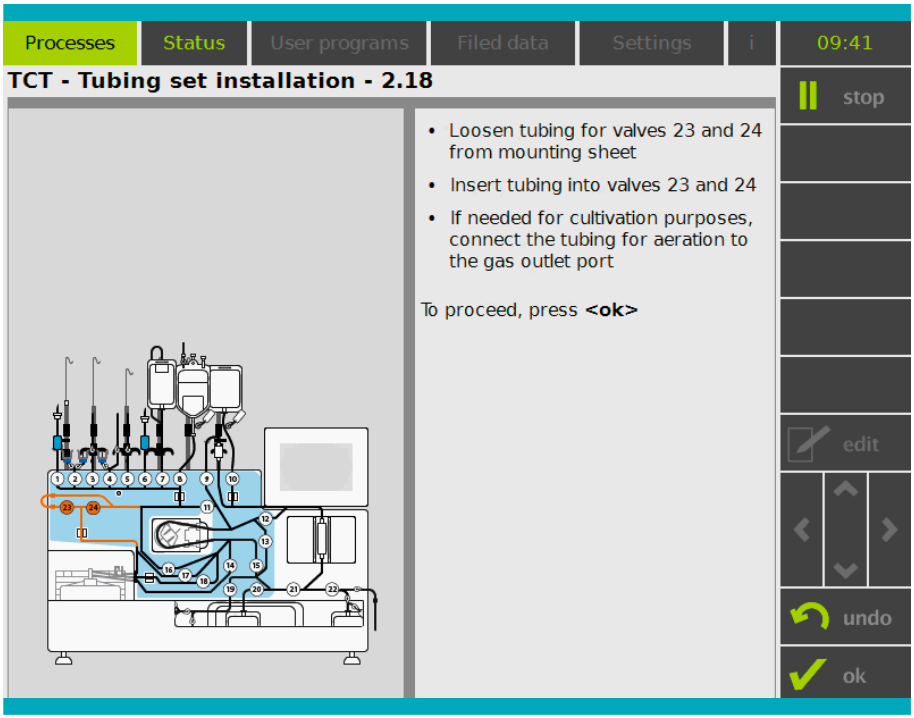
Figure 6.8: Guidance of tubing for separation column and valves 12



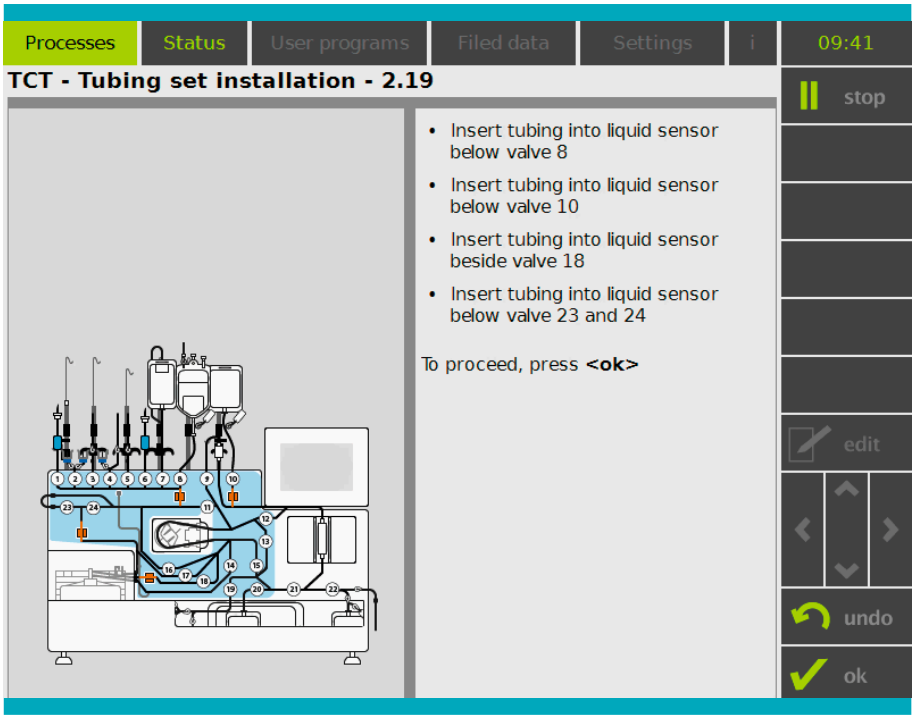
Screen 6.22: Insertion of tubing into valves 14 to 20



Screen 6.23: Insertion of tubing into valves 21 and 22 and placement of Target Cell Bags into bag compartment

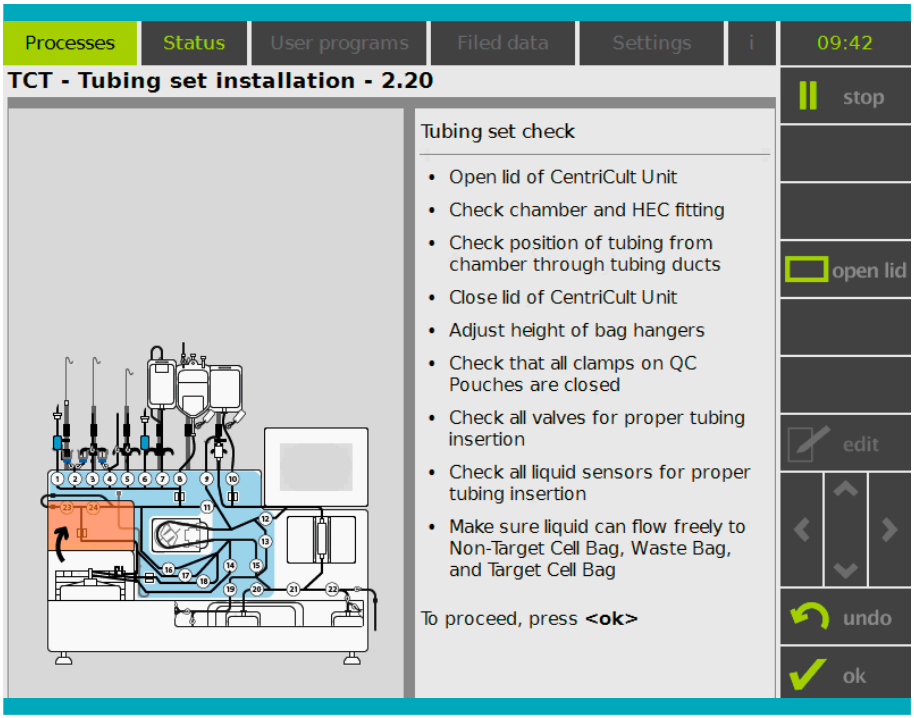


Screen 6.24: Insertion of tubing into valves 23 and 24 and connection of tubing for aeration to the gas outlet port



Screen 6.25: Insertion of tubing into liquid sensors

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



Screen 6.26: Insertion of tubing into liquid sensors

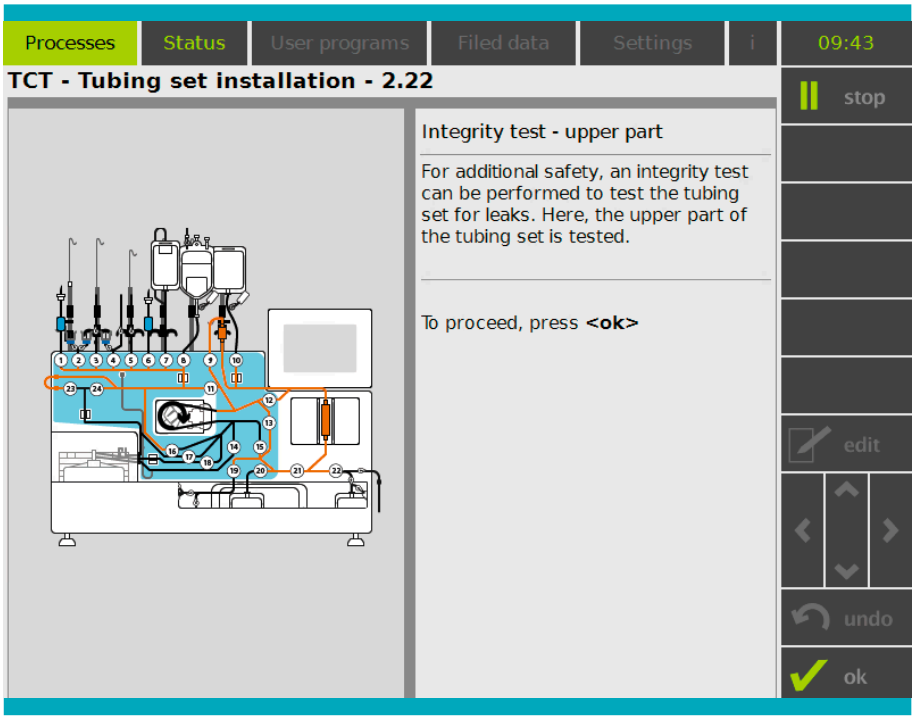
It is important to check all tubing for kinks or severe bending that could restrict the flow of liquid through the tubing. If the tubing has to be adjusted, remove the tubing by using the valve button to open the valve (Figure 6.7). If necessary, the height of the bag hanger can be adjusted to the size of the bag by raising or lowering the hanger. Ensure that the bag is positioned high enough to prevent severe bending of the tubing and that it is low enough to avoid the tubing or connections from being stretched. In addition, check the tightness of all Luer Lock connections within the tubing set.

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

Upon completion of the tubing set installation, all valves will open and close twice to confirm they are working properly. In addition, the movement of the magnet backwards and forwards inside the magnet unit will be checked. If a valve or the magnet does not operate correctly, refer to chapter 8 of the user manual to contact Miltenyi Biotec Technical Support.

IMPORTANT

For additional safety, an integrity test should be performed to test the tubing set for leaks. The test consists of two automated sequences which pressurize the upper and lower parts of the tubing set separately.



Screen 6.27: Insertion of tubing into liquid sensors

⚠ WARNING

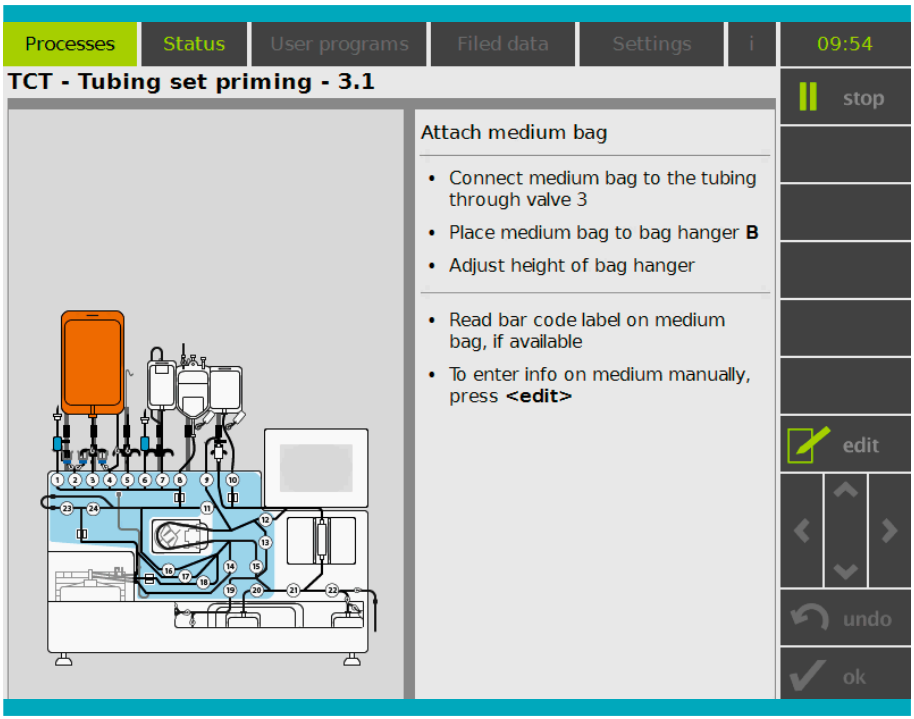
If the tubing set fails the integrity test, it must be replaced by a new tubing set. Contact Miltenyi Biotec Technical Support for further assistance and handling instructions.

Follow the instructions on Screen 2.22 to start the integrity test for the upper and lower part of the tubing set. It is possible, **but not recommended**, to skip the integrity test by pressing **no** on the display.Perform separate integrity tests for both upper and lower parts.

6.4 Tubing set priming

6.4.1 Connection of medium and buffer

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



Screen 6.28: Connection of medium bag

Screens 3.1 to 3.5 will guide the user through the priming phase.

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. Two medium bags may be connected to the CliniMACS Prodigy TS 520 at once.

Read the bar code label on the medium bag or press **edit** to enter part number (P/N) and batch code (LOT) manually by using the keyboard displayed on the screen.

IMPORTANT

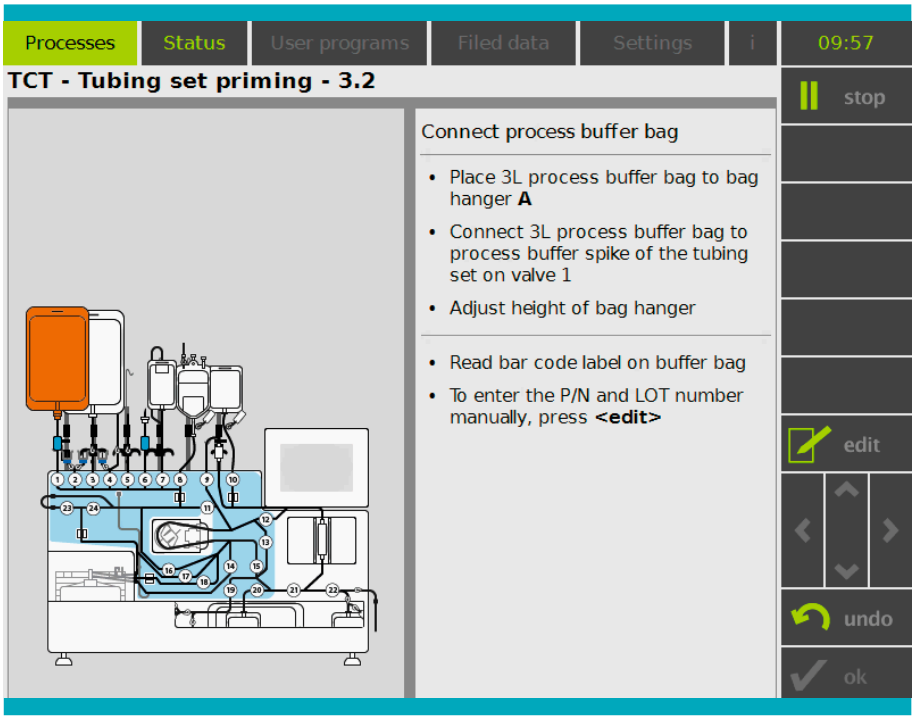
Confirm that the clamp of the medium bag is open at this point.

Information on supplements can be entered by pressing edit.

If necessary, the height of the bag hanger can be adjusted by raising or lowering the hanger. Ensure that the bag is positioned at the right height to prevent severe bending or overstretching of the tubing.

IMPORTANT

It is recommended to use TexMACS GMP Medium for the T Cell Transduction Process. Using other media could result in a lower performance.



Screen 6.29: 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 and spike it into the process buffer bag. Place the process buffer bag on bag hanger A. Alternatively, process buffer bag can be connected to the CliniMACS Prodigy TS 520 by sterile welding.

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

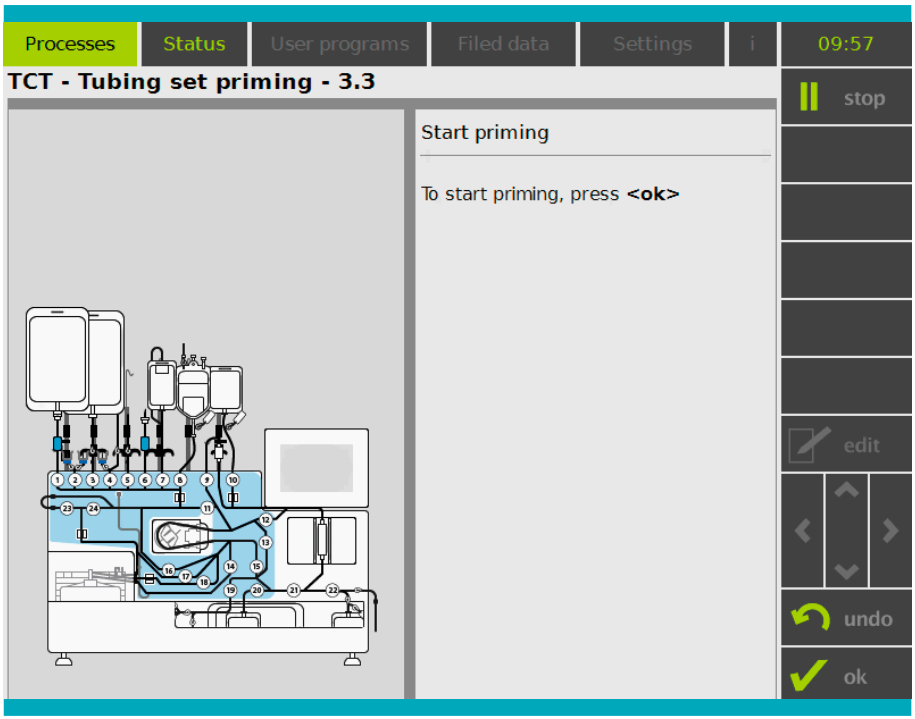
Information on the HSA can be entered by pressing **edit**. If necessary, the height of the bag hanger can be adjusted by raising or lowering the hanger. Ensure that the bag is positioned at the right height to prevent severe bending or overstretching of the tubing.

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 enrichmen

6.4.2 Priming phase

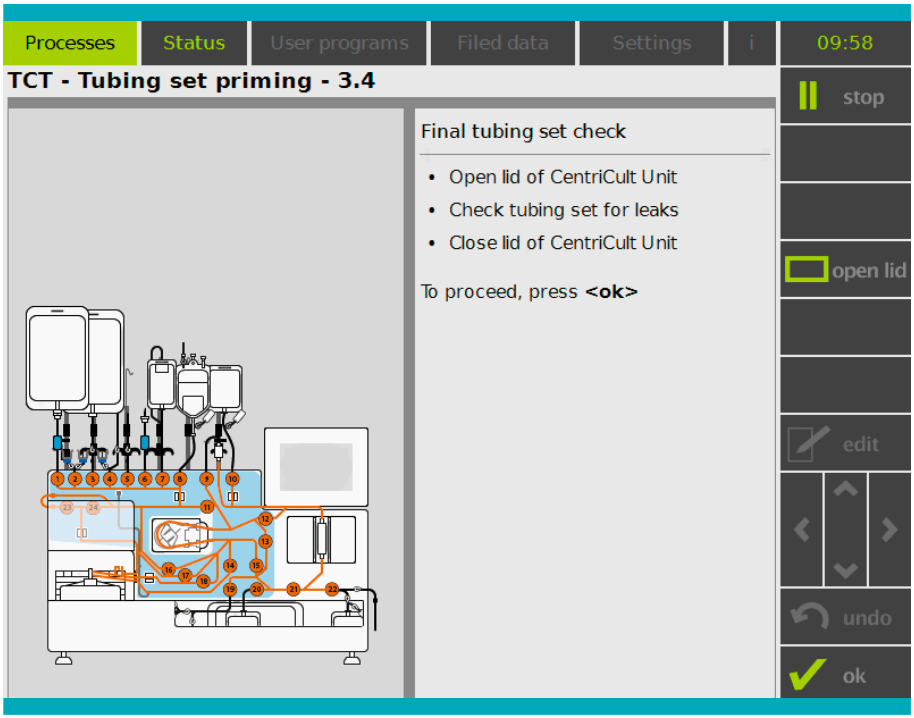


Screen 6.30: Start of priming phase

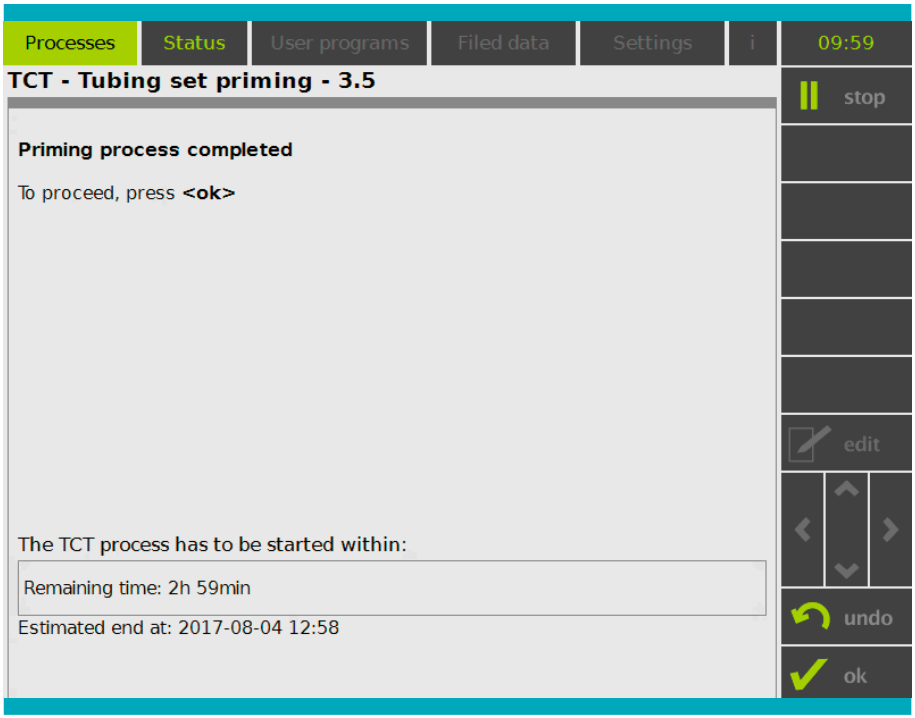
During the priming phase, the Reapplication Bag is filled with medium and the tubing set is filled with process buffer. The priming includes repeating cycles. The time for the priming phase will be displayed as a process bar on the screen.

When the priming phase is completed, check all tubing, fittings, valves, chamber, and columns for leaks or kinks that may block the fluids. Restart the process with a new tubing set, medium, and buffer if there is any leakage.

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



Screen 6.31: Final tubing set check after priming phase



Screen 6.32: End of priming phase

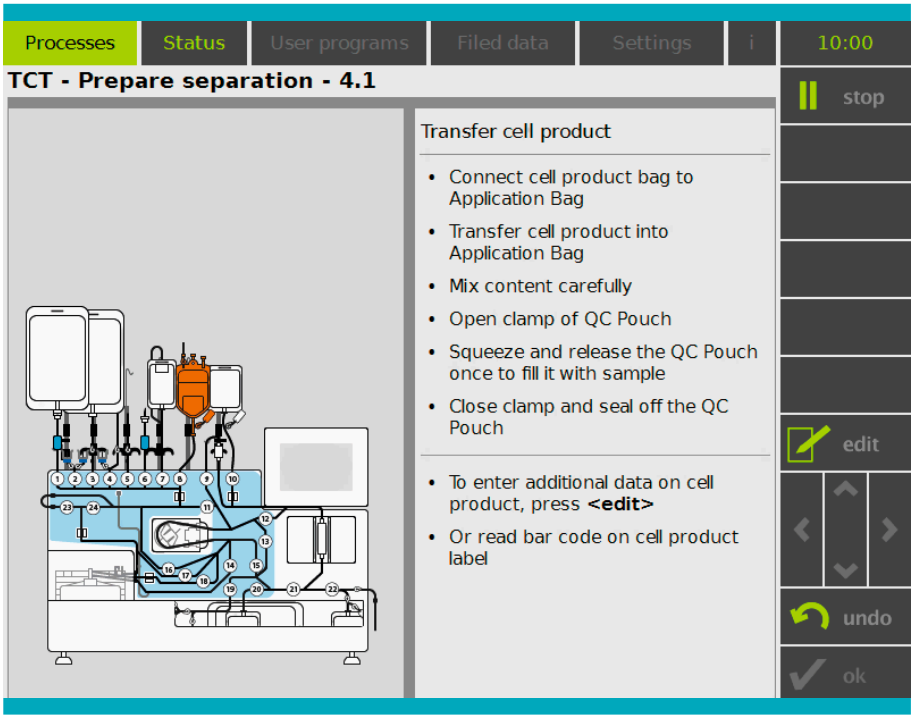
IMPORTANT

It is recommended to perform the tubing set priming directly before the enrichment process. 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 Transfer of cellular starting product

Before connecting the cellular starting product bag to the tubing set, determine the volume, the total WBC count and the target cell frequency. If the total number of target cells is higher than 3×10^9 cells, the process capacity is exceeded and target cells will be lost in the negative fraction. For details regarding how to calculate the number of target cells refer to section 5.1.



Screen 6.33: Transfer of cellular starting product to Application Bag

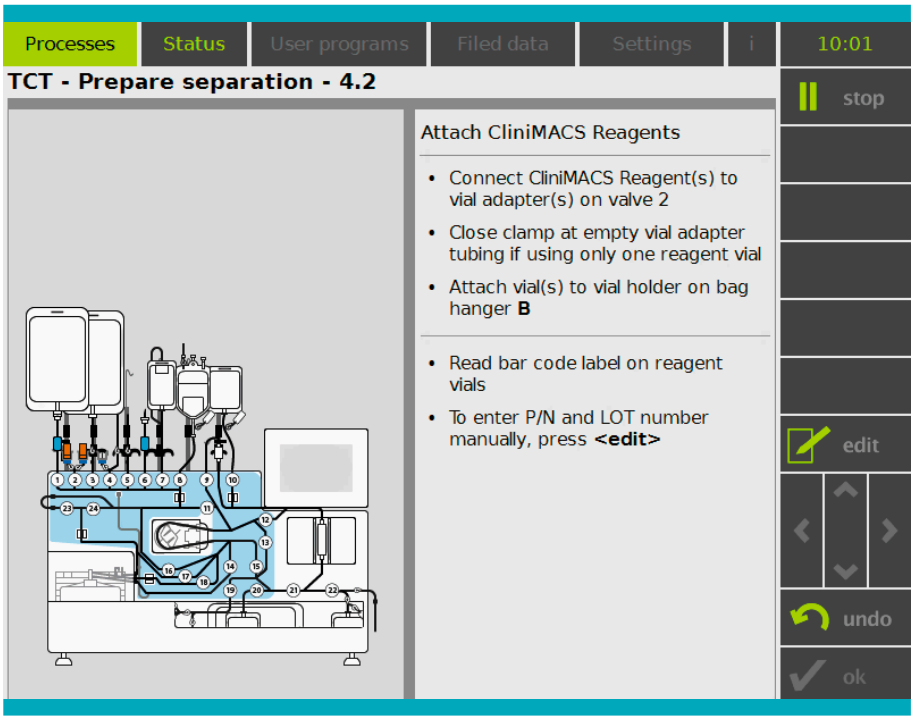
To transfer the cellular starting product to the Application Bag, follow the institution's standard operating procedures. For example, connect the container using either a sterile tubing connection, a Luer Lock connection, or a spike port connection. Transfer the cellular starting product into the Application Bag and seal off the empty bag using a heat sealer.

If a sample has not already been taken from the blood product, it can be taken from the Application Bag using the QC Pouch at this time: After opening the clamp, the QC Pouch is sufficiently filled with cellular starting product by squeezing and releasing the pouch once to obtain a QC sample. 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.

Information regarding the cellular starting product can then be entered by pressing **edit**.

6.5.2 Connection of CliniMACS GMP MicroBeads/CliniMACS Reagents

According to prior process selection, connect either one vial of the CliniMACS CD62L GMP MicroBeads/CliniMACS CD62L[3] Reagent or one vial of each CliniMACS CD4 GMP MicroBeads/CliniMACS CD4 Reagent and CliniMACS CD8 GMP MicroBeads/CliniMACS CD4[4] Reagent to the vial adapters on valve 2.



Screen 6.34: Transfer of cellular starting product to Application Bag

3 **Note:** CliniMACS CD62L Reagent/Microbeads are not approved for use in Canada.
4 **Note:** Only CliniMACS CD4 Reagent and CliniMACS CD8 Reagent are approved for use in Canada.

If connecting one vial of CliniMACS CD62L GMP MicroBeads/CliniMACS CD62L Reagent:

- 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/CliniMACS CD62L Reagent 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.
- Ensure that the Luer Lock connection below the vial adapter is still tightly closed. Otherwise, there is the risk of incomplete reagent load.
- In one smooth motion, turn the vial and vial adapter back again and insert the CliniMACS CD62L GMP MicroBeads/CliniMACS CD62L Reagent vial into the vial holder on bag hanger B. For correct connection, refer to Figure 6.9.
- Adjust height of vial holder.

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

- 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.
- Ensure that the Luer Lock connections below the vial adapters are still tightly closed. Otherwise, there is the risk of incomplete reagent load.
- In one smooth motion, turn the vials upside down and insert both vials into the vial holder on bag hanger B. For correct connection, see Figure 6.9.
- 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 by using the keyboard displayed on the screen.

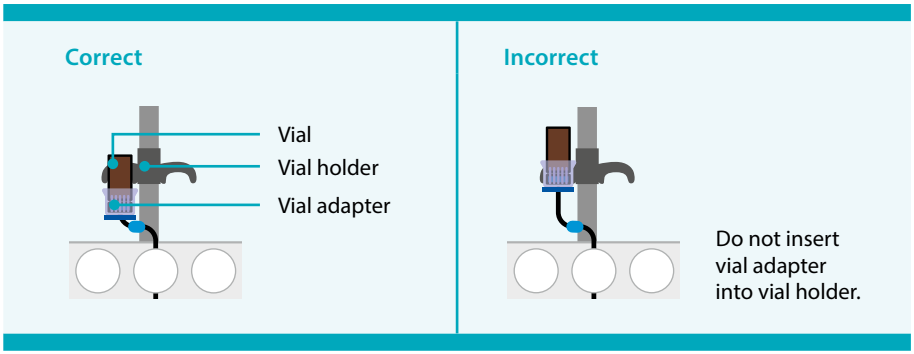
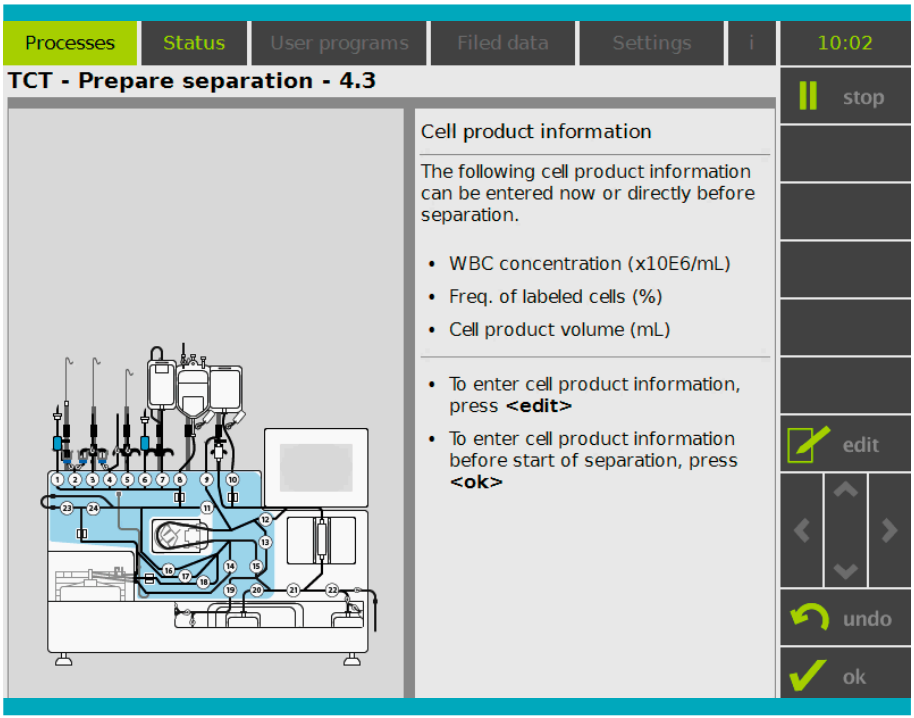


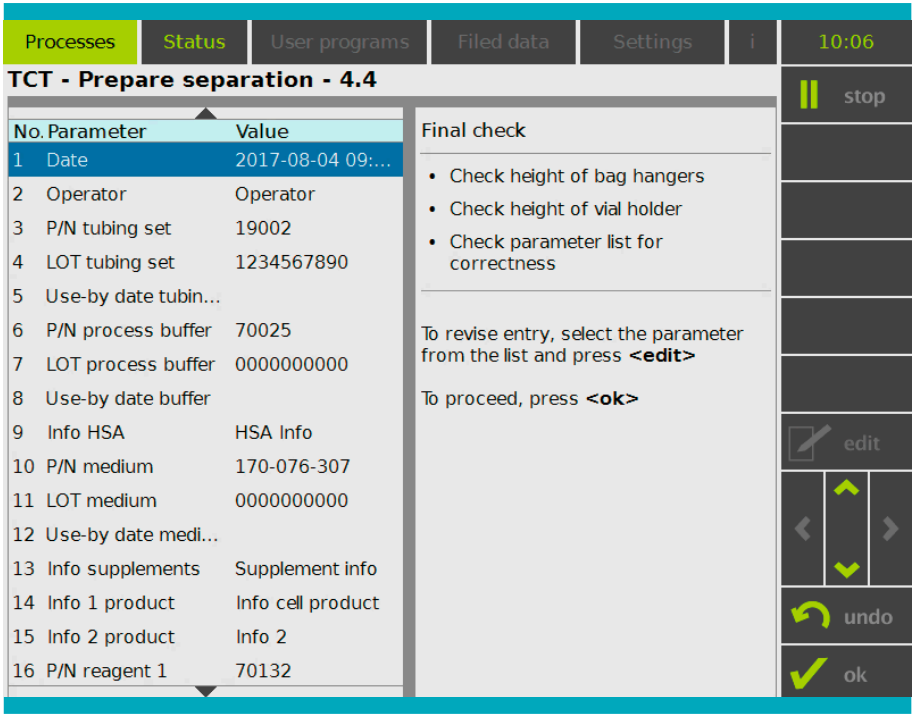
Figure 6.9: Insertion of vials into vial holder



Screen 6.35: Entry of cell product information

Enter the cell concentration (×10^6/mL), the frequency of labeled cells (i.e. percentage (%) CD62L positive cells or sum of percentage (%) CD4 and CD8 positive cells) and the volume of the cellular starting product that was transferred to the Application Bag (see section 6.5.1).

6.5.3 Final set-up check



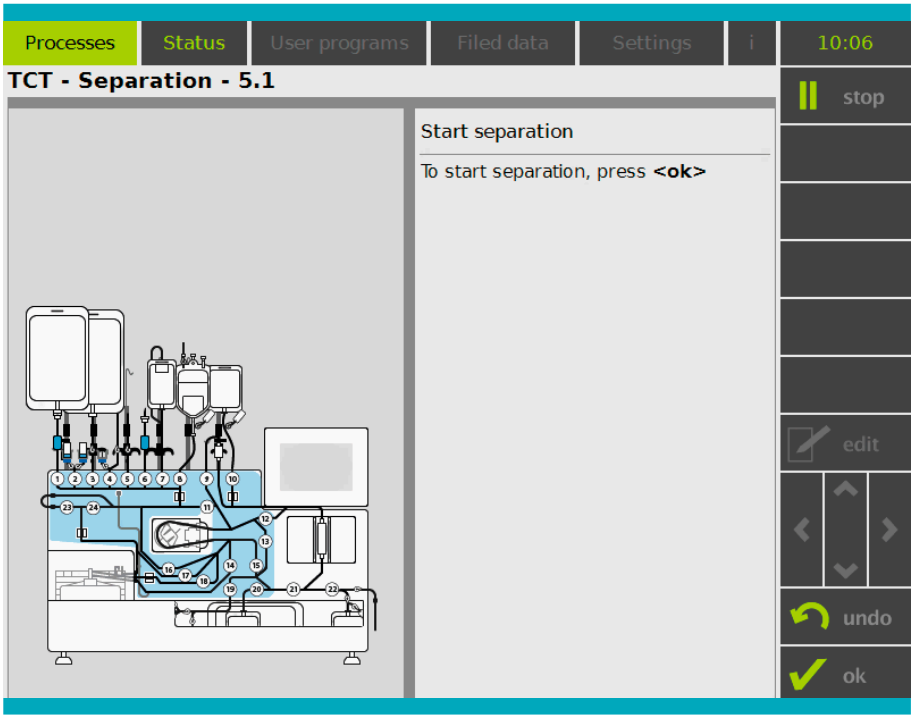
Screen 6.36: Final set-up check

The parameter list can be revised manually by choosing the parameter from the list and pressing **edit**. The keyboard will be displayed on the screen.

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

6.6 Automated enrichment process

6.6.1 Start of enrichment process



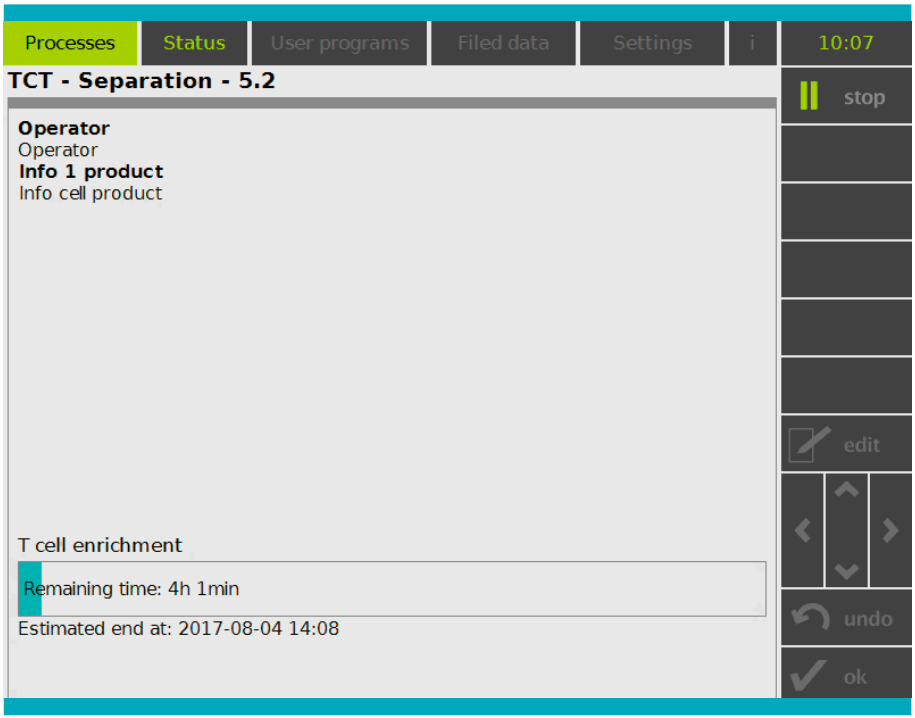
Screen 6.37: Start of automated enrichment process

Note: After pressing **ok**, a pop-up window will ask the user to confirm the start of the enrichment process by pressing **yes**.

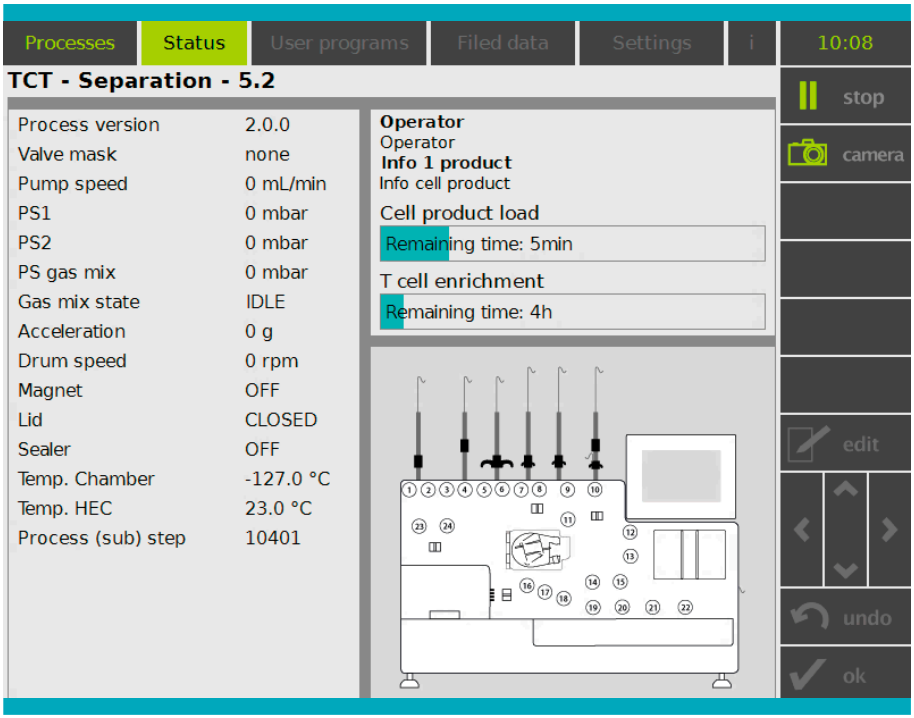
The fully automated enrichment process is divided into several phases:

1) Sample loading
2) Red blood cell reduction
3) Washing/volume adjustment
4) Labeling
5) Removal of excess reagent
6) Cell filtration
7) Magnetic separation
8) Elution

The different phases can be monitored during the process by pressing **Status** on the menu bar. Based on the screens displayed, the phases can be followed during the automated enrichment process. For details, refer to CliniMACS Prodigy User Manual (Instrument).



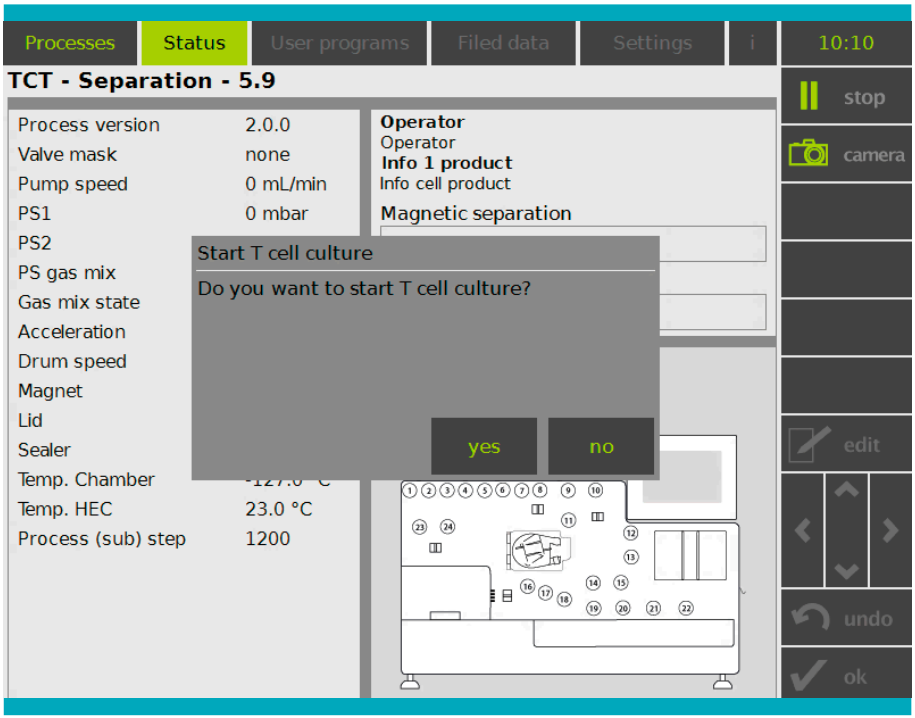
Screen 6.38: (Processes): Generic information screen



Screen 6.39: (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.

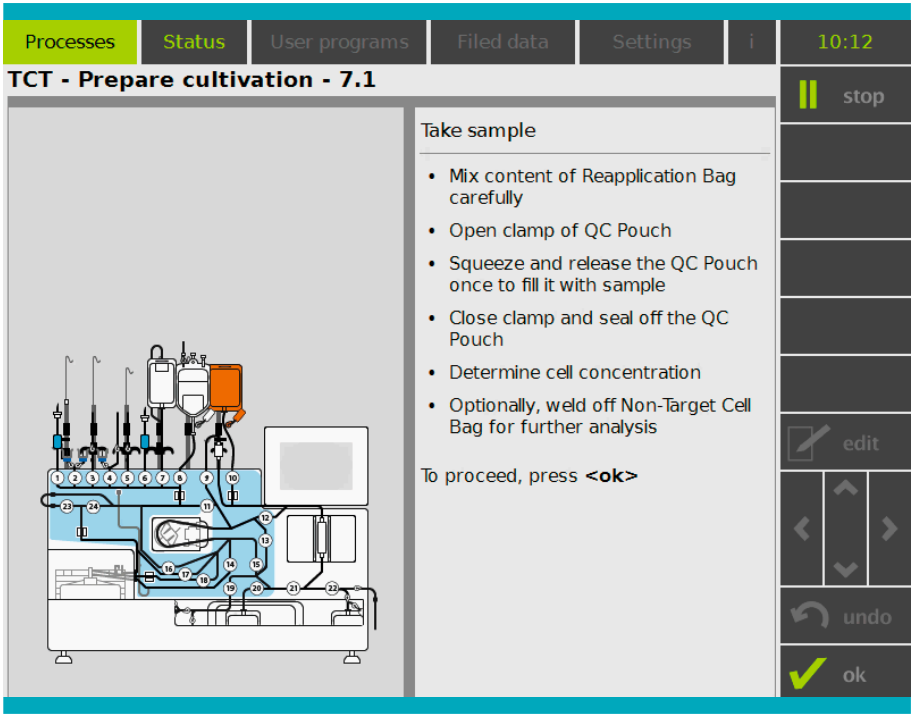


Screen 6.40: (Status): End of enrichment process

If cultivation is to be performed, press **yes** and proceed to the analysis of the target cell fraction (section 6.6.3).

If no cultivation is to be performed press **no** and proceed to final handling (see section 7.5). The Reapplication Bag above valve 10 contains the enriched target cell fraction. Weld off this bag to retrieve the enriched cells.

6.6.3 Analysis of enriched target cell fraction



Screen 6.41: Removal of sample from target cell fraction

For analysis of the enriched target cell fraction, a sample can be taken from the Reapplication Bag using the QC Pouch: After opening the clamp, the QC Pouch is sufficiently filled with sample by squeezing and releasing the pouch once to obtain a QC sample. Multiple squeezes and releases will increase the volume in the QC Pouch. The maximum volume of the QC Pouch is 4 mL. Close the clamp at the top end of the tubing and seal off the tubing. After the QC Pouch is sealed off, the QC sample can be analyzed regarding the concentration of the target cells.

The enriched target cells should be examined with regard to quality and quantity in view of their intended use. All QC samples should be stored at +4 °C to +8 °C (+39 °F to +46 °F) and analyzed according to the institution's standard operating procedures.

Additionally, the non-target-cell fraction can be analyzed. To do so, weld off the Non-Target Cell Bag below valve 20.

7

Cultivation of T Cell

7.1 General remarks

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

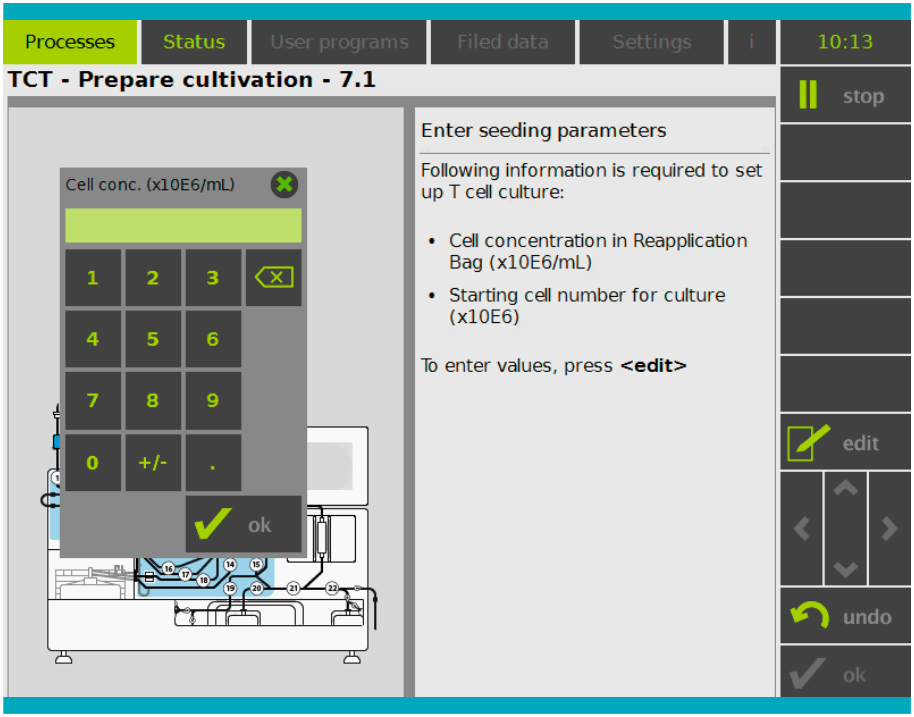
> *Every 10 hours, the tubing set is rinsed with 10 mL of medium connected to valve 3.* ***Therefore, sufficient medium should always be connected to the tubing on valve 3*** *(e.g. if the device is running for 72 hours, a minimum of 80 mL medium has to be connected).*

Each Waste Bag connected to the CliniMACS Prodigy TS 520 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 Bags are 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 Process. Each screen displays the instructions supported by a picture. At any step during the setup the **undo** key may be pressed to return to previous steps.

7.2.1 Input of cell seeding parameters



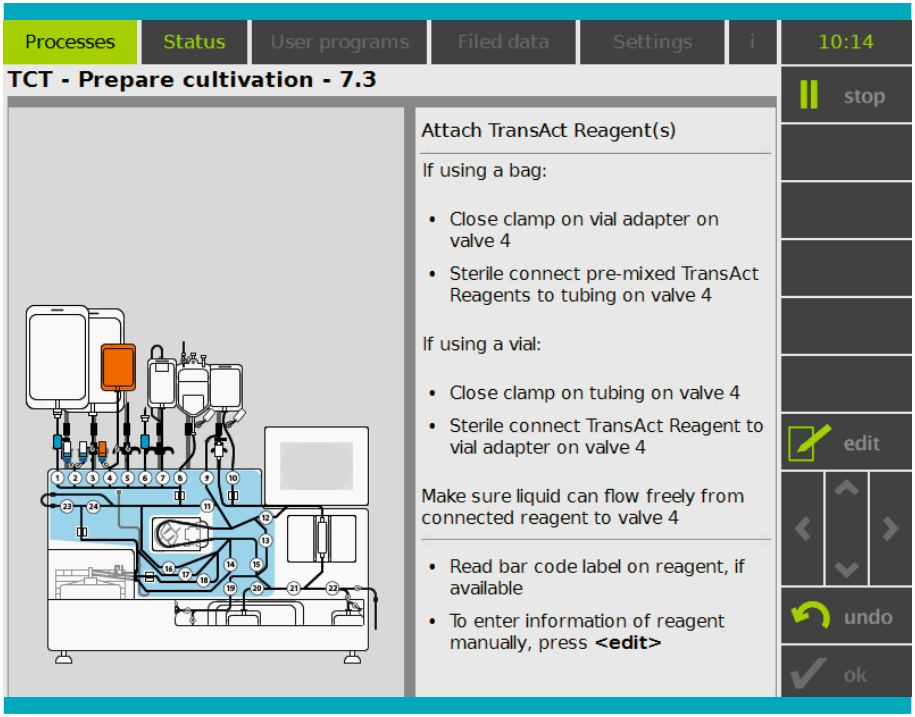
Screen 7.1: Input of seeding parameters

Enter the cell concentration of the enriched target cell fraction in the Reapplication Bag determined in section 6.6.3 and the total number of cells to be seeded.

It is recommended to seed a total cell number of 100×10^6. This corresponds to an approximate cell seeding density of 1×10^6 cells per cm^2. The volume to be transferred is automatically calculated.

Note: The remaining enriched target cells can be retrieved (e.g. to be frozen as back-up) by sealing off the Reapplication Bag **after the automatic setup of cultivation is completed** (see section 7.2.7).

7.2.2 Connection of activation reagent

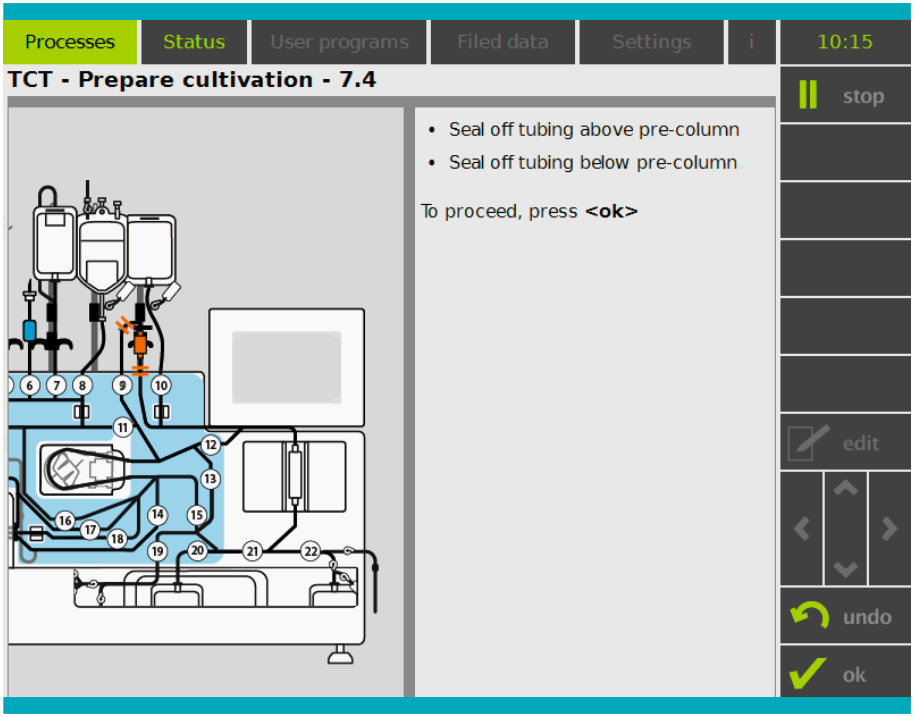


Screen 7.2: Connection of the activation reagent

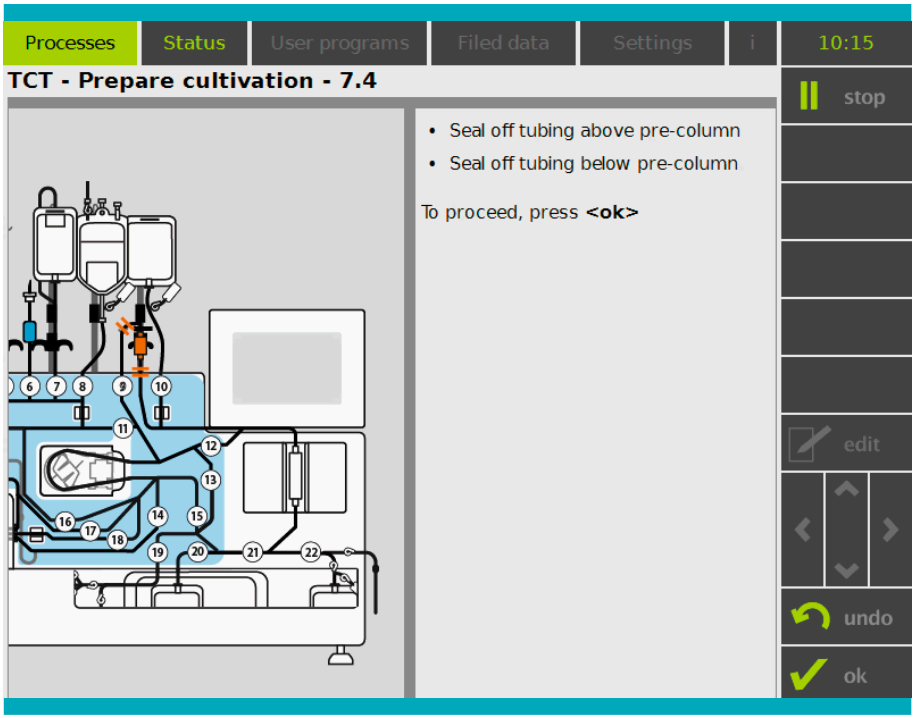
Connect the MACS GMP T Cell TransAct vial following the instructions on Screen 7.3. Read the bar code label on the reagent vial(s) or press **edit** and enter part number(s) (P/N) and batch code(s) (LOT) manually by using the keyboard displayed on the screen. Choose the TransAct format on the following popup, for MACS GMP T Cell TransAct choose **vial**.

7.2.3 Sealing off the pre-column and separation column

In order to avoid diffusion of cells from the columns during the cultivation step, the pre-column and the separation column of the mounted tubing set should be sealed off or clamped off as shown on Screen 7.4 and Screen 7.5.



Screen 7.3: Sealing off the pre-column

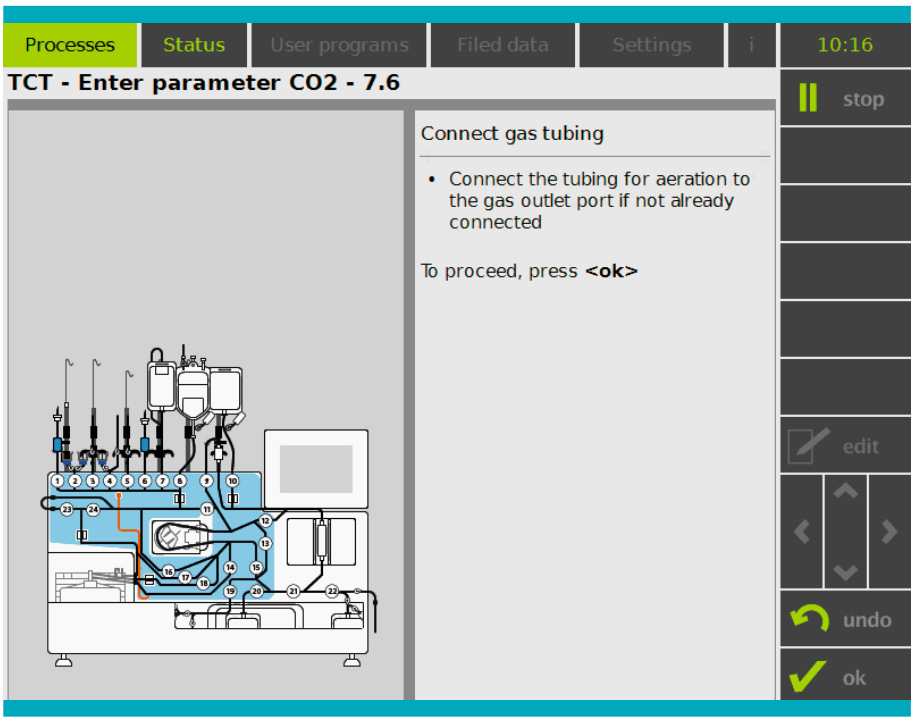


Screen 7.4: Sealing off the separation column

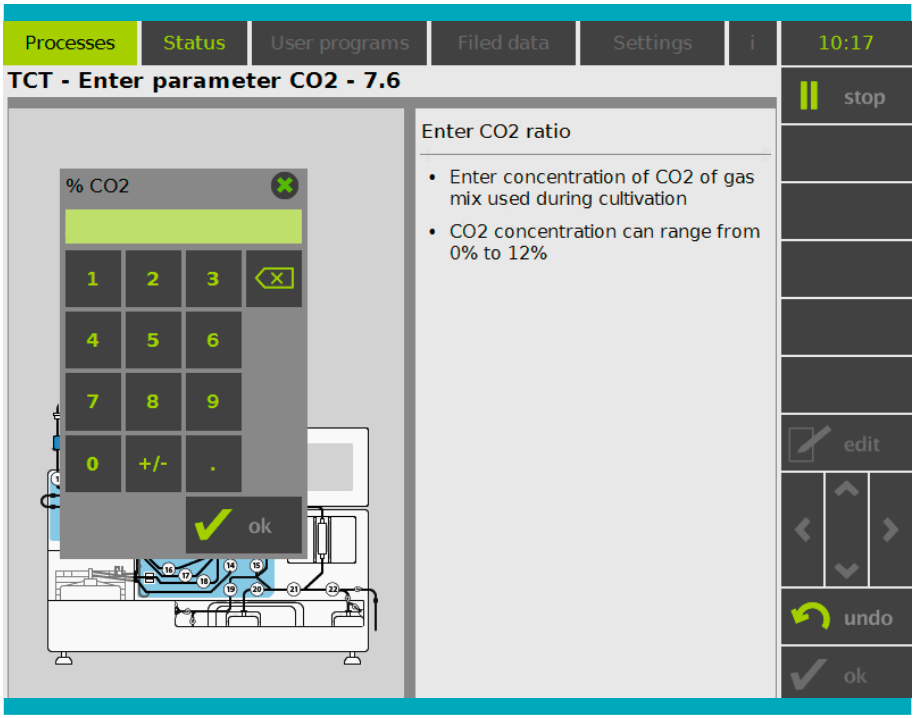
7.2.4 Input of CO2 and temperature parameters

If running the full process (case 1), the gas tubing has already been connected (see section 6.3.2), press **ok** to proceed. For the cultivation of the cells, the values for CO_2 concentration has to be entered. For TexMACS GMP Medium enter **5% CO_2**.

The temperature parameter only has to be entered manually, if the instrument has no temperature offset values. In this case, a warning will appear stating that no temperature offset values could be detected on the instrument. It is recommended to receive a temperature adjustment and validation procedure for the instrument, contact Miltenyi Biotec Technical Support. To continue without the temperature adjustment settings, confirm the warning and enter the temperature manually: Enter +39 °C (+102 °F) (or a value specifically calibrated for the instrument).



Screen 7.5: Connection of gas tubing

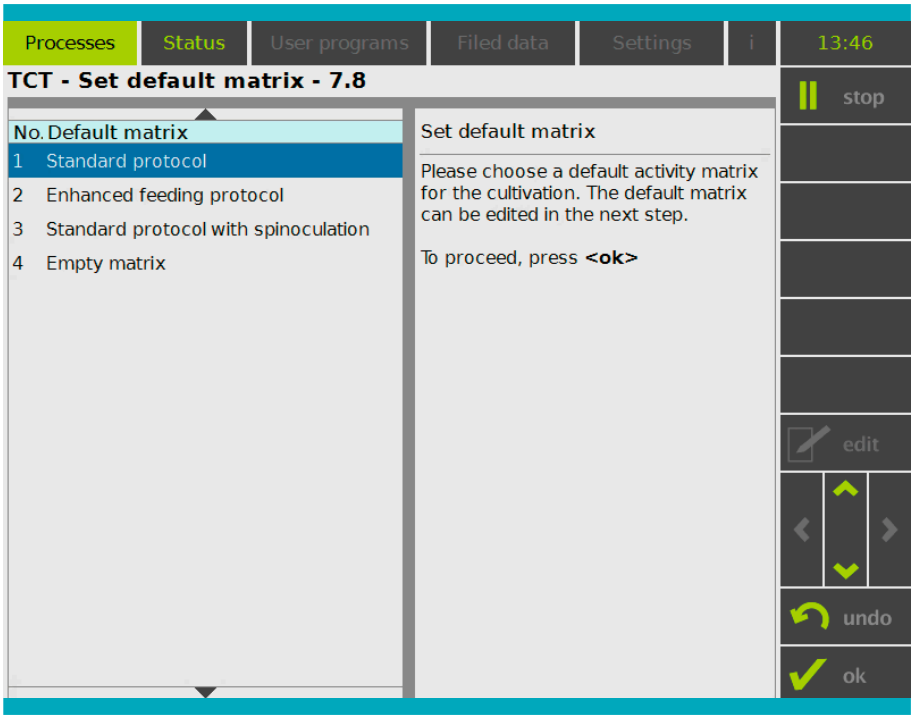


Screen 7.6: Input of CO_2 concentration

7.2.5 Setup of activity matrix

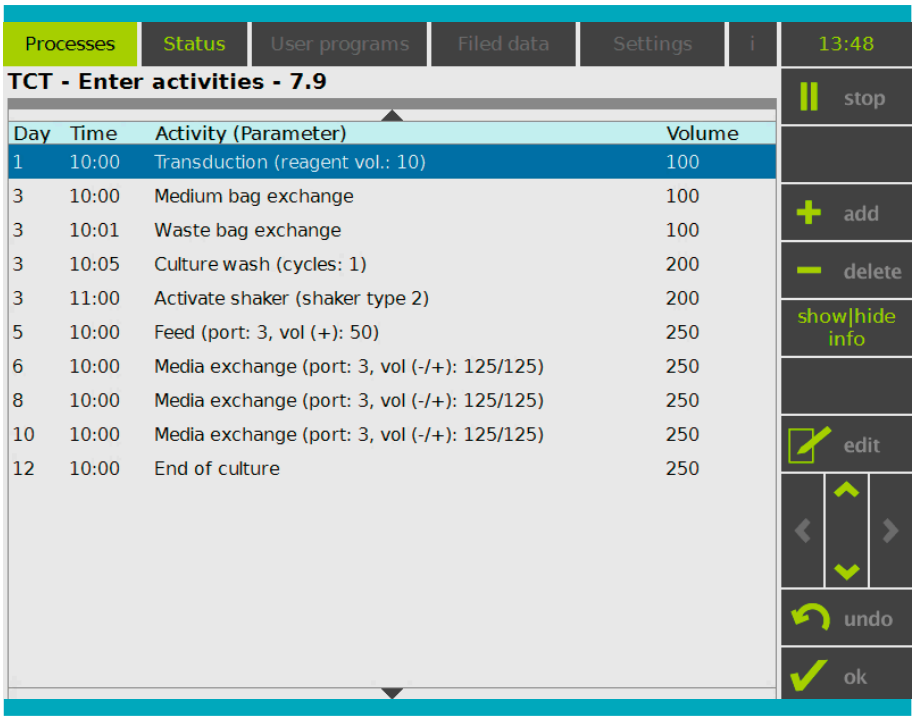
The activity matrix of the T Cell Transduction Process allows customized programming of T cell cultivation steps.

Choose the default activity matrix best suiting the needs of your application, which can later be adapted accordingly.



Screen 7.7: Set default matrix

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



Screen 7.8: Default activity matrix - Enhanced feeding protocol

When pressing **show/hide** info extra information on the entered parameters and instructions to edit set up of the activity matrix are displayed.

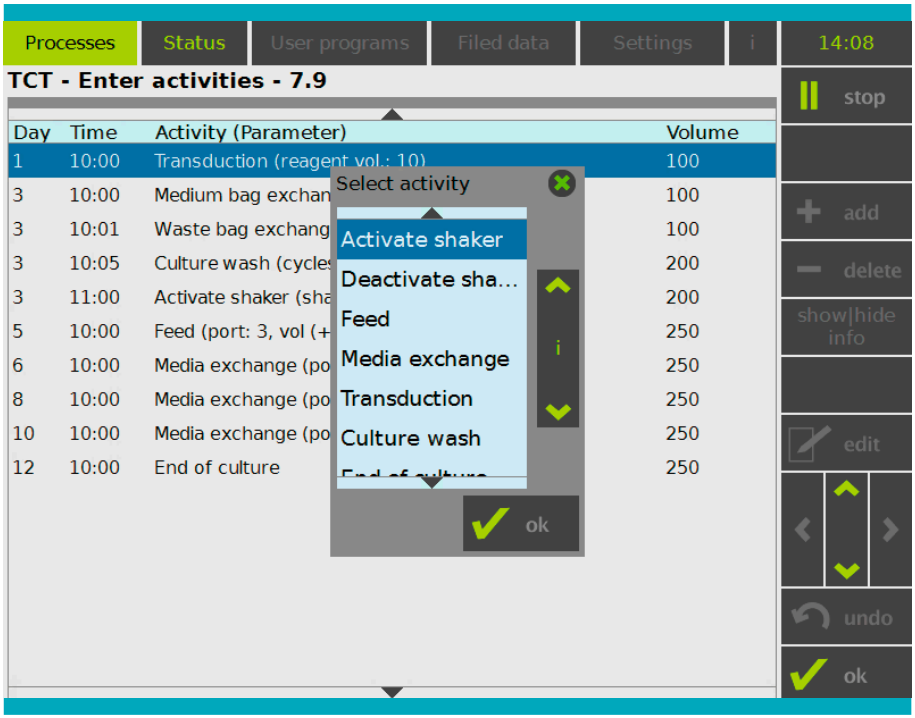
IMPORTANT

All steps programmed in the activity matrix are performed after the automated culture setup. The culture setup is performed automatically in one step (see section 7.2.7) and is not part of the activity matrix. It cannot be modified.

After the automated culture setup the volume in the chamber will be approximately 70 mL. The different activities that will be performed from this point on have an influence on the volume in the chamber. This volume is theoretically calculated in the activity matrix in the column “Volume”. The total volume in the chamber has to be maintained between 10 mL and 250 mL. If these values are exceeded, during the setup of the activity matrix, a warning will be displayed at the final confirmation step.

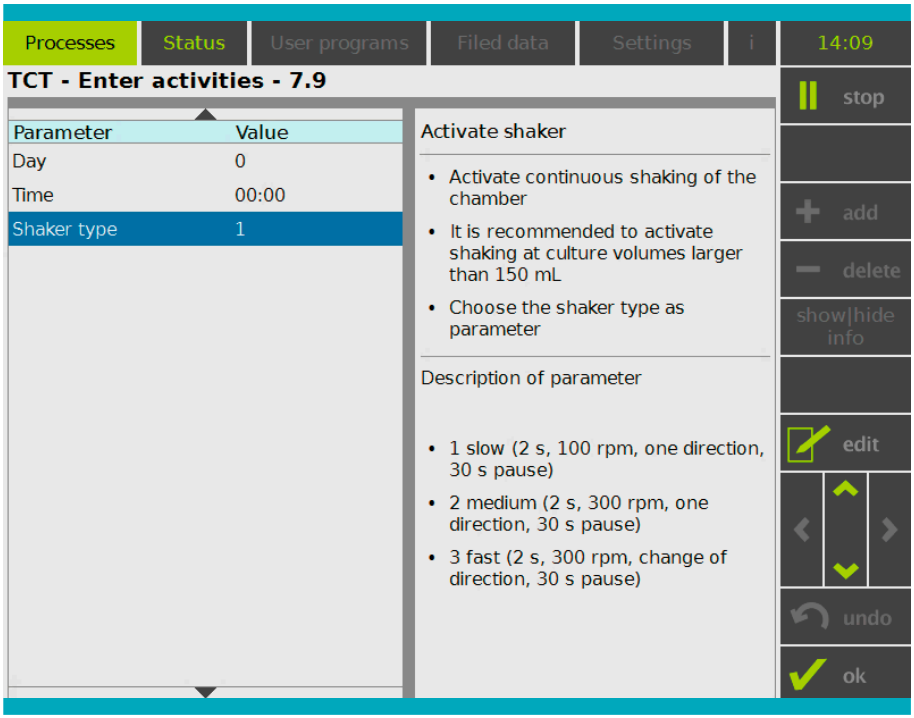
Activity	Description	Remark
Activate shaker	Start a continuous shaking of the chamber for suspension culture	Continuous in background
Deactivate shaker	Switches off a previously activated shaker	Automatic execution
Feed	Add fresh media	Automatic execution
Media exchange	Remove old media and add fresh media	Automatic execution
Volume reduction	Volume reduction of culture, e.g., to be used before transduction with large viral vector volumes	Automatic execution
CliniMACS PBS/ EDTA Buffer	3 liters	Use 1×3,000 mL bag
Spin	Centrifugation step to be used after addition of viral vector for spinoculation protocol	Automatic execution
Culture wash	Wash of culture to remove TransAct and/or viral vector	Automatic execution
Medium bag exchange	Prompt and user guidance to exchange medium bag	User confirmation/ Interaction upon execution required
Waste bag exchange	Prompt and user guidance to exchange waste bag	User confirmation/ Interaction upon execution required
End of culture	Wash and re-suspend the cells in formulation solution	User confirmation/ Interaction upon execution required

Table 7.1: Available activities for cultivation



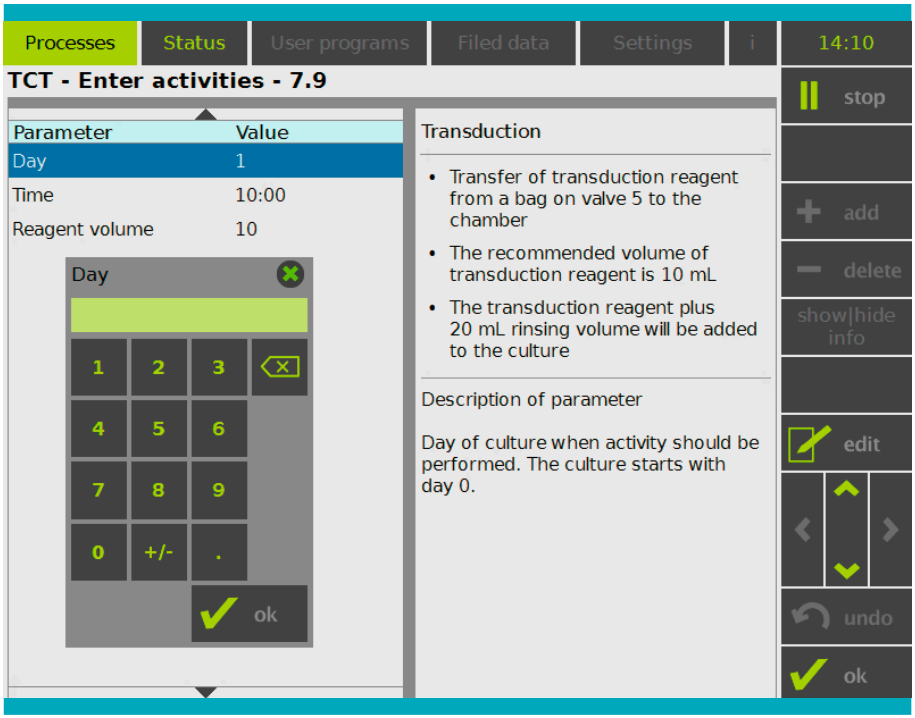
Screen 7.9: Addition of an activity

To add an activity, press **add** and select the activity from the pop-up list.



Screen 7.10: Addition of an activity

A screen specific for that activity opens, where the parameters of this activity can be changed by pressing **edit**. To confirm and to return to the matrix press **ok**.



Screen 7.11: Editing of parameter "Transduction"

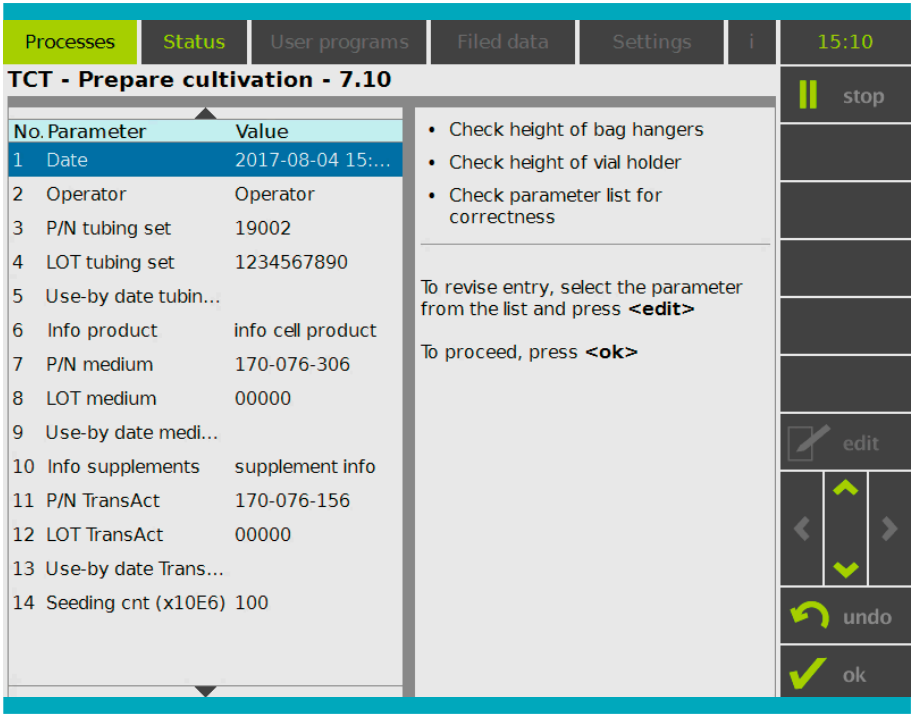
To edit an activity, select the activity of choice and press **edit**. A screen specific for that activity opens, where the parameters of the activity can be changed by pressing **edit**. To confirm and to return to the matrix press **ok**.

Note: When planning the process, consider that user interaction is required prior to the execution of the activities **"Transduction", "Medium bag exchange", "Waste bag exchange",** and **"End of culture"** (see Table 6.1).

Note: The replacement of the medium bag should be planned according to the activity matrix and the corresponding usage of medium. The exchange of the Waste Bags should be planned depending on the activity matrix and the corresponding filling of the Waste Bags.

When the activity matrix is created and all editing is completed, proceed to the final setup check by pressing **ok** and –for confirmation– by pressing **yes**.

7.2.6 Final setup check



Screen 7.12: Final setup check

The user is asked to check the height of the bag hangers and the vial holders as well as the parameter list. The parameter list can be revised manually by choosing the relevant parameter from the list and pressing **edit**.

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

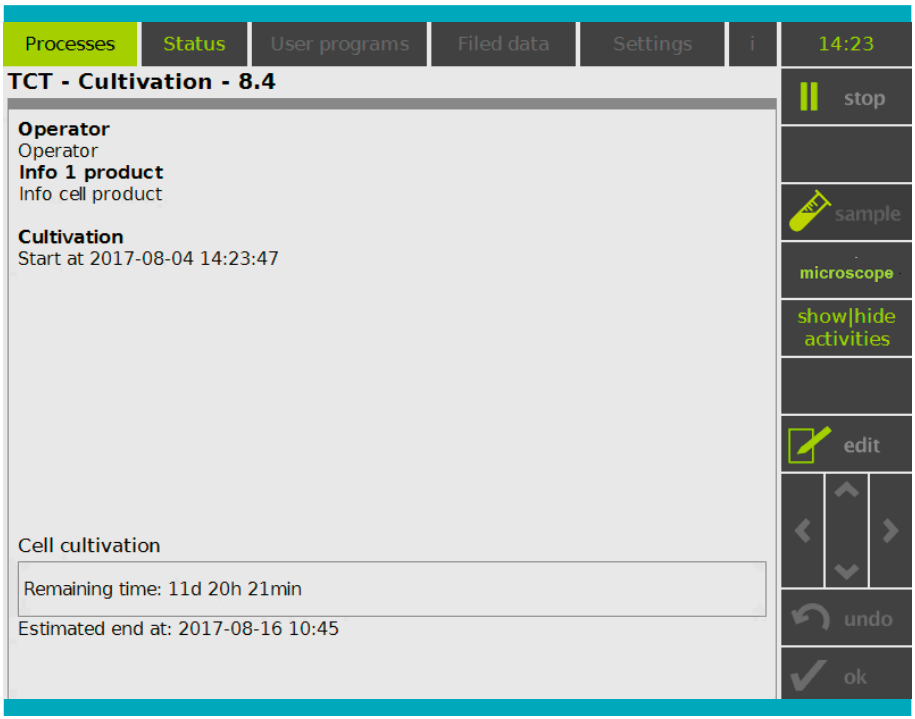
After pressing **ok** to proceed and confirming the setup by pressing **yes**, the automated culture setup is initialized (see Screen 8.4).

7.2.7 Automated culture setup

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

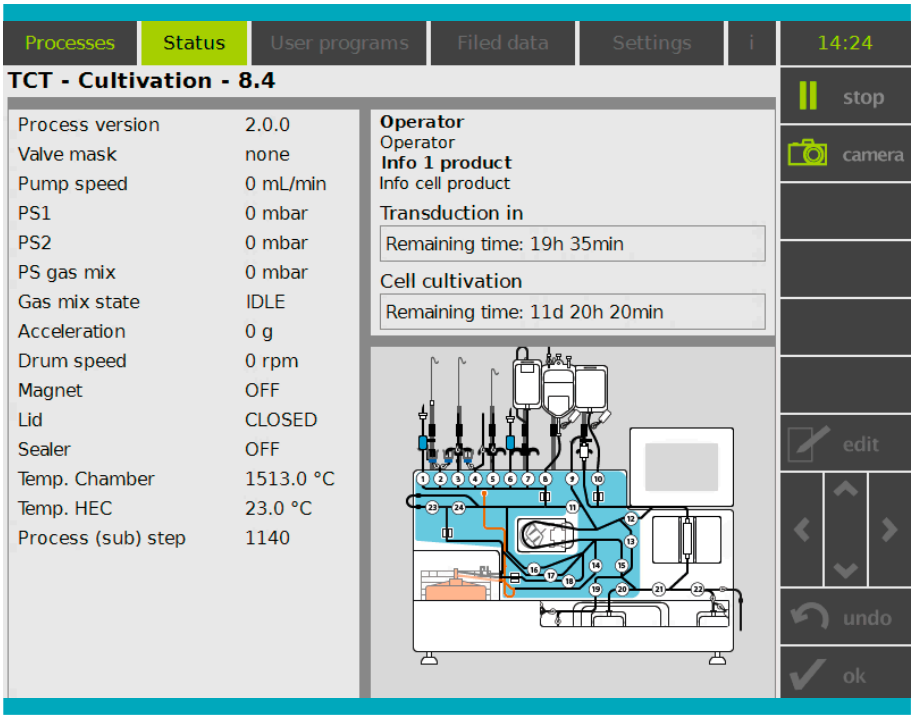
- rinsing and priming of the chamber and the tubing set with medium
- transfer of the selected amount of cells to the chamber
- washing of the cells with medium
- transfer of TransAct Reagent to the chamber
- setup of aeration and temperature

7.3 Cultivation



Screen 7.13: Cultivation (Processes)

During the cultivation, the activity matrix can be changed by pressing **edit**. To show the current activity matrix, press the **show/hide** button. Furthermore it is possible to make a photographic record of cells by pressing the **microscope** button. To take a sample during cultivation, press sample. For details refer to the following sections.



Screen 7.14: Cell cultivation (Status)

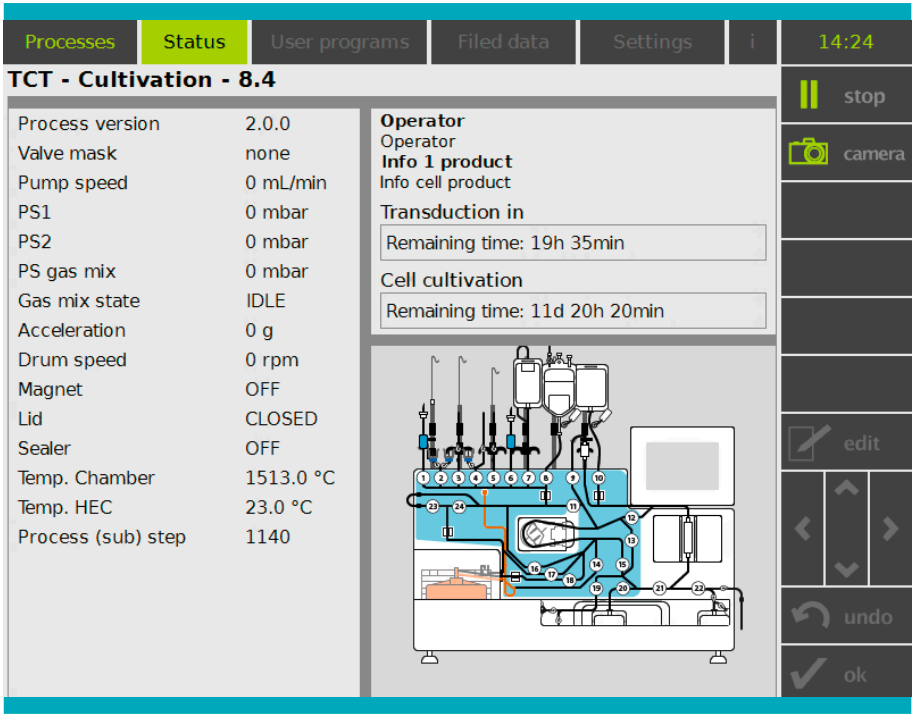
After initial setup of the cultivation, the monitor displays the current step and the remaining time of the cultivation process in the **Status** screen.

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 7.15: Taking a sample

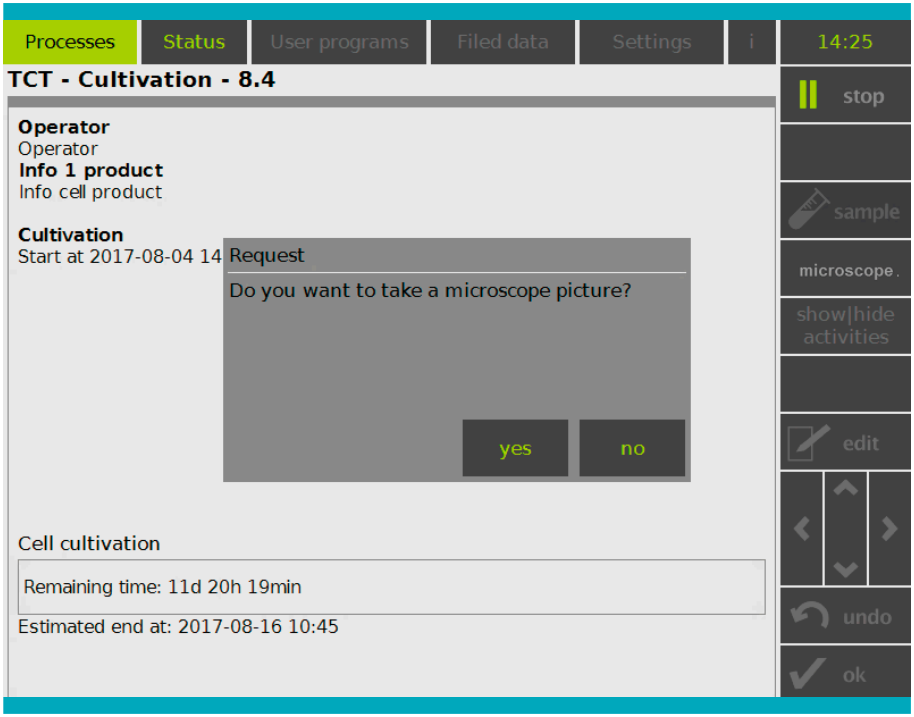
To take a sample from the chamber during cultivation, change to the **Processes** screen and press the button **sample**. Follow the instructions in the pop-ups.

Note: Hold the sampling pouch with the sterile filter facing up to prevent filter clogging.

After the sampling is completed, weld off the pouch and collect the sample by unscrewing the sterile filter.

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

7.3.3 Taking microscope pictures during cultivation



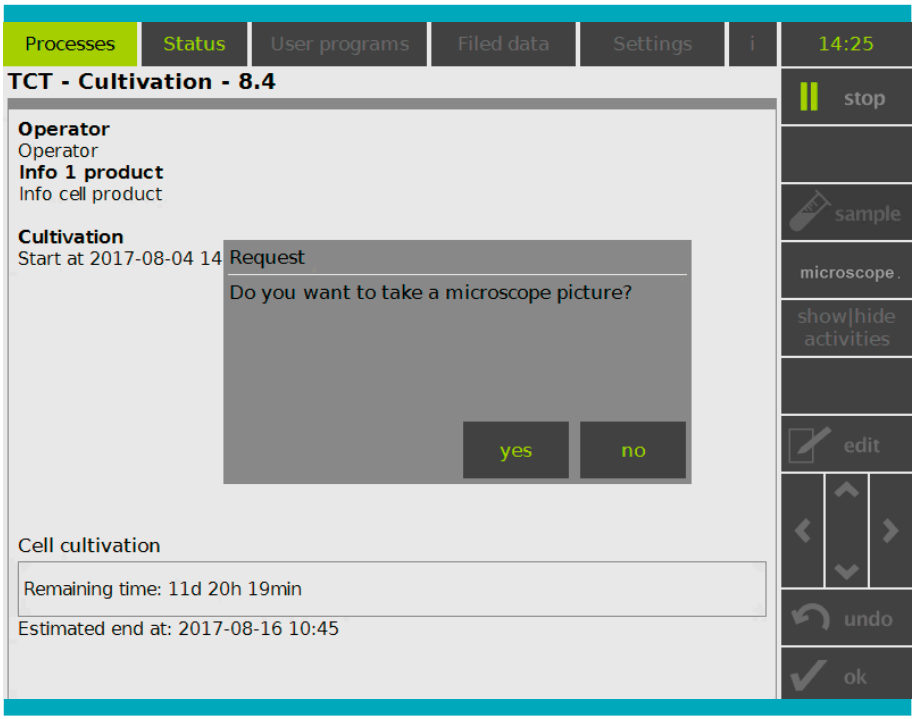
Screen 7.16: Taking microscope picture

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

- Press **Processes**.
- Press the button **microscope**.
- Confirm taking microscope picture by pressing **yes**.
- 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 due to the light dispersion and due to the fact that the cells are in suspension and not settled on the bottom of the chamber.

7.3.4 Transduction



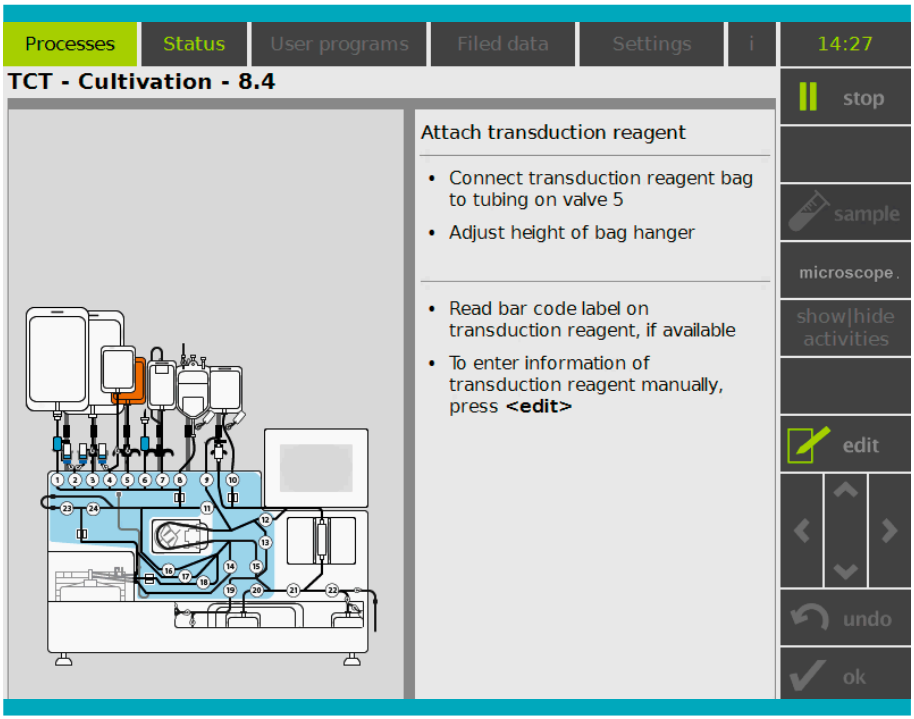
Screen 7.17: Transduction

Note: The activity **"Transduction"** will not start without confirmation by the user.

For transduction of the activated T cells, the activity "Transduction" has already been programmed into the activity matrix (see section 7.2.5). When the scheduled time point is reached, user interaction is required. Follow the instructions on the screen for connection of the viral vector bag.

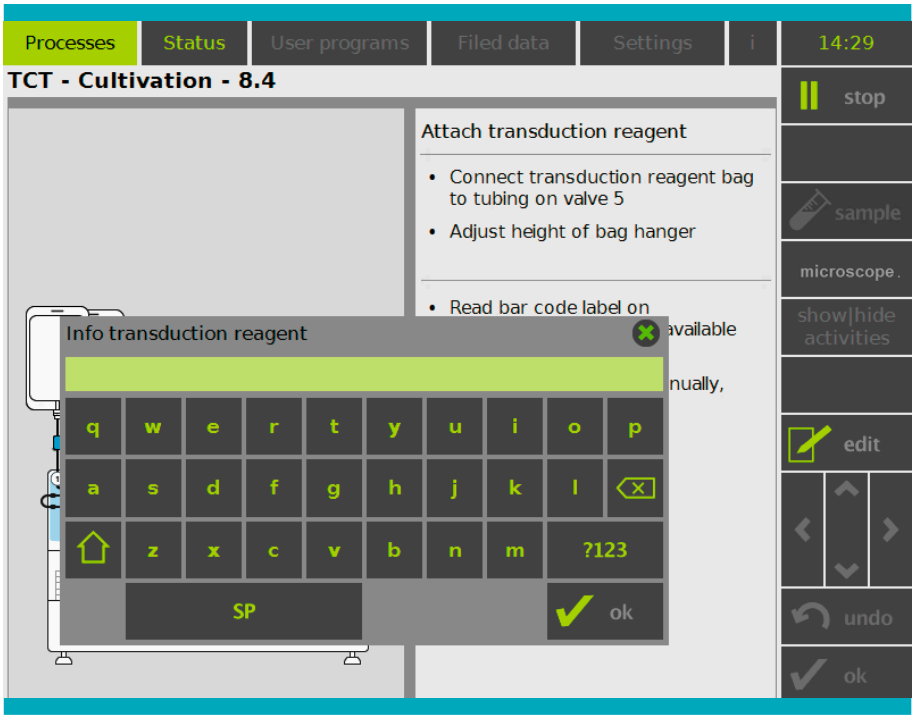
The viral vector should be prepared under sterile conditions in a bag that can be connected to the tubing set via sterile welding. The viral vector has to be in a volume between 5 mL and 100 mL. It is recommended to use concentrated viral vector in a volume of 10 mL. If the vector is in a much larger 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 7.18: Attachment of viral vector

Connect the viral vector to the tubing on valve 5 by using a sterile tubing welder.



Screen 7.19: Entering information on viral vector

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

7.3.5 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. When the programmed time point is reached, user interaction is required. Follow the instructions on the screen.

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 volume of 600, 900, and 1200 shall be available in the bag on valve 3. Use the activity “Medium bag exchange” to attach a new medium bag.

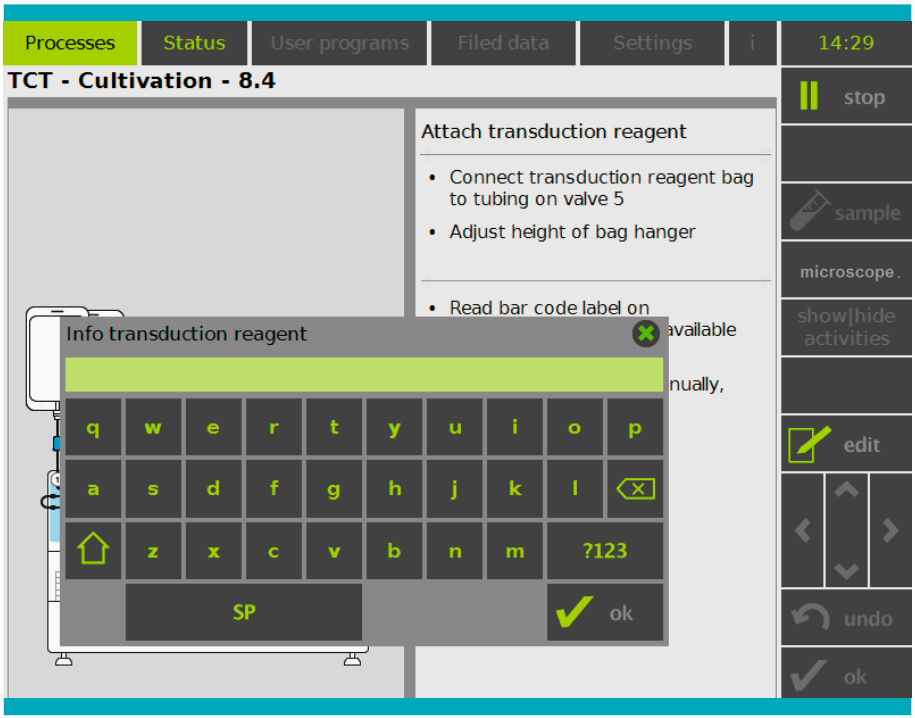
7.3.6 Volume reduction and spin

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

For the activity “Volume reduction” confirm there is sufficient medium connected to port 3 and for target volumes smaller than 30 mL also to the port selected in the activity (50 mL).

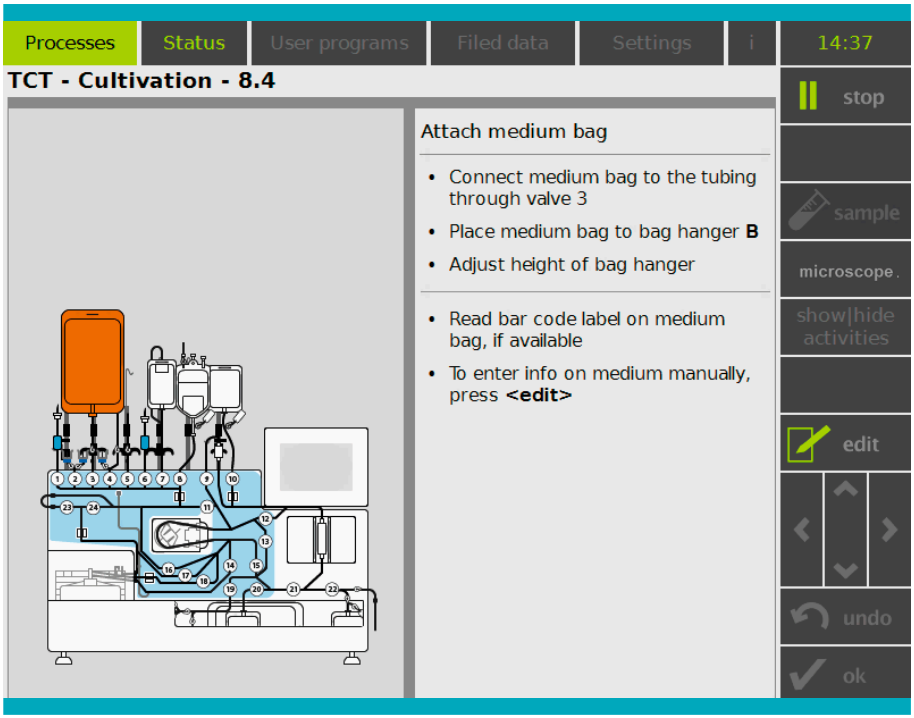
7.3.7 Medium and waste bag exchange

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



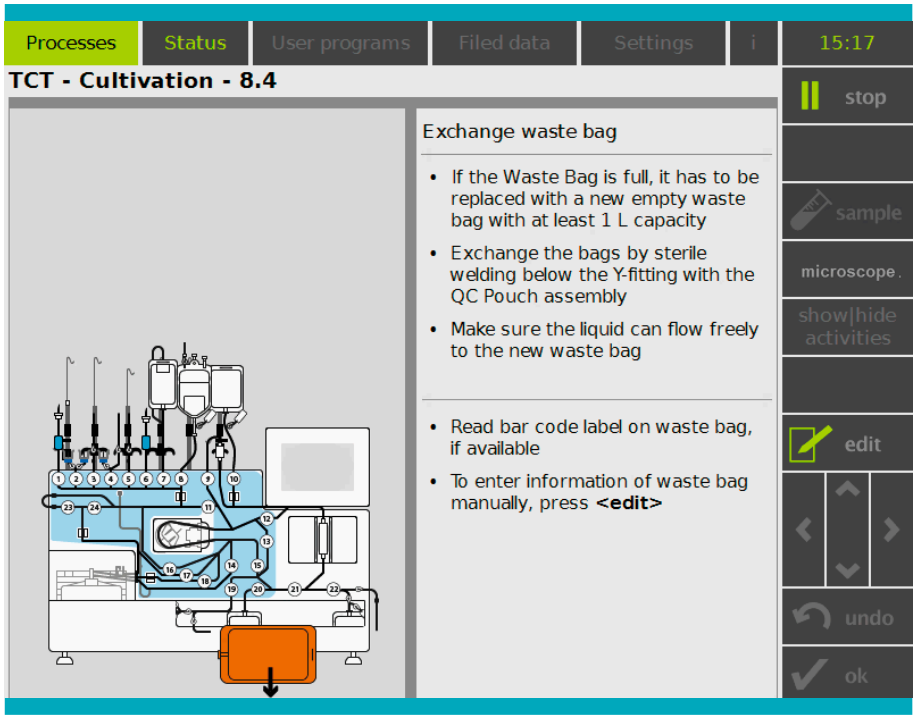
Screen 7.20: Start of activity medium bag exchange and waste bag exchange

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



Screen 7.21: 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 section 6.4.1). Read the bar code label on the medium bag or press **edit** to manually enter the information, then press **ok**. Information regarding supplements can be entered by pressing **edit**. Confirm entered values with **ok** to complete the activity and return to cultivation. It is strongly recommended that the user monitors the medium bag capacity to prevent any T cell transduction errors.



Screen 7.22: 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. Read the bar code label on the waste bag or press **edit** to manually enter the information, then press ok. Confirm entered values with **ok** to complete the activity and return to cultivation. Depending on the cultivation protocol a waste bag exchange may not be required. Ensure waste liquid can flow freely to available waste bag in use.

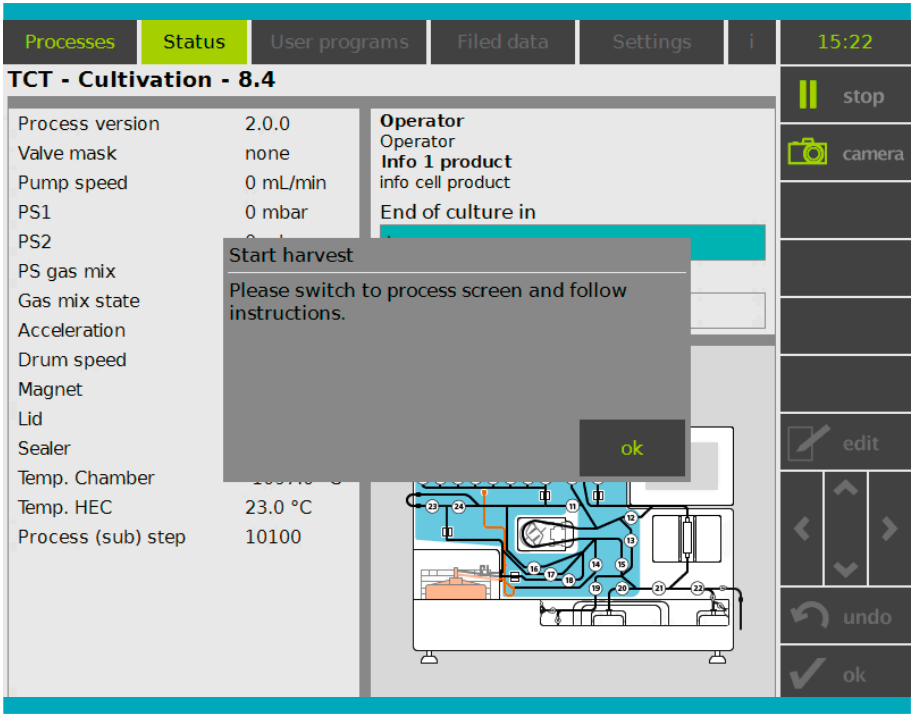
7.3.8 Feeding/Exchange of media

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

When the programmed time for activity “Feed” and “Media exchange” is reached (see section 7.2.5), 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.

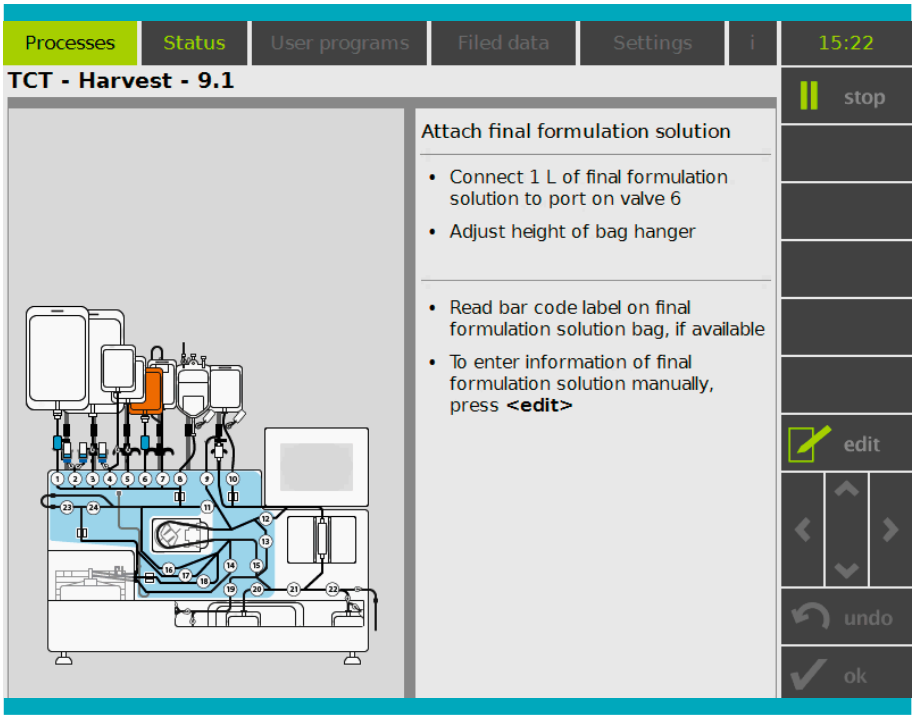
7.4 Formulation and harvest



Screen 7.23: Harvesting of final cell product

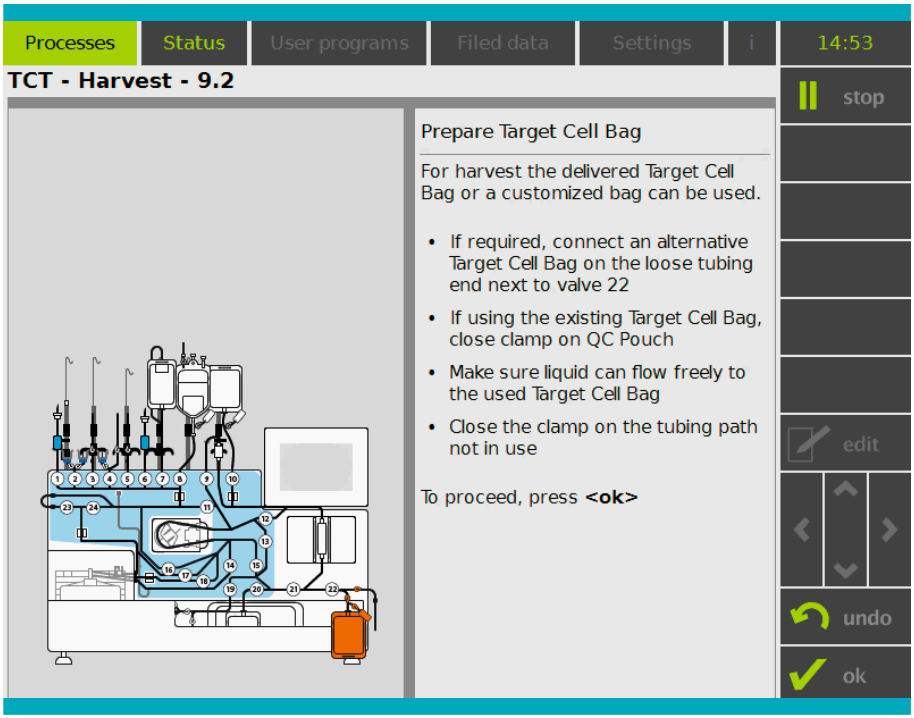
Note: The **harvest** will **not** start without confirmation by the user. Immediately start the harvest when asked in the pop-up.

When the programmed time for “End of culture” is reached (see section 7.2.5) user interaction is required. Prepare 1 liter of formulation solution (e.g. sodium chloride solution for injection with 0.5% HSA) and follow the instructions on the screen.



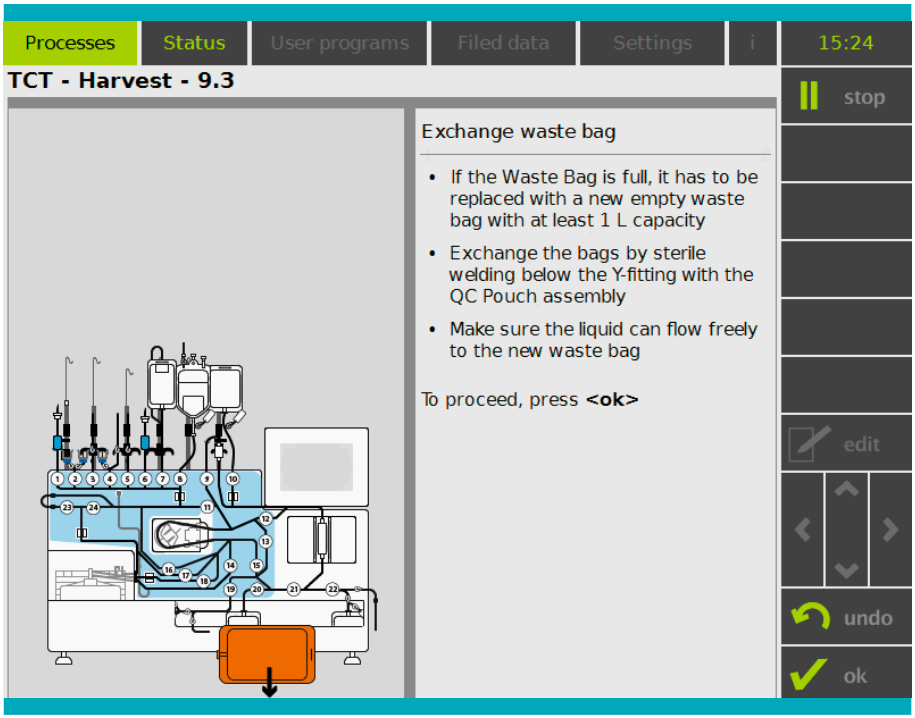
Screen 7.24: Attachment of formulation solution

Connect the formulation solution to the spike port on valve 6. Read the bar code label on the formulation solution bag or press **edit** to manually enter the information. If using the in-line filter, replace the separation column that has been sealed and removed from the magnet unit with the in-line filter. Continue by pressing **ok**.



Screen 7.25: Preparation of Target Cell Bags

Filtration is an option when harvesting target cells. This is only available with the pre-assembled in-line filter connected to the designated Target Cell Bag. If using the in-line filter on the Target Cell Bag, make sure that the in-line filter is in an upright position. If an alternative Target Cell Bag is required, sterile connect alternative Target Cell Bag between valve 22 and the in-line filter. Close clamp for the Target Cell Bag that will not be used. Follow the instructions on the screen to prepare the Target Cell Bags. The volume of the final product will be 100 mL.

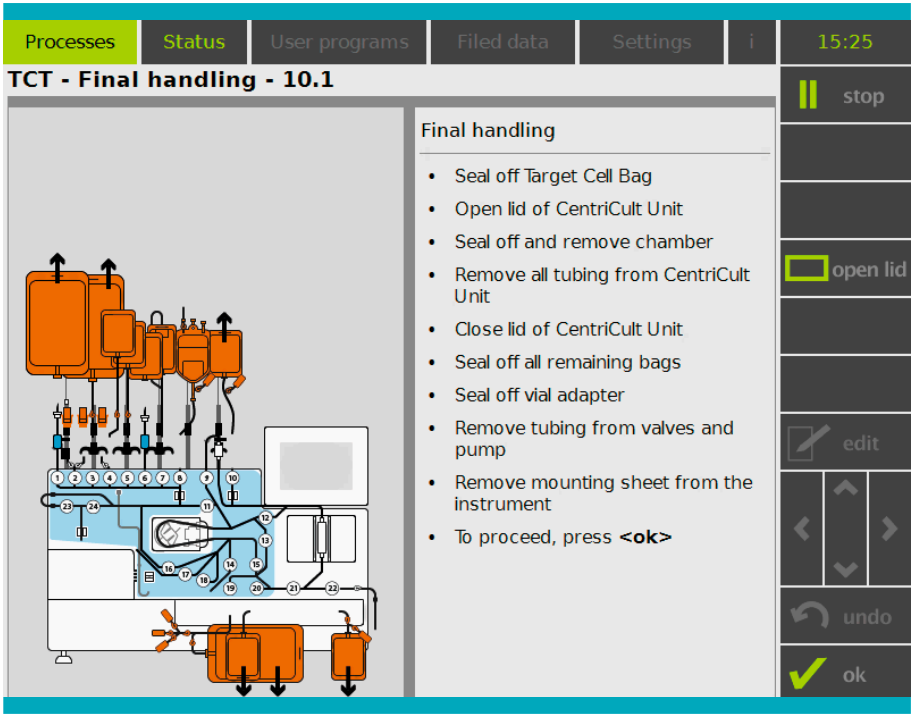


Screen 7.26: Exchange of waste bag

With the next screen the user is asked to exchange the Waste Bag. This is only necessary if the capacity of the current bag(s) is nearly reached and additional waste liquid capacity is required. In this case, connect a new empty waste bag via sterile welding. Follow the instruction given on Screen 9.3 “Exchange waste bag”. Continue by pressing **ok**. If not required, press directly **ok**.

The automated formulation 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 selected Target Cell Bag on valve 22.

7.5 Final handling



Screen 7.27: Final handling

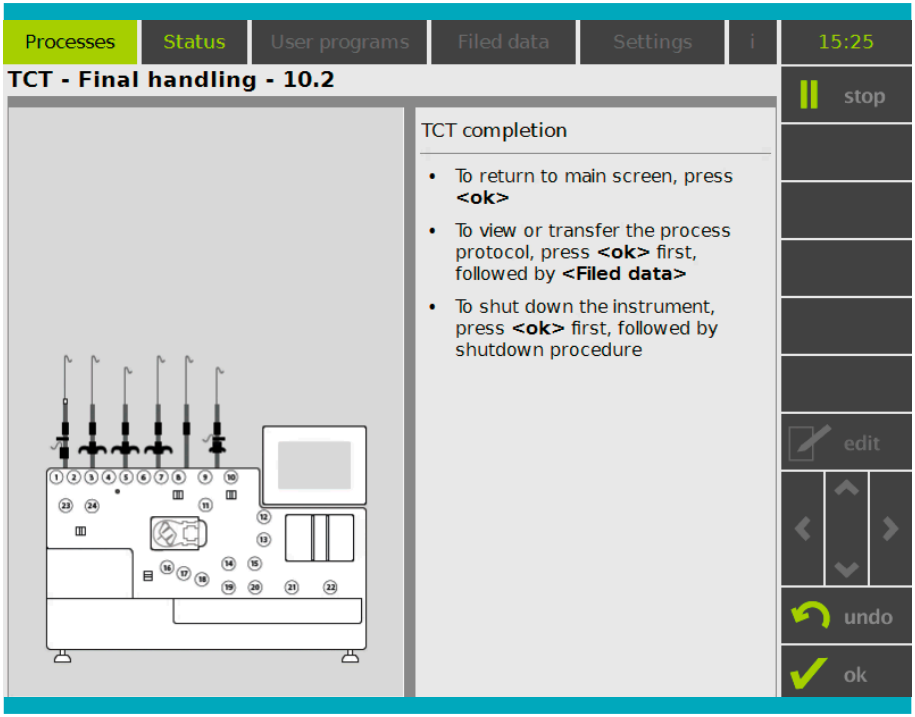
Seal off the Target Cell Bag containing the final product. Take a sample from the Target Cell Bag for further analysis. Store samples at +4 °C to +8 °C (+39 °F to +46 °F).

Note: If no cultivation was performed and the final handling takes place directly after enrichment (coming from section 6.6.2), seal off the Reapplication Bag containing the enriched target cells and the Non-Target Cell Bag. Take a sample from the Reapplication Bag and Non-Target Cell Bag for further analysis. Store samples at +4 °C to +8 °C (+39 °F to +46 °F).

Follow the instructions given on the screen to remove the tubing set and to finalize the process. 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 tool 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 7.28: Process completion

The process protocol is stored in **Filed data** and can be transferred to other media by using 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 it is recommended to follow the institution's standard procedures for the treatment of infectious materials.

The target cells should be examined regarding quality and quantity in view of 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 T Cell Transduction Process 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, process failure, or the necessity of cell rescue, contact the Miltenyi Biotec Technical Support:

+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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38024/06 | Issued: 2023-04