Miltenyi Biotec

CliniMACS Prodigy®
HSC Enrichment 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 CliniMACS Tubing Set LS, and the CliniMACS PBS/EDTA Buffer, is FDA approved as a Humanitarian Use Device (HUD), authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this indication has not been demonstrated. Other products of the CliniMACS Product Line are available for use only under an approved Investigational New Drug (IND) application, Investigational Device Exemption (IDE) or FDA approval. CR/GMP products are for *ex vivo* cell processing only, and are not intended for human *in vivo* applications. For the manufacture and use of cellular products, national and international legislation and regulations must be followed. Unless otherwise specifically indicated, Miltenyi Biotec products and services are for research use only and not for therapeutic or diagnostic use.

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

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

www.miltenyibiotec.com

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

Essential information

This user manual provides information for the use of the CliniMACS Prodigy HSC Enrichment 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 cellular starting products are described in the CliniMACS Prodigy user manual (instrument). For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Prodigy HSC Enrichment 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 users 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), section 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 user must adhere to all instructions and procedures at all times during the operation of the instrument, confirming that all safety information, warnings, precautions, and instructions are observed. Failure to follow the safety information, warnings, precautions, and instructions contained in the instructions for use can result in instrument malfunction, property damage, loss of target cells, personal injury, and/or death.

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

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1 Introduction

1.1 General information

The CliniMACS Prodigy HSC Enrichment System including the CliniMACS Prodigy, the CliniMACS CD34 Reagent, the CliniMACS Prodigy TS 320, and the CliniMACS PBS/EDTA Buffer is intended for the *in vitro* pre-enrichment of human CD34-positive cells from leukapheresis harvest as preparative step for the subsequent manipulation of these cells.

The CliniMACS Prodigy HSC Enrichment System uses CD34-monoclonal antibodies conjugated to super-paramagnetic beads. In an automated procedure, the CD34-positive cells are initially washed and afterwards specifically labeled by incubation with the CliniMACS CD34 Reagent. After the excess reagent has been removed, the antibody-labeled cell suspension is passed through the separation column in which strong magnetic gradients are generated. The separation column retains the magnetically labeled CD34-positive target cells while unwanted cells (non-target cells) flow through the column and are collected in the Non-Target Cell Bag. The magnetically labeled CD34-positive cells are released from the column by removing the magnetic field and eluting them into the Target Cell Bag.

1.2 Separation process

The labeling and enrichment procedure of CD34-expressing cells with the CliniMACS Prodigy HSC Enrichment System is automated and requires only minimal manual handling steps:

- Install the CliniMACS Prodigy TS 320 on the instrument.
- Attach process buffer and elution solution.
- Enter separation-related cell product information after priming of the tubing set.
- Transfer the starting cell product into the Application Bag.
- Transfer the required volume of CliniMACS CD34 Reagent and IgG solution (optional) into the dedicated reagent bags.
- Enter a dedicated process end time (optional).
- Mix Application Bag.

Once the process has been started, subsequent steps of the separation process are automated. After process completion, the target cells (CD34-enriched cells) are provided in elution solution, ready for further use.

Samples for quality control can be taken at the beginning and at the end of the process.

After process completion, only minimal handling steps are required to remove target and non-target fraction and to deinstall the tubing set.

Refer to section 6 ´Separation process´ for details on process setup and completion.

IMPORTANT
The CliniMACS Prodigy HSC Enrichment System is a scalable application. The volume of the starting cell product must be between 50 mL and 600 mL.

Normal-scale process:
The normal-scale process is capable of enriching up to 0.6×10^9 CD34-positive cells from up to 60×10^9 total white blood cells (WBC) using one vial of CliniMACS CD34 Reagent.

Large-scale process:
The large-scale process is capable of enriching up to 1.2×10^9 of CD34-positive cells from up to 120×10^9 total WBC using two vials of CliniMACS CD34 Reagent.

2
Glossary

2.1 Graphical depiction

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

⚠ WARNING

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

⚠ CAUTION

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

NOTICE

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

IMPORTANT

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

2.2 Glossary of symbols and terms

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

3 Materials

The following section outlines the materials and consumables that are required for use of the CliniMACS Prodigy HSC Enrichment System.

3.1 System components

The following CliniMACS Materials are required:

CliniMACS Materials	Quantity	
	Normal scale	Large scale
CliniMACS Prodigy	1	1
CliniMACS CD34 Reagent	1	2
CliniMACS Prodigy TS 320	1	1
CliniMACS PBS/EDTA Buffer, 3L bags	2	3

Table 3.1: CliniMACS Materials required

3.2 Accessories

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

CliniMACS Accessories	Quantity		Comment
	Normal scale	Large scale	
Transfer Set Coupler/Coupler	1	2	Additionally others, if necessary for connection of elution solution

Table 3.2: CliniMACS Accessories required

3.3 Additional materials and equipment

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

Materials & equipment
Human serum albumin (HSA), pharmaceutical grade, to be added to the CliniMACS PBS/EDTA Buffer and to the elution solution to a final concentration of 1.0% (w/v)
Elution solution (500 mL, e.g., sodium chloride solution, for infusion), preferably in bag
Transfer Bag(s) 600 mL (optional)
5% IgG solution (10 mL) (optional)
Appropriate Luer Lock syringes (10 mL, 30 mL, and 50 mL) and hypodermic 20 gauge needles
Uninterruptable power supply unit (optional)
Sterile docking device
Cell counter
Flow cytometer

Table 3.3: Additional materials and equipment required

IMPORTANT

This protocol requires the use of components and materials which are not part of the CliniMACS Prodigy HSC Enrichment System (e.g., human serum albumin [HSA]). Therefore, either components, e.g., elution buffer and HSA, which are registered as drugs in the country of the user must be used or, if not available as registered drugs, all risks arising from these components must be evaluated by the user before application of the target cell product to humans. Special attention should be given to the stability of the target cell product in the chosen elution solution.

4

Warnings and precautions

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

Instructions, warnings, precautions, and other important information for the use of the CliniMACS Prodigy as well as warnings and precautions concerning the handling of biohazardous materials and 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 HSC Enrichment System components, refer to the instructions for use provided for the respective component.

5 Preparative steps

The enrichment of CD34-expressing cells with the CliniMACS Prodigy HSC Enrichment System is automated and requires only a few preparative steps.

⚠ WARNING

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

5.1 Testing of the starting cell product

⚠ WARNING

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

The leukapheresis product used for the process needs to be tested for the following parameters:

- WBC concentration ($\times 10^6$/mL)
- frequency of CD34-positive cells (%)
- cell product volume (mL)

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

To test the starting cell product, a fluorochrome staining for CD45 and CD34 is recommended. The percentage of all CD34-positive cells is used for parameter input. Contact Miltenyi Biotec Technical Support for analysis support, if required.

⚠ CAUTION

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

Dead cell exclusion must not be performed when determining WBC concentration and frequency of labeled cells. All CD34-positive cells are to be included irrespective of potential non-specific binding of fluorochrome antibodies. Based on the cell parameter input, total WBC and labeled cell numbers are automatically calculated and the appropriate process scale is indicated.

5.2 Preparation of process buffer

Six liters (2×3L) CliniMACS PBS/EDTA Buffer are required for the normal-scale process while nine liters (3×3L) are required for the large-scale process. Refer to section 1.2 ´Separation process´ for normal and large-scale process specifications. Supplement the required amount of CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 1.0% (w/v), i.e., add 50 mL of 20% HSA per liter of buffer. The HSA-supplemented buffer is henceforth referred to as "process buffer".

IMPORTANT

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

5.3 Preparation of elution solution

Supplement 500 mL of suitable solution for elution (e.g., 0.9% sodium chloride solution, for infusion) with HSA to a final concentration of 1.0% (w/v), i.e., add 25 mL of 20% HSA to 500 mL of elution solution. The HSA-supplemented solution is henceforth referred to as "elution solution".
If the elution solution container is not suitable for connection to the CliniMACS Prodigy TS 320, transfer the elution solution volume required into a suitable transfer bag prior to connection. Note the HSA-relevant information in the Important section in 5.2 ´Preparation of process buffer´.

IMPORTANT

The 0.9% sodium chloride solution is not a component of this system. Therefore, the recommendation is to use 0.9% sodium chloride solution which is registered as a drug in the user's country. Special attention should be given to the stability of the target cell product in the chosen elution solution.

5.4 Preparation of IgG solution (optional)

If this option is selected, 10 mL of 5% IgG solution are required for the process, independent of the process scale. If only 10% IgG solution is available, IgG must be diluted to a 5% solution (w/v) with process buffer (as described in section 5.2 ´Preparation of process buffer´). The 5% IgG solution must be transferred to the dedicated bag at the CliniMACS Prodigy TS 320 using a syringe.

IMPORTANT

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

6

Separation process

6.1 Initialization of the CliniMACS Prodigy

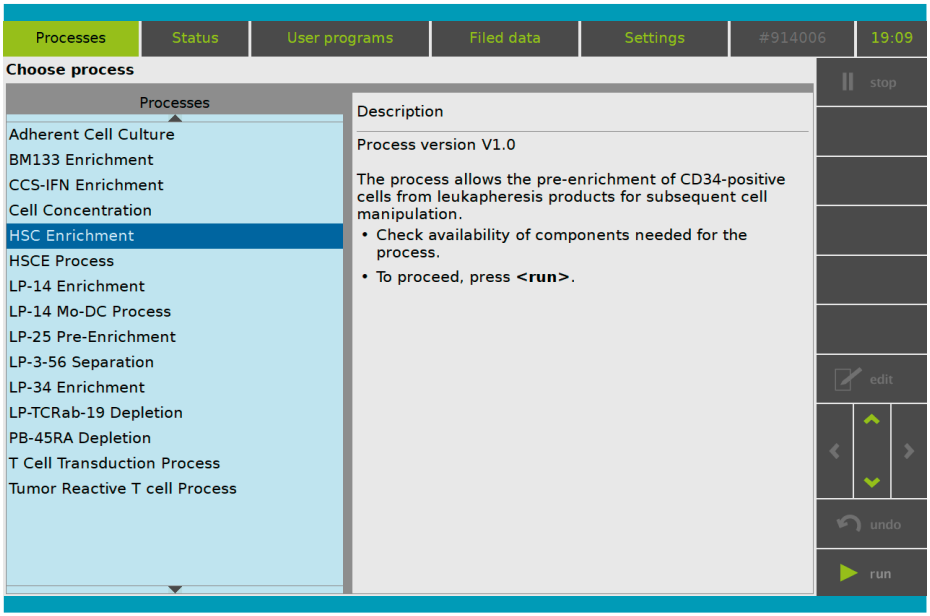
Switch on the CliniMACS Prodigy with the power switch located on the rear side of the instrument.

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

After the instrument initialization phase is completed, the main screen is shown. Select the process "HSC Enrichment". Tap **<run>** to confirm the selection.

⚠ CAUTION

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



Screen 6.1: HSC Enrichment process selection

⚠ CAUTION

Risk of process failure. If an incorrect drive shaft is installed on the instrument, the process is not executed. The process is designed for use with the CliniMACS Prodigy TS 320 only. The CliniMACS Prodigy TS 320 contains a large CentriCult™ Chamber (MaxSize Chamber) requiring a specific drive shaft. Make sure that the drive shaft is installed by Miltenyi Biotec Instrument Service before using the tubing set.

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

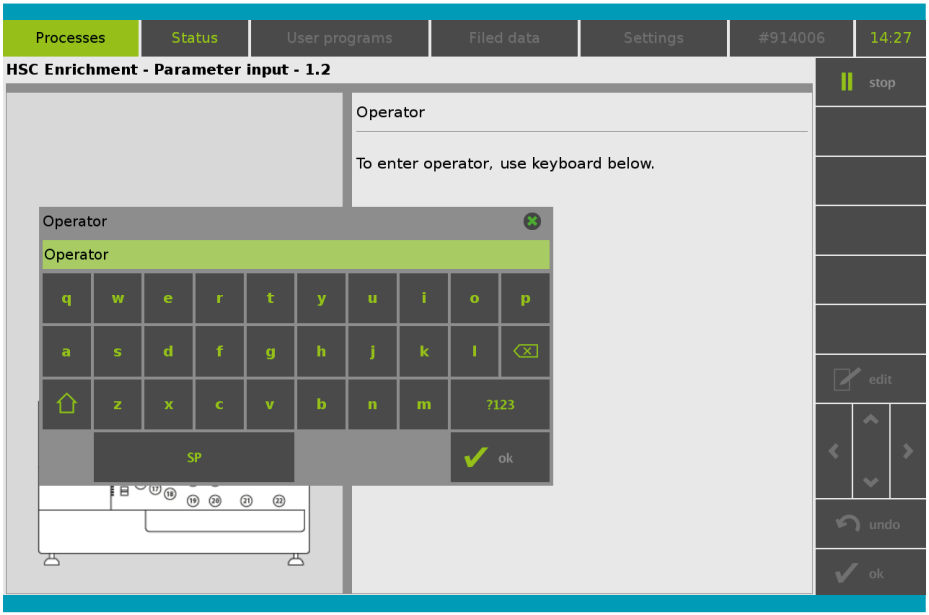
Contact Miltenyi Biotec Technical Support if a drive shaft exchange is required.

6.2 Parameter input

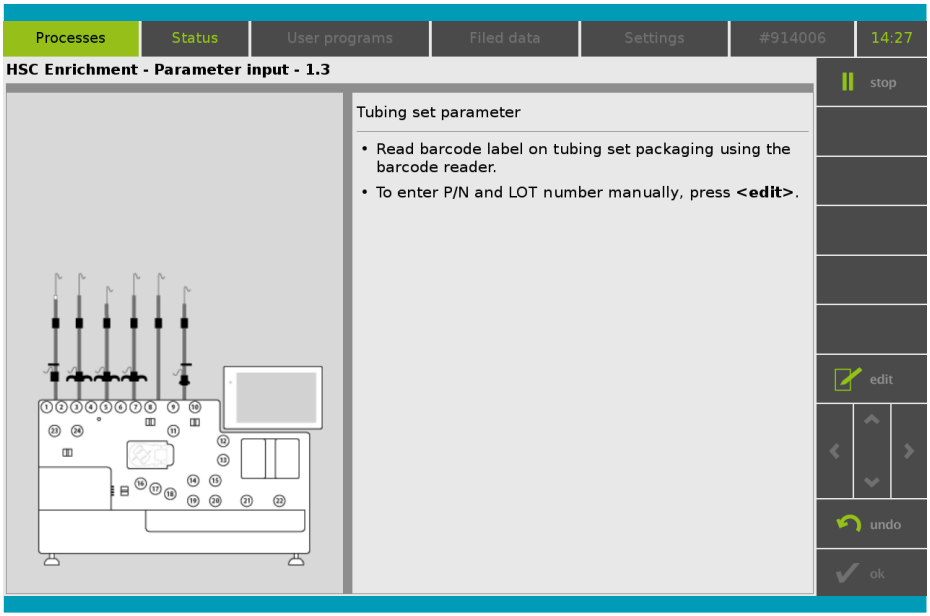
Multiple parameters must be entered for the process either with the barcode reader connected to the CliniMACS Prodigy or the keyboard shown on the screen. Tap <ok> to confirm.

All entered parameters are saved in the process-related protocol.

For the manual entry of capital letters tap <⇧>, for space tap <SP> for numbers tap <?123>, and for backspace tap <⌫>.



Screen 6.2: Entry of user-related information



Screen 6.3: Entry of CliniMACS Prodigy TS 320 part number (P/N) and LOT number

6.3 Tubing Set installation

6.3.1 Components of the CliniMACS Prodigy TS 320

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

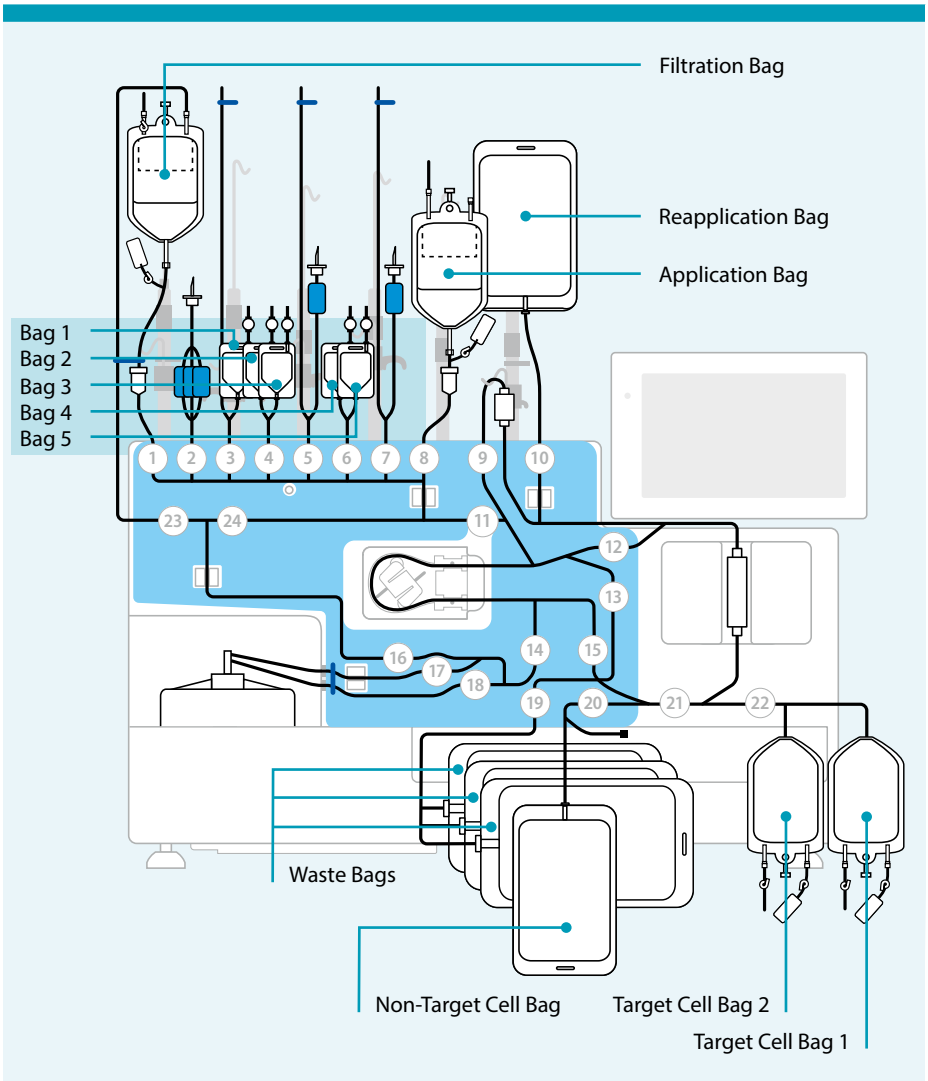


Figure 6.1: Components of the CliniMACS Prodigy TS 320

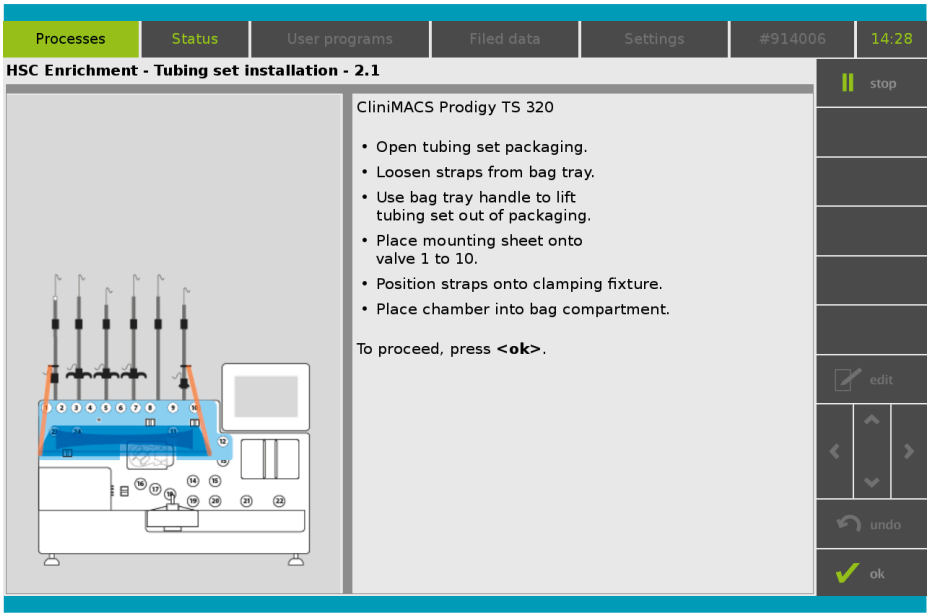
6.3.2 Installation steps

This section guides step-by-step through the installation of the CliniMACS Prodigy TS 320 (Screen 6.4 to Screen 6.23).

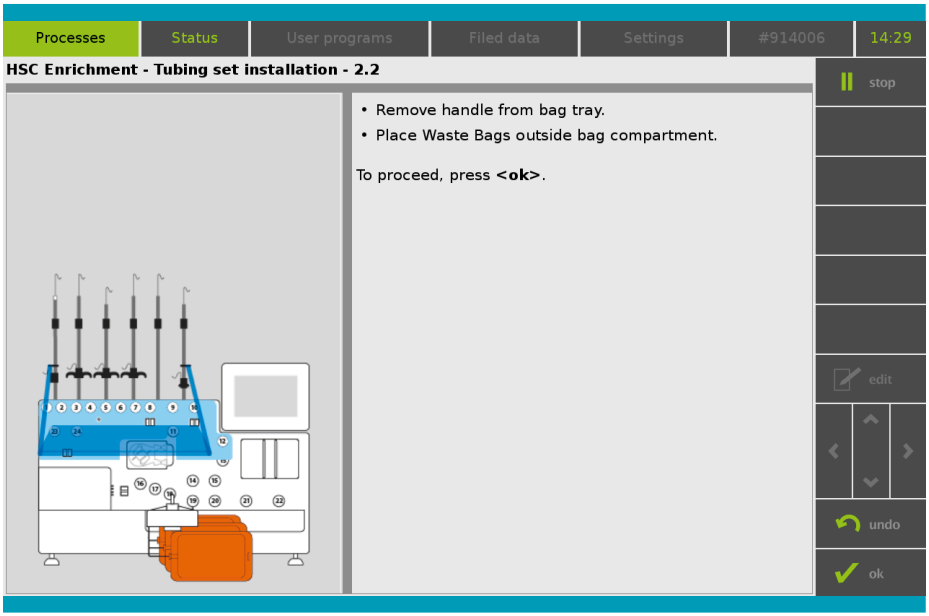
Each screen shows instructions supported by an informative image. Blinking and orange features in an image indicate areas of attention. At any step during the installation tap <undo> to return to a previous step.

⚠ 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 the package is undamaged and sealed.



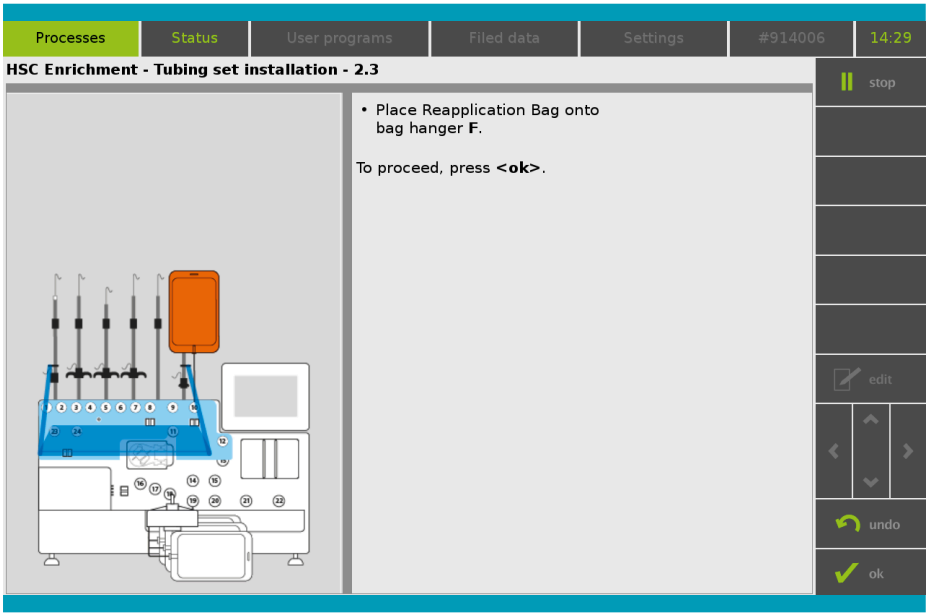
Screen 6.4: Installation of bag tray and mounting sheet onto the instrument



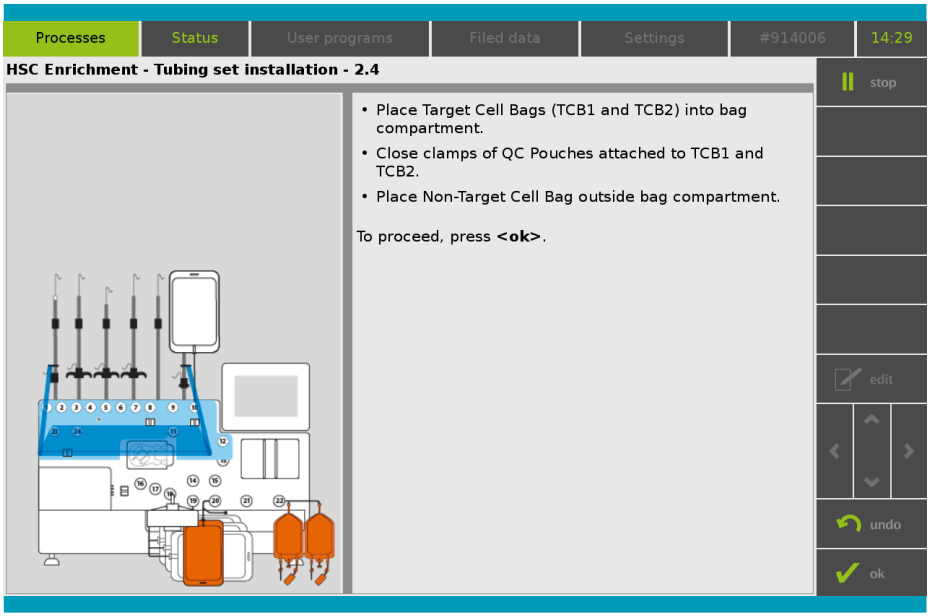
Screen 6.5: Positioning of the three Waste Bags

IMPORTANT

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



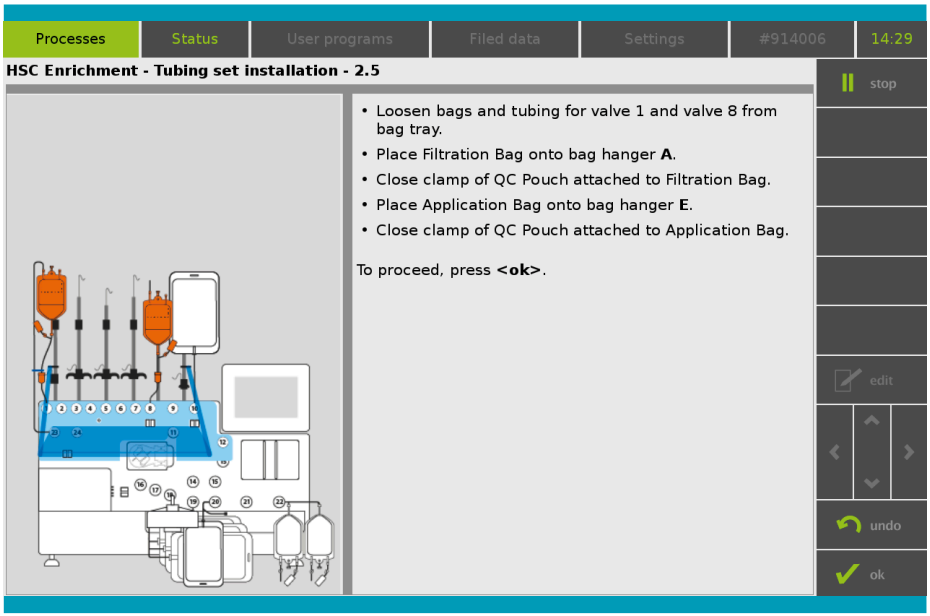
Screen 6.6: Positioning of the Reapplication Bag



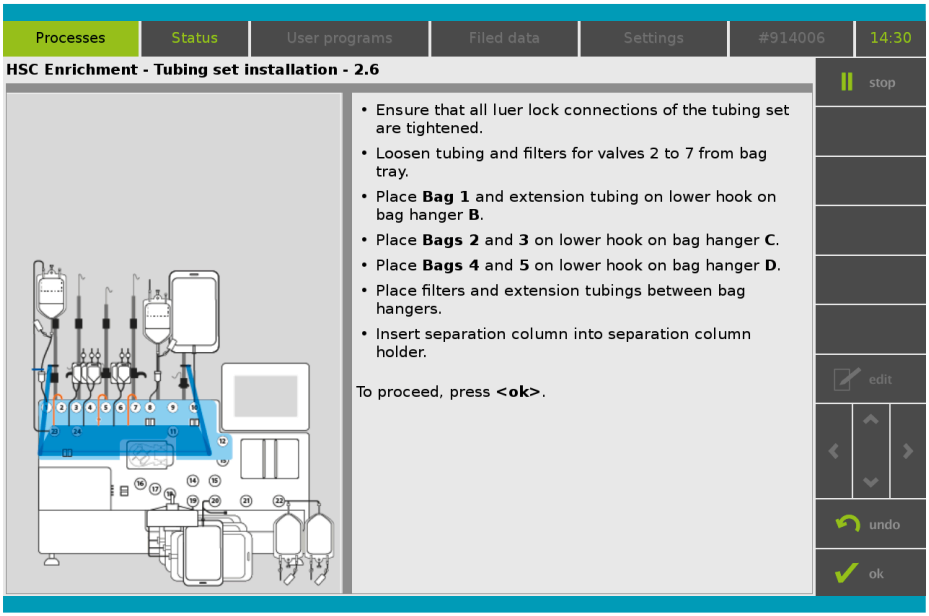
Screen 6.7: Positioning of Target Cell Bags (TCB1 and TCB2) and Non-Target Cell Bag (NTCB)

IMPORTANT

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



Screen 6.8: Positioning of Filtration Bag and Application Bag



Screen 6.9: Attachment of Bags 1 – 5 and insertion of the separation column

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

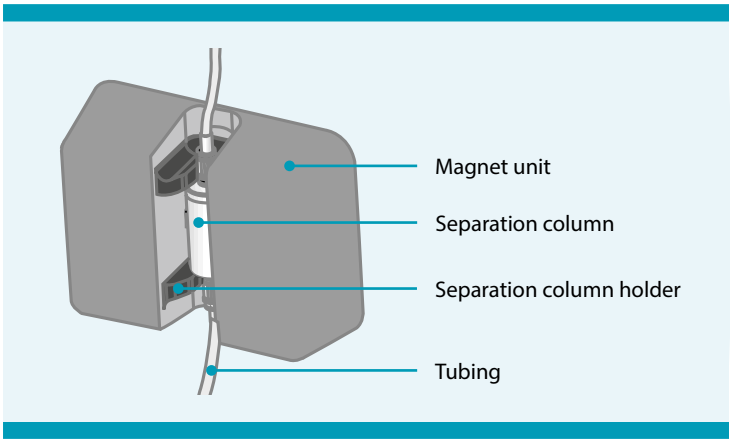
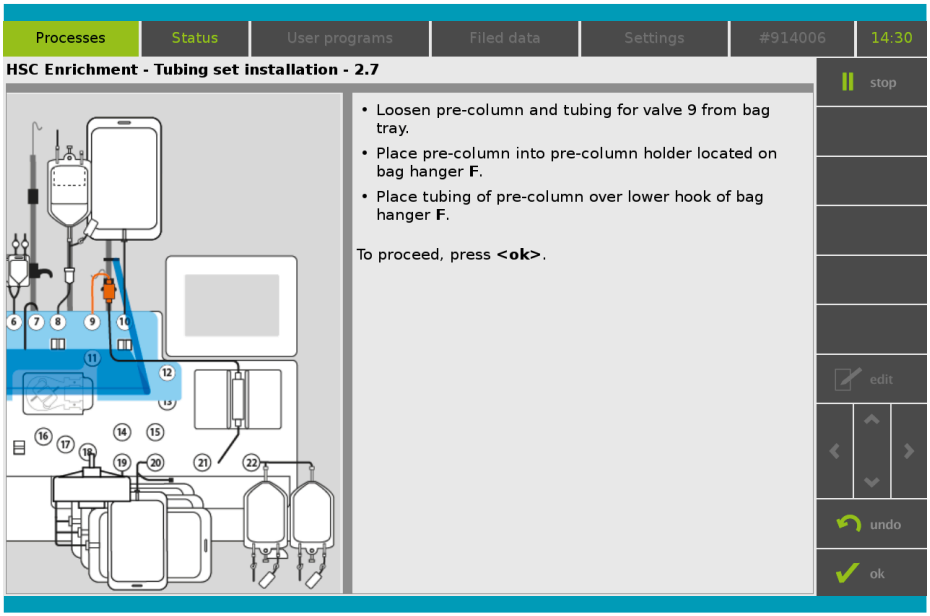


Figure 6.2: Separation column inserted into magnet unit

⚠ WARNING

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

Contact Miltenyi Biotec Technical Support for assistance if required.



Screen 6.10: Insertion of the pre-column

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

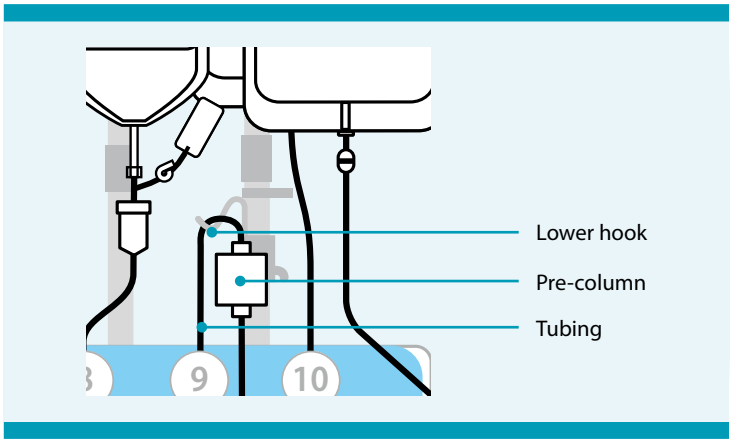
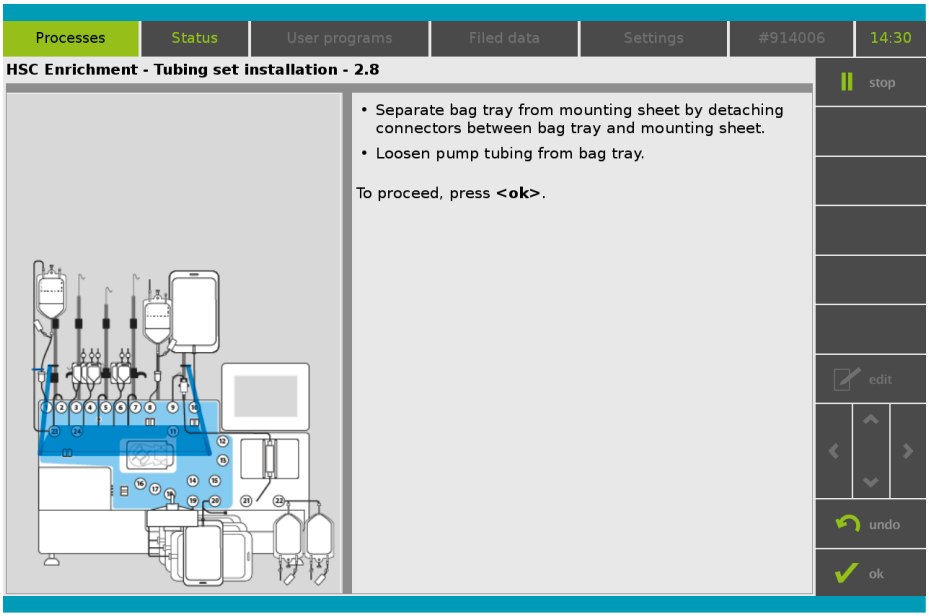
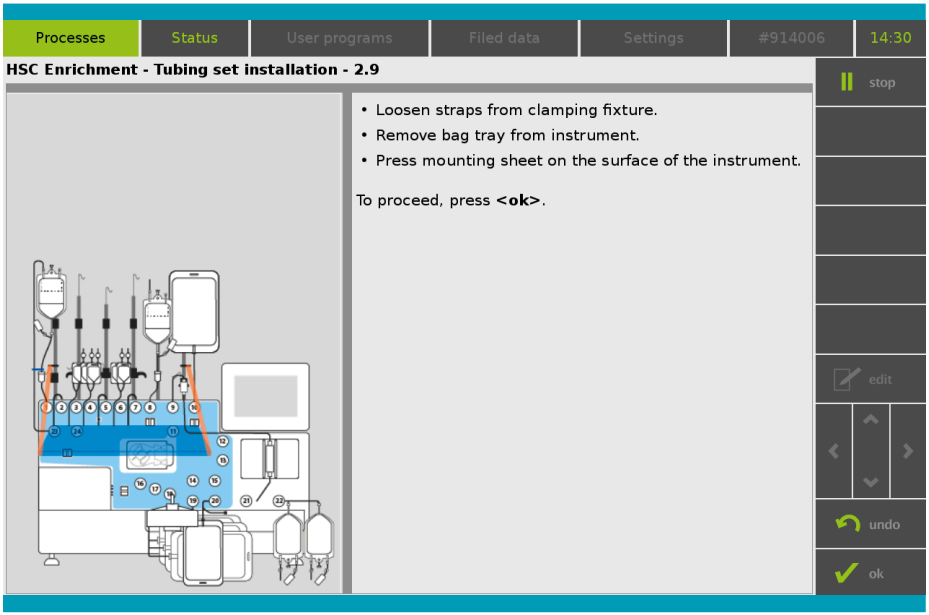


Figure 6.3: Guidance of pre-column-associated tubing



Screen 6.11: Separation of bag tray from mounting sheet



Screen 6.12: Removal of bag tray from instrument

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

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

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

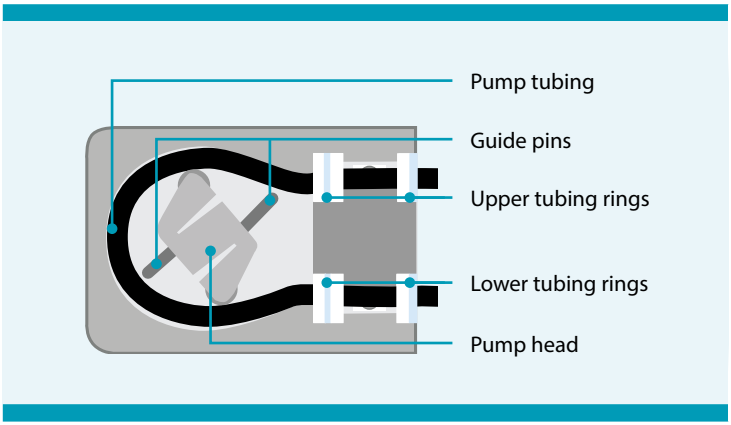
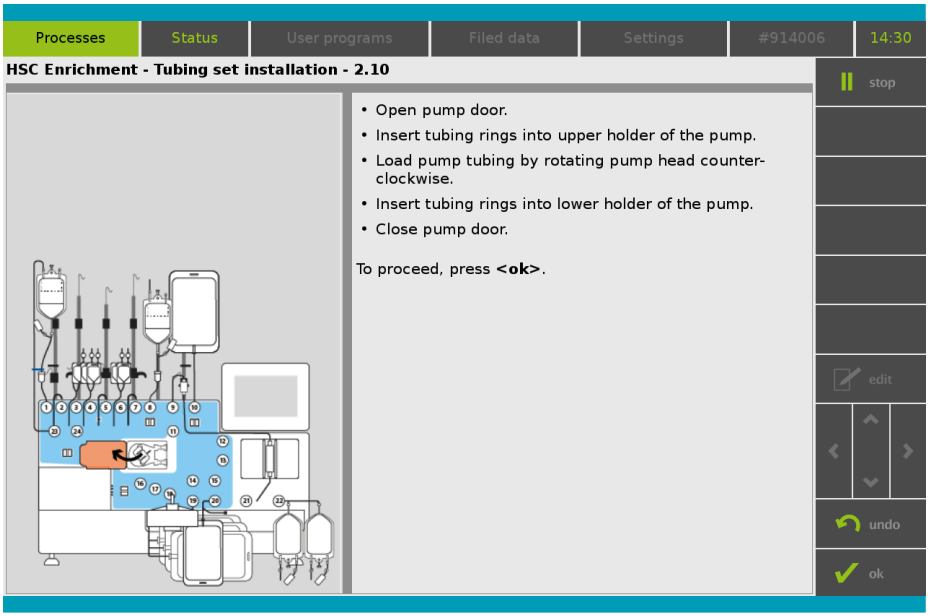
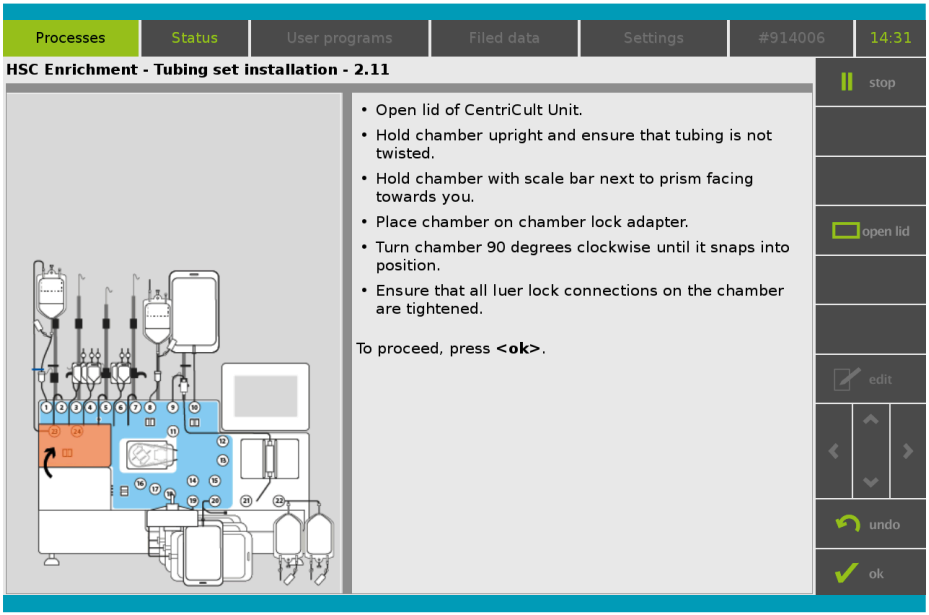


Figure 6.4: Pump tubing inserted into pump



Screen 6.13: Insertion of pump tubing into pump



Screen 6.14: Installation of the chamber in the CentriCult Unit

Figure 6.5 shows the components of the CentriCult Unit.

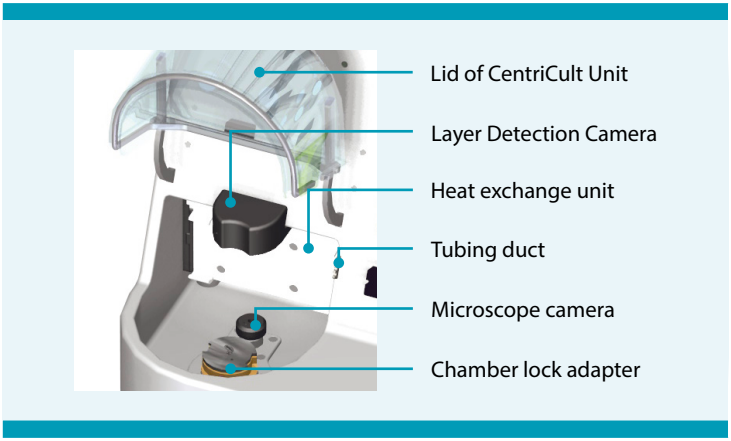


Figure 6.5: Components of the CentriCult Unit

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

IMPORTANT

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

Before closing the lid, make sure that no tubing is pinched by the lid.

NOTICE

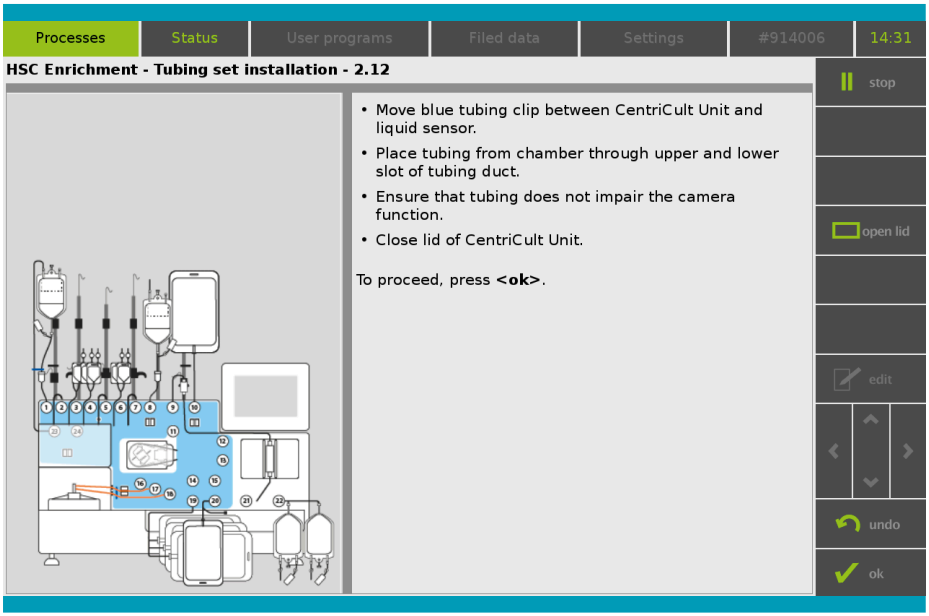
Risk of process delay. An incorrectly installed chamber tubing can cause a process delay. Before positioning the chamber onto the chamber lock adapter, make sure that the tubing attached to the chamber is in a parallel position and not twisted or bent. The chamber must not be detachable from the chamber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

⚠ CAUTION

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

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

If a twist or bending of the chamber tubing is recognized after the chamber is already locked, follow the instructions in the **IMPORTANT** paragraph connected to Screen 6.20 and correct the tubing position during that step.



Screen 6.15: Installation of chamber-associated tubing

Figure 6.6 illustrates how to insert the two chamber tubing lines through the tubing duct. Make sure that the tubing is not twisted or pinched by the lid of the CentriCult Unit.

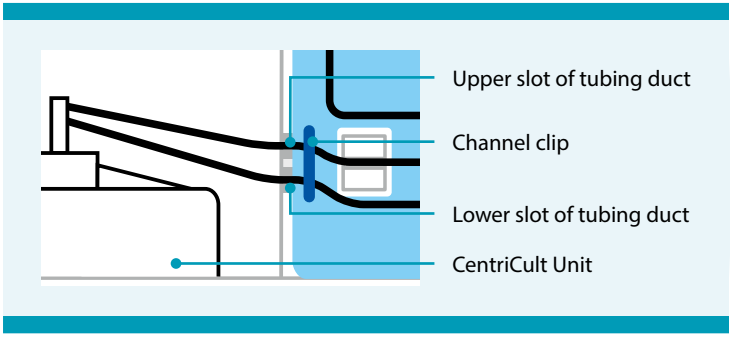
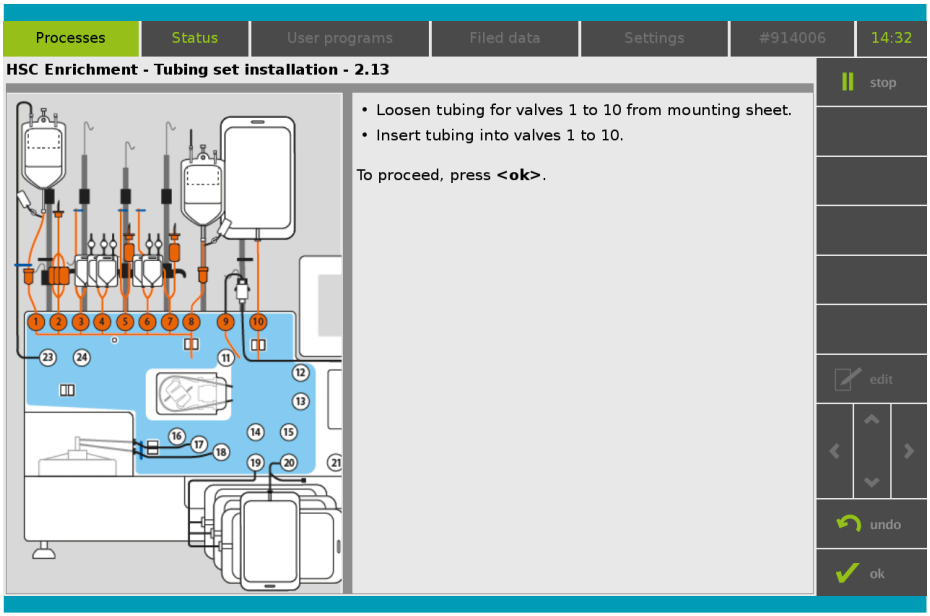


Figure 6.6: Guidance of tubing through tubing duct



Screen 6.16: Insertion of tubing into valves 1 – 10

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

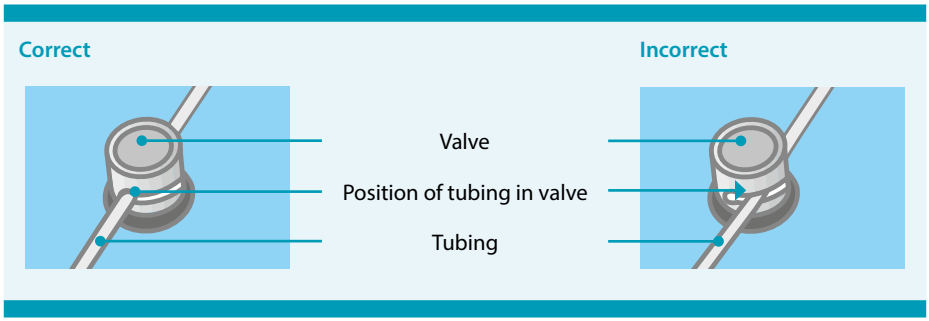
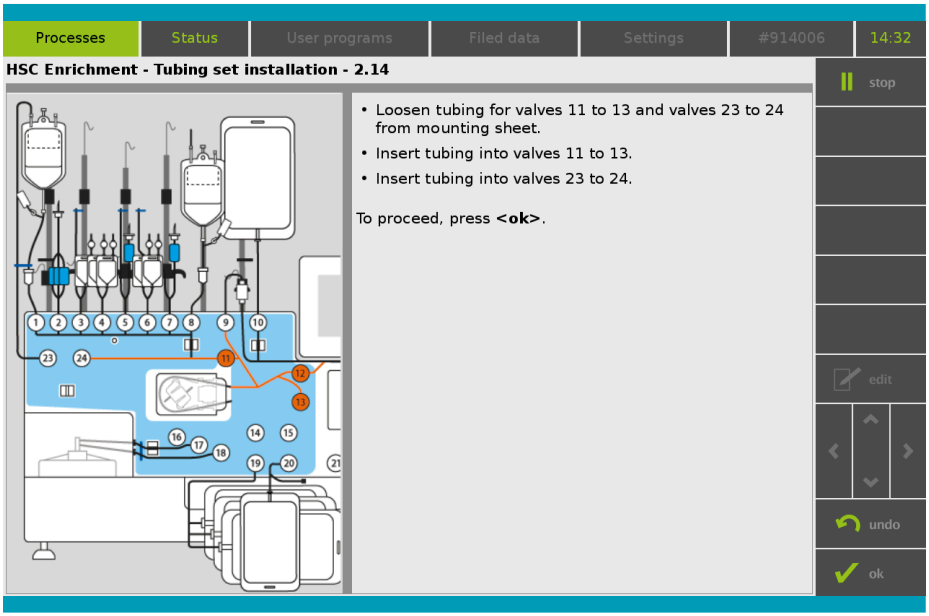


Figure 6.7: Insertion of tubing into valves



Screen 6.17: Insertion of tubing into valves 11 – 13 and 23 – 24

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

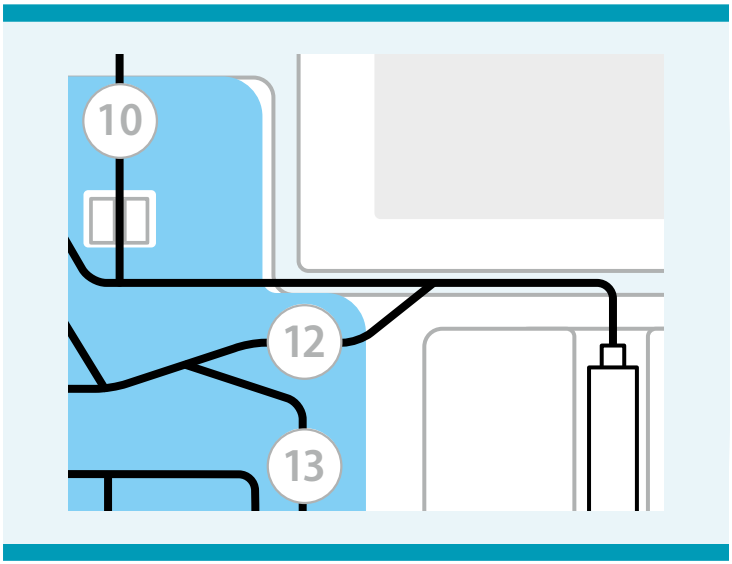
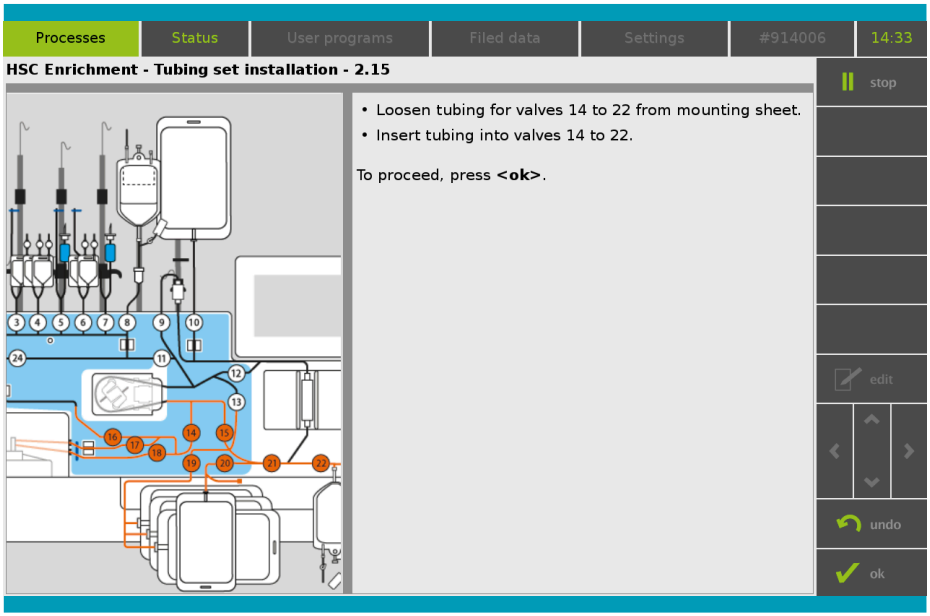
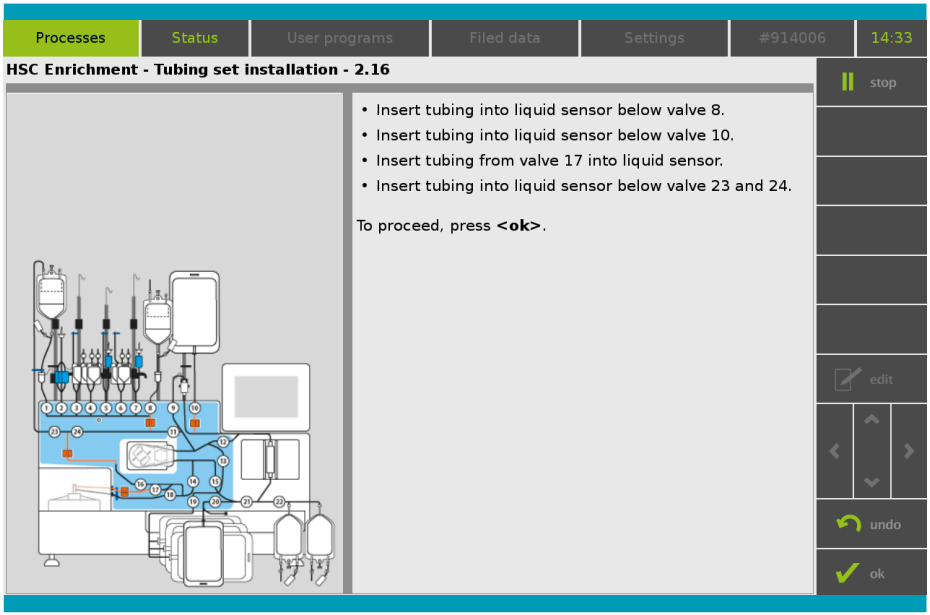


Figure 6.8: Tubing positioning for valves 12 and 13



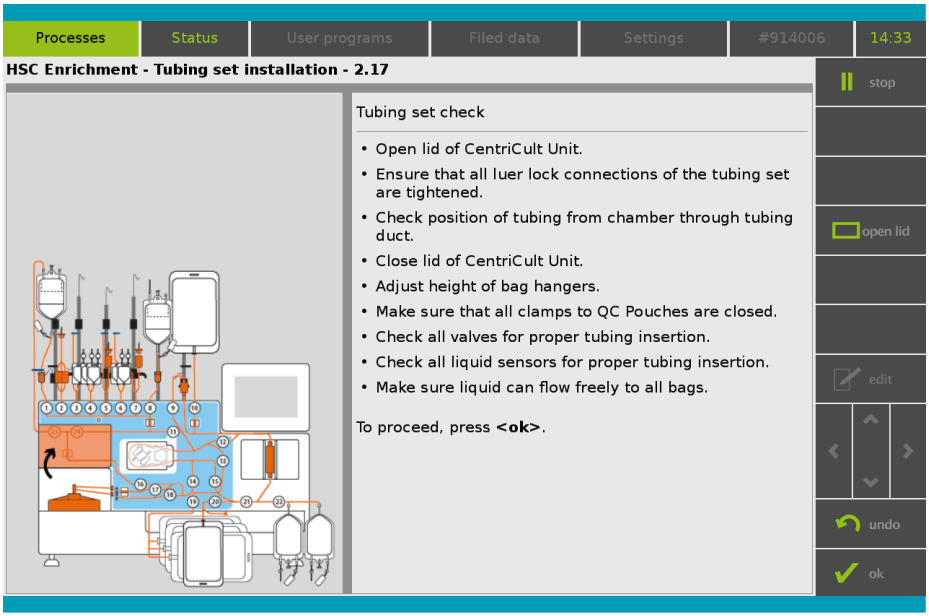
Screen 6.18: Insertion of tubing into valves 14 – 22



Screen 6.19: Insertion of tubing into liquid sensors

IMPORTANT

To ensure 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 6.20: Final check of tubing set installation

IMPORTANT

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

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

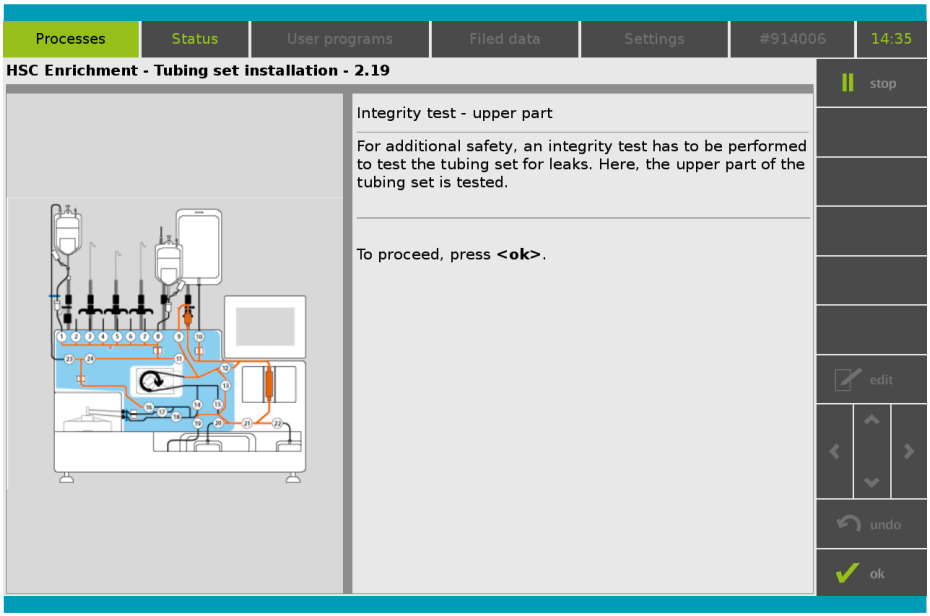
If necessary, raise or lower the bag hanger to adjust the height of the hanger. Remove the attached bag from the hook first before changing the height of a bag hanger. For raising or lowering make sure that the bags are positioned high enough to prevent severe bending of the tubing and low enough to avoid tubing or connections being stretched.

In case any irregularities are observed, contact Miltenyi Biotec Technical Support for further assistance.

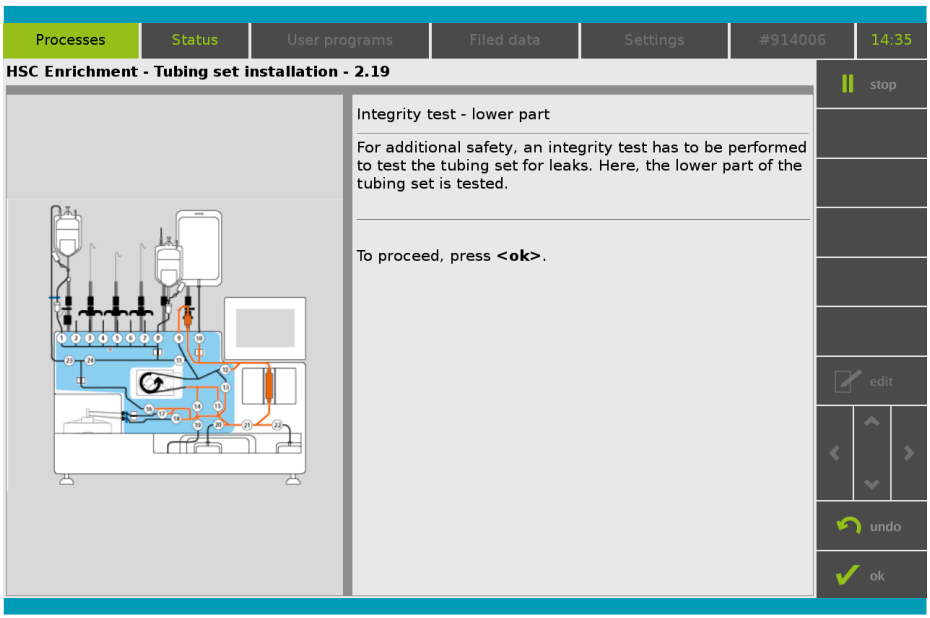
When the tubing set installation is complete, all valves are automatically opened and closed twice to verify proper functionality. In addition, the forward and backward movement of the magnet inside the magnet unit is tested. If a valve or the magnet does not operate correctly, refer to section 7 ´Troubleshooting´ of this user manual.

IMPORTANT

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



Screen 6.21: Integrity test for the upper part of the tubing set



Screen 6.22: Integrity Test for the lower part of the tubing set

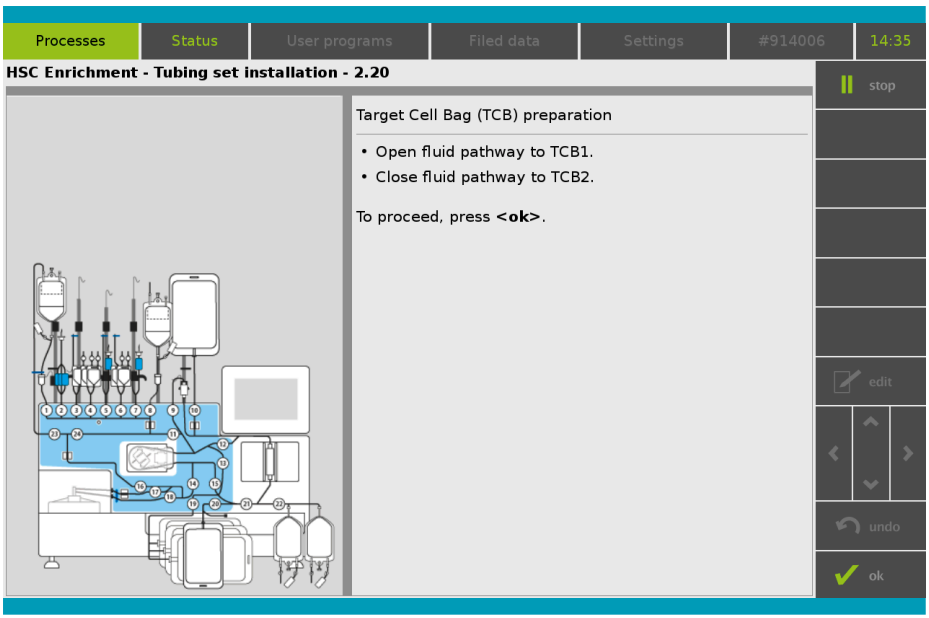
Follow the instructions on the screen to start the integrity test for the upper and lower part of the tubing set.

⚠ WARNING

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

Contact Miltenyi Biotec Technical Support for assistance if required.

After successful completion of the integrity test, make sure that the pathway to TCB1 is open. Close the pathway to TCB2 using the small integrated clamp as instructed on the screen.



Screen 6.23: Preparation of TCB1 and TCB2

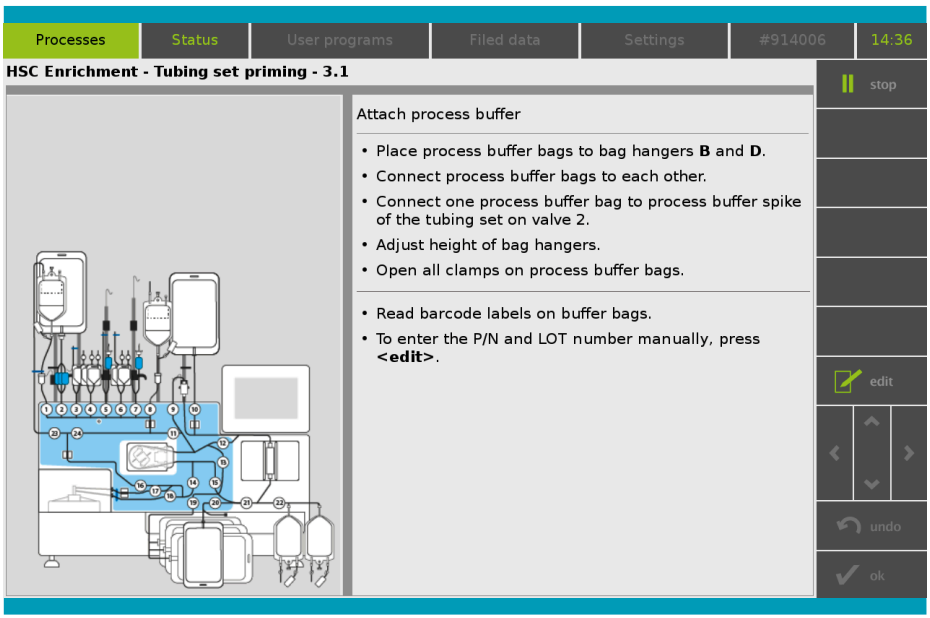
6.4 Tubing set priming

6.4.1 Connection of process buffer and elution solution

NOTICE

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

Screen 6.24 to Screen 6.29 guide through priming preparation and priming phase.



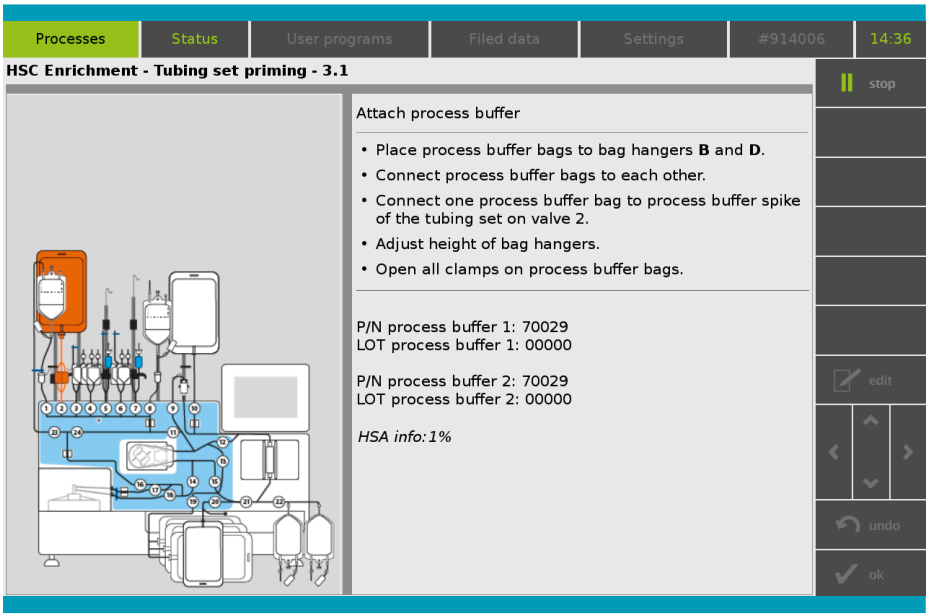
Screen 6.24: Connection and positioning of process buffer bags

IMPORTANT

Two 3L process buffer bags must be connected for the priming sequence. Place bags as indicated on the screen. Connect the bags to each other using a Transfer Set Coupler/Coupler. Close the clamp at the transfer set prior to interconnection. After interconnection, the bag placed on bag hanger B is connected to the tubing set spike of the buffer line at valve 2.

To ensure optimal liquid flow, adjust the process buffer bag at bag hanger B a bit lower than the process buffer bag at D. Take particular care not to stretch the triple sterile filters at the buffer line and make sure that the triple sterile filter is in a vertical position. After height adjustment of the bag hangers, open the clamp at the interconnection between the process buffer bags.

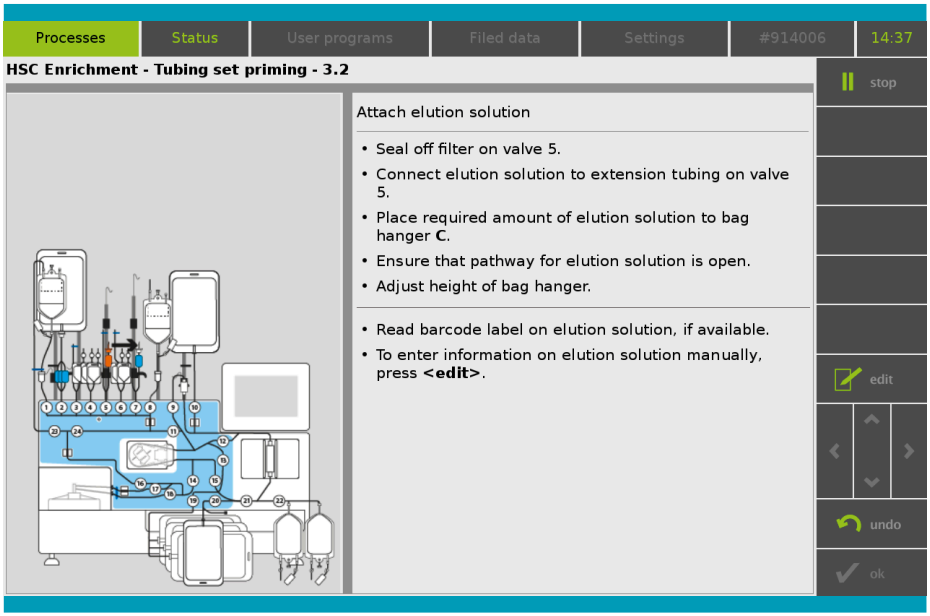
Depending on the cell parameter input (see Screen 6.30 to Screen 6.32), the software will then automatically determine and indicate if a third process buffer bag is required (see Screen 6.32).



Screen 6.25: Input of part number (P/N) and LOT numbers of the process buffer bags and HSA information

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

Tap <edit> to enter information regarding the HSA used for the process.



Screen 6.26: Connection of elution solution

Figure 6.9 depicts the connection of elution solution to the correct tubing line at valve 5.

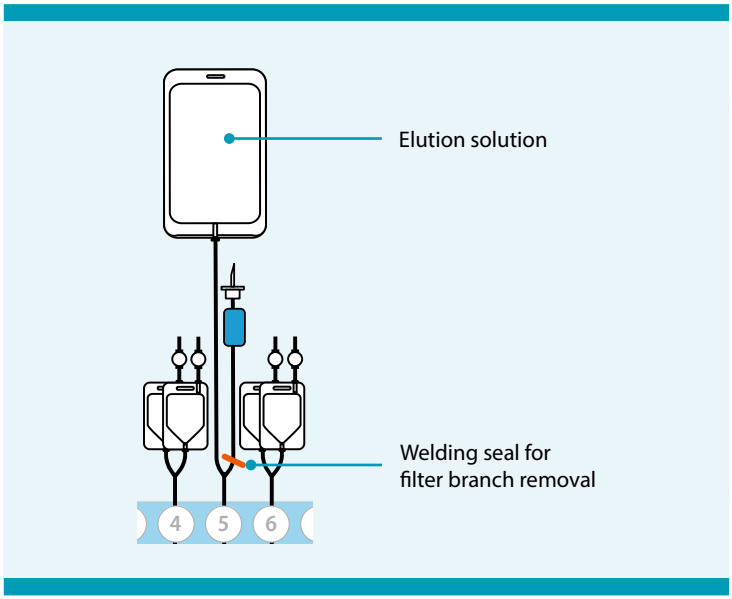


Figure 6.9: Connection of elution solution

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

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

IMPORTANT

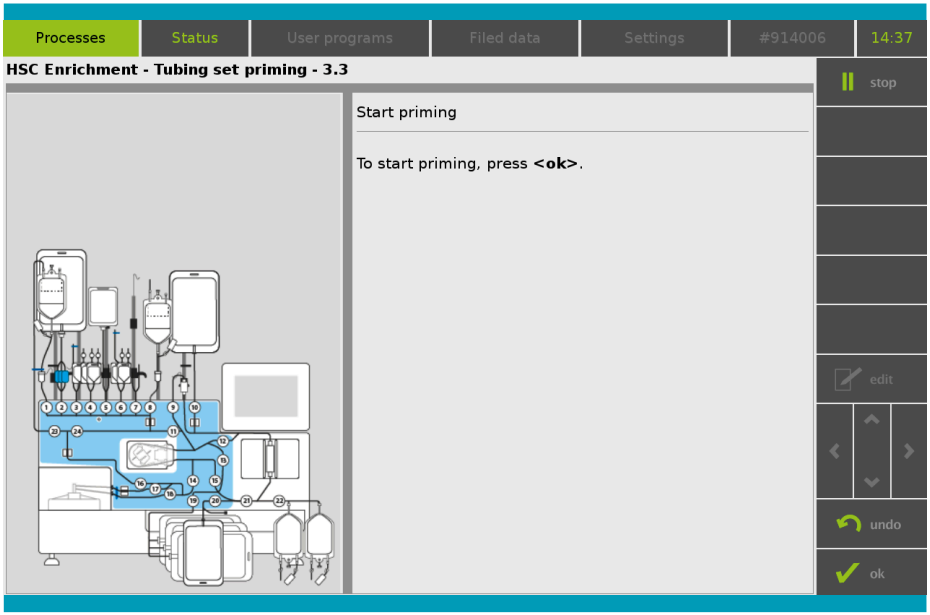
500 mL of elution solution are required for the HSC Enrichment process, independent of the process scale. The elution solution should be provided in a suitable bag.

If the elution solution bag cannot be welded directly to the extension tubing at valve 5, use a Transfer Set Coupler/Coupler for sterile connection of the elution solution bag and the extension tubing.

If necessary, raise or lower the bag hanger to adjust the height for the elution solution.

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

6.4.2 Priming phase

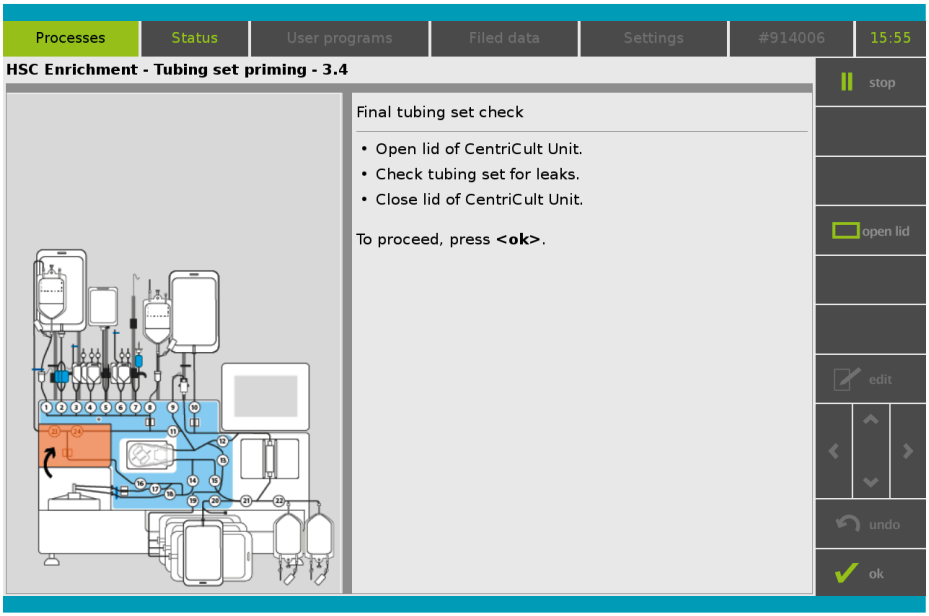


Screen 6.27: Start of the priming phase

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

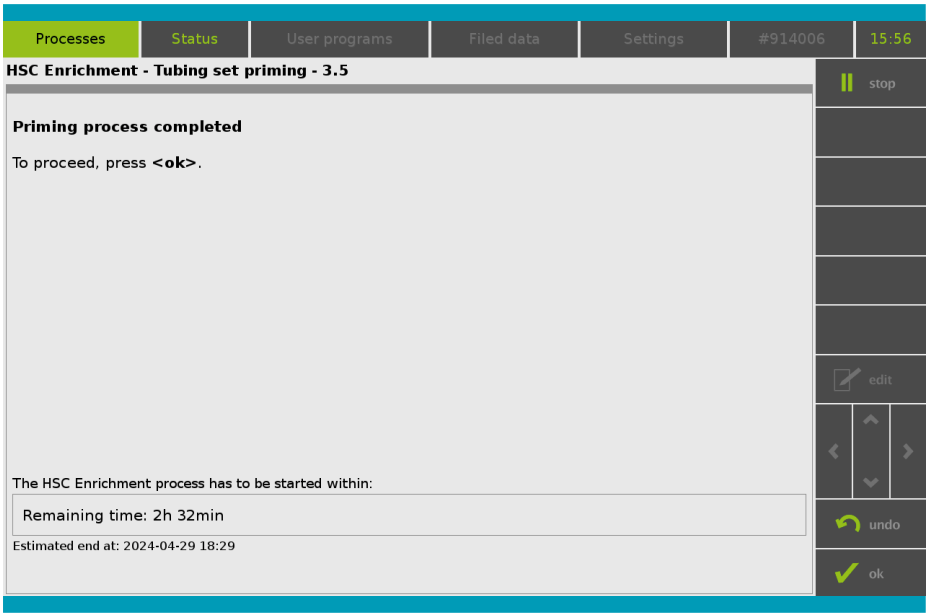
When the priming phase is completed, check all bags, tubing, fittings, valves, chamber and columns for the appearance of leaks or the presence of kinks which may block fluid pathways. If leaks are observed, the tubing set must not be used. In this case, restart the process using a new tubing set, process buffer and elution solution.

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



Screen 6.28: Final tubing set check after priming phase

Screen 6.29 is shown when the priming phase is completed.



Screen 6.29: End of priming phase

Before proceeding with the separation process, make sure that all materials required for the separation process are available. Make sure as well that the starting cell product, analyzed as described in section 5.1 ´Testing of the starting cell product´, is available.

6.5 Preparation for the automated enrichment process

The HSC Enrichment process is scalable (normal and large scale). According to the total numbers of WBC and CD34-positive cells entered during cell parameter input, the software automatically calculates and recommends the appropriate process scale and the required amount of CliniMACS CD34 Reagent. Furthermore, the software indicates if a third process buffer bag is required. (see Table 6.1).

	Normal scale	Large scale
Process capacity	WBC: ≤60×10^9 CD34: ≤0.6×10^9	WBC: >60-120×10^9 CD34: >0.6-1.2×10^9
CliniMACS CD34 Reagent	1 vial	2 vials
Process buffer	2×3L	3×3L

Table 6.1: Process capacities, CD34 reagent and process buffer requirements

6.5.1 Cell parameter input

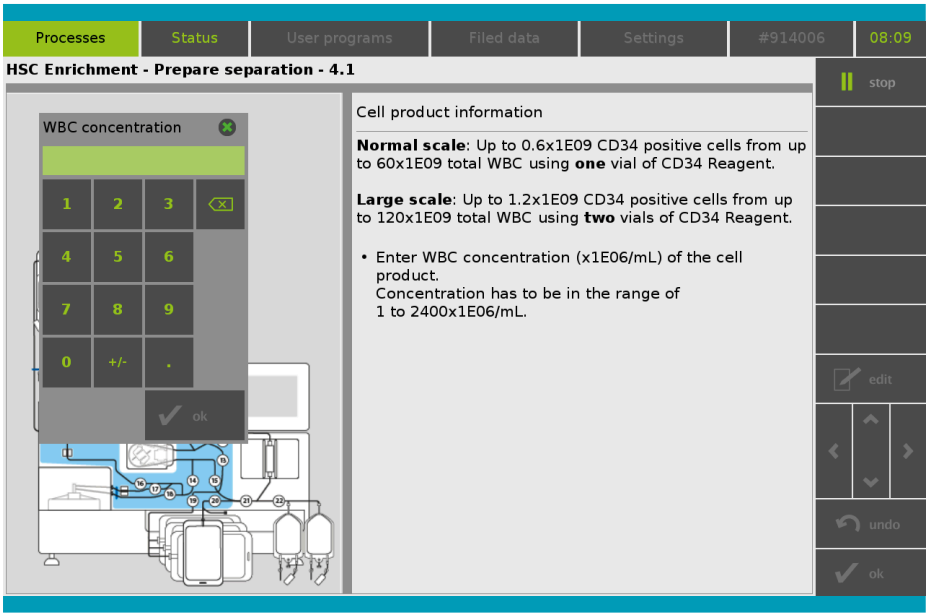
IMPORTANT

Process scale and duration depend on the numbers of WBC and labeled cells within the starting cell product. The input of respective cell parameters (i.e., WBC concentration, frequency of labeled cells and cell product volume) is always required prior to process execution. Refer to section 5.1 ´Testing of the starting cell product´ for details on analytical testing.

Process specification for normal and large-scale processing and related reagent requirements are indicated in Table 6.1 and on Screen 6.30.

Enter the WBC concentration (×10^6/mL) of the starting cell product to be transferred to the Application Bag of the CliniMACS Prodigy TS 320.

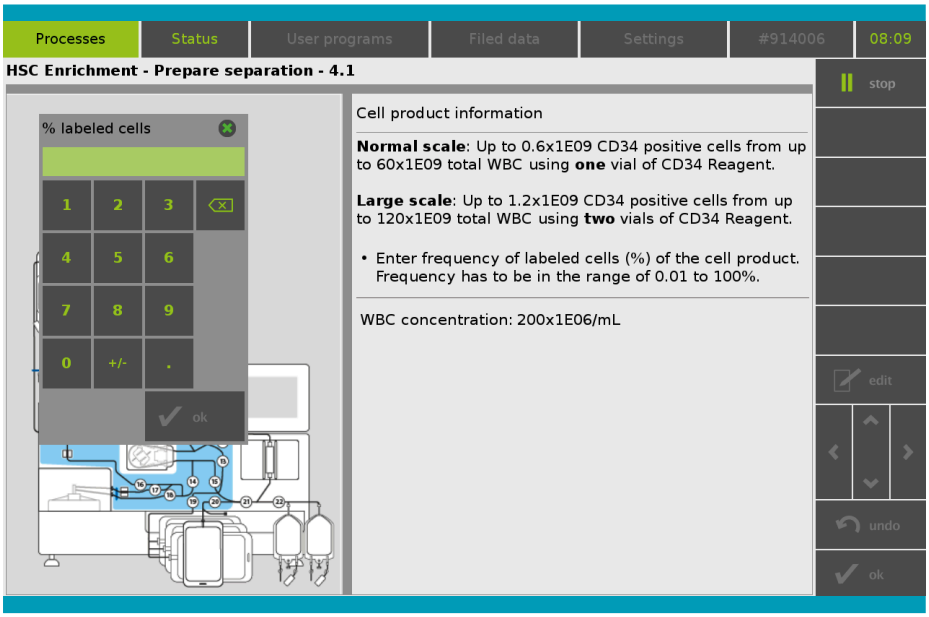
The acceptable input range is shown on the screen.



Screen 6.30: Entry of WBC concentration

After the WBC concentration of the starting cell product has been entered and confirmed, type in the frequency of labeled (CD34-positive) cells.

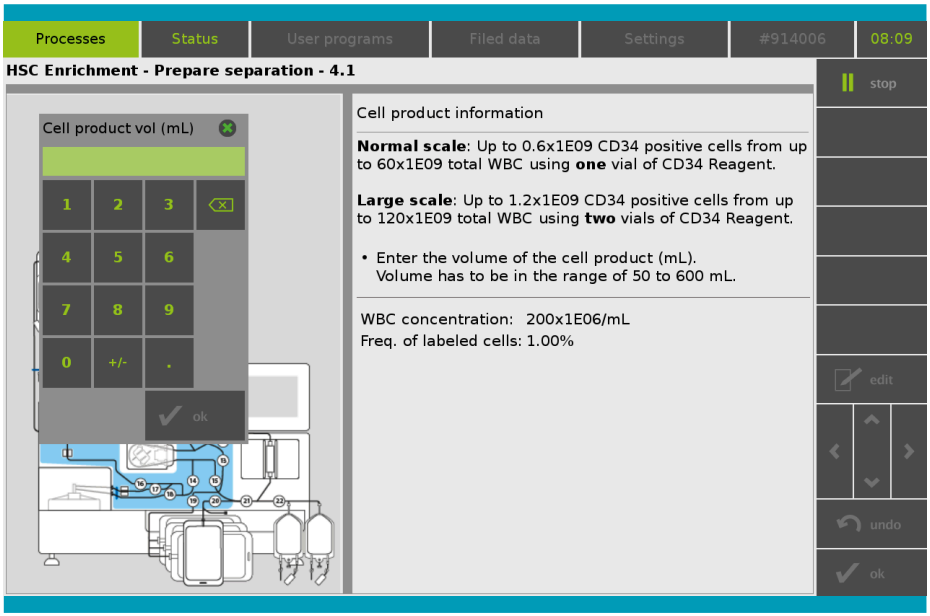
The acceptable input range is shown on the screen.



Screen 6.31: Entry of frequency of labeled cells

After the frequency of CD34-positive cells of the starting cell product has been entered and confirmed, type in the volume of the starting cell product.

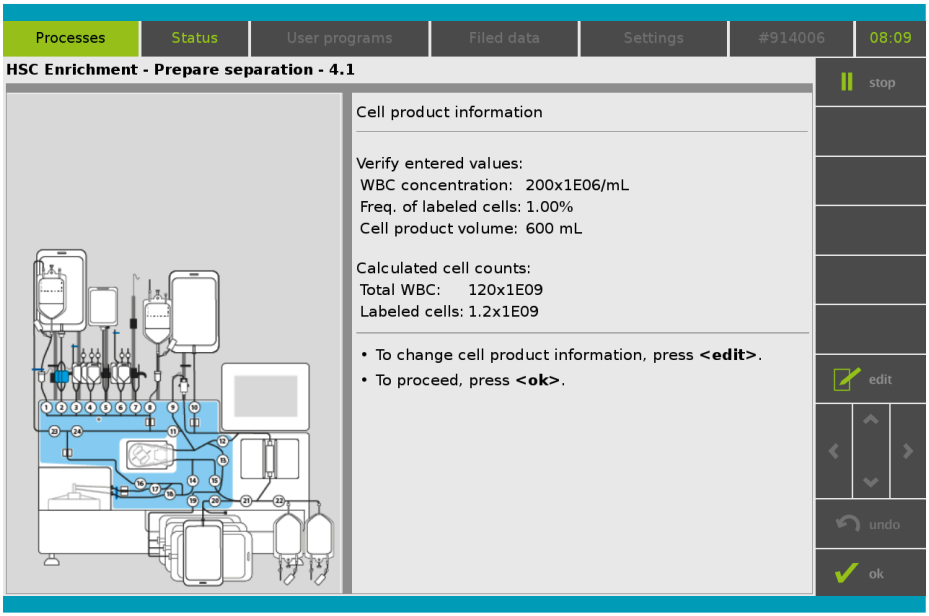
The acceptable input range is shown on the screen.



Screen 6.32: Entry of cell product volume

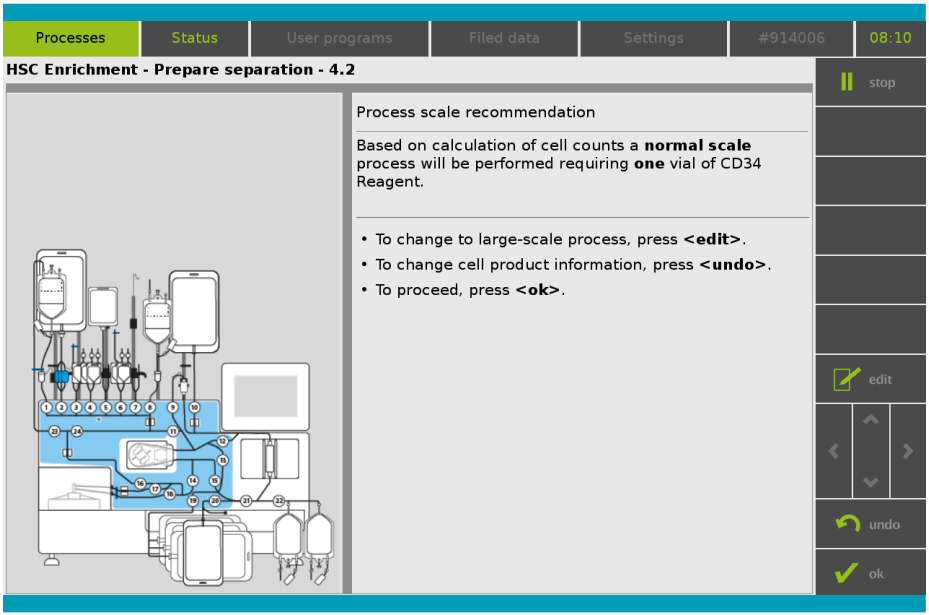
Based on the starting cell product information entered, total cell numbers are calculated automatically and compared to the given process specifications.

If required, tap <edit> to return to the WBC concentration entry screen and change the cell product information input.

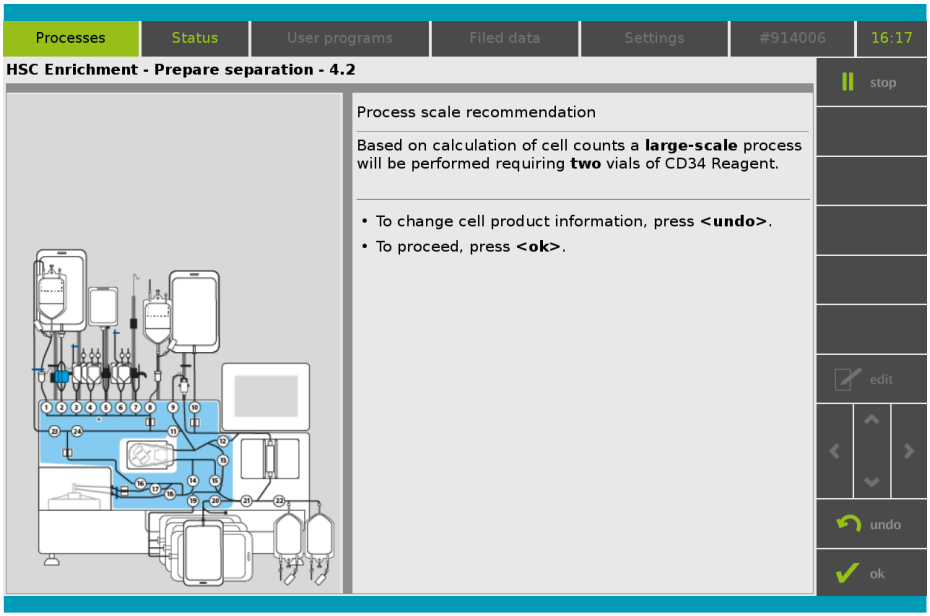


Screen 6.33: Verification of entered values

After confirmation of the cell product information entered, the appropriate process scale and related reagent requirements are automatically calculated and indicated on the screen.



Screen 6.34: Process scale recommendation for normal-scale processing



Screen 6.35: Process scale recommendation for large-scale processing

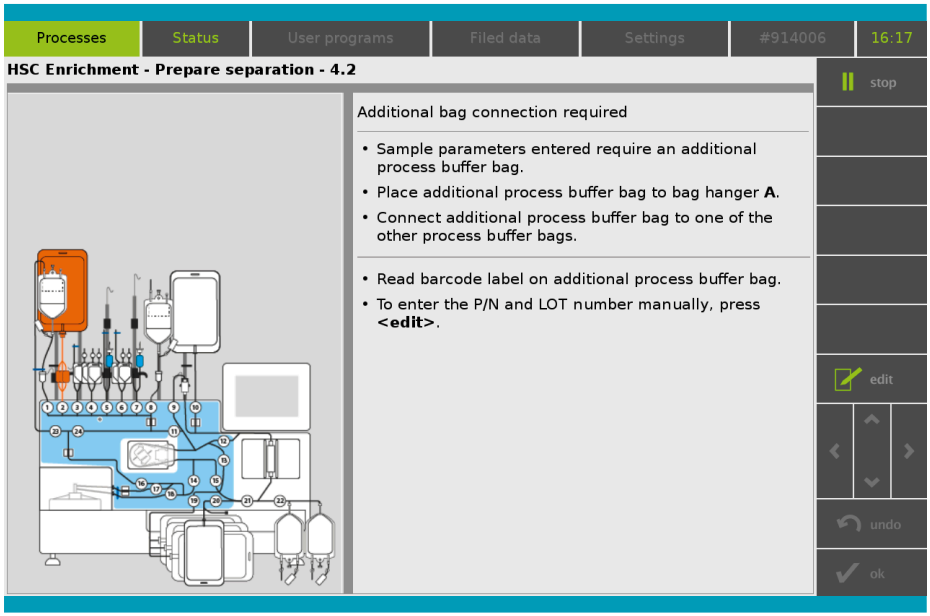
If required, tap <undo> to return to the WBC concentration entry screen and change the cell product information input. Subsequently, all process-related parameters are updated accordingly.

IMPORTANT

- Strictly comply with the reagent requirements as outlined on Screen 6.34 and Screen 6.35.
- Tap <edit> on Screen 6.34 to change from normal to large-scale processing, even if normal scale would be sufficient.
- If a large-scale process is recommended (Screen 6.35), it is impossible to change to a normal-scale process.
- If the total numbers for WBC or CD34-positive cells exceed the given process specifications for large-scale processing, split the starting cell product for two procedures. Alternatively, adjust the volume of the cell product used in order to meet the process specifications. Tap <undo> to change the cell product information accordingly and continue within the process specifications.
- Process buffer consumption depends on the process scale. Two (for normal scale) or three (for large scale) process buffer bags are required. The application software automatically calculates the actual buffer consumption after cell parameter input and indicates if a third process buffer bag is required.

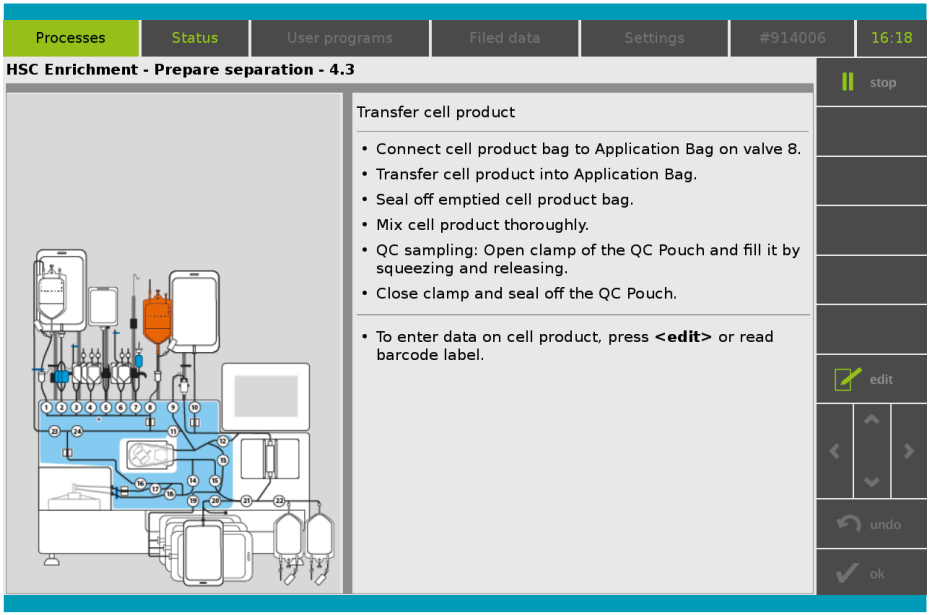
If a large-scale process is performed, the connection of an additional process buffer bag (3 L) is required. This is automatically calculated and indicated after cell parameter input (see Screen 6.36).

If the use of an additional process buffer bag is indicated, use a Transfer Set Coupler/Coupler for interconnection with one of the already installed process buffer bags. Clamp the transfer set prior to connection. Follow the instructions given on the screen to enter product-specific information and place the bag as instructed. Take care to open the clamp once the additional process buffer bag is connected safely.



Screen 6.36: Connection of additional process buffer

6.5.2 Transfer of the starting cell product



Screen 6.37: Transfer of the starting cell product into the Application Bag

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

IMPORTANT

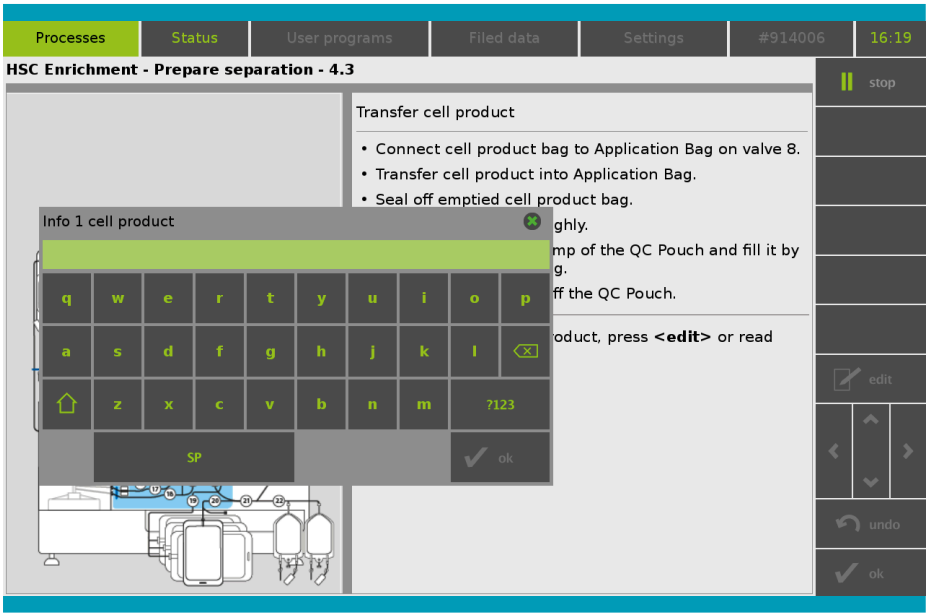
Gently but thoroughly mix the cells before sampling to ensure representative samples.

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

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

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

Tap <edit> to enter cell product identification.



Screen 6.38: Enter cell product identification

6.5.3 Connection of CliniMACS CD34 Reagent

For transfer of the CliniMACS CD34 Reagent into reagent bag 1, one (normal scale) or two vials (large scale) of the same kind are required.

IMPORTANT

To avoid blocking of the sterile filter, pool two vials of CliniMACS CD34 Reagent required for large-scale processing in a suitable syringe first before transfer to the corresponding reagent bag.

Figure 6.10 illustrates how to transfer the contents from the reagent vial(s) into the related reagent bag. The following preparative steps must be performed subsequently:

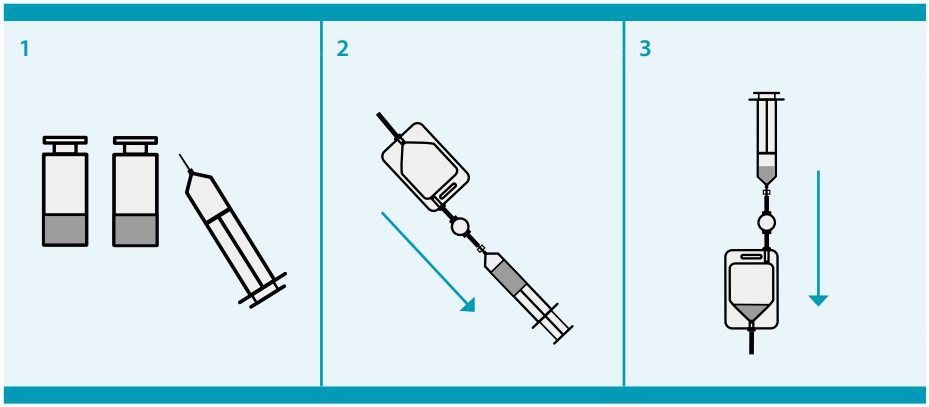


Figure 6.10: Transfer of one or two reagents into the reagent bag of the tubing set

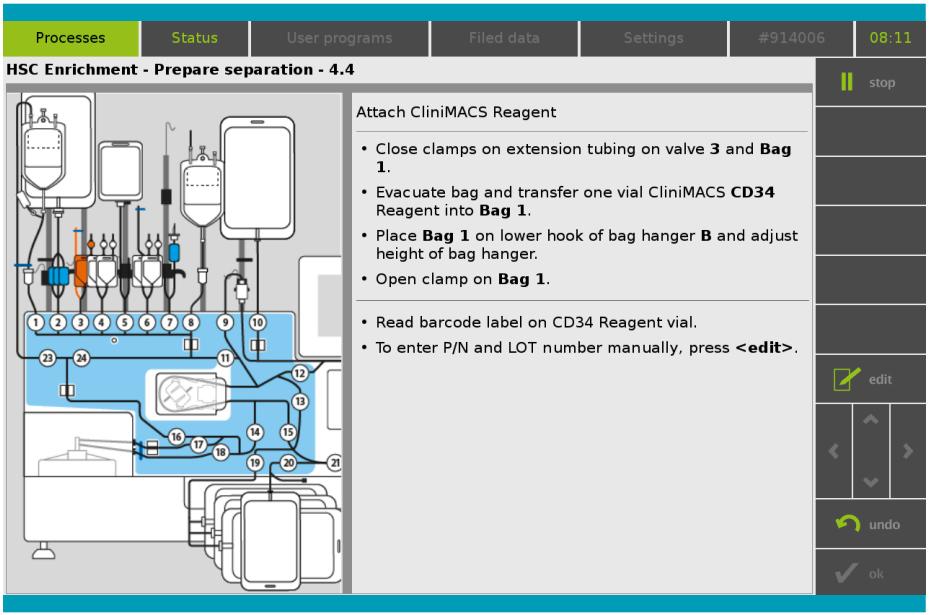
1. Remove the plastic cap(s) from the reagent vial(s). Remove the contents of the vial(s) using a suitable Luer Lock syringe equipped with a needle. If two vials of CliniMACS CD34 Reagent are required (large-scale process), combine the contents of the vials in the Luer Lock syringe (see Figure 6.10, part 1). Carefully remove the needle from the syringe.
2. Connect the syringe via Luer Lock connection to the respective reagent bag of the tubing set. Initially, evacuate the air from the bag (see Figure 6.10, part 2).
3. Turn around bag and syringe and transfer the reagent (without air) in one go through the sterile filter into the bag (see Figure 6.10, part 3). Remove the syringe. Attach the reagent bag to the hook on the respective bag hanger. Make sure that the liquid can flow freely.

For details on appropriate reagent transfer and bag positioning, refer to Figure 6.10. and Table 6.2. Note that Bag 3 to Bag 5 are not used for the HSC Enrichment process.

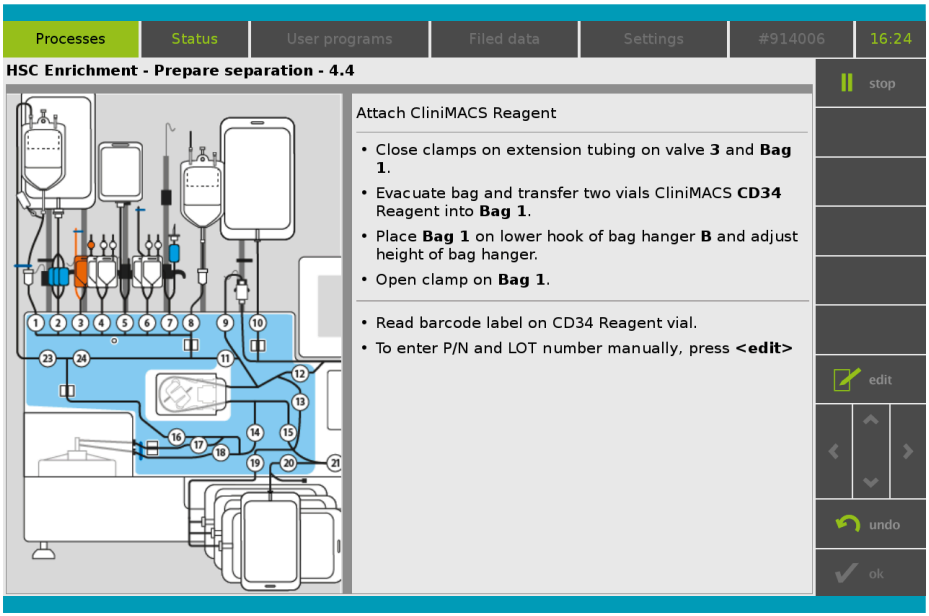
Reagent	Bag no.	Bag hanger
CliniMACS CD34 Reagent	1	B
IgG solution (optional)	2	C
Not used	***3***	***C***
Not used	***4***	***D***
Not used	***5***	***D***

Table 6.2: Overview reagent bag setup

Before reagent transfer, make sure that the clamps on the extension tubing and on Bag 1 (at valve 3) are closed. Open the clamp on Bag 1 only after reagent transfer.



Screen 6.39: Connection of one vial CliniMACS CD34 Reagent for normal-scale processing

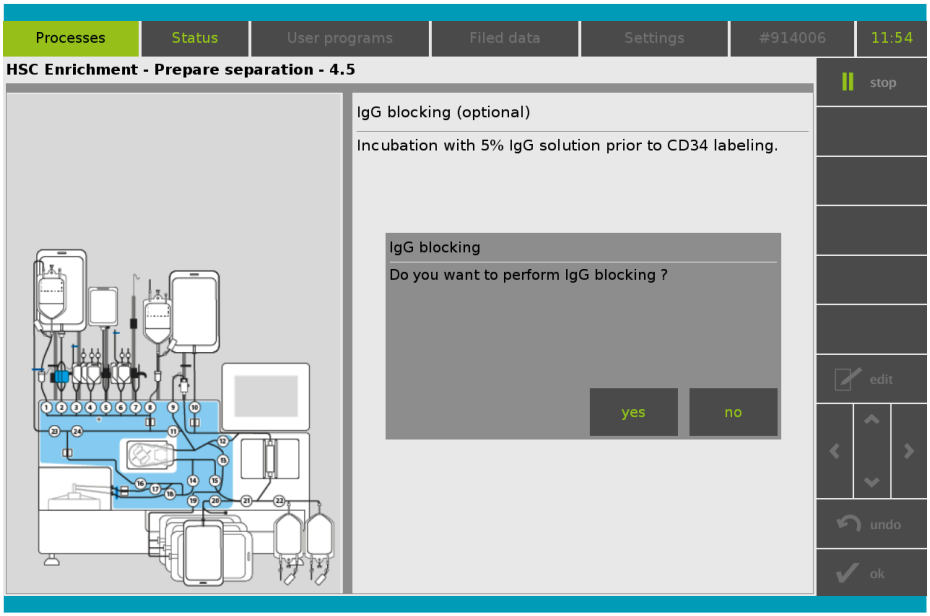


Screen 6.40: Connection of two vials CliniMACS CD34 Reagent for large-scale processing

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

6.5.4 Connection of IgG solution (optional)

The use of IgG solution for blocking of potentially unspecific binding is an option in the HSC Enrichment process. If selected, 10 mL of 5% IgG solution are required independent of the process scale. Refer to 5.4 ´Preparation of IgG solution (optional)´ for details on preparation.

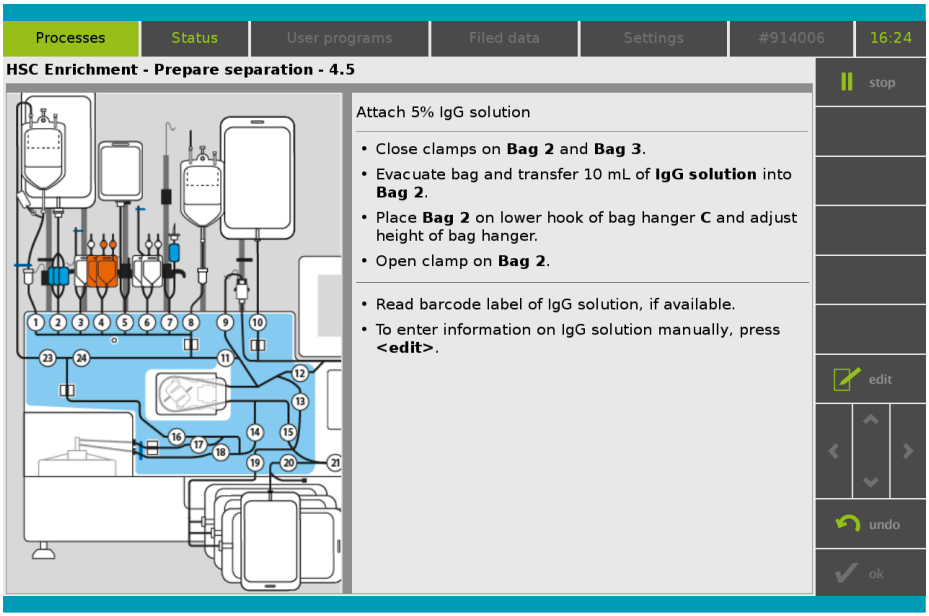


Screen 6.41: Selecting the IgG blocking option

If blocking with IgG solution is desired, tap <yes> on Screen 6.41 to proceed to Screen 6.42 for connection.

If IgG blocking should not be performed, tab <no> and proceed to section 6.5.5 ´Delay of the process end time (storage option)´.

Before IgG solution transfer, make sure that the clamps on Bag 2 and 3 are closed. Open clamp on Bag 2 only after IgG solution transfer.



Screen 6.42: Connection of 5% IgG solution

If applicable, read the barcode label on the IgG solution with the barcode reader. Alternatively, tap <edit> to enter IgG solution-specific information manually with the keyboard shown on the screen.

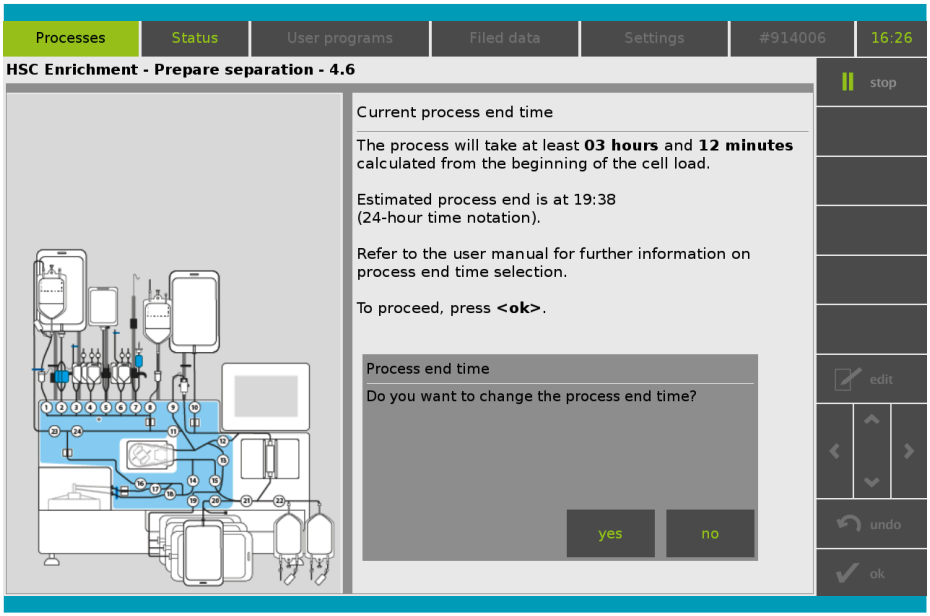
For details on appropriate IgG solution transfer and bag position, refer to Figure 6.10. and Table 6.2.

6.5.5 Delay of the process end time (storage option)

After cell product and reagent connections are completed, the application software calculates process duration and completion time automatically. In case the calculated completion time is not desirable, the user has the option to delay the time the target cells (purified CD34-positive cells) are provided.

If a delay of the automatically calculated process end time is desired, tap <yes> on Screen 6.43. Enter the desired end time for the process with the keyboard shown on the screen.

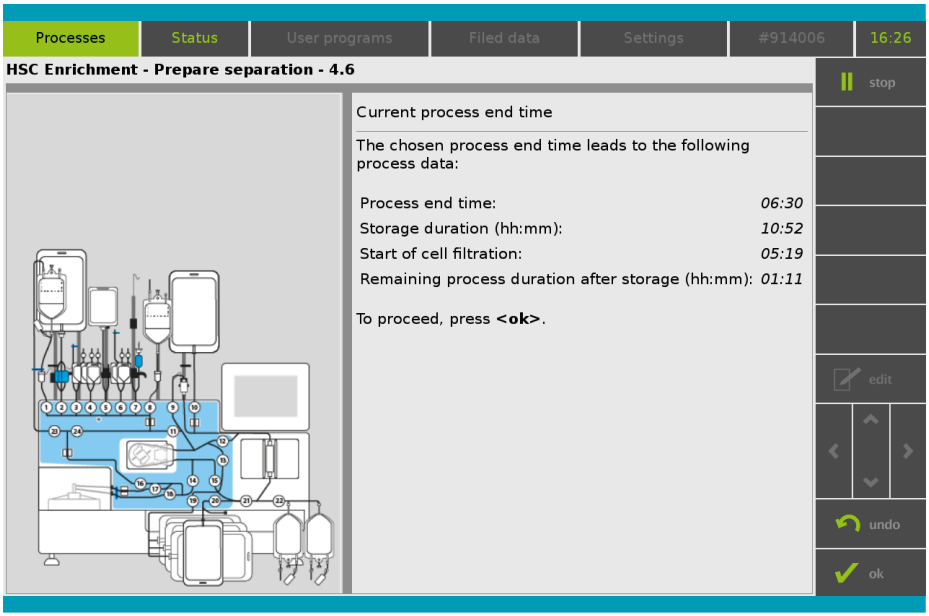
In order to provide the CD34-enriched target cells at the time defined by the user, the process is paused after CD34 labeling and removal of excess reagent. The labeled cells are adjusted to a WBC concentration of max. 2.5×10^8/mL and transiently stored at a temperature of +6 °C (+43 °F) in the chamber while shaking in intervals.



Screen 6.43: Process end time entry option

If a delay of the automatically calculated process end time is not desired, tap <no> on Screen 6.43.

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



Screen 6.44: Important information after process end time entry

To modify the entered process end time, tap <undo> and enter the new desired end time using the keyboard shown on the screen.

IMPORTANT

Make sure to enter a process end time later than the automatically calculated process end time. If the entered process end time input exceeds the maximum storage time of 16 hours, the end time entry is not valid and needs to be readjusted. If the entered process end time is before the automatically calculated end time has elapsed, the entered time is shifted by 24h. As this shift leads to a storage of >16h, the end time entry is not valid and needs to be readjusted.

6.5.6 Final process preparation

Figure 6.11 shows the tubing set with all connections required for the HSC Enrichment process.

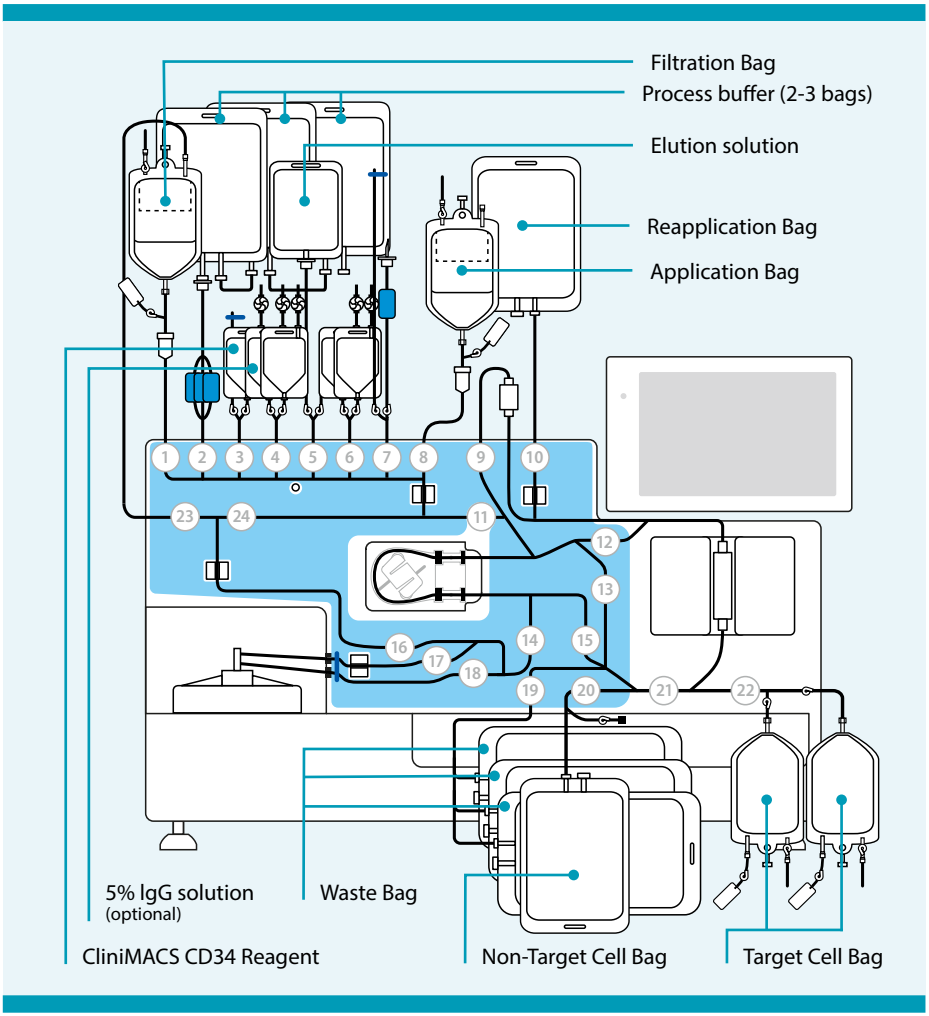
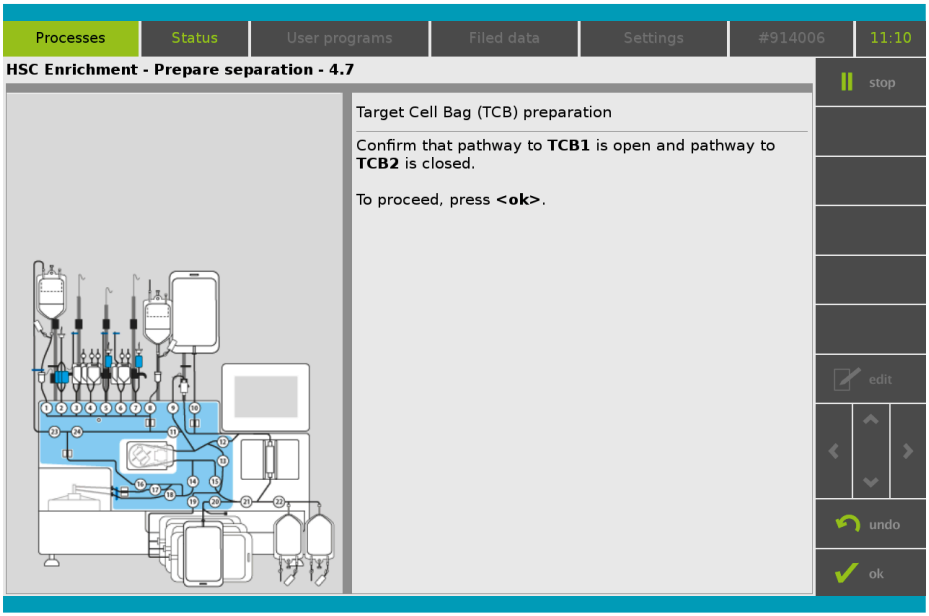


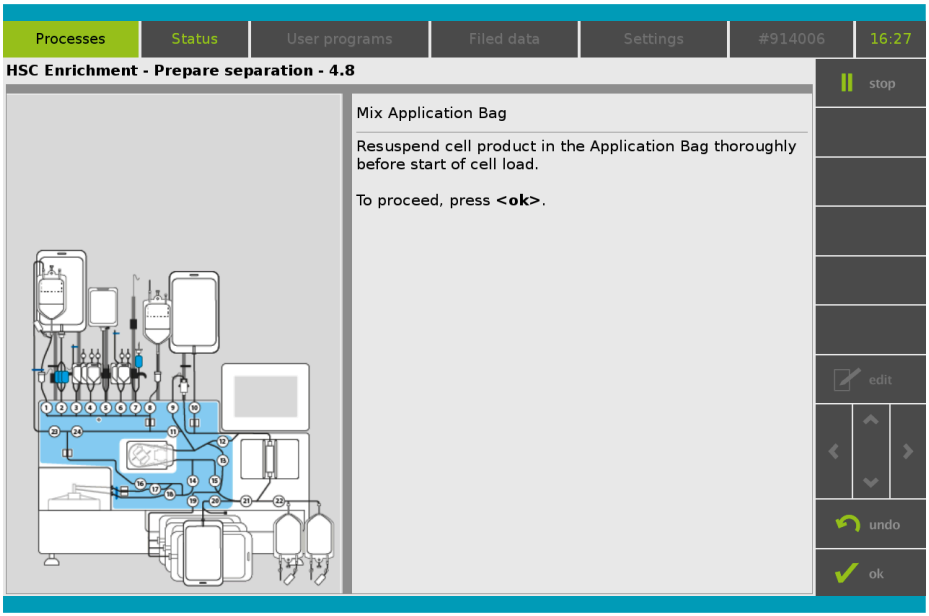
Figure 6.11: CliniMACS Prodigy TS 320 with all connections required for a HSC Enrichment process

Make sure that the pathway to Target Cell Bag 1 (TCB1) is open and the pathway to Target Cell Bag 2 (TCB2) is closed.



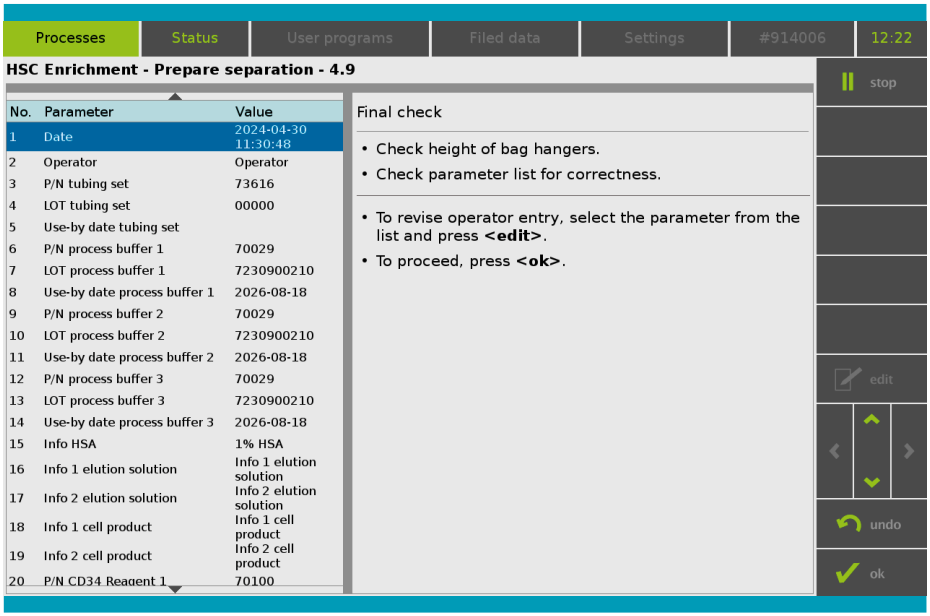
Screen 6.45: Target Cell Bag (TCB) preparation

Mix the Application Bag gently but thoroughly.



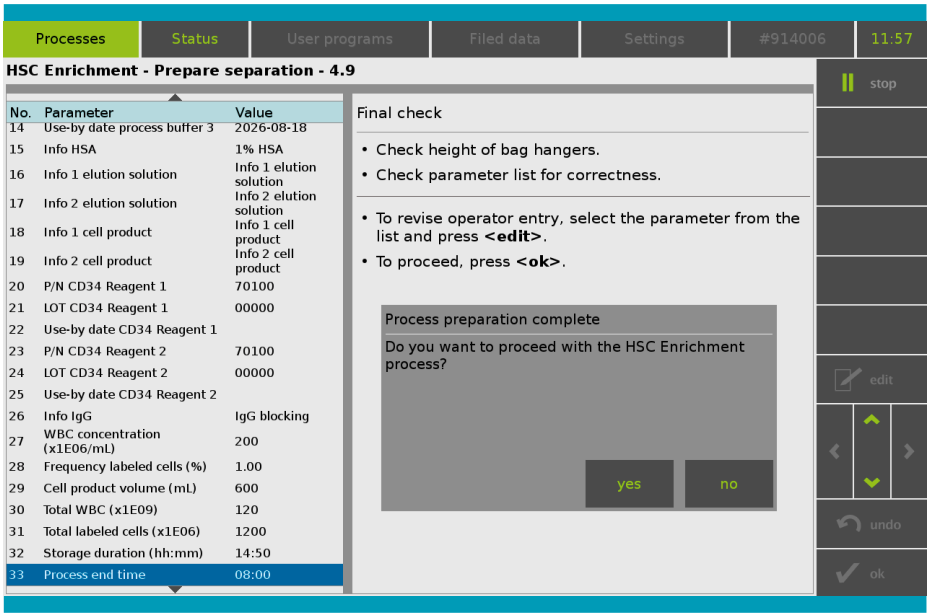
Screen 6.46: Mix Application Bag

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



Screen 6.47: Final process setup check

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



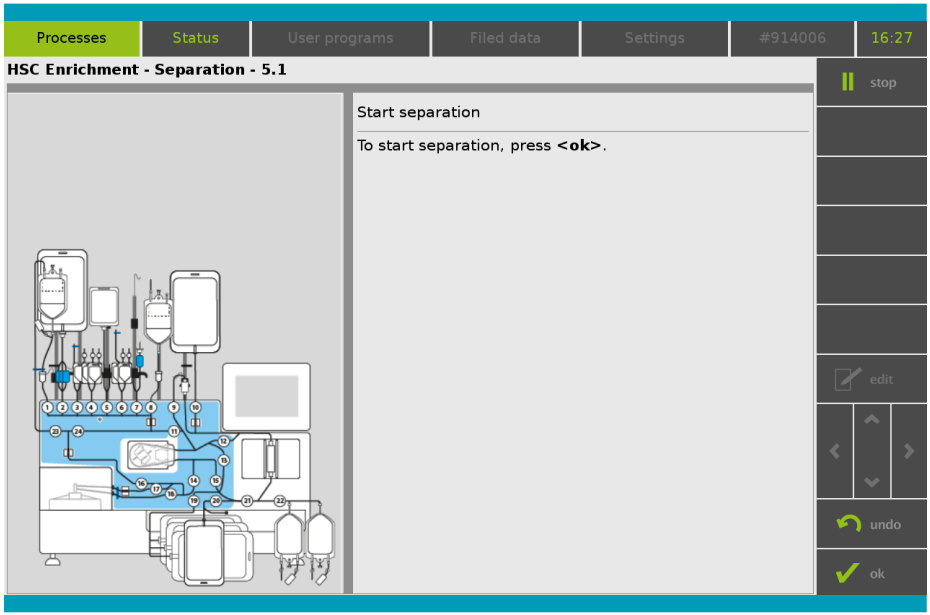
Screen 6.48: Completion of process preparation

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

6.6 Automated enrichment process

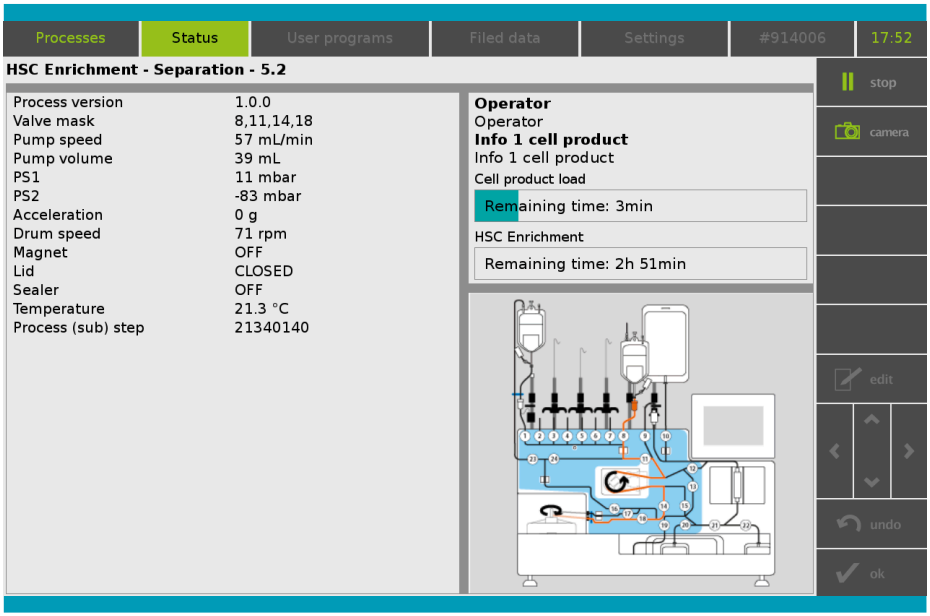
6.6.1 Start of the enrichment process

To start the enrichment process, tap <ok> as shown in Screen 6.49.



Screen 6.49: Start of the automated separation process

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



Screen 6.50: Status screen – process information screen after start of the separation process

For a detailed description of the status screen, also refer to the CliniMACS Prodigy user manual (instrument).

Important process phases include:

- cell product load
- cell wash
- IgG labeling (optional)
- reagent labeling
- reagent wash
- storage (optional)
- cell filtration
- cell separation

In detail:
The starting cell product is loaded into the chamber (in one or two batches according to the WBC number). Each batch is washed separately and after merging, the washed cell product is prepared for labeling. IgG is (optionally) added prior to CD34 labeling in order to minimize unspecific binding. After incubation with the CliniMACS CD34 Reagent, the excess of reagent is removed and the labeled cells are adjusted to a WBC concentration of max. 2.5×10^8/mL. If a delayed end time is desired (optional), the cells are temporarily stored in the chamber at +6 °C (+43 °F) for as long as is necessary to comply with the entered process end time.
For enrichment, the cells are filtered first before they are loaded on the separation column. Several column washes and release/reloading steps are applied in order to maximize purity of the target cells. Finally, the purified CD34-positive cells are eluted in elution solution (0.9% sodium chloride supplemented with 1% HSA) into the Target Cell Bag (TCB1), ready for further use.

6.7 Post-process handling steps

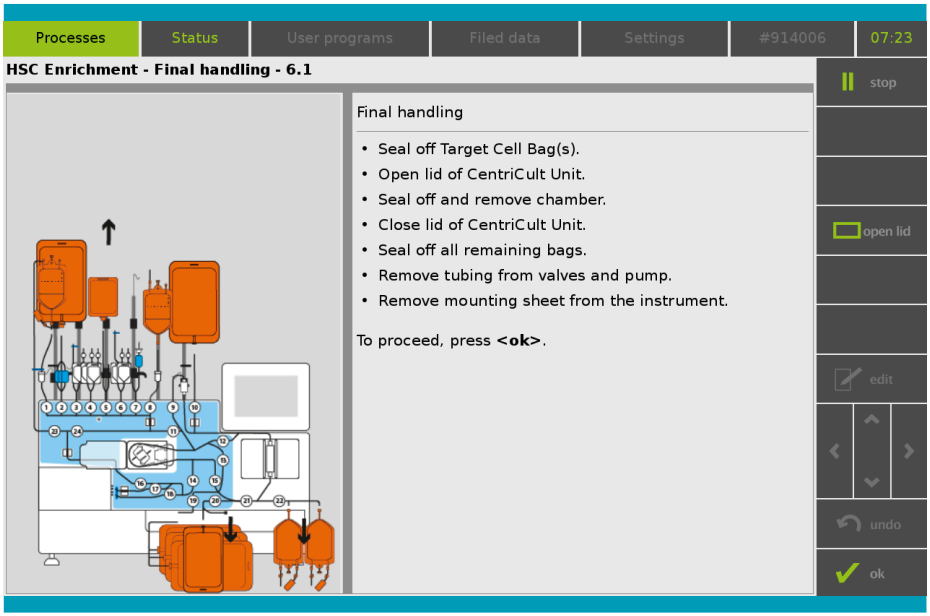
6.7.1 Completion of the enrichment process

After completion of the enrichment process, seal off Target Cell Bag 1 and the Non-Target Cell Bag. Use the QC Pouch to take a sample from the Target Cell Bag for QC analysis. Optionally, take and analyze a sample from the Non-Target Cell Bag as well.

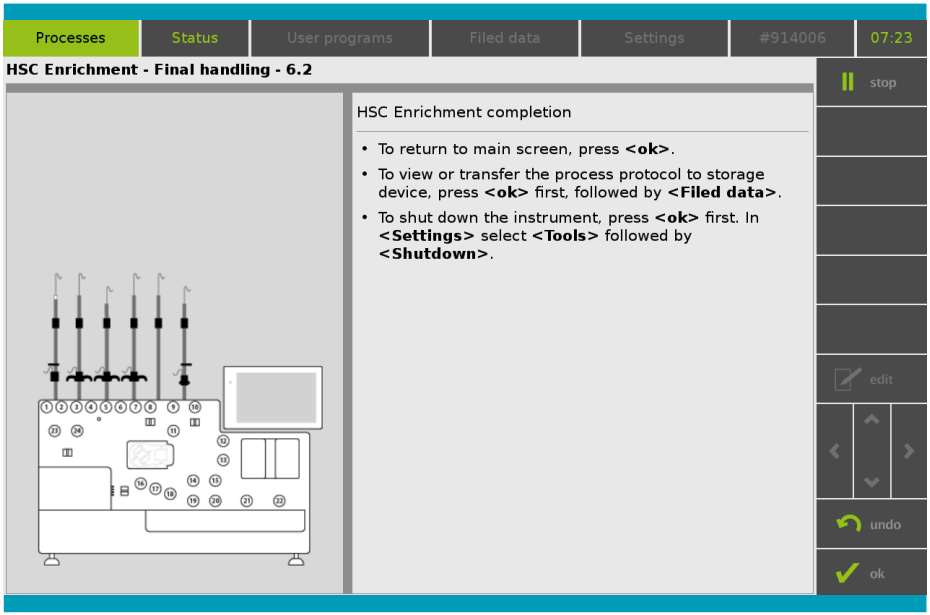
Store the samples according to institutional guidelines prior to analysis.

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

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



Screen 6.51: Final handling after separation process completion



Screen 6.52: Final handling after tubing set removal

6.7.2 Download of process log files

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

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

6.7.3 Shutdown and switch off the instrument

Always perform a shutdown procedure before switching off the instrument. In the <Settings> menu, select the program <Shutdown> in the <Tools> group and tap <run>. Once the shutdown is completed, the instrument can be switched off safely.

6.7.4 Cleaning and disinfection

⚠ CAUTION

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

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

6.7.5 Analysis of cells

⚠ CAUTION

Risk of reduced quality of target cells. The target cells must be analyzed and confirmed to meet user requirements, otherwise the suitability for clinical application can be compromised. Examine the target cells regarding quality and quantity according to their intended use.

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

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

IMPORTANT

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

Miltenyi Biotec as the provider of the CliniMACS Prodigy HSC Enrichment 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 Troubleshooting

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

+49 2204 8306-3803
technicalsupport@miltenyi.com

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

8 Legal notes

8.1 Limited warranty

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

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

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

inappropriate consumables, accessories, or work materials.

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

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

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NOTES

Miltenyi Biotec

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

www.miltenyibiotec.com

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