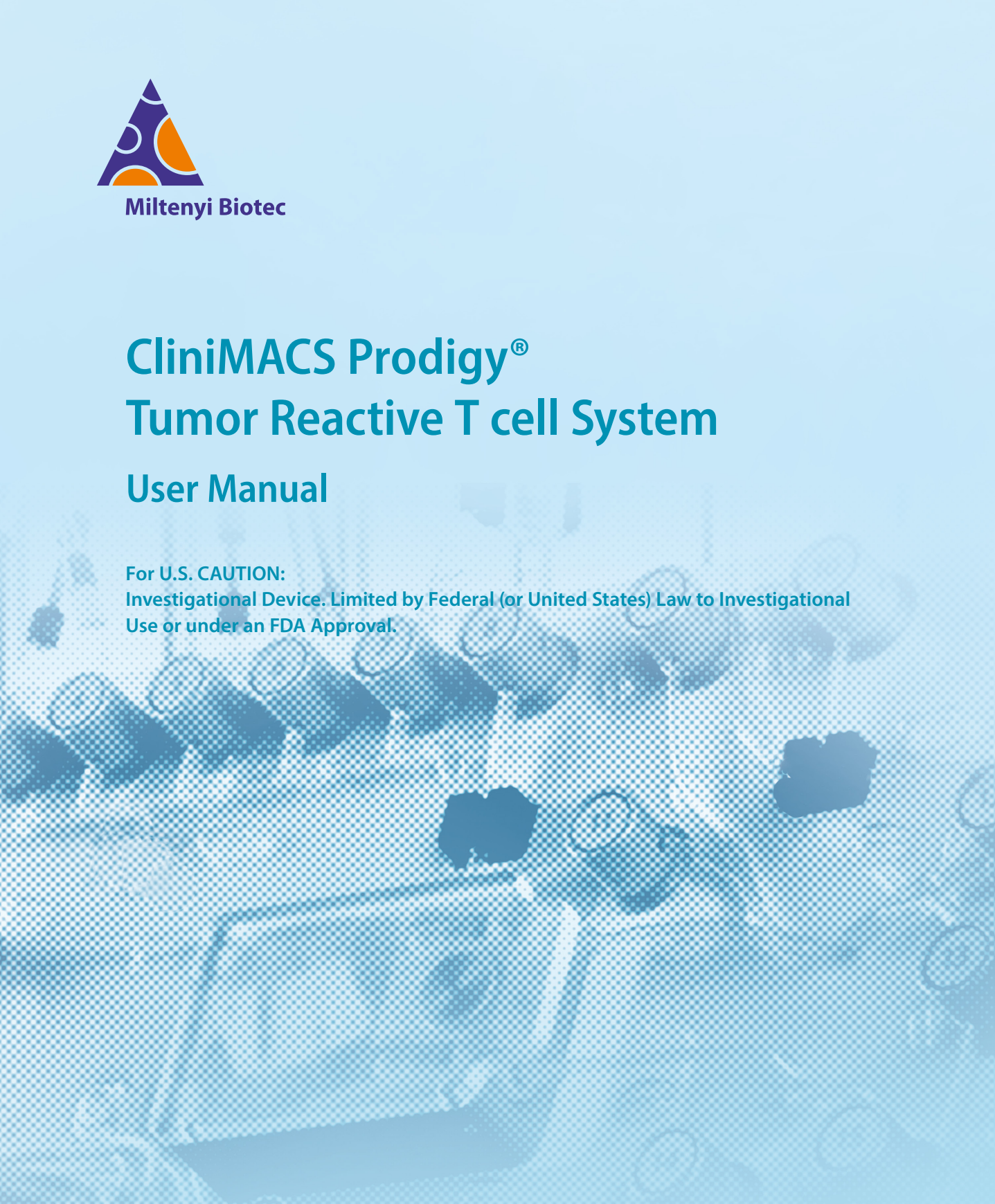
Miltenyi Biotec

CliniMACS Prodigy®
Tumor Reactive T cell System

User Manual

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

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CliniMACS Prodigy®
Tumor Reactive T cell System

User Manual

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Essential information

This user manual provides information for the use of the CliniMACS Prodigy Tumor Reactive T cell 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 product are described in the CliniMACS Prodigy User Manual (Instrument). For instructions for use, e.g.,, warnings and precautions, concerning the CliniMACS Prodigy Tumor Reactive T cell System components, refer to the instructions for use provided for the respective component.

⚠ WARNING

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

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

Table of contents

1	**Introduction**	**17**
1.1	General information	17
1.2	Process	18
1.2.1	Case 1: Stimulation, Enrichment and Expansion (complete process)	20
1.2.2	Case 2: Enrichment and Expansion	20
1.2.3	Case 3: Expansion	21
1.2.4	Case 4: Resume cases	21
2	**Glossary**	**23**
2.1	Graphical depiction	23
2.2	Glossary of symbols and terms	24
3	**Materials required**	**25**
3.1	Components of the CliniMACS Prodigy Tumor Reactive T cell System	25
3.2	Additional materials	27
4	**Warnings and precautions**	**29**
5	**Preparative steps**	**31**
5.1	Preparative steps for case 1 (Stimulation, Enrichment and Expansion)	31
5.1.1	Preparation and analysis of starting material	32
5.1.2	Preparation of process buffer	33
5.1.3	Preparation of medium for stimulation and enrichment	34
5.1.4	Preparation of supplements and/or peptide(s)	34
5.1.5	Preparation of enriched T cells for expansion	35
5.1.6	Analysis of T cells for expansion	35

Table of contents (continued)

5.1.7	Preparation of expansion medium	36
5.1.8	Preparation of feeder cells for expansion	37
5.1.9	Preparation of activation reagent (MACS GMP CD3 pure)	38
5.1.10	Optional preparation of transduction reagent (viral vector)	38
5.2	Preparative steps for case 2 (Enrichment and Expansion)	39
5.2.1	Preparation and analysis of starting material	39
5.2.2	Preparation of process buffer	40
5.2.3	Preparation of medium for enrichment	40
5.2.4	Preparations for expansion	40
5.3	Preparative steps for case 3 (Expansion)	41
5.3.1	Preparation and analysis of starting material	41
5.3.2	Preparations for expansion	42
6	**Starting the process**	**43**
6.1	Initialization of the CliniMACS Prodigy	43
6.2	Parameter input and process setup choice	46
6.3	Tubing set installation	49
6.3.1	Components of the CliniMACS Prodigy TS 520	49
6.3.2	Installation steps	51
6.4	Tubing set priming	72
6.4.1	Connection of buffer and medium	72
6.4.2	Priming phase	77
7	**T cell stimulation**	**81**
7.1	Preparation for the stimulation culture	81
7.1.1	Transfer of cellular starting product	83
7.1.2	Connection of stimulation peptide(s)	88

Table of contents (continued)

7.1.3	Input of CO_2 and temperature parameters	90
7.1.4	Setup of the activity matrix	93
7.1.5	Final setup check	96
7.2	Automated culture setup	98
7.3	Stimulation culture	99
7.4	End of stimulation culture	101
8	**CD137 enrichment**	**103**
8.1	Preparation for the automated CD137 enrichment	103
8.1.1	Connection of CliniMACS Reagents	103
8.1.2	Final check	106
8.2	Automated enrichment process	108
8.2.1	Start of enrichment process	108
8.2.2	Optional sampling of the original fraction	110
8.3	Completion of enrichment process	114
8.3.1	Analysis of enriched target cell fraction	115
9	**Expansion**	**117**
9.1	General remarks	117
9.2	Preparation for the expansion culture	118
9.2.1	Sealing off the pre-column and separation column	118
9.2.2	Rinsing of chamber and tubing set	120
9.2.3	Connection of medium	121
9.2.4	Input of cell seeding parameters	122
9.2.5	Input of CO_2 and temperature parameters	127
9.2.6	Setup of the activity matrix	130
9.2.7	Final setup check	136

Table of contents (continued)

9.2.8	Automated culture setup	137
9.3	Expansion culture	138
9.3.1	Addition of feeder cells	140
9.3.2	Activation	145
9.3.3	Feed	146
9.3.4	Sampling to monitor the culture	146
9.3.5	Taking microscope pictures during cultivation	148
9.3.6	Media exchange	150
9.3.7	Activate/Deactivate shaker	150
9.3.8	Medium Bag exchange	151
9.3.9	Waste Bag exchange	152
9.3.10	Transduction	154
9.3.11	Spin	155
9.3.12	Culture wash	155
9.3.13	Volume reduction	156
9.4	Final formulation and harvest	156
9.5	Final handling	159

10	**Cases 2 + 3**	**163**
10.1	Case 2 (Enrichment and Expansion)	163
10.1.1	Process setup and differences to case 1	163
10.1.2	Transfer of cellular starting product	165
10.1.3	CD137 enrichment (case 2)	169
10.1.4	Expansion (case 2)	170
10.2	Case 3 (Expansion)	170
10.2.1	Process setup and differences to case 1	170
10.2.2	Transfer of cellular starting product	171
10.2.3	Expansion (case 3)	177

Table of contents (continued)

11 Resume cases 4.1–4.3 179

11.1 General remarks 180

11.2 Case 4.1 (Resume Stimulation) 181

11.3 Case 4.2 (Resume after Enrichment) 183

11.4 Case 4.3 (Resume Expansion) 184

12 Troubleshooting 187

13 Legal notices 189

13.1 Limited warranty 189

13.2 Trademarks 190

1 Introduction

1.1 General information

Tumor-reactive T cells can be present within the patients, for example as tumor-infiltrating lymphocytes (TIL), or circulating in the peripheral blood and lymphatics. Tumor-reactive T cells usually exist in low frequencies in patients and often require complex *ex vivo* culturing to reach suitable cell numbers necessary for therapeutic application. The CliniMACS Prodigy Tumor Reactive T cell System has been developed to allow the efficient enrichment and expansion of large numbers of tumor-reactive T cells in a versatile and semi-automated manner.

The CliniMACS Prodigy Tumor Reactive T cell System is modular in design and can be used in part, or in whole, to support the user needs. Starting material that can be used in this process includes leukapheresis material (LP), tumor digests or any material that contains potentially tumor-reactive T cells.

Following a stimulation culture, tumor-reactive T cells can be enriched by automated magnetic selection via the activation marker CD137 using the CliniMACS Prodigy Tumor Reactive T cell System. Alternatively, tumor-reactive T cells can be obtained by FACS sorting on the MACSQuant Tyto or by T cell outgrowth from tumor fragments or digest. These cells can then be entered into a Rapid Expansion Protocol (REP) for further cell expansion. At this stage it is also possible to transduce the cells.

Depending on the user demands, the CliniMACS Prodigy Tumor Reactive T cell System allows parts of all of the entire process to be performed in an automated and closed system. The entire process includes the stimulation, enrichment, expansion, transduction and final formulation of the product (see Figure 1.1). Samples for quality control can be obtained during manufacturing in a sterile

fashion.
The components that are required for the CliniMACS Prodigy Tumor Reactive T cell System are described in chapter 3 of this user manual.

This user manual provides guidance for the use of the CliniMACS Prodigy Tumor Reactive T cell System in order to manufacture tumor-reactive and potentially genetically modified T cells for clinical application.

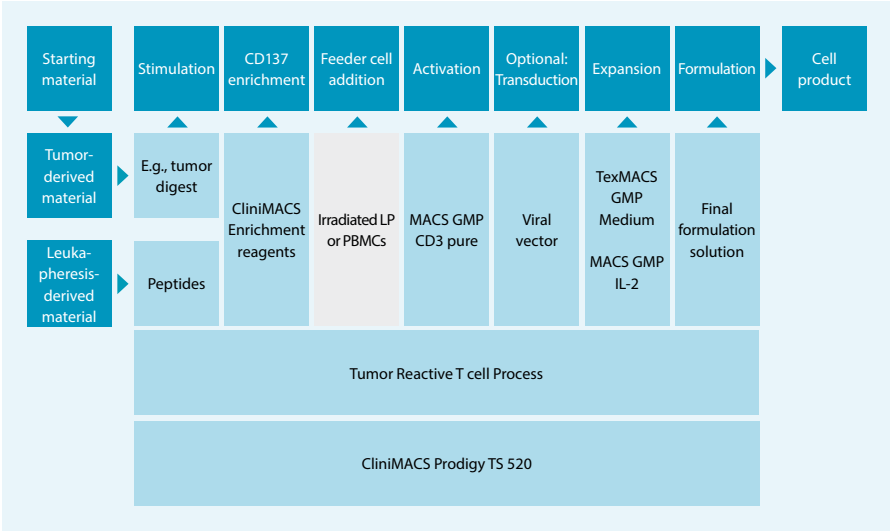


Figure 1.1: Generic process scheme using the CliniMACS Prodigy Tumor Reactive T cell System

IMPORTANT

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

1.2 Process

The Tumor Reactive T cell Process includes the following steps:

1. Install the CliniMACS Prodigy TS 520 on the CliniMACS Prodigy.

2. Prepare the process buffer (CliniMACS® PBS/EDTA Buffer supplemented with 0.5% human serum albumin (HSA)).

3. Prepare the medium (TexMACS™ GMP Medium supplemented with 3% human AB serum).

4. Connect the process buffer and medium to the tubing set.
5. The tubing set is primed.

6. Choose one of the following options:

   - Stimulation of tumor-derived material: if addition of supplements is desired (e.g., cytokines or tumor peptides to the stimulation culture) select the option addition of supplements and/or peptide(s).

   - Stimulation of leukapheresis-derived material: the addition of supplements and/or peptides is mandatory.

7. Connect the starting material and the supplements/peptides to the tubing set.

8. The stimulation is started. The cells are washed and, if selected, the supplements are added to the culture.

   The maximal upregulation of the activation marker CD137 usually occurs within 18–36 hours. The kinetics of CD137 upregulation can vary depending on the starting material. Further investigation is recommended to define the optimal stimulation culture period.

9. At the end of this stimulation culture, connect CliniMACS enrichment reagents to the tubing set for the CD137 enrichment.

10. Initial washing, cell labeling, and magnetic separation are performed automatically.

11. Enriched cells will be directed to a dedicated bag (Reapplication Bag) from which a sample can be taken for analysis.

12. To start the expansion of the isolated cells, connect a rinsing solution (sterile water) to the tubing set to rinse the tubing set and cultivation chamber.

13. Replace the TexMACS GMP Medium bag supplemented with 3% human AB serum with a bag which additionally contains high doses of IL-2 (3,000 IU/mL IL-2 recommended).

14. A user-defined number of isolated $CD137^+$ T cells is seeded into the chamber for the subsequent cell expansion.

15. The user is then prompted to plan the expansion culture using an activity matrix which includes the addition of irradiated feeder cells followed by addition of the activation reagent, MACS® GMP CD3 pure.

16. The Tumor Reactive T cell Process allows additional activities including viral transduction, culture washes, feeding, medium exchanges, chamber shaking and final formulation for harvest. The activities will be executed automatically according to the user-defined activity matrix, which can also be edited during the expansion culture period of typically 12–14 days.

17. The final product is then ready for clinical applications or applications desired by the user.

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

The status of the previously described process steps can be monitored on the screen of the CliniMACS Prodigy.

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

In order to permit a more flexible use of the Tumor Reactive T cell Process, several options named "cases" are available (see Figure 1.2). One of four cases must be selected at the beginning of a manufacturing run. For more details see sections 5.1.1.

1.2.1 Case 1: Stimulation, Enrichment and Expansion (complete process)

Case 1 is the complete Tumor Reactive T cell Process and includes the installation of the tubing set, stimulation, CD137 enrichment, irradiated feeder cell addition, T cell activation, viral transduction (optional), and expansion as described above.

1.2.2 Case 2: Enrichment and Expansion

Choose case 2 if the cell stimulation is performed manually and only the T cell enrichment and subsequent expansion are performed in the CliniMACS Prodigy. Manual stimulation could be performed in e.g., bags or plates when cell numbers are too low or too high for the cell culture chamber of the tubing set. See section 5.2.1 for more details.

Alternatively, choose this option if $CD137^+$ cells should be isolated directly, e.g., from tumor-derived material without the stimulation step. This case includes tubing set installation prior to cell processing.

1.2.3 Case 3: Expansion

Use case 3 in order to perform only the expansion part of the process without prior T cell stimulation or enrichment. For example, if T cells are isolated by different means (e.g., cell sorting) or when CD137 enrichment is not possible due to low starting cell numbers. See section 5.3.1 for more details. Case 3 includes the tubing set installation prior to the cell processing.

1.2.4 Case 4: Resume cases

If the Tumor Reactive T cell Process was interrupted (e.g., due to a power failure) and needs to be resumed, then use this option. There are 3 different resume cases available:

- Case 4.1: resume the stimulation culture of case 1
- Case 4.2: resume after enrichment of case 1 or case 2
- Case 4.3: resume the expansion culture of case 1, case 2 or case 3

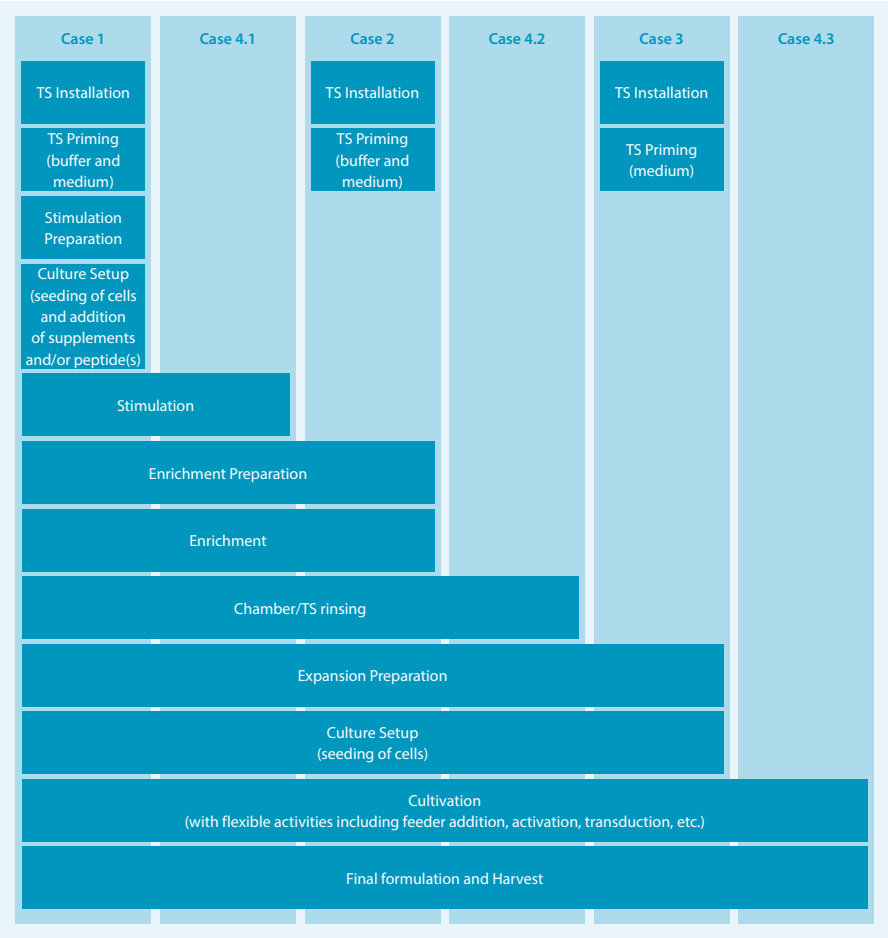


Figure 1.2: Versatility of the Tumor Reactive T cell Process

This user manual describes the entire process (case 1: Stimulation, Enrichment and Expansion) in detail (sections 6–9). For additional remarks about the cases 2–4, see sections 10 and 11.

IMPORTANT

The CliniMACS Prodigy Tumor Reactive T cell System has a maximum capacity with respect to cell stimulation, cell enrichment and cell expansion. Details are described in section 5.

2
Glossary

2.1 Graphical depiction

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

⚠ WARNING

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

⚠ CAUTION

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

NOTICE

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 (Customer information, warnings, and precautions). The glossary of symbols depicts the symbols used for information in the user manuals and labeling of the CliniMACS Products.

3

Materials required

The following section outlines the materials and consumables required for the generation of tumor-reactive T cells with the CliniMACS Prodigy Tumor Reactive T cell System.

3.1 Components of the CliniMACS Prodigy Tumor Reactive T cell System

The following components are used in the CliniMACS Prodigy Tumor Reactive T cell System. Depending on the selected process case not all components are required (see Table 3.1).

Material	Quantity	Comment
CliniMACS Materials		
CliniMACS Prodigy	1	
CliniMACS Prodigy TS 520	1 set	
CliniMACS CD137 GMP Biotin	1 vial	Used for case 1/case 2
CliniMACS Anti-Biotin GMP MicroBeads/ CliniMACS Anti-Biotin Reagent	1 vial	Used for case 1/case 2
CliniMACS PBS/EDTA Buffer	3 liters	Use 1×3 L bag for case 1/case 2
CliniMACS PBS/EDTA Buffer	1 liter	Use additionally 1×1 L bag for case 1

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

Material	Quantity	Comment
CliniMACS Materials		
Luer/Spike Interconnector	several	For transfer bags and buffer preparation
Triple Sampling Adapter	several	In case more than 3 in-process controls are required during the process
20 mL Reagent Bag	2–3 pieces	• 1 bag for supplements and/or peptide(s) (case 1) • 1 bag for MACS GMP CD3 pure (all cases) • 1 bag for viral vector, if needed
Transfer Set Coupler/Coupler	1 piece	• Used to connect the two process buffer bags required for stimulation and enrichment (case 1)
5 L Collection Bag	1 piece	In case more than 2 Waste Bags are required during the process
MACS GMP Materials		
TexMACS GMP Medium	6–8 liters	At least 3x2 L bag (case 1/case 2) At least 2x2 L bag (case 3) Depending on the cell growth additional medium may be required
MACS GMP Cytokines	x vials	Optional: can be used for the T cell stimulation (e.g., human IL-7 and IL-15 – needs to be tested for your own indication) (case 1)
MACS GMP Recombinant Human IL-2	x vials	If IL-2 is used for T cell expansion; the amount depends on the cytokine activity and size of the vial (recommended: 3,000 IU/mL) (all cases)
MACS GMP CD3 pure	1 vial 0.2 mg in 1 mL	Used for T cell expansion; 30 µL is needed per run when starting the expansion in 200 mL culture volume (all cases)
MACS GMP Vectofusin®-1	1 vial	Optional, in case of transduction with retroviral vectors; the amount depends on the volume of viral vector as it will be pre-mixed with viral vector (all cases)
Software		
Tumor Reactive T cell Process		

Table 3.1: Materials recommended for the CliniMACS Prodigy Tumor Reactive T cell System

IMPORTANT

The materials referenced above are recommended for use in combination with the Tumor Reactive T cell Process. Since the user is responsible for the validation of the process, the user can ultimately decide on the source of the material.

3.2 Additional materials

Besides the CliniMACS Prodigy Tumor Reactive T cell System components, additional materials are required for the Tumor Reactive T cell Process (see Table 3.2):

Material	Comment
Cell sources	
Tumor-derived or leukapheresis-derived material	• Tumor-derived: preferably freshly digested cells, if using thawed cells, the yield may be reduced • Leukapheresis-derived: only fresh material not older than 24 hours • Cell count and volume depend on the chosen case: • Stimulation (case 1): recommended to use ~4×10^8 total cells (tumor-derived) or 1×10^9 total white blood cells (leukapheresis-derived) in 20–200 mL For stimulation of higher or lower cell numbers see section 5.2.1 • Enrichment (case 2): recommended to use up to 1×10^9 total cells (tumor-derived) or 1×10^9 total white blood cells (leukapheresis-derived) 20–200 mL • Expansion (case 3): recommended to use 1×10^6 viable T cells in 20–200 mL
Leukapheresis product (LP) or PBMCs (for irradiated feeder cells)	• Cells can be autologous or allogeneic (e.g., mix of 3 donors) • Either fresh or thawed • 4×10^8 viable white blood cells in 50–100 mL
Reagents and biological materials	
Antibiotics	For the medium of the entire process, if desired (recommended for tumor digest)
Human serum albumin (HSA)	• Use only pharmaceutical grade HSA approved in your country • Final concentration: 0.5% HSA in a total of 3 or 4 liter CliniMACS PBS/EDTA Buffer (case 2/case 1)
Human AB serum	• Use only GMP grade product • Final concentration: 3% human AB serum for media preparation; 60 mL for stimulation/enrichment and 60 mL for first expansion medium bag
Sterile water for injection	• Pharmaceutical grade • 300 mL in a weldable bag (rinsing solution) • Additional x mL required to reconstitute cytokines
Viral vector	Lentiviral or gamma-retroviral vector in a bag in 5–100 mL (recommended: 10 mL)
Final formulation solution	1 liter in a weldable bag, (e.g., CliniMACS Formulation Solution, 0.9% NaCl supplemented with HSA or cytokines of choice)

Material	Amount required	Comment
Consumables		
Syringes	several	• For transfer of cells or reagents in a transfer bag • 10 mL, Luer-Lock Tip • 60 mL, Luer-Lock Tip
Transfer bag, 150 mL	2 bags	• 1 bag for starting material of stimulation or enrichment (case 1/case 2) • 1 bag for start of expansion with isolated target cells (case 3) • 1 bag for irradiated feeder cells for start of expansion (all cases)
Transfer bag, 600 mL	1 bag	Used for sterile water (rinsing solution, needed after enrichment if expansion is performed)
Equipment		
Uninterruptable power supply		
CO_2 and compressed air supply		
Sterile tubing welder, e.g., Terumo Sterile Connecting Device and sterile wafers		
Device to measure glucose and lactate levels, e.g., Accutrend Plus and test strips (Roche)		
Flow cytometer, e.g., MACSQuant Analyzer 10		
Laminar flow hood		
X-Ray device for preparation of feeder cells		

Table 3.2: Additional materials required for the CliniMACS Prodigy Tumor Reactive T cell System

IMPORTANT

This protocol requires the use of components and materials which are not part of the CliniMACS Prodigy Tumor Reactive T cell System, e.g., human serum albumin 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 before application of the target cell product to humans.

4

Warnings and precautions

⚠ CAUTION

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

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 products are described in the CliniMACS Prodigy User Manual (Instrument). For instructions for use, e.g., warnings and precautions, concerning the CliniMACS Prodigy Tumor Reactive T cell System components, refer to the instructions for use provided for the respective component.

5
Preparative steps

⚠ WARNING

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

The process using the CliniMACS Prodigy Tumor Reactive T cell System requires a few preparative steps.

5.1 Preparative steps for case 1 (Stimulation, Enrichment and Expansion)

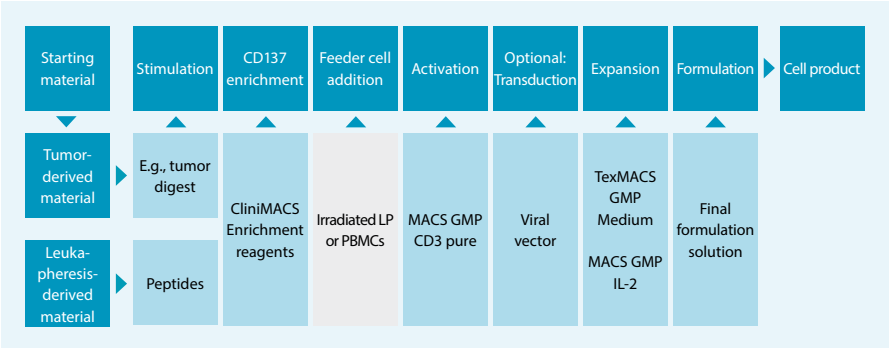


Figure 5.1: Generic process scheme of case 1 using the CliniMACS Prodigy Tumor Reactive T cell System

5.1.1 Preparation and analysis of starting material

It is recommended to analyze the starting material with regard to cell count and available T cells before starting the process. Especially when using tumor-derived material as starting material, this information is required to determine which case is most suitable.

5.1.1.1 Tumor-derived material

When starting with tumor-derived material, a single-cell suspension is required. This can be done using the GentleMACS Octo together with the Human Tumor Dissociation kit, for example.

It is recommended to analyze the digest for the presence of T cells as well as total count of all cells, viable cells and viable T cells.

Tumor material is highly variable in its content, viability, T cell infiltration and the reactivity of those T cells. Because of this heterogeneity only general recommendations can be given. The total cell count is critical for the decision which case to use.

As a general recommendation, a minimum of ~2×10^6 total T cells within the tumor digest is required to obtain relevant numbers of CD137-enriched cells for expansion for clinical use.

Note: This is only a rough assumption as it is highly dependent on the number of CD137-expressing T cells within the sample after the stimulation culture.

When sufficient T cells are present, and the tumor-derived material contains 2×10^8 to 4×10^8 total cells, it is recommended to use **case 1 (Stimulation, Enrichment and Expansion)** (see Figure 5.1) followed by the option tumor-derived material in which the cells are cultured in the CliniMACS Prodigy.

When using lower or higher cell numbers, follow the instructions described in section 5.2.1 to perform manual stimulation in combination with **case 2 (Enrichment and Expansion).**

When only very few T cells are present, optionally expand the T cells without previous enrichment. Follow the instructions described in section 5.3.1 to perform **case 3 (Expansion).**

5.1.1.2 Leukapheresis-derived material

When using leukapheresis-derived material, analyze the cellular starting material by determining the white blood cell concentration as well as white blood cell viability.

Recommendation:

- Use 1×10^9 total white blood cells.
- Select case 1 to perform the stimulation culture in the CliniMACS Prodigy (see Figure 5.1) followed by the option leukapheresis-derived material.

A more extensive washing procedure than for tumor-derived material will be performed prior to stimulation setup.

5.1.1.3 Remarks for both tumor- and LP-derived material

Volume of the starting material for setup of the stimulation in case 1:

- Minimal volume: 20 mL.
- Maximum volume: 200 mL.

The required volume is calculated based on the cell concentration of the starting material and the desired starting cell number.

If necessary, dilute or concentrate the cell product and transfer the cells into a suitable transfer bag before connecting it to the Application Bag of the tubing set.

5.1.2 Preparation of process buffer

Supplement a 3 L bag of CliniMACS PBS/EDTA Buffer and an additional 1 L bag of CliniMACS PBS/EDTA Buffer each with HSA to a final concentration of 0.5% (v/v), e.g., add 20 mL of 25% HSA per 1 L of buffer.

The supplemented buffer is henceforth referred to as 'process buffer'.

IMPORTANT

HSA is not a component of the CliniMACS Prodigy Tumor Reactive T cell System. Use only pharmaceutical-grade HSA approved in your country. Carefully read the package insert of the HSA used; in particular the section regarding hypersensitivity reactions and the risk of infection associated with HSA as a blood-derived product.

Evaluate all risks arising from this material.

5.1.3 Preparation of medium for stimulation and enrichment

For the stimulation and enrichment part of case 1 and case 2, one medium bag is required in total. Supplement a 2 L bag of TexMACS GMP Medium with 3% human AB serum and optionally antibiotics. Antibiotics are highly recommended for tumor-derived starting material as the risk for contamination is increased.

To prepare the medium bag:

- Supplement the TexMACS GMP Medium with 60 mL (3%) human AB serum using the Luer Lock port of the medium bag.
- Optional: add the appropriate amount of antibiotics to the medium bag using the Luer Lock port of the medium bag.
- Optional: remove 40 mL from the medium bag in a vessel of choice. Store at +4 °C to +8 °C (+39 °F to +46 °F) to use for dilution of the supplements and/or peptide(s) (case 1) and optional small-scale controls.

IMPORTANT
Human AB serum and antibiotics are not components of the CliniMACS Prodigy Tumor Reactive T cell System. Use only GMP grade human AB serum and pharmaceutical-grade antibiotics.

5.1.4 Preparation of supplements and/or peptide(s)

For leukapheresis-derived material, add peptides of interest (e.g., predicted neo-antigens) or MACS GMP PepTivators® to the stimulation culture.

When tumor-derived material is used (e.g., tumor digest), the tumor cells within the preparation can stimulate the T cells. Therefore, addition of e.g., peptides is not necessary but optional.

If desired, cytokines such as MACS GMP Recombinant Human IL-7 and/or IL-15 can be used as supplements.

Note: It is not recommended to use IL-2 for the stimulation culture as it induces unspecific CD137 upregulation.

Prepare a 20 mL Reagent Bag containing supplements and/or peptide(s).

The minimal volume is 1 mL and the maximum volume is 20 mL (recommended: 5-10 mL):

- Reconstitute lyophilized peptides or MACS GMP PepTivators as described in the respective data sheet. E.g., reconstitute one vial MACS GMP PepTivator containing 60 nmol/peptide in 2 mL sterile water for injection.
- Reconstitute the lyophilized MACS GMP Cytokines as described in the respective data sheet. Further dilute according to the biological activity or vial-filled mass to get the desired concentration. E.g., reconstitute a 25 µg vial of human IL-7 with 1 mL sterile water for injection.
- Optional: If necessary, dilute using the medium from the medium bag.
- Add the appropriate amount of the desired supplements and/or peptide(s) to a 20 mL Reagent Bag using the Luer Lock port.

IMPORTANT
Reagents such as predicted neo-antigenic peptides are not components of the CliniMACS Prodigy Tumor Reactive T cell System. Use only GMP grade reagents.

5.1.5 Preparation of enriched T cells for expansion

After enrichment, the cells are eluted into the Reapplication Bag. To analyze the cells, take a sample using the sample pouch. For the analysis, see section 5.1.6. For expansion of previously enriched T cells (case 1 or case 2):

- The minimal volume of the starting cell product is 10 mL.
- The maximum volume of the cell suspension present in the Reapplication Bag is 40 mL.

See section 9.2.4 for further handling instructions.

5.1.6 Analysis of T cells for expansion

It is recommended to analyze the starting material in regard to cell count and viability of $CD3^+$ T cells before starting the process. Determine the number of viable $CD3^+$ cells within the starting material on a MACSQuant Analyzer. Alternatively, determine the total viable white blood cell count and multiply this count by the percentage of $CD3^+$ cells within the starting material.

It is recommended to start the expansion culture with 1×10^6 viable responder T cells and 4×10^8 viable, irradiated feeder cells. When more T cells are available, up to 1×10^7 viable responder T cells together with $4x10^8$ viable, irradiated feeder cells can be expanded maximally.

Note: When using such high starting numbers, T cells can potentially reach the maximum cell number of ~$3–4\times10^9$ earlier than the standard expansion duration of 14 days.

When the number of responder T cells is low (e.g., 1×10^6 viable T cells or less), it is recommended to transfer all T cells from the Reapplication Bag to the chamber for expansion.

5.1.7 Preparation of expansion medium

For expansion, supplement a 2 L bag of TexMACS GMP Medium with 3% human AB serum and IL-2 (MACS GMP Recombinant Human IL-2).

The recommended dose is 3,000 IU/mL IL-2.

The respective TexMACS GMP Medium bag is referred to as 'expansion medium bag'.

Further medium bags are required during the expansion depending on the expansion activities.

If starting from tumor material, it is recommended to supplement all medium bags with antibiotics.

Note: When using active shaking for agitated T cell expansion in the CliniMACS Prodigy, it is highly recommended to supplement the TexMACS GMP Medium with 3% human AB serum. It is possible to adapt the T cell culture and reduce the human AB serum by using 3% supplementation only in the first 2 L bag and using the subsequent 2 L bag(s) of TexMACS GMP Medium without supplementation of human AB serum.

To prepare medium bags:

- Reconstitute the lyophilized MACS GMP Cytokines with 1 mL sterile water for injection. Further dilute with TexMACS GMP Medium according to the biological activity or vial-filled mass to get the desired concentration. E.g., reconstitute a 500 µg vial of human IL-2 with an activity of 6×10^6 IU/mg in a total volume of 3 mL to get a stock solution of 1×10^6 IU/mL. Prepare a stock solution of the cytokines.

- Supplement the TexMACS GMP Medium with the appropriate amount of IL-2 (recommended: 3,000 IU/mL IL-2) using the Luer Lock port of the medium bag.

- Add 60 mL (3%) human AB serum to the medium bag using the Luer Lock port of the medium bag.

- Optional: add the appropriate amount of antibiotics to the medium bag using the Luer Lock port of the medium bag.

- Remove approximately 200 mL (case 1 + 2) or 250 mL (case 3) from the medium bag in a vessel of choice. Store at +4 °C to +8 °C (+39 °F to +46 °F) to use for dilution of the activation reagent (MACS GMP CD3 pure), preparation of the feeder cells, preparation of starting T cells (only case 3), and optional small-scale controls.

IMPORTANT

Human AB serum and antibiotics are not components of the CliniMACS Prodigy Tumor Reactive T cell System. Use only GMP grade human AB serum and pharmaceutical-grade antibiotics.

5.1.8 Preparation of feeder cells for expansion

Tumor-reactive T cells must be activated with MACS GMP CD3 pure in the presence of a large excess of irradiated feeder cells to support the T cell growth.

Prepare the feeder cells from either leukapheresis material (allogeneic or autologous) or from PBMC generated from buffy coats (it is recommended to mix several PBMC donors, total recommended amount required: 4×10^8 viable white blood cells in order to confluently cover the chamber surface).

Feeder cells can be used freshly or directly after thawing in medium with minimum 10% human AB serum. Following centrifugation (recommended: 300×g for 10 min with low brake), resuspend cells in sterile TexMACS GMP Medium supplemented with 3% human AB serum and IL-2 that was withdrawn from the prepared expansion medium bag (see section 5.1.7).

Determine the viable white blood cell count within the feeder cell suspension. Irradiate the cells at a recommended dose of 40 Gy to prevent them from growing out during the expansion.

Feeder cell product volumes:

- The minimal volume is 50 mL.
- The maximum volume is 100 mL.

The volume is calculated based on the cell concentration and the desired starting cell number (recommended: 4×10^8 viable white blood cells).

If necessary, dilute or concentrate the cell suspension. Transfer the cells into a suitable transfer bag (e.g., 150 mL transfer bag). Then connect the transfer bag to the Reapplication Bag of the tubing set.

See section 9.3.1 for further handling instructions.

Note: After transfer of the feeder cells to the chamber, a volume reduction to 40 mL will be performed automatically.

5.1.9 Preparation of activation reagent (MACS GMP CD3 pure)

For expansion, MACS GMP CD3 pure is needed to activate the T cells in the presence of irradiated feeder cells.

The recommended final concentration in the cell culture is 30 ng/mL.

- Add 30 µL of the 0.2 mg/mL MACS GMP CD3 pure to 5 mL sterile TexMACS GMP Medium for a recommended total starting culture volume of 200 mL.

- Transfer the solution into a 20 mL Reagent Bag using the Luer Lock port of the Reagent Bag.

- Alternatively, use a vial instead of a bag, e.g., using MACS GMP T Cell TransAct™. **Note:** If the vial port has already been used for the CliniMACS Anti-Biotin Reagent/GMP MicroBeads in case 1 or case 2, this option is not feasible. To define optimal concentration further investigation is required. Therefore, a Reagent Bag with diluted MACS GMP CD3 pure is recommended.

5.1.10 Optional preparation of transduction reagent (viral vector)

Viral vectors should be prepared in a suitable bag, e.g., a 20 mL Reagent Bag. The volume should be in range of 5–100 mL (recommended: 10 mL).

If desired, prepare the viral vector in a mixture with MACS GMP Vectofusin®-1 as described in the respective data sheet.

5.2 Preparative steps for case 2 (Enrichment and Expansion)

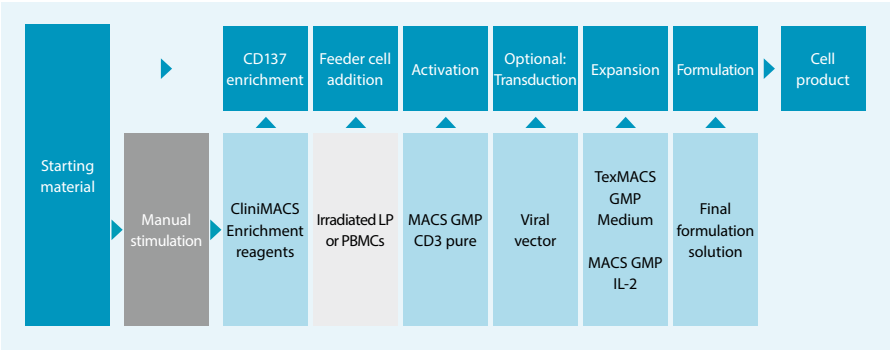


Figure 5.2: Generic process scheme of case 2 using the CliniMACS Prodigy Tumor Reactive T cell Process

5.2.1 Preparation and analysis of starting material

Case 2 performs the CD137 enrichment upon transfer of the starting material (see Figure 5.2) followed by the expansion. Select this case if no stimulation is desired or when the stimulation has been performed outside the CliniMACS Prodigy. For this, culture the starting material either in bags or plates at around 4×10^6 total cells/cm^2 in TexMACS GMP Medium with 3% human AB serum. Optionally, add desired cytokines and antibiotics.

When starting with tumor-derived material, it is required to generate a single-cell suspension. This can be done using the GentleMACS Octo using the Human Tumor Dissociation kit.

Before starting the process it is recommended to analyze the starting material in regard to cell count and available T cells (see section 5.1.1). Analyze the sample for the presence of T cells as well as total count of all cells, viable cells and viable T cells.

Additional staining of CD137 will provide an estimation how many cells can potentially be isolated from the starting material (considering that a certain amount of cell loss will occur during the enrichment process).

It is recommended to use 1×10^9 total white blood cells (leukapheresis-derived material) or up to 1×10^9 total cells (tumor-derived material).

Volume of the starting material for setup of the enrichment in case 2:

- Minimal volume: 20 mL.
- Maximum volume: 200 mL.

The required volume is calculated based on the cell concentration of the starting material and the desired starting cell number.

If necessary, dilute or concentrate the cells. Transfer the cells into a suitable transfer bag before connecting it to the Application Bag of the tubing set. See section 10.1.2 for further handling instructions.

5.2.2 Preparation of process buffer

Supplement a 3 L bag of CliniMACS PBS/EDTA Buffer with HSA to a final concentration of 0.5% (v/v), e.g., add 20 mL of 25% HSA per 1 L of buffer.

The supplemented buffer is henceforth referred to as 'process buffer'.

IMPORTANT
HSA is not a component of the CliniMACS Prodigy Tumor Reactive T cell System. Use only pharmaceutical-grade HSA approved in your country. Carefully read the package insert of the HSA used; in particular the section regarding hypersensitivity reactions and the risk of infection associated with HSA as a blood-derived product. The user must evaluate all risks arising from this material.

5.2.3 Preparation of medium for enrichment

Follow the instructions as described in section 5.1.3.

5.2.4 Preparations for expansion

Follow the instructions described in section 5.1;

- Section 5.1.5: for preparation of enriched T cells
- Section 5.1.6: for the analysis of enriched T cells
- Section 5.1.7: for preparation of the expansion medium
- Section 5.1.8: for preparation of feeder cells
- Section 5.1.9: for preparation of activation reagent
- Section 5.1.10: for preparation of optional transduction reagent

5.3 Preparative steps for case 3 (Expansion)

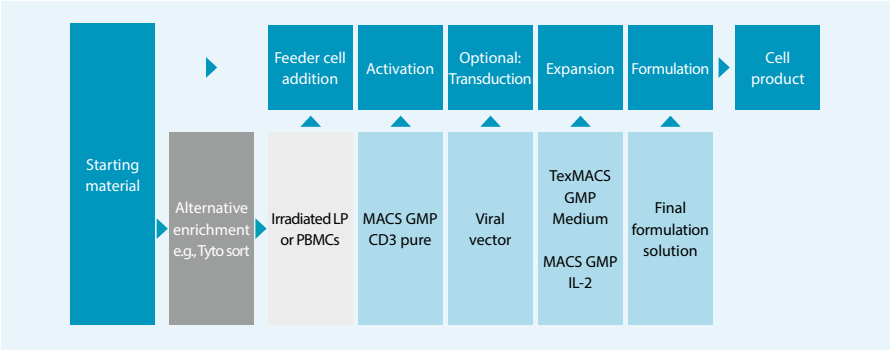


Figure 5.3: Generic process scheme of case 3 using the CliniMACS Prodigy Tumor Reactive T cell Process

5.3.1 Preparation and analysis of starting material

Select case 3 to directly expand the starting material without a previous enrichment (see Figure 5.3). This could be relevant for example:

- In samples where tumor-reactive T cells have been isolated from starting material via other means (e.g., sorting on surface marker expression, such as on the MACSQuant Tyto).
- In samples where the tumor-derived starting material contains very few T cells.
- When small numbers of tumor-infiltrating lymphocytes have previously been grown out of a tumor sample.

When starting with tumor-derived material, it is required to generate a single-cell suspension. This can be done using the GentleMACS Octo using the Human Tumor Dissociation kit.

Before starting the process, it is recommended to analyze the starting material in regard to cell count and viability of $CD3^+$ T cells (see section 5.1.1). The required testing and recommendations regarding cell count of the starting material are described in section 5.1.6.

Volume of the starting cell product for the setup of case 3:

- Minimal volume: 50 mL.
- Maximum volume: 100 mL.

The required volume is calculated based on the cell concentration of the starting material and the desired starting cell number.

If necessary, dilute or concentrate the cell product. Transfer the cells into a suitable transfer bag (e.g., 150 mL transfer bag) before connecting it to the Reapplication Bag of the tubing set. See section 10.2.2 for further handling instructions.

5.3.2 Preparations for expansion

Follow the instructions described in section 5.1;

- Section 5.1.7: for preparation of the expansion medium
- Section 5.1.8: for preparation of feeder cells
- Section 5.1.9: for preparation of activation reagent
- Section 5.1.10: for preparation of optional transduction reagent

6

Starting the process

6.1 Initialization of the CliniMACS Prodigy

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

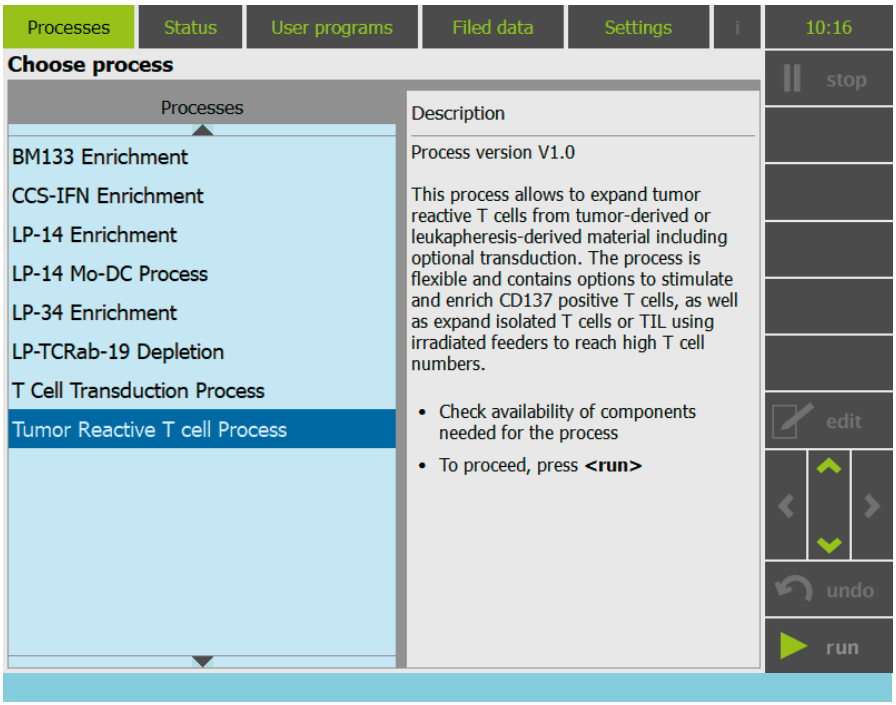
Note: A user interface showing screens with instructions and pictures will guide through the procedure. Parts of the text presented on the screens in the user manual may differ slightly from the screens displayed on the instrument.

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

After the instrument initialization phase the main screen is shown. Select the process 'Tumor Reactive T cell Process' and tap **run** to confirm the selection (see Main screen).

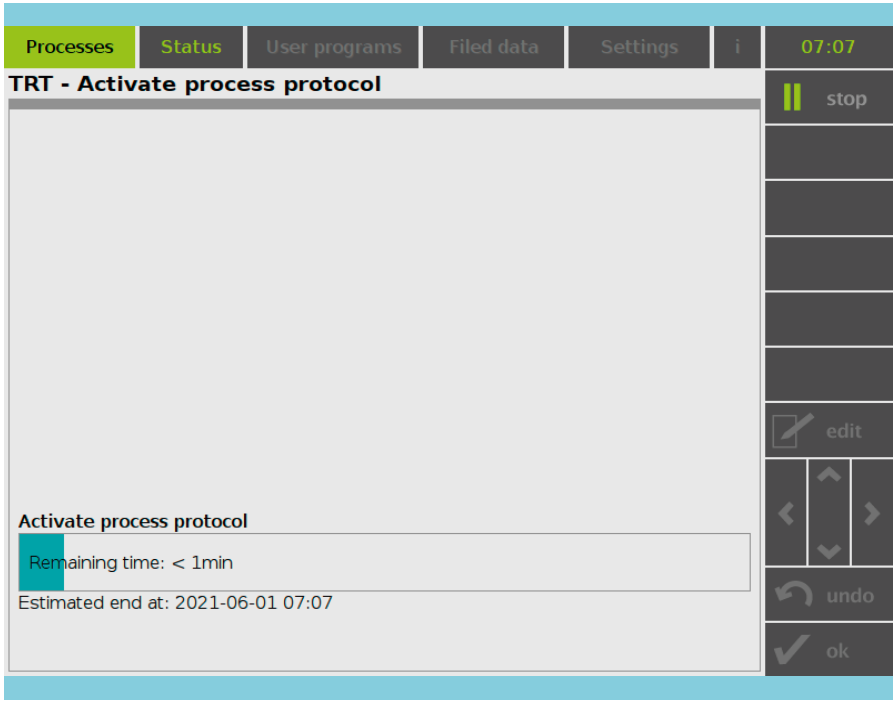
⚠ CAUTION

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



Main screen (Tumor Reactive T cell Process): Choice of process

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



Screen 1.1: Self-test

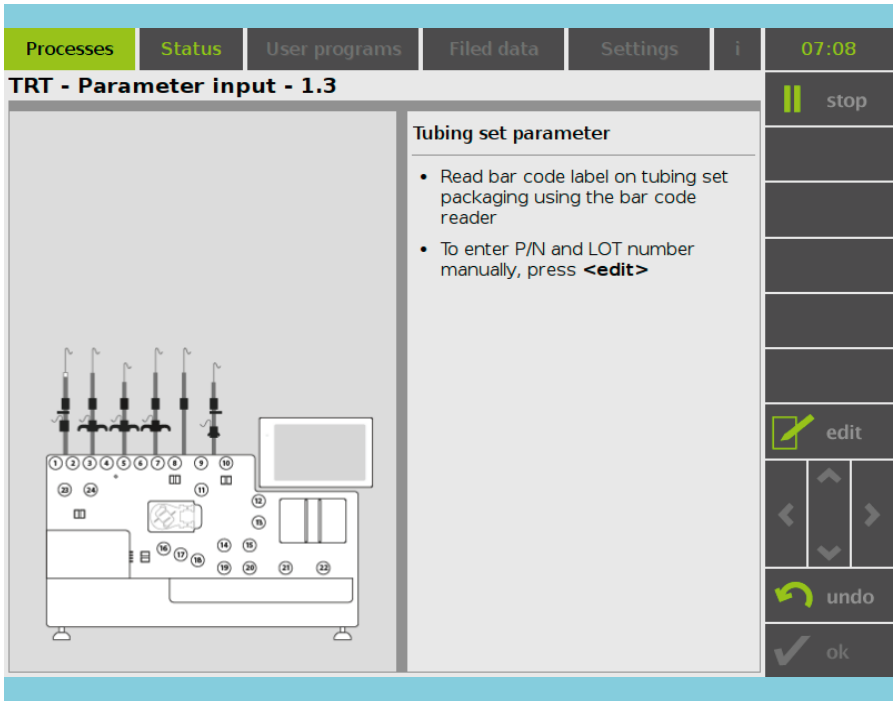
6.2 Parameter input and process setup choice

Multiple parameters can be entered for the Tumor Reactive T cell Process using either the bar code reader attached to the CliniMACS Prodigy or the keyboard shown on the screen. Tap **ok** to confirm. All entered parameters are saved in a process-related protocol, which can be transferred on an USB storage device after the process is completed.

During the next steps of the process, enter information related to the operator (see Screen 1.2) and the CliniMACS Prodigy TS 520 (see Screen 1.3). For the manual entry of capital letters, tap ⌂, for space tap **SP**, for numbers tap **?123**, and for backspace tap ⌫.

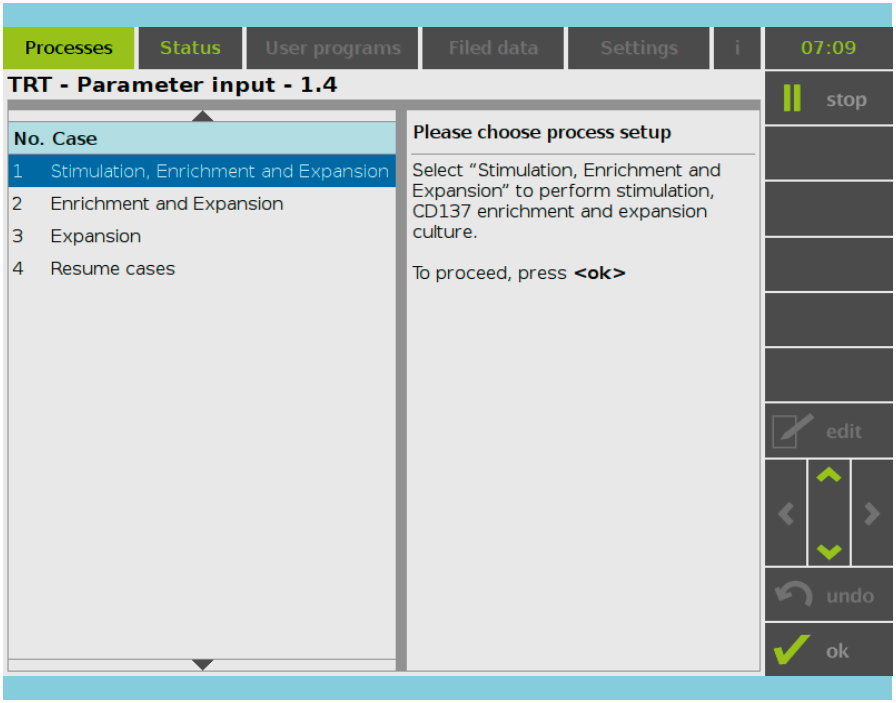


Screen 1.2: Entry of operator-related information



Screen 1.3: Entry of CliniMACS Prodigy TS 520 part number (P/N) and batch code (LOT)

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



Screen 1.4: Process setup of case 1 'Stimulation, Enrichment and Expansion'

After parameter input, choose between four different cases explained in section 1.2.

This user manual describes the complete process using case 1 (Stimulation, Enrichment and Expansion) as the default process. Tap **ok** to confirm the case selection.

The other cases have similar screens but might have a slightly changed order compared to the screens shown of process case 1. Nevertheless, the screens themselves guide through the process case like in the complete process. See section 10 for further details on case 2 (section 10.1) and case 3 (section 10.2) as well as section 11 for the resume cases.

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.

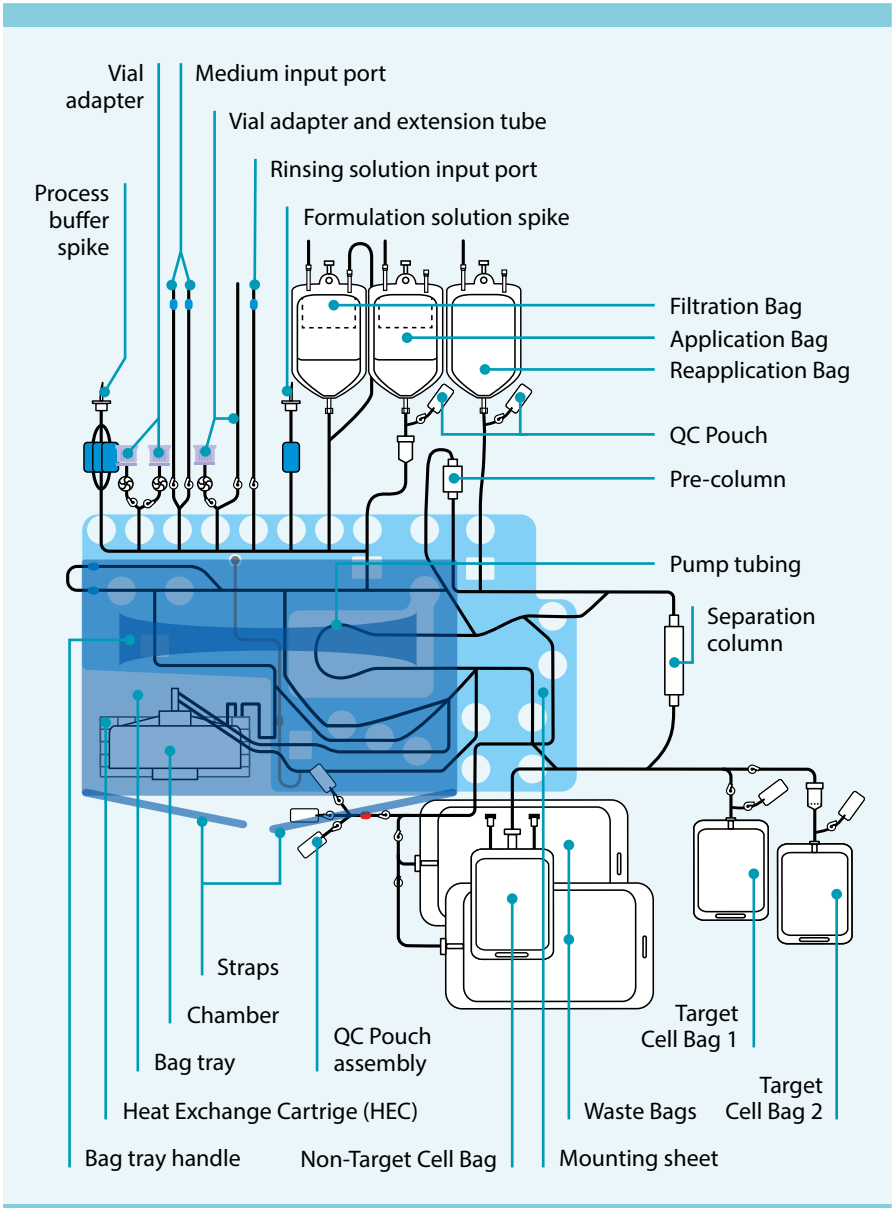


Figure 6.1: Components of the CliniMACS Prodigy TS 520

6.2 shows which process components are connected to which port of the TS 520 for the different process steps.

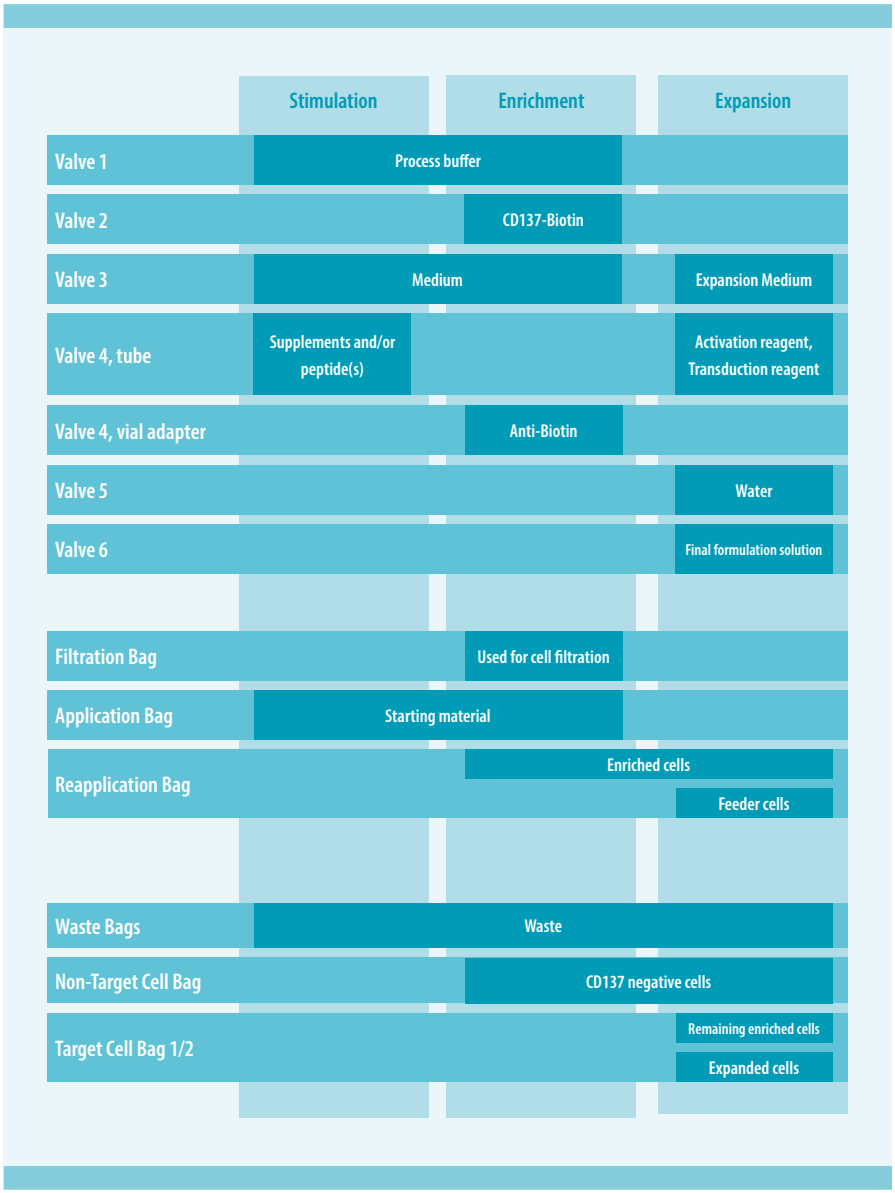


Figure 6.2: Components for the stimulation, enrichment, and expansion part of the CliniMACS Prodigy Tumor Reactive T cell Process

6.3.2 Installation steps

This section guides step by step through the installation of all tubing set components (see Screens 2.1 to 2.22).

Each screen shows the instructions supported by a picture.

Blinking and orange features on the screen indicate areas of attention.

During the installation tap **undo** to return to a previous step.

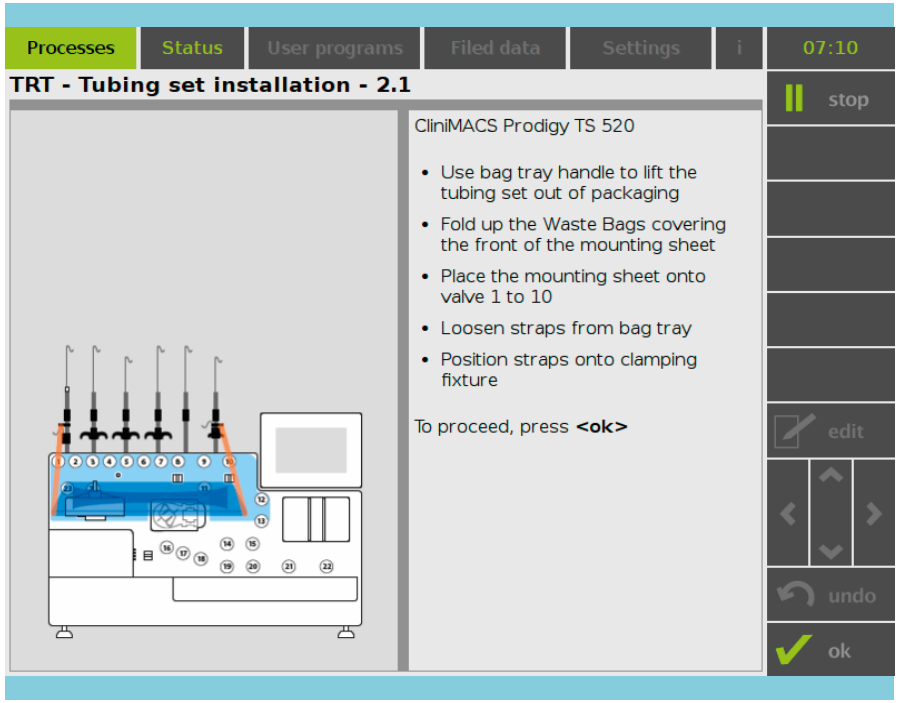
NOTICE

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

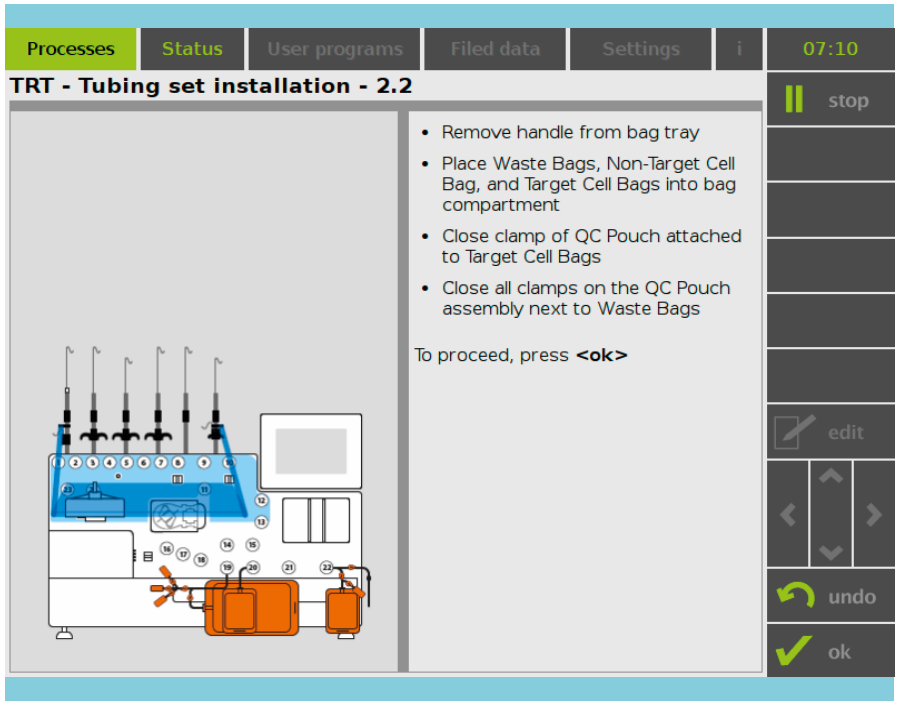
⚠ WARNING

Risk of contamination. Damaged packaging indicates that the tubing set may no longer be sterile and therefore must not be used. Carefully inspect the packaging for damage, puncture or tears. Use the tubing set only if package is undamaged and sealed.

Note: Check the tightness of all Luer Lock connections within the tubing set whenever a Luer Lock connection is present before installing the corresponding part of the tubing set.



Screen 2.1: Installation of bag tray and mounting sheet onto the instrument



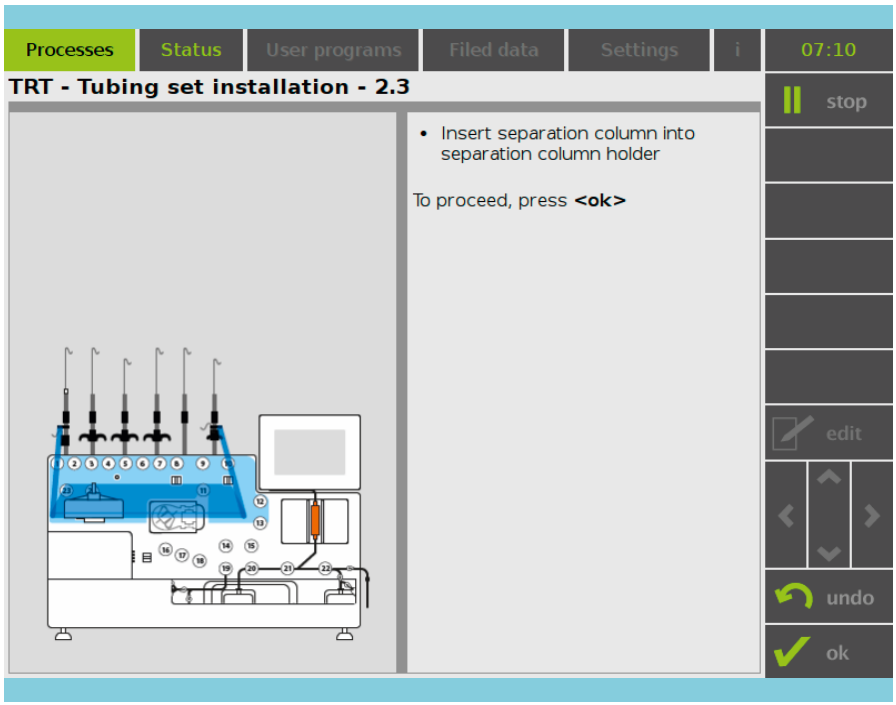
Screen 2.2: Placement of bags into bag compartment and closure of clamps

The CliniMACS Prodigy TS 520 contains two Waste Bags, which do not fit both into the bag compartment when completely filled. Therefore, use one of the following options:

- Store one bag in the bag compartment and keep the clamp on the other bag closed until replacement is required.

- Put both bags in a separate fitting bag compartment on the left-hand side of the CliniMACS Prodigy to be filled simultaneously.

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



Screen 2.3: Insertion of separation column into magnet unit

Figure 6.3 shows how to insert the separation column into the column holder of the magnet unit.

⚠ CAUTION

Risk of pinch injury. If the column is not inserted correctly, there is the risk of pinch injury. Hold the top and the bottom part of the column between thumb and index finger. The column label should be facing towards the user. Place the column carefully into the column holder. Ensure that no tubing is pinched between the column and the column holder.

⚠ 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.

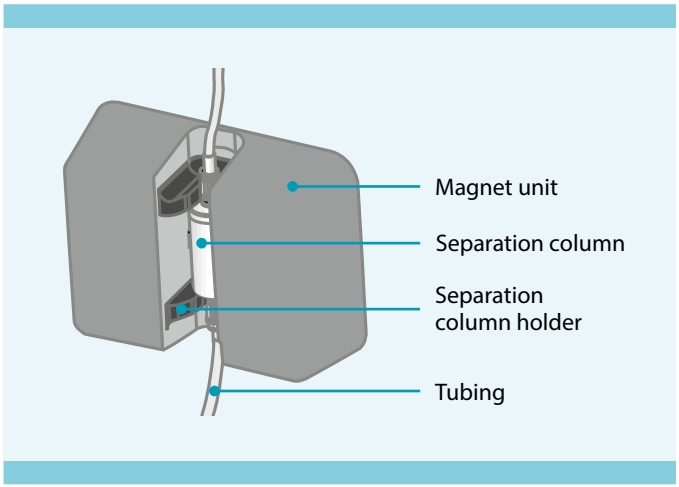
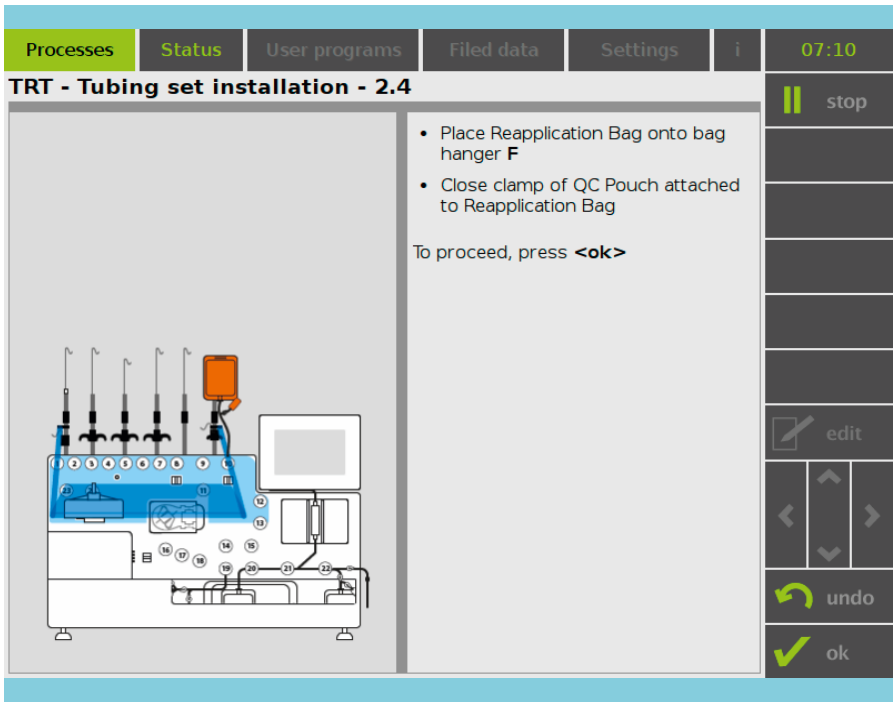
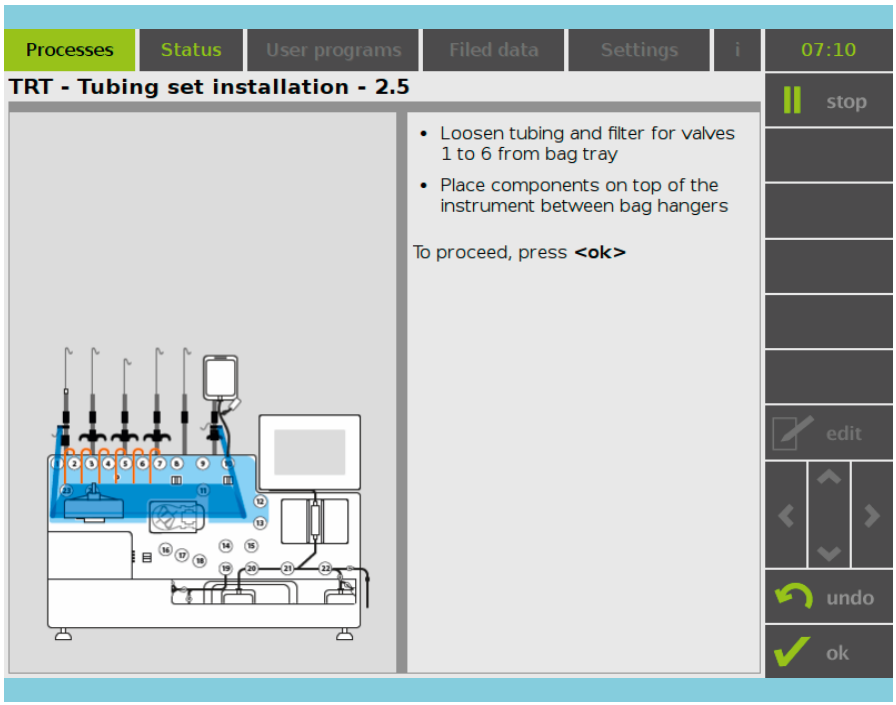


Figure 6.3: Separation column inserted into magnet unit

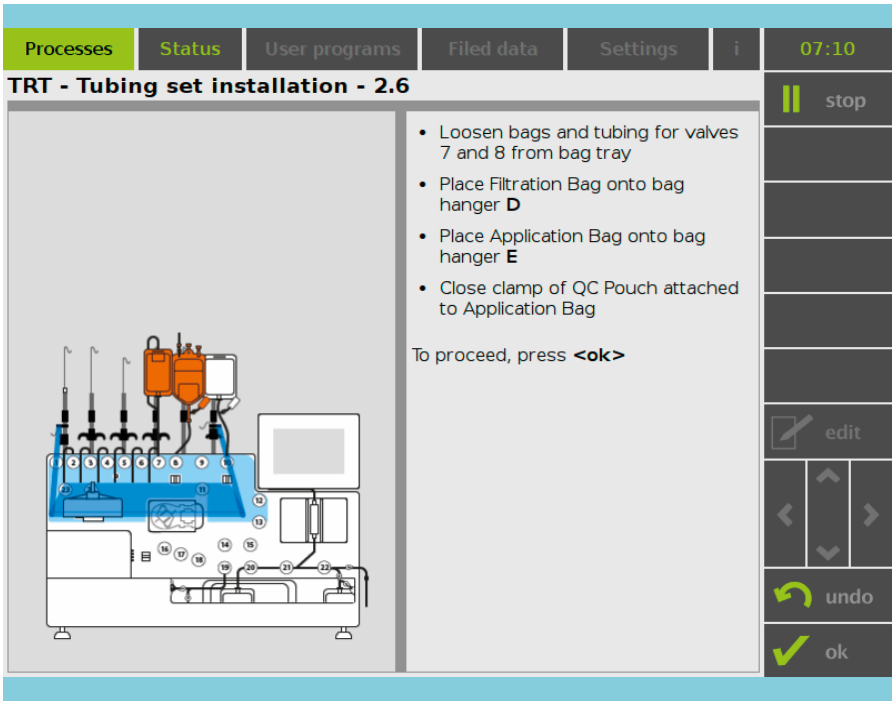


Screen 2.4: Installation of Reapplication Bag



Screen 2.5: Placement of tubing set components between bag hangers

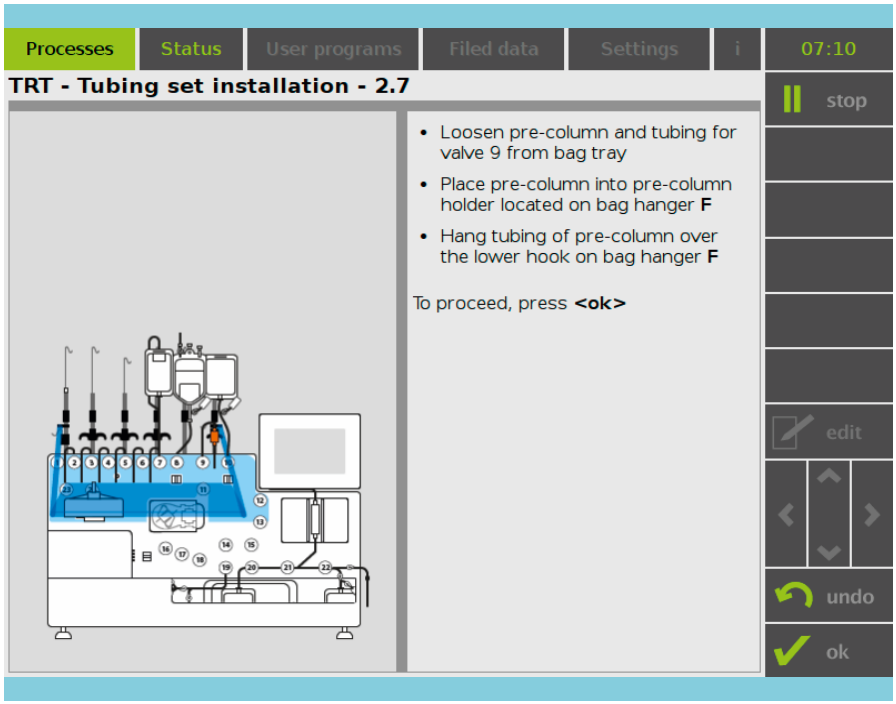
Note: Place each vial adapter in the vial holder temporarily. The final position of the vial adapters is described in Figure 8.1 (see section 8.1.1).



Screen 2.6: Installation of Filtration Bag and Application Bag

IMPORTANT

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



Screen 2.7: Installation of pre-column into pre-column holder on bag hanger (F)

Figure 6.4 shows how to place the tubing over the lower hook on bag hanger (F).

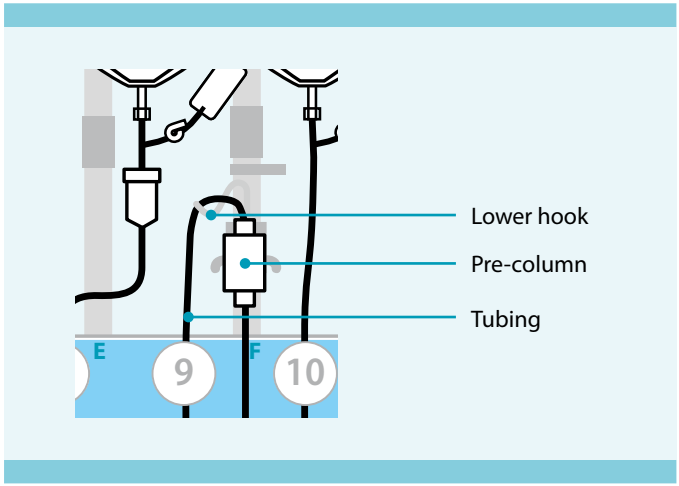
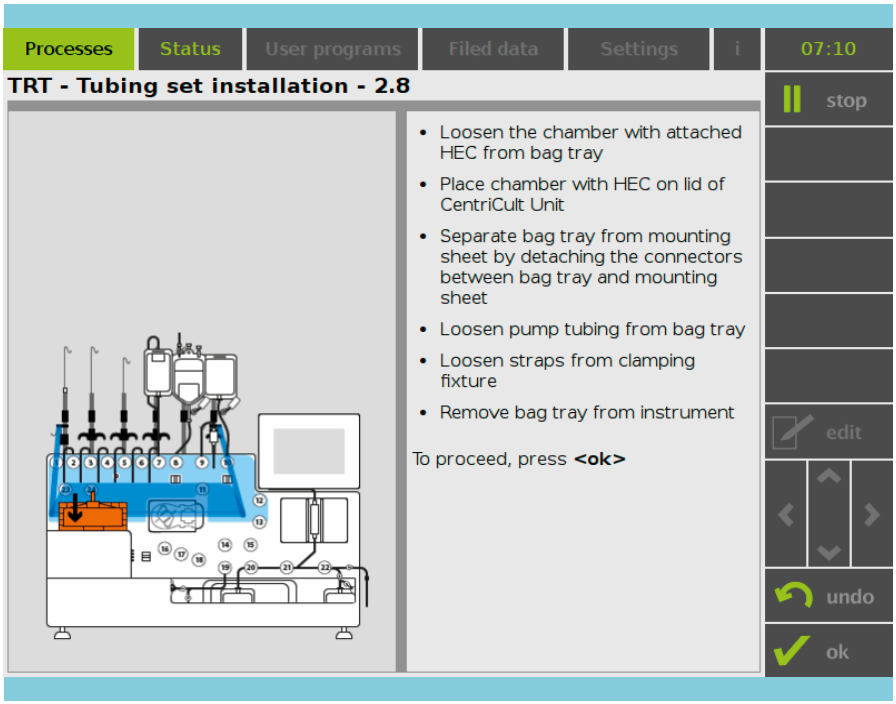
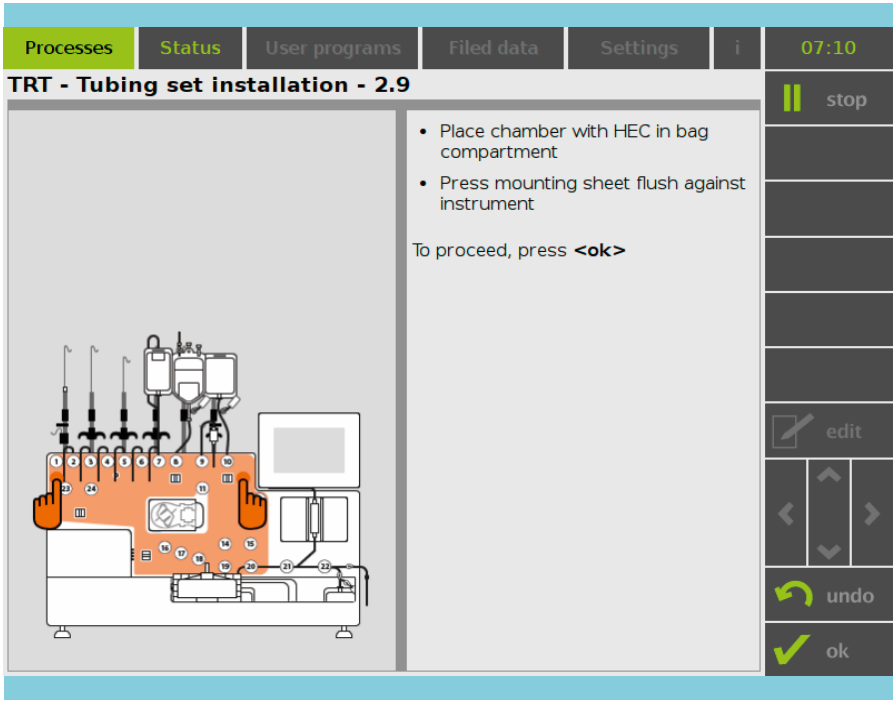


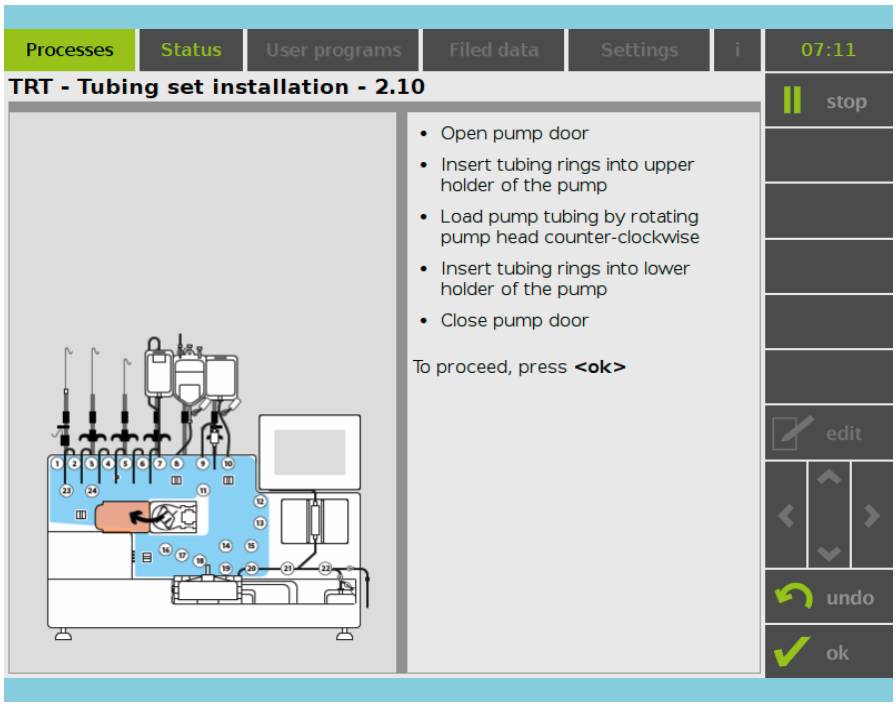
Figure 6.4: Guidance of tubing over the lower hook on bag hanger (F)



Screen 2.8: Removal of bag tray from instrument



Screen 2.9: Placement of chamber with Heat Exchange Cartridge (HEC) in bag compartment



Screen 2.10: Insertion of pump tubing into pump

Figure 6.5 shows how to insert the pump tubing into the pump.

1. Insert the upper outer tubing ring next to the edge of the pump.

2. Rotate the pump head counter-clockwise until the tubing is threaded between both sets of tubing guide pins.

3. Then insert the lower outer tubing ring next to the edge of the pump.

4. Check that the pump tubing is not pinched between the guide pins.

5. 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.

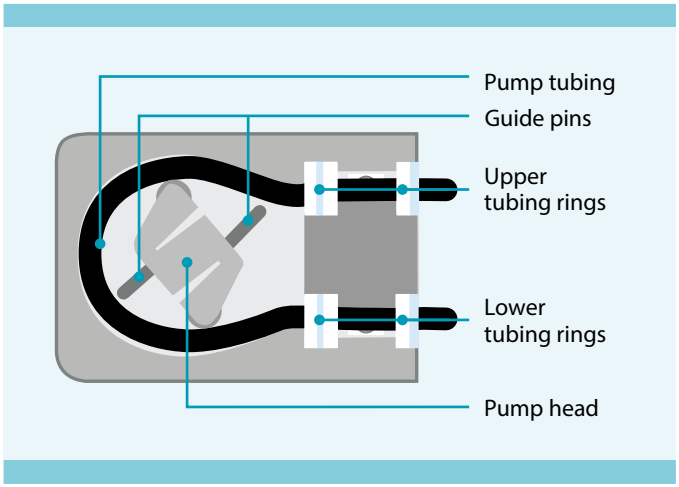
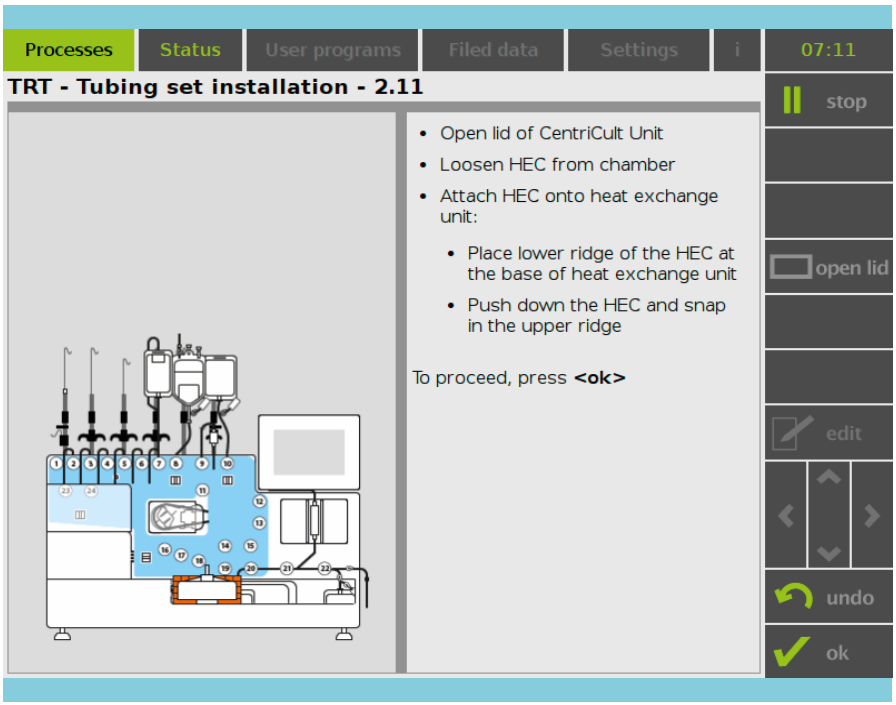


Figure 6.5: Pump tubing inserted into pump

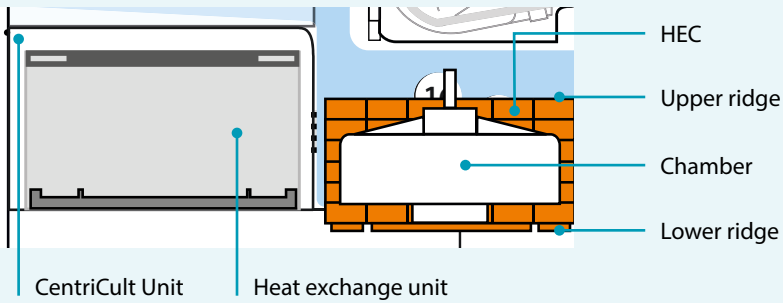


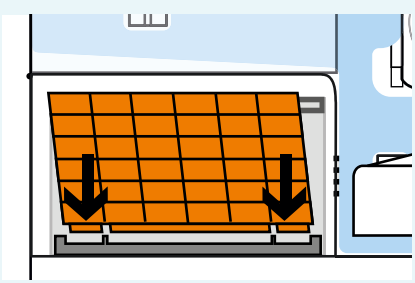
Screen 2.11: Installation of HEC onto heat exchange unit

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

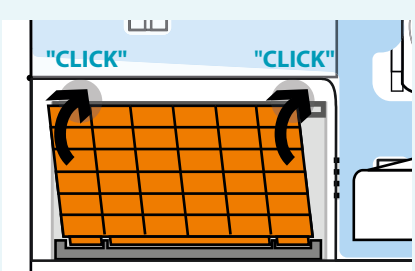
Figure 6.6 illustrates the installation of the Heat Exchange Cartridge (HEC) onto the heat exchange unit. Confirm the HEC attaches tightly to the heat exchange unit to allow heating and cooling of liquid inside the HEC.

1.
Loosen the HEC from chamber.



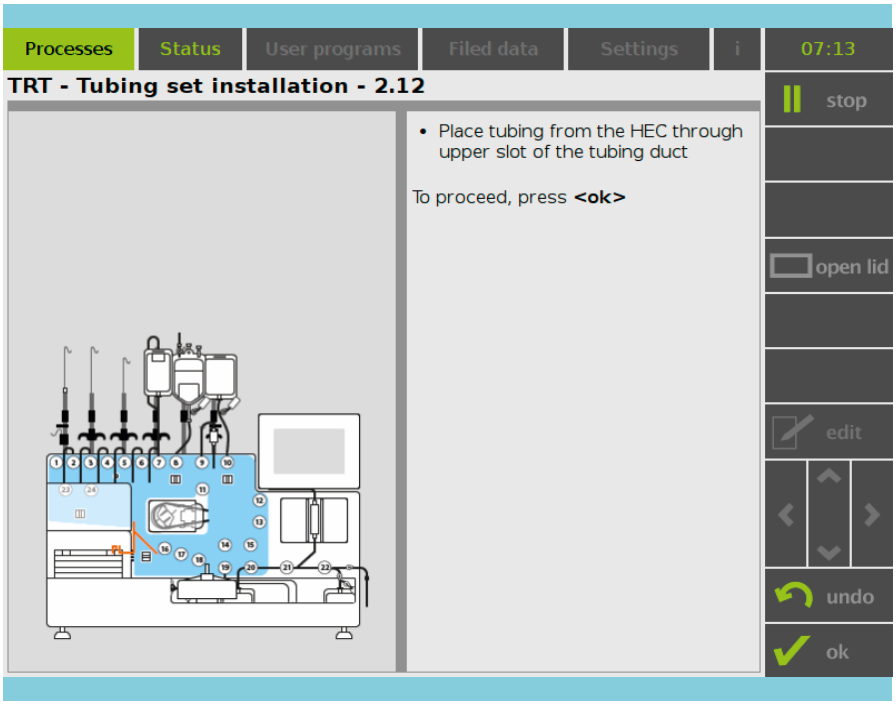


2.
Place lower ridge of the HEC
at the base of heat exchange unit.

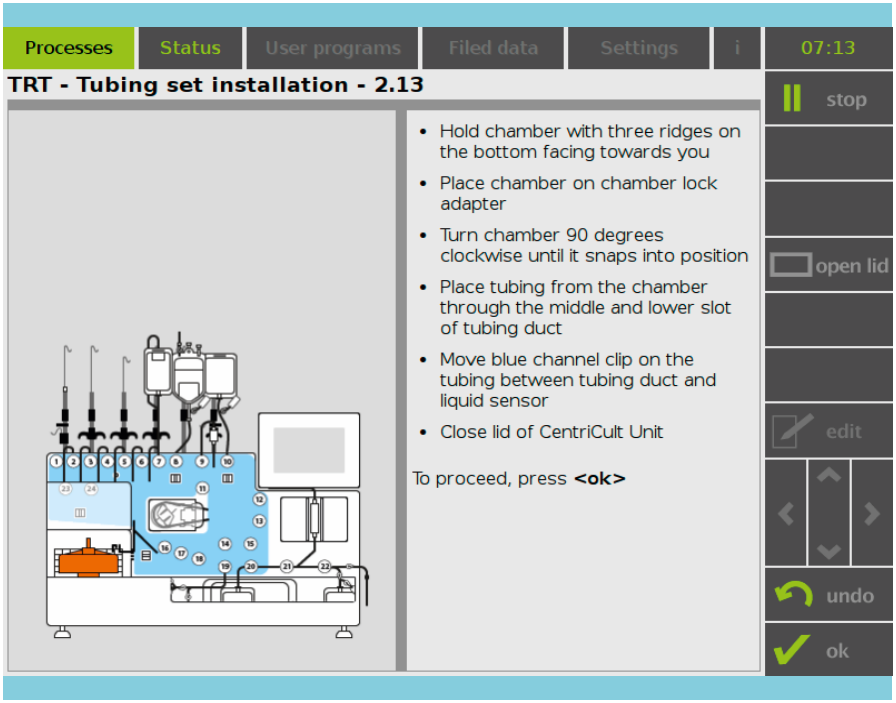


3.
Push down the HEC
and snap in upper
ridge.

Figure 6.6: Installation of HEC onto heat exchange unit



Screen 2.12: Guidance of tubing from HEC through upper slot of tubing duct



Screen 2.13: Installation of chamber into CentriCult Unit

NOTICE

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

Figure 6.7 shows how to insert the three tubes through the tubing duct. Ensure that the tubing is not crossed or pinched by the lid of the CentriCult Unit.

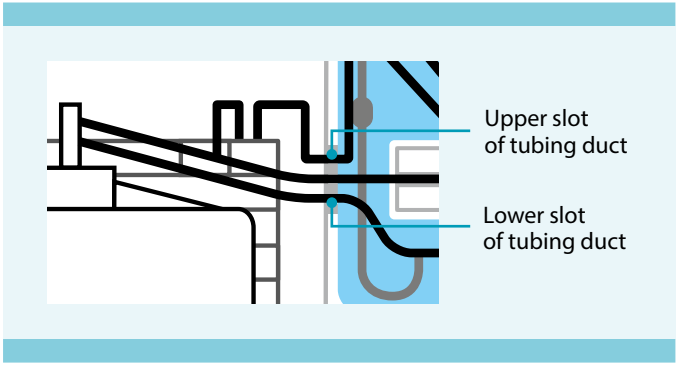
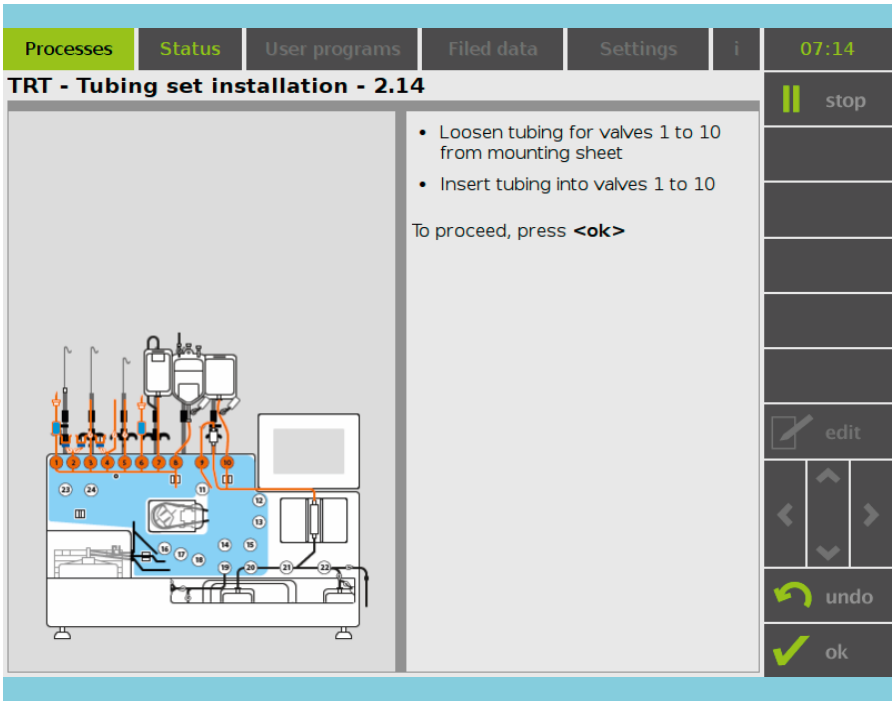


Figure 6.7: Guidance of tubing through tubing duct



Screen 2.14: Insertion of tubing into valves 1 to 10

Figure 6.8 shows how to insert the tubing into the valves correctly.

Only insert the tubing into open valves (when the 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.

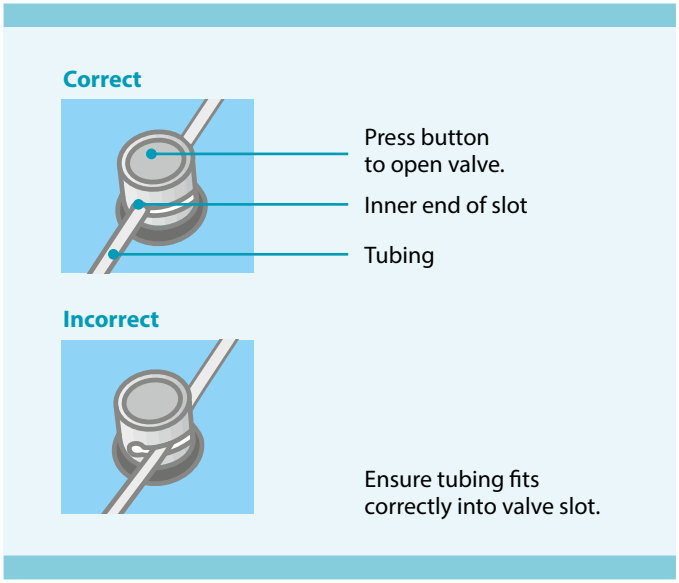
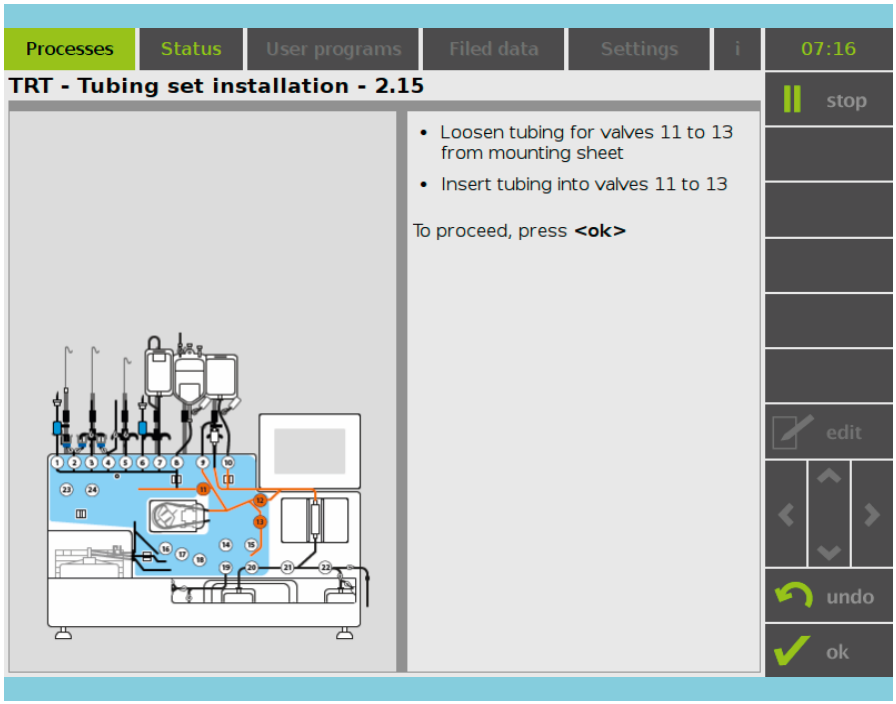


Figure 6.8: Insertion of tubing into valves

Fix the tubing with double vial adapters on valve 2 to the vial holders on bag hanger B. Fix the tubing with single vial adapter on valve 4 to the left vial holder on bag hanger C.



Screen 2.15: Insertion of tubing into valves 11 to 13

Figure 6.9 shows how to insert the tubing correctly into valves 10, 12, and 13.

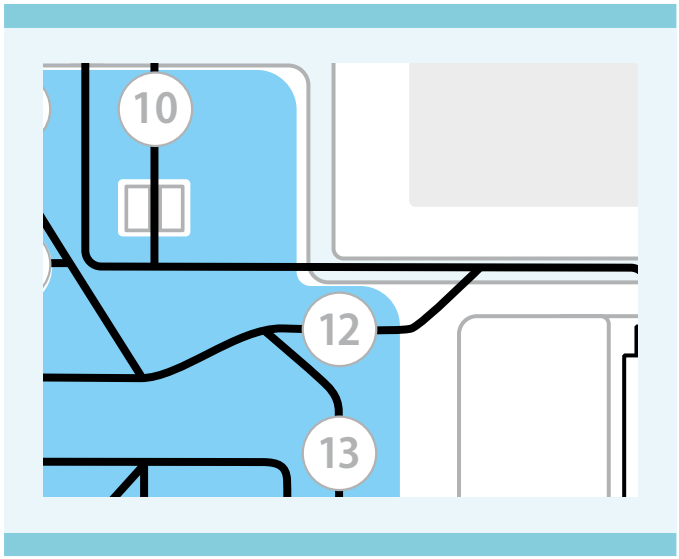
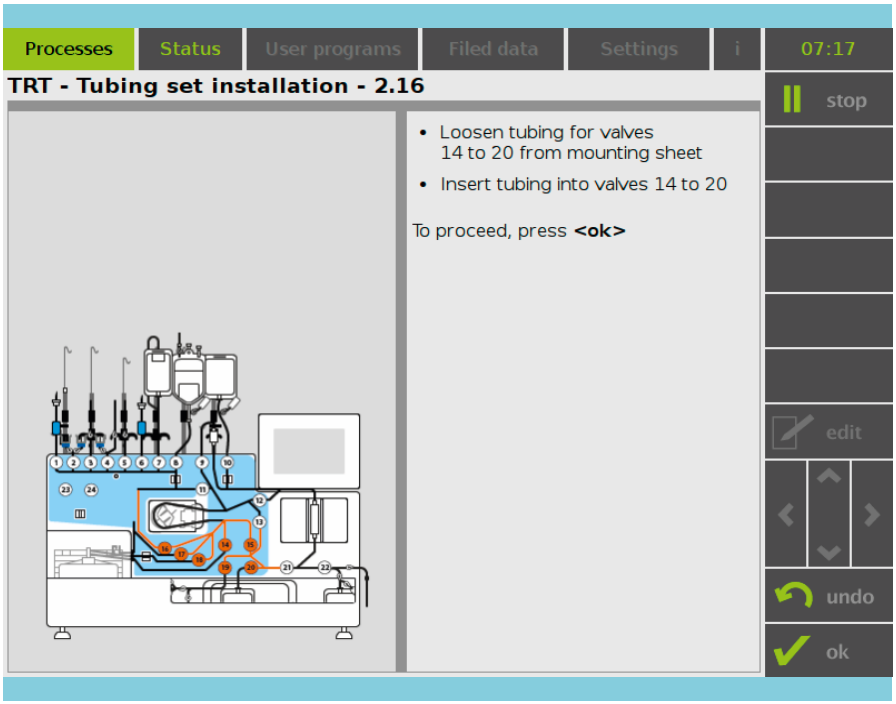
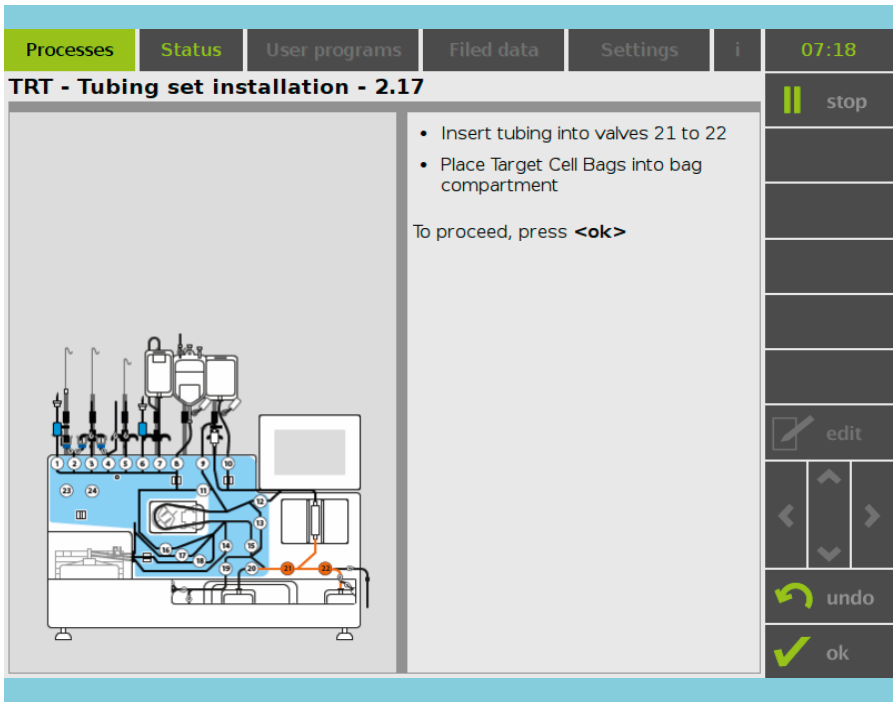


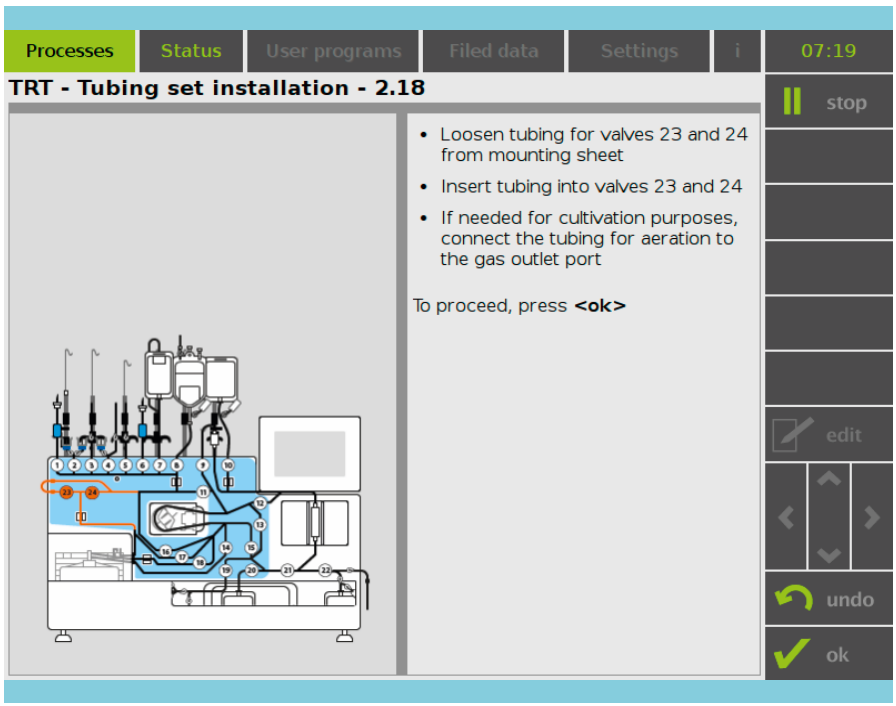
Figure 6.9: Guidance of tubing for separation column and valves 10, 12, and 13



Screen 2.16: Insertion of tubing into valves 14 to 20



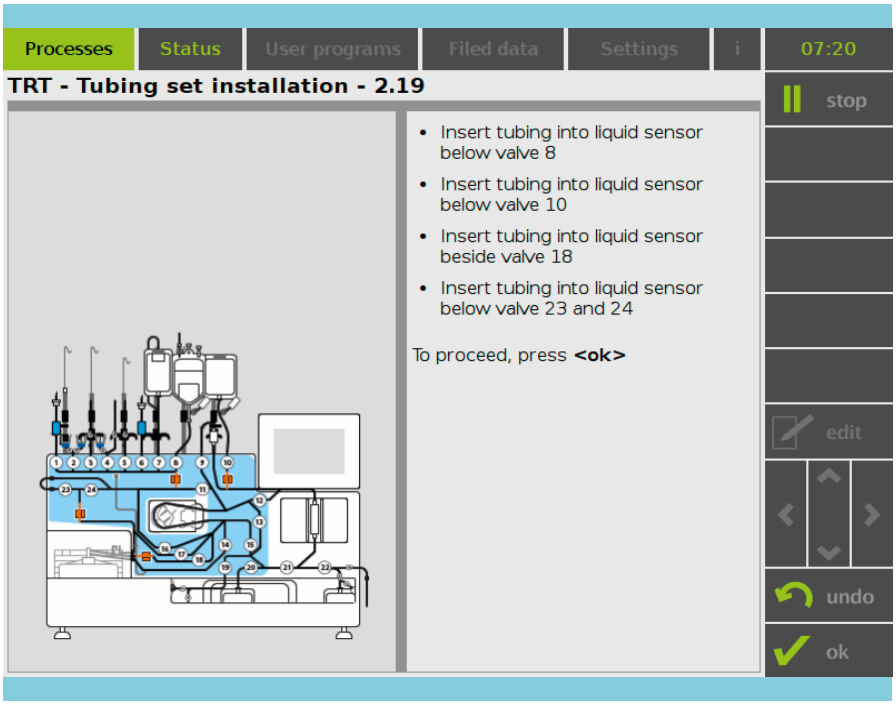
Screen 2.17: Insertion of tubing into valves 21 and 22 and placement of Target Cell Bag into bag compartment



Screen 2.18: Insertion of tubing into valves 23 and 24 and connection of tubing for aeration to the gas outlet port

⚠ CAUTION

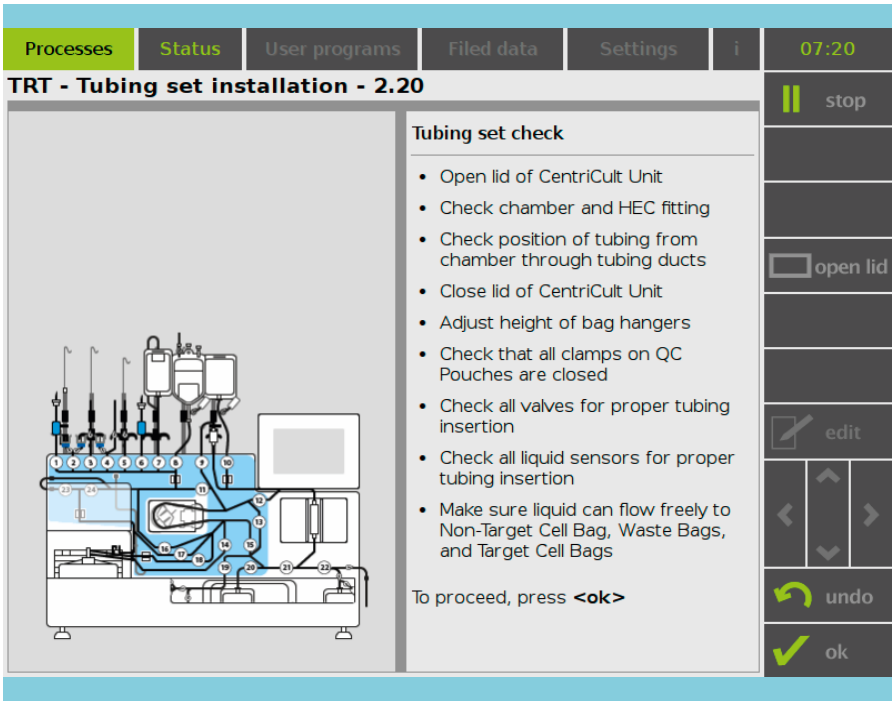
Risk of process failure. Improper gas flow can cause process failure. The tubing for aeration should be tightly connected to the gas outlet to ensure a proper gas flow into the cultivation chamber.



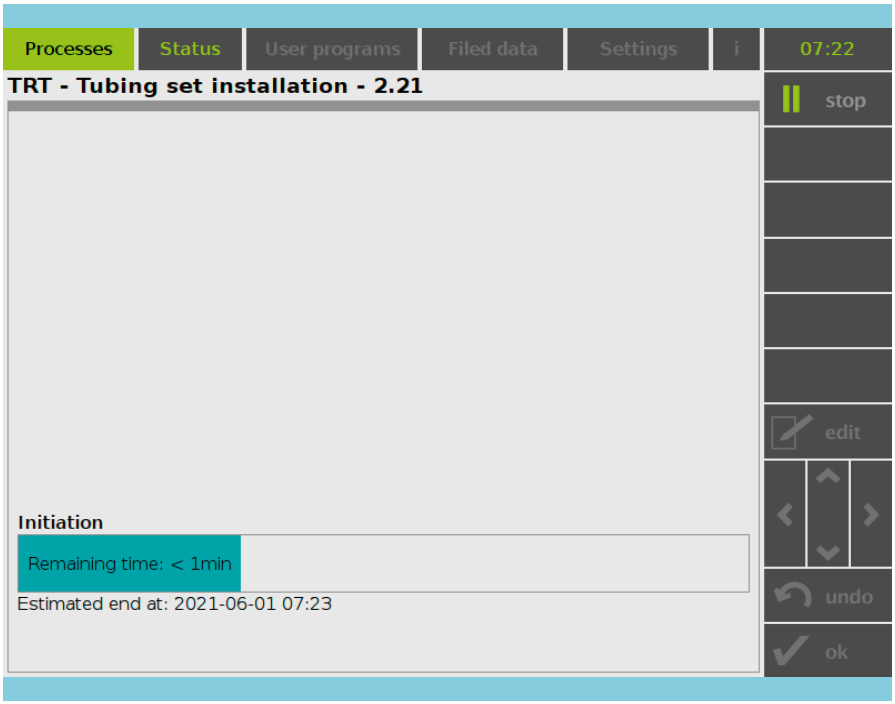
Screen 2.19: Insertion of tubing into liquid sensors

IMPORTANT

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



Screen 2.20: Final check of tubing set installation



Screen 2.21: Initiation of tubing set installation

Note: 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, press the valve button to open the valve (see Figure 6.8) before adjusting the tubing.

If necessary, raise or lower the bag hanger to adjust the height of the hanger. Ensure that the bag is positioned at a suitable height to prevent severe bending or overstretching of the tubing. 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, tap **open lid** on the menu bar. In case the lid cannot be opened, contact Miltenyi Biotec Technical Support.

When the tubing set installation is complete, all valves will open and close twice to 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, contact Miltenyi Biotec Technical Support.

6.3.2.1Integrity test

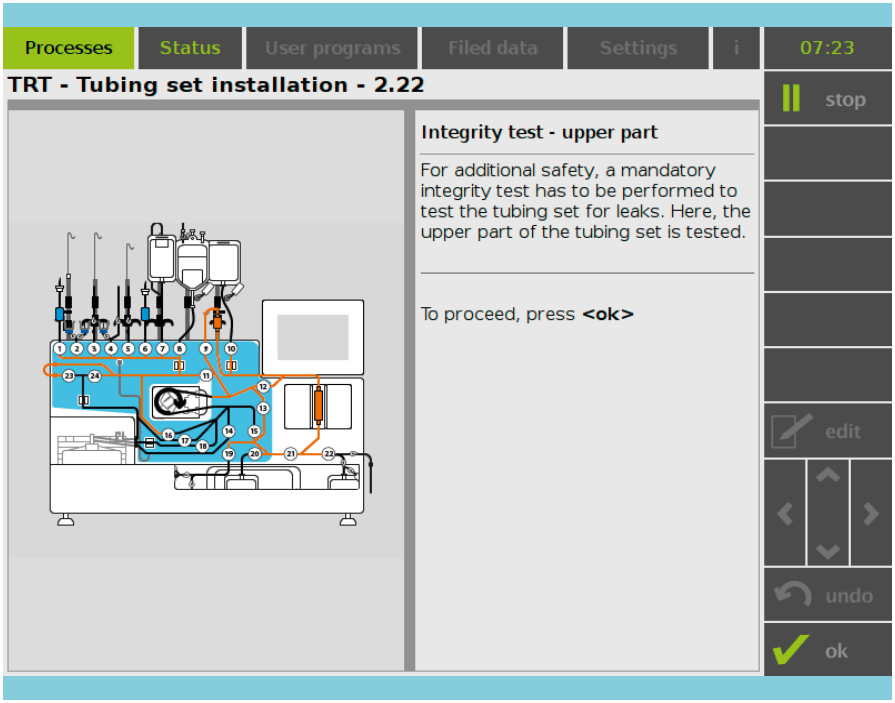
IMPORTANT

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

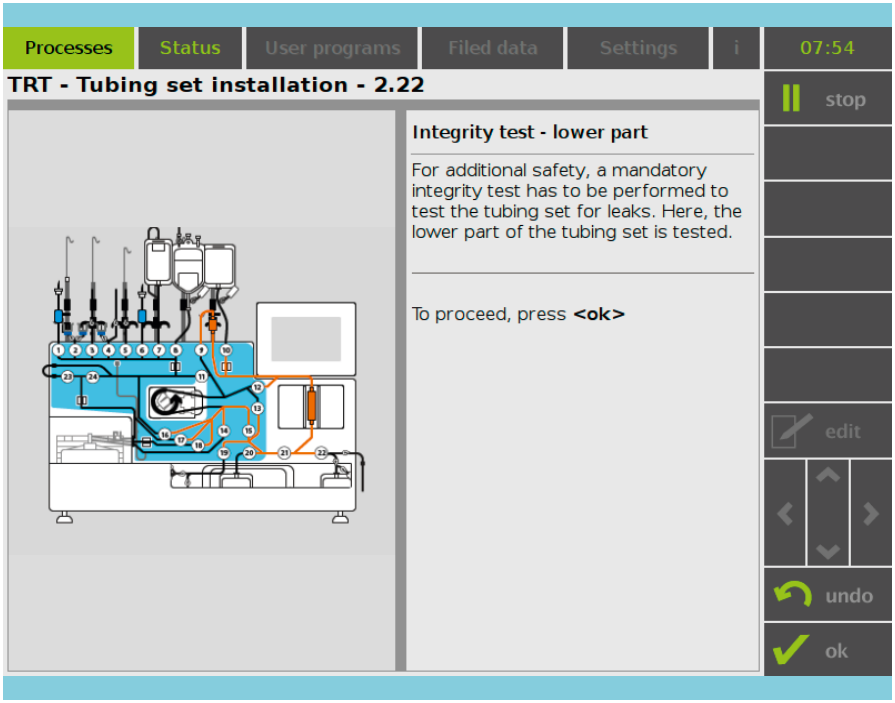
⚠ WARNING

Risk of cell loss or contamination. If the tubing set fails the integrity test there is a risk of cell loss or contamination. 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 part and for the lower part of the tubing set.



Screen 2.22: Integrity test for upper part



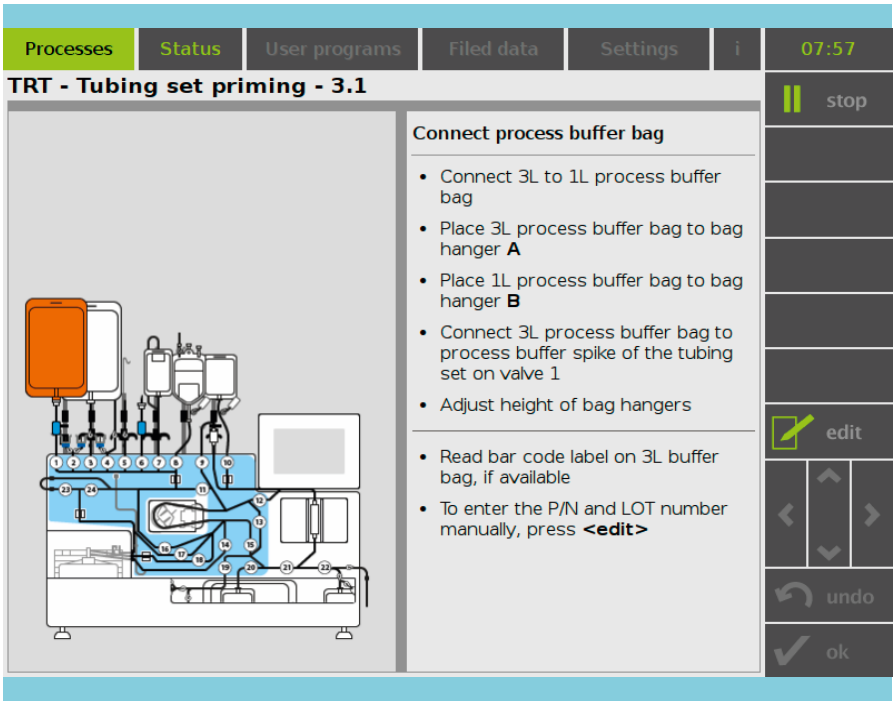
Screen 2.22: Integrity test for lower part

6.4 Tubing set priming

6.4.1 Connection of buffer and medium

Refer to sections 5.1.2 and 5.1.3 for the preparation of the process buffer and medium.

Screens 3.1 to 3.5 will guide through the priming phase.



Screen 3.1: Connection of process buffer bags and enter P/N and LOT number of the 3 L process buffer bag

Connect the 3 L process buffer bag to the 1 L process buffer bag with a Transfer Set Coupler/Coupler by spiking both bags. Make sure the tubing between the two bags is open.

Place the 3 L process buffer bag on bag hanger A and the 1 L process buffer bag on bag hanger B. To connect the process buffer bags to the tubing set on valve 1:

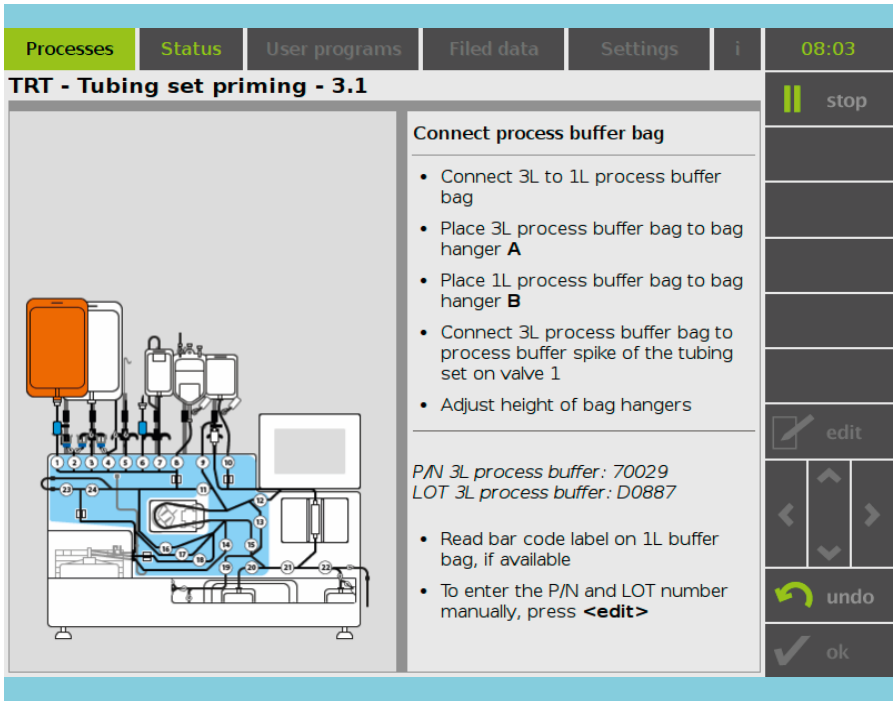
- Remove the capping of the spike of the tubing connected to valve 1.
- Spike it into the 3 L process buffer bag.

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

IMPORTANT

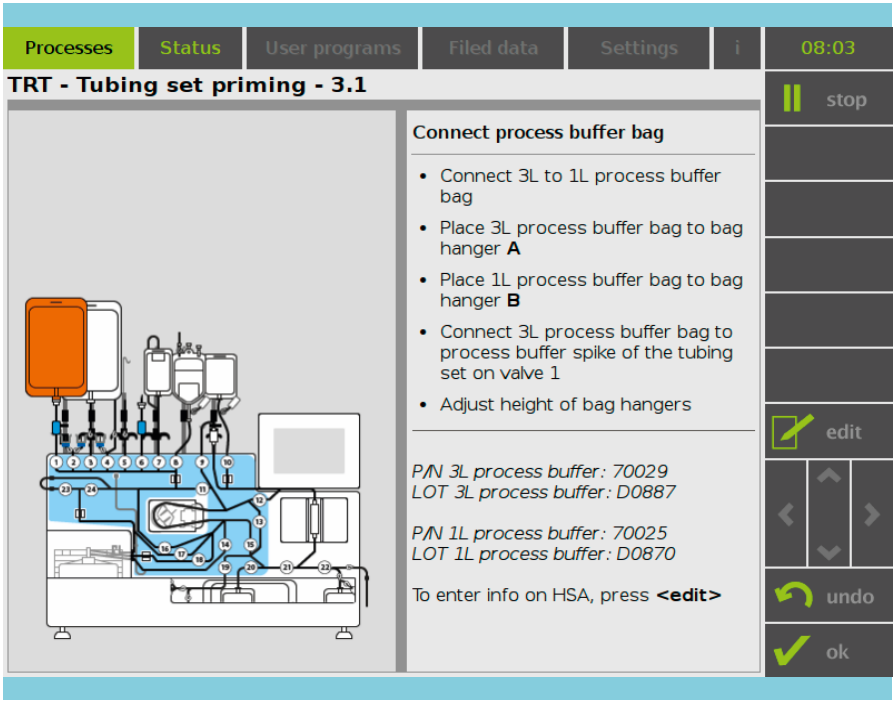
Only hang bags with a maximal total volume of 4 L on one bag hanger.

Read the bar code label on the 3 L process buffer bag with the bar code reader. Alternatively, tap **edit** to enter the part number (P/N) and batch code (LOT) manually with the keyboard (see Screen 3.1).



Screen 3.1: Enter P/N and LOT number of the 1 L process buffer bag

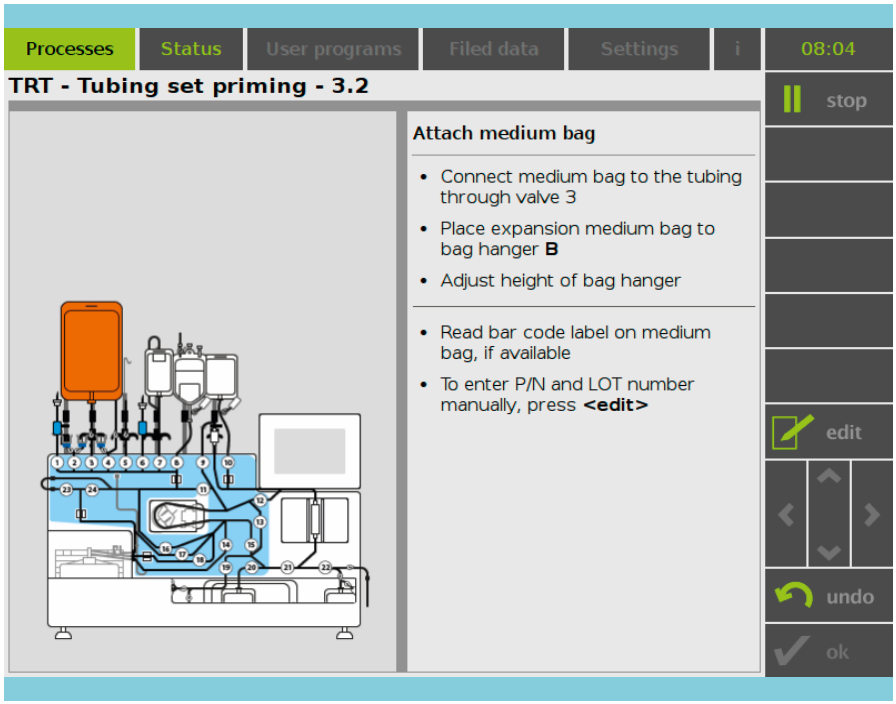
Then, read the bar code label on the 1 L process buffer bag. Alternatively, tap **edit** to enter the part number (P/N) and batch code (LOT) manually.



Screen 3.1: Enter information on the HSA of the process buffer

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

Place a sterile tubing welder next to the instrument to connect the medium bag to the tubing set. Weld the medium to one of the two tube extensions on valve 3 by sterile welding. Place the medium bag on bag hanger B.

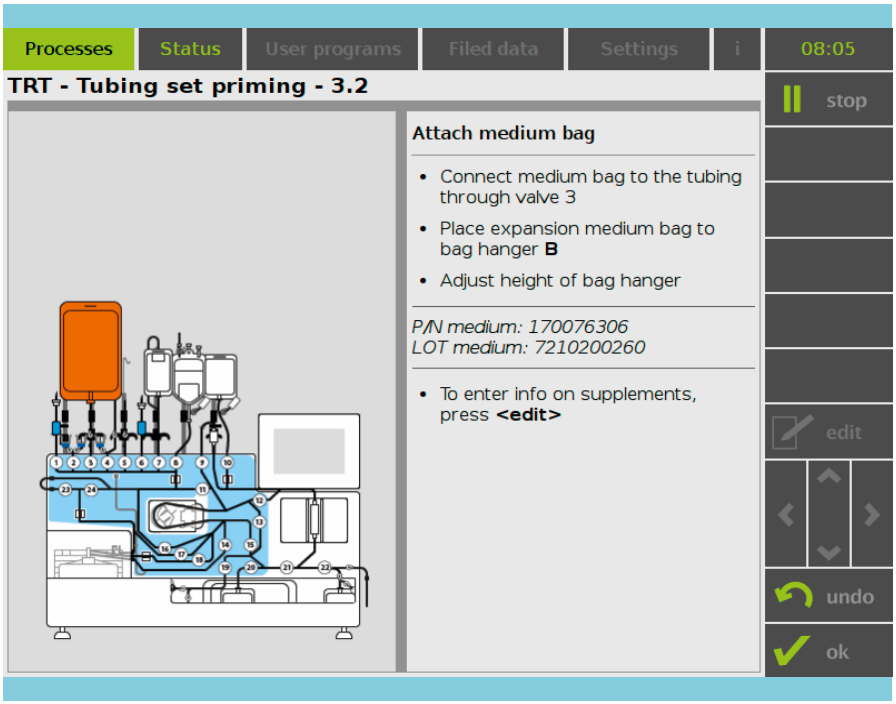


Screen 3.2: Connection of medium bag and enter P/N and LOT number of the medium bag

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

IMPORTANT

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



Screen 3.2: Enter Information on the supplements of the medium

Tap **edit** to enter information on supplements (e.g., 3% human AB serum). Up to 3 different texts on the supplements can be entered.

If necessary, adjust the height of the bag hanger to prevent severe bending or overstretching of the tubing.

IMPORTANT

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

Table 6.1 provides an overview of the positioning of the different bags on the bag hangers.

Bag hanger ID	Bag
A	3000 mL process buffer bag
B	1000 mL process buffer bag, 2000 mL medium bag
C	–
D	Filtration Bag
E	Application Bag
F	Reapplication Bag

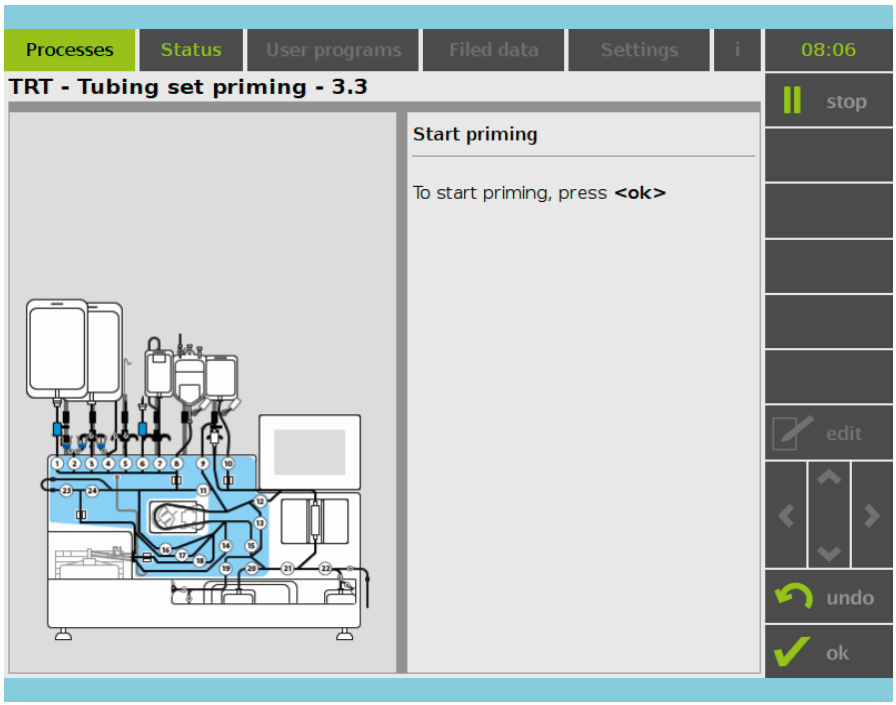
Table 6.1: Positions of bags on bag hangers during priming and enrichment

6.4.2 Priming phase

During the priming phase, the tubing set is filled with medium (case 1 and 3) and with buffer (case 2).

The priming includes repeating cycles.

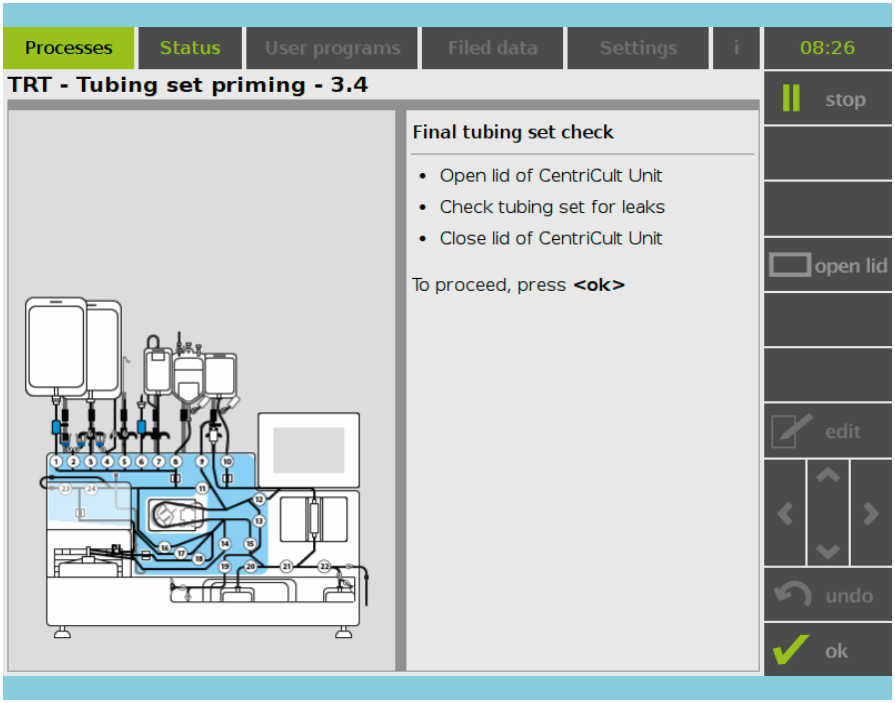
The remaining time for the priming phase will be shown as a process bar on the screen (total time: around 15 minutes).



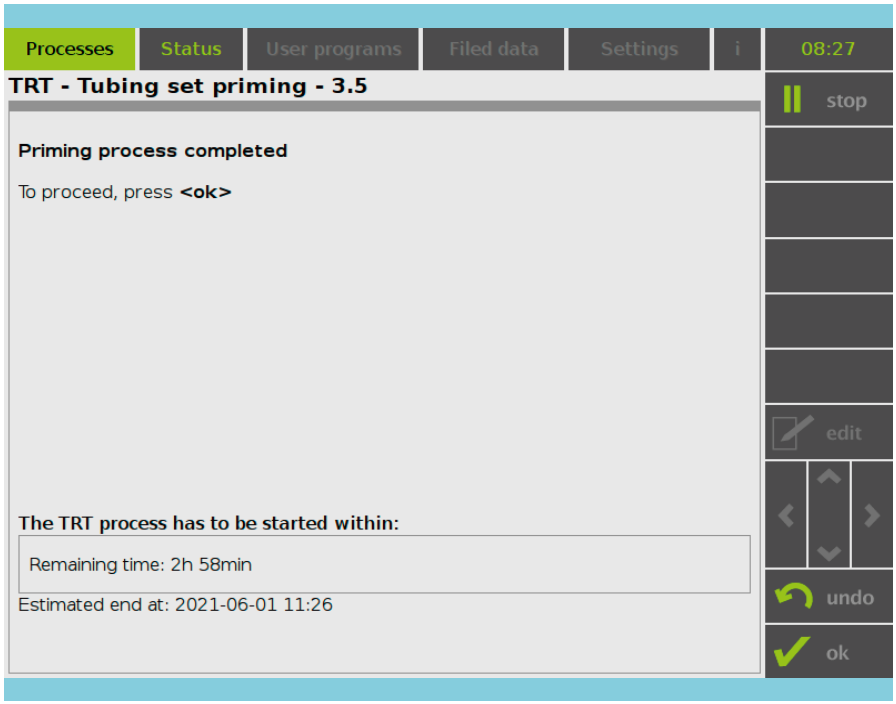
Screen 3.3: Start of priming phase

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

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



Screen 3.4: Final tubing set check after priming phase



Screen 3.5: End of priming phase

Tap **ok** to start the process.

IMPORTANT

It is recommended to perform the tubing set priming directly before starting the process. Check that the cellular starting product and the necessary reagent(s) are available for the process.

7

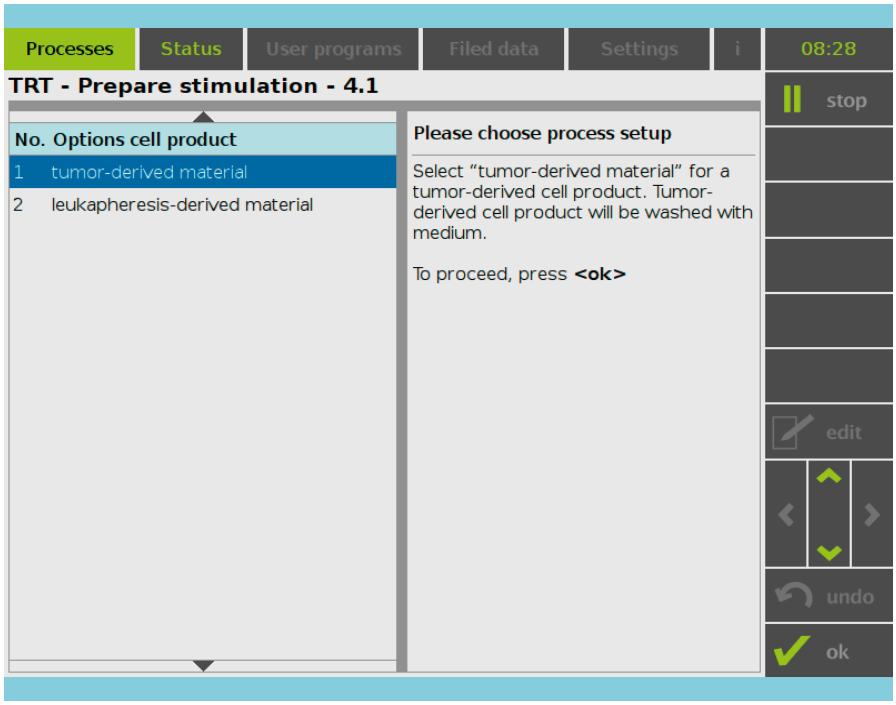
T cell stimulation

7.1 Preparation for the stimulation culture

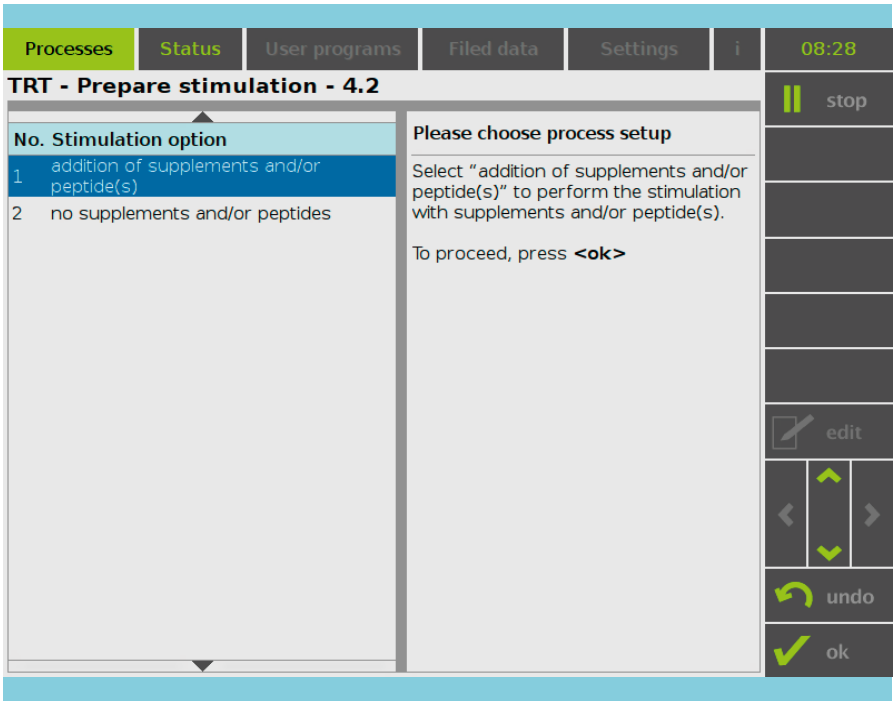
After tubing set installation and priming, the automated stimulation can be started.

Initially choose between stimulation of tumor-derived material and leukapheresis-derived material (see Screen 4.1).

Tap **ok** to confirm the case selection.



Screen 4.1: Stimulation setup 'tumor-derived material' or 'leukapheresis-derived material'



Screen 4.2: Stimulation setup 'addition of supplements and/or peptide(s)' or 'no supplements and/or peptide(s)'

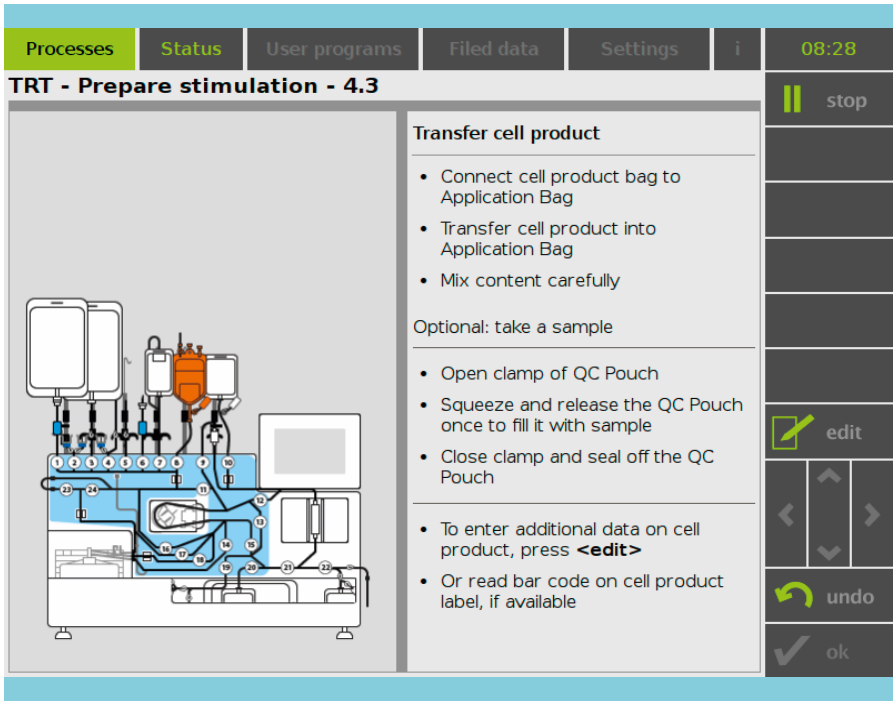
When choosing tumor-derived material, select between addition of supplements and/or peptide(s) or no supplements and/or peptides.

Tap **ok** to confirm the case selection.

In case of leukapheresis-derived material the addition of supplements and/or peptide(s) is mandatory.

This user manual describes the stimulation with addition of supplements and/or peptide(s). Without addition of supplements and/or peptide(s) screen 4.5 and screen 4.6 are skipped.

7.1.1 Transfer of cellular starting product



Screen 4.3: Connection and transfer of cellular starting product to Application Bag

See section 5.1.1 for more details on the preparation of the starting material and e.g., volume requirements.

Connect the bag containing the cells to the Application Bag by sterile welding.

Transfer the cellular starting product into the Application Bag and seal off the empty bag using the heat sealer.

Optional: take a sample of the filtered starting product from the Application Bag with the QC Pouch:

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

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

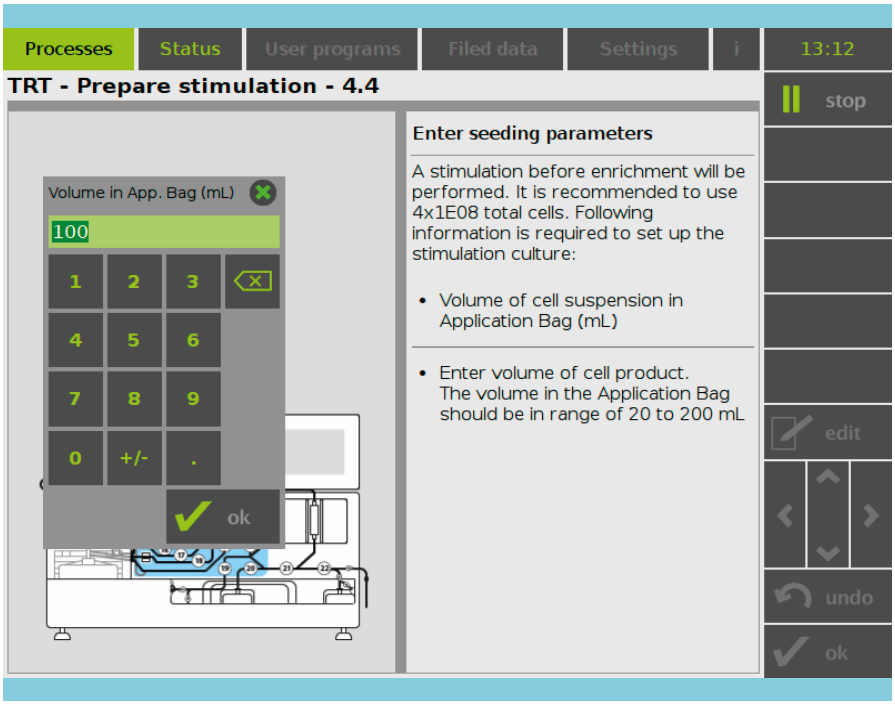
Determine the cell concentration (x1E6/mL) of the filtered cell suspension within the Application Bag.

Tap **edit** to enter information on the cellular starting product (see Screen 4.3). Up to two different texts can be entered.

Enter the following values:

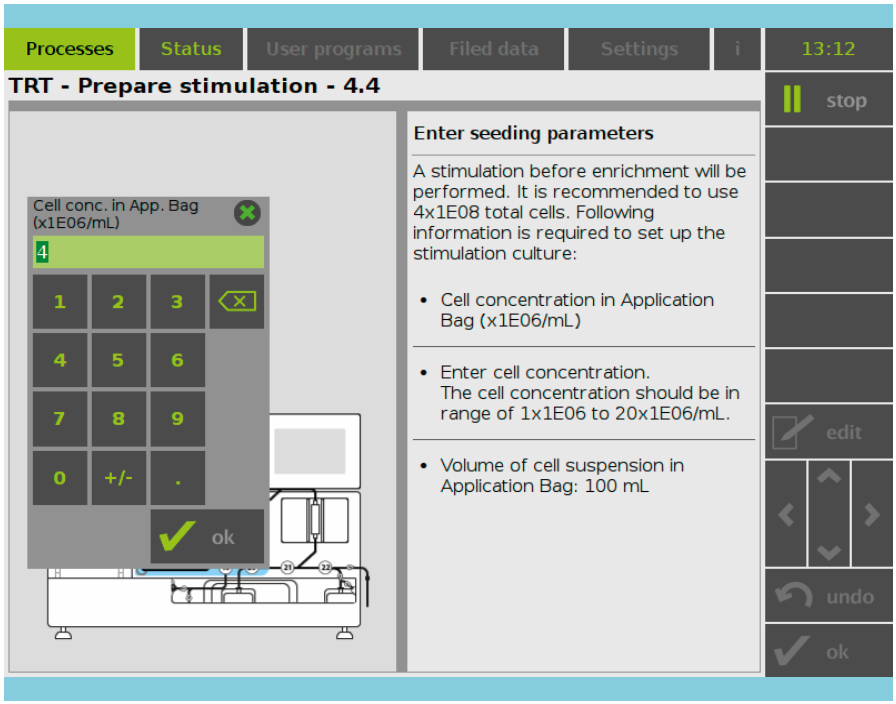
- Volume of the cell suspension in the Application Bag (mL).
- Cell concentration that is present in the Application Bag (x1E06/mL).
- Desired starting cell number (x1E06 total cells) for the stimulation culture.

For culture setup, 20-200 mL cells can be transferred from the Application Bag to the chamber.



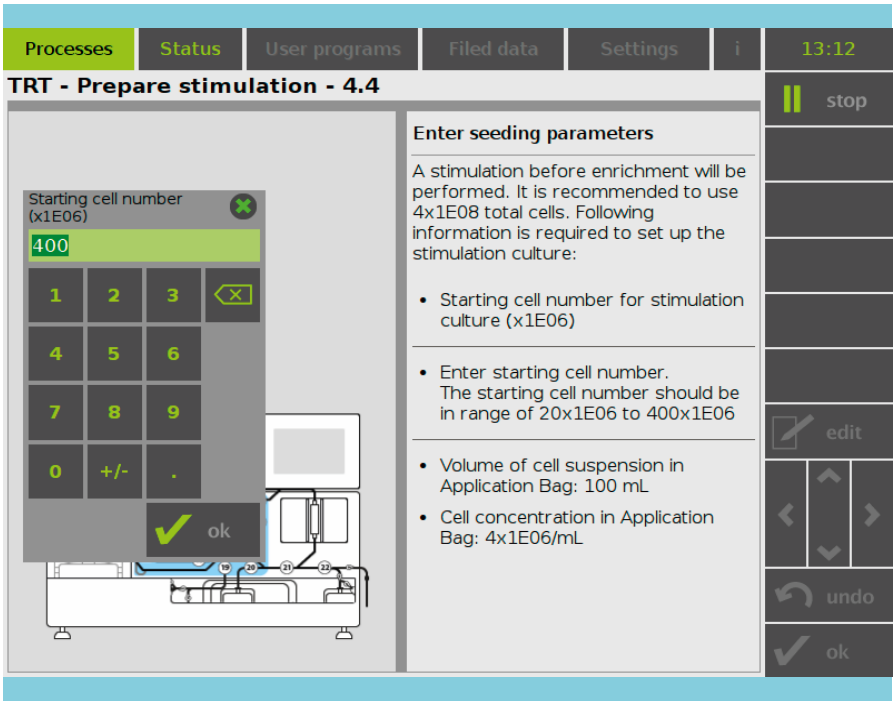
Screen 4.4: Input of seeding parameters for the stimulation culture – Volume in Application Bag (tumor-derived material)

The volume of the cell suspension should be in the range of 20-200 mL.



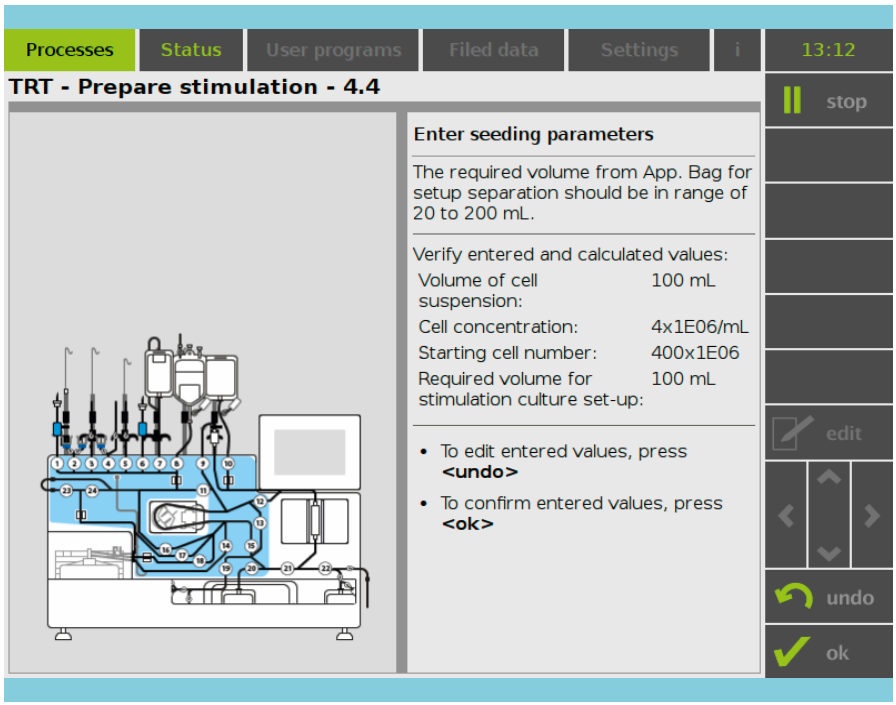
Screen 4.4: Input of seeding parameters for the stimulation culture – Cell concentration (tumor-derived material)

The cell concentration should be in the range of 1×10^6 to 2×10^7 total cells/mL for tumor-derived material or 1×10^6 to 5×10^7 total white blood cells /mL for leukapheresis-derived material.



Screen 4.4: Input of seeding parameters for the stimulation culture – Starting cell number (tumor-derived material)

The recommended starting cell number for culture is 4×10^8 total cells for tumor-derived material or 1×10^9 total white blood cells for leukapheresis-derived material.



Screen 4.4: Calculation of the required volume for culture setup of the stimulation culture

The volume of the cellular starting product in the Application Bag that is transferred for the stimulation culture is calculated automatically.

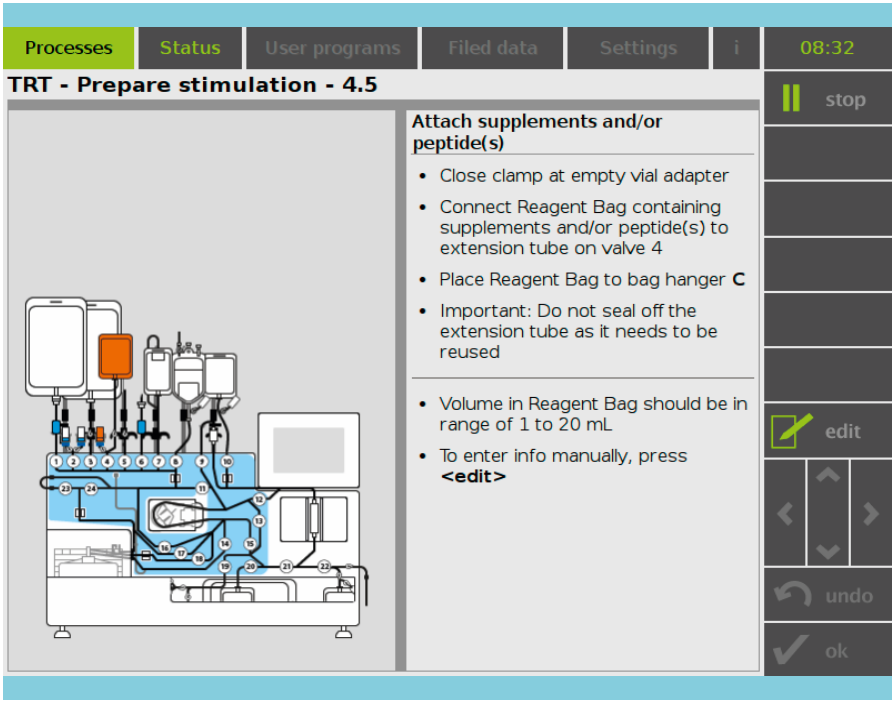
In case the volume in the Application Bag is higher than the volume that is calculated to be transferred into the chamber, tap **ok** to confirm the entered parameters. Alternatively, tap **undo** to change the entered parameters.

7.1.2 Connection of stimulation peptide(s)

Connect the bag with the supplements and/or peptide(s) for the stimulation culture to the tubing of valve 4 by sterile welding.

Hang the bag on bag hanger C. Make sure that the clamp of the connected bag is open.

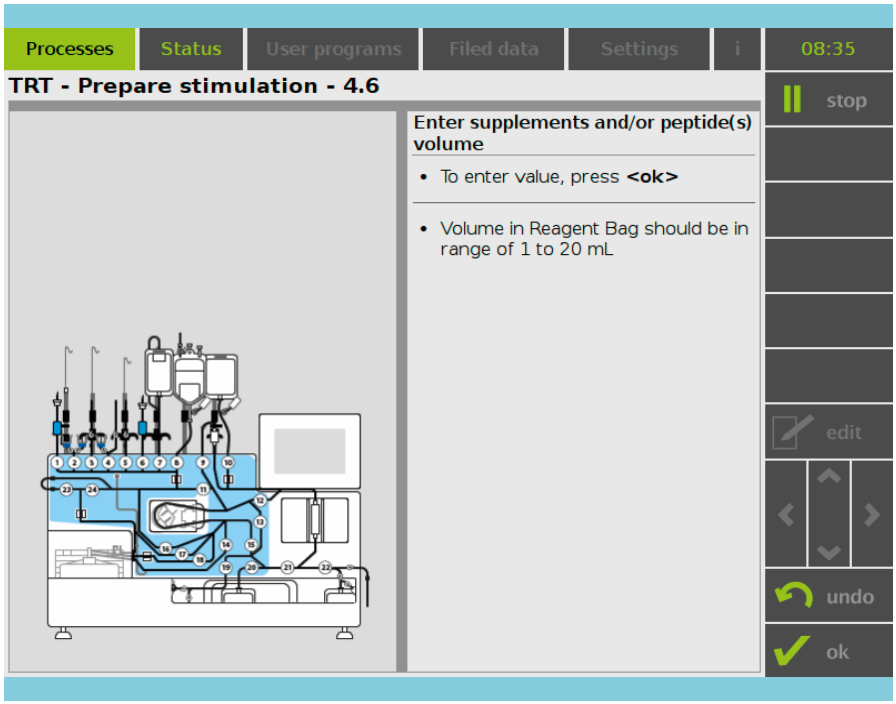
Close the clamp at the vial adapter (will not be used).



Screen 4.5: Connection of supplements and/or peptide(s)

Tap **edit** to enter information on the supplements and/or peptide(s). Up to 3 different texts can be entered (e.g., kind of reagent, P/N and LOT). Alternatively, read the bar code label of the reagent, if available. Only one information can be saved in this case.

Tap **ok.** Enter the volume of the supplement and/or peptide solution.



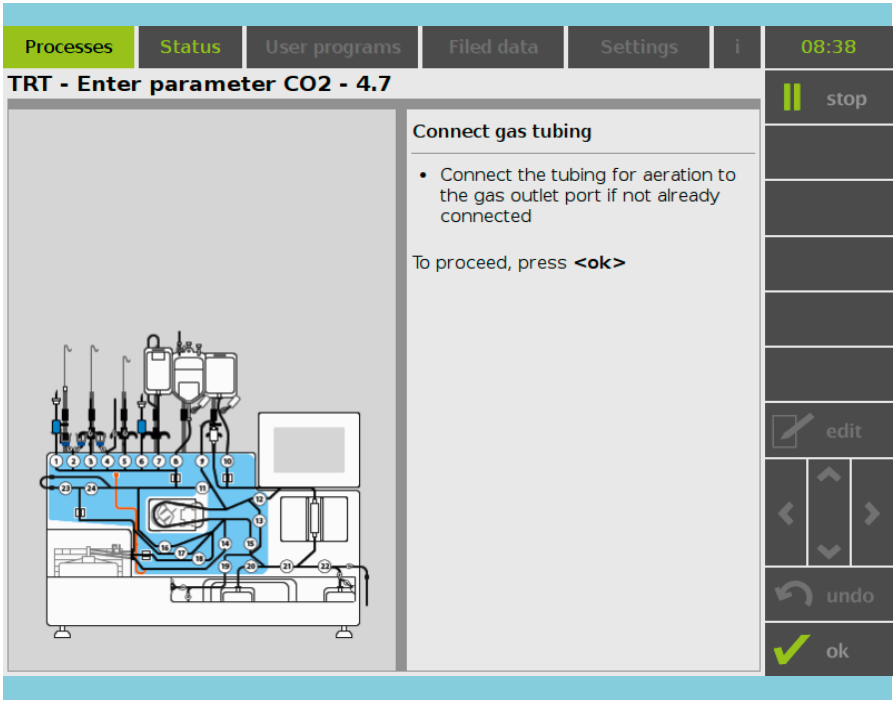
Screen 4.6: Input of the volume of stimulation supplements and/or peptide(s)

The volume can range between 1 and 20 mL.

IMPORTANT

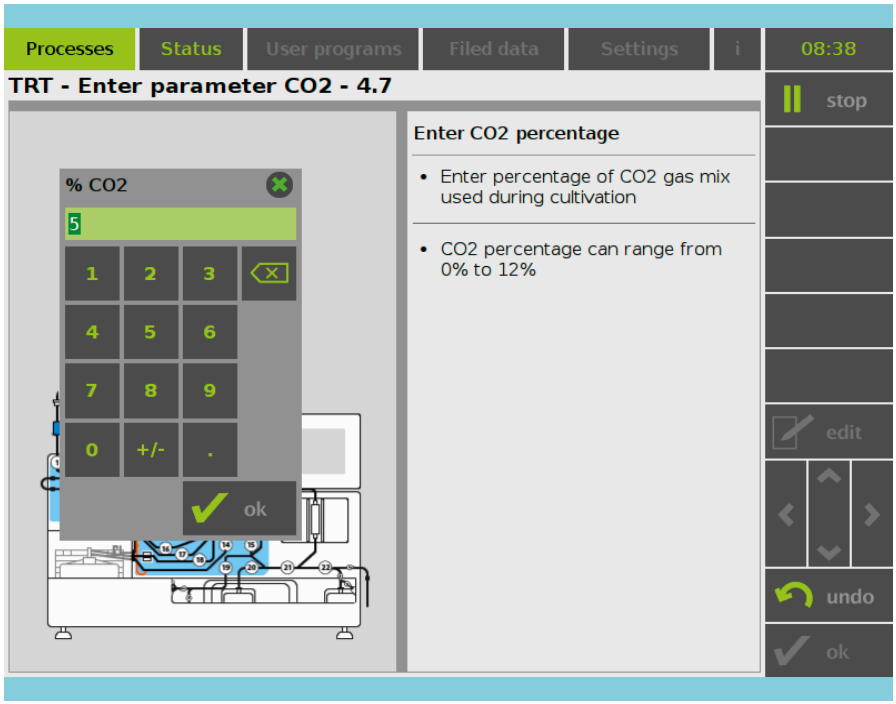
Do not seal off the extension tube after the reagent transfer of the supplements and/or peptide(s) is performed as the tubing may need to be reused. Alternatively, if it is desired to seal off the Reagent Bag after reagent transfer, make sure to keep the tubing long enough to be able to weld on another bag at a later time point.

7.1.3 Input of CO_2 and temperature parameters



Screen 4.7: Connection of gas tubing

During tubing set installation, the gas outlet port was already connected (see Screen 2.18 in section 6.3.2). Double check if the tubing for aeration is connected tightly.



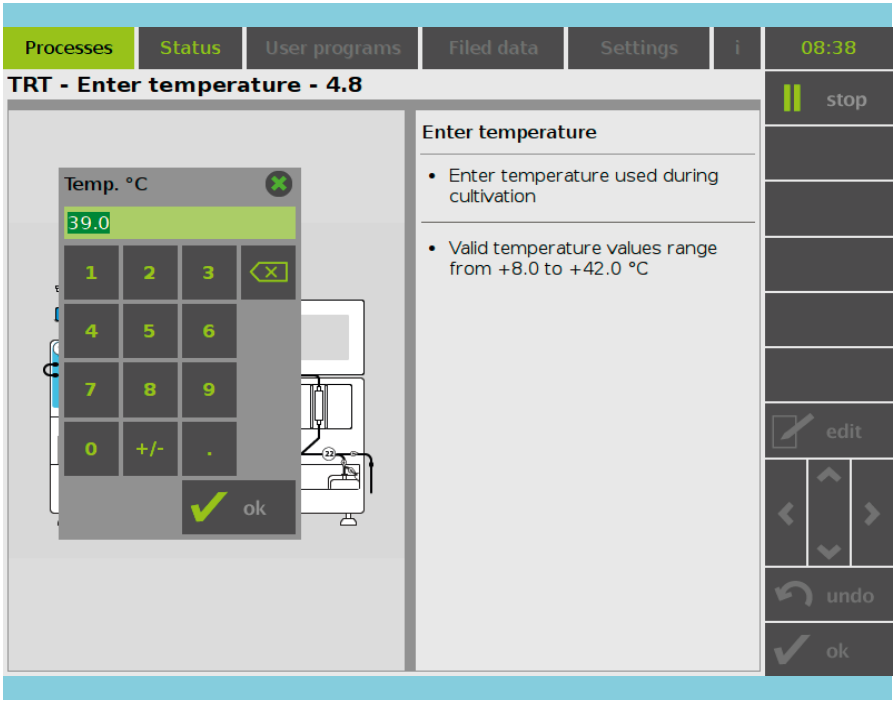
Screen 4.7: Input of CO_2 percentage

For the cultivation of the cells, the value for CO_2 percentage must be entered. For TexMACS GMP Medium enter 5% CO_2.

The temperature parameter has to be entered manually only 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).

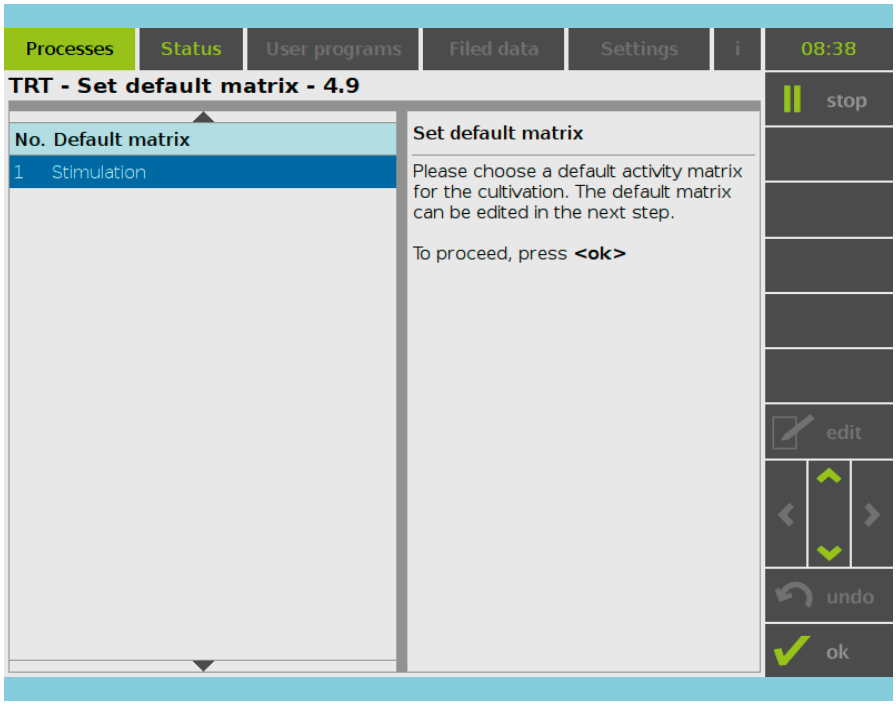


Screen 4.8: Input of temperature

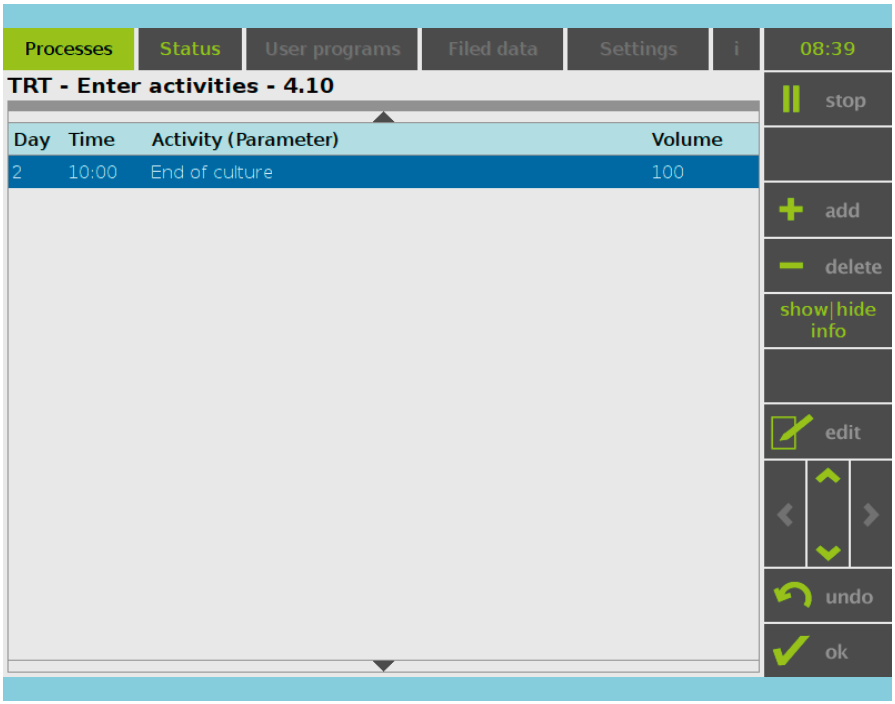
7.1.4 Setup of the activity matrix

During setup of the activity matrix, the end of the stimulation culture can be defined. Choose the default matrix 'stimulation' and tap **ok** to confirm (see Screen 4.9).

The maximal upregulation of the activation marker CD137 usually occurs within 18–36 hours. The kinetics of CD137 upregulation can vary depending on the starting material. Therefore, further investigation is recommended to define the optimal stimulation culture period.

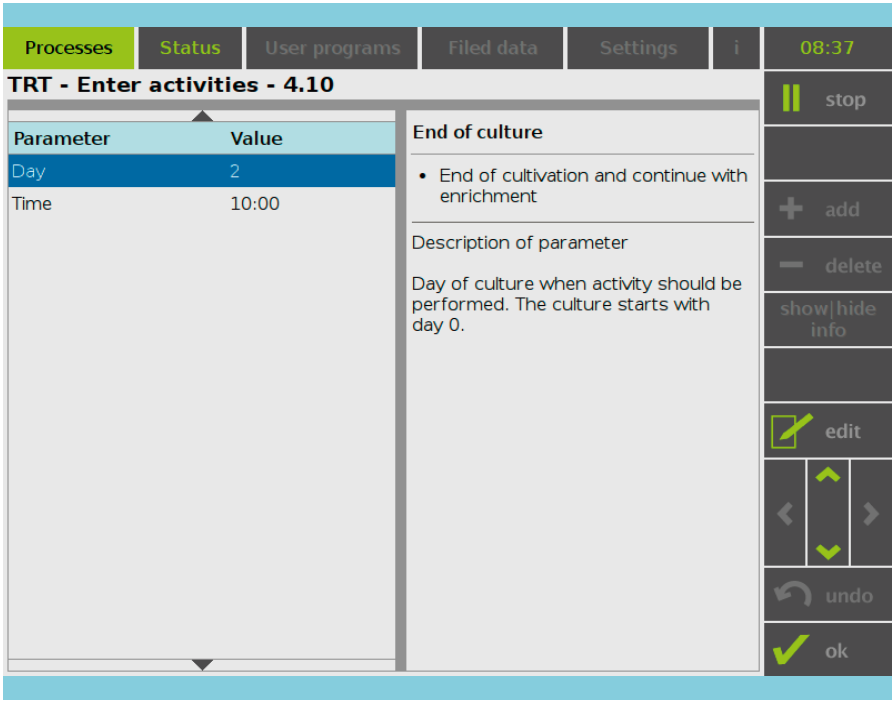


Screen 4.9: Set default matrix for stimulation culture



Screen 4.10: Set end of stimulation culture

Tap **edit** to edit the activity 'end of culture' in the matrix.



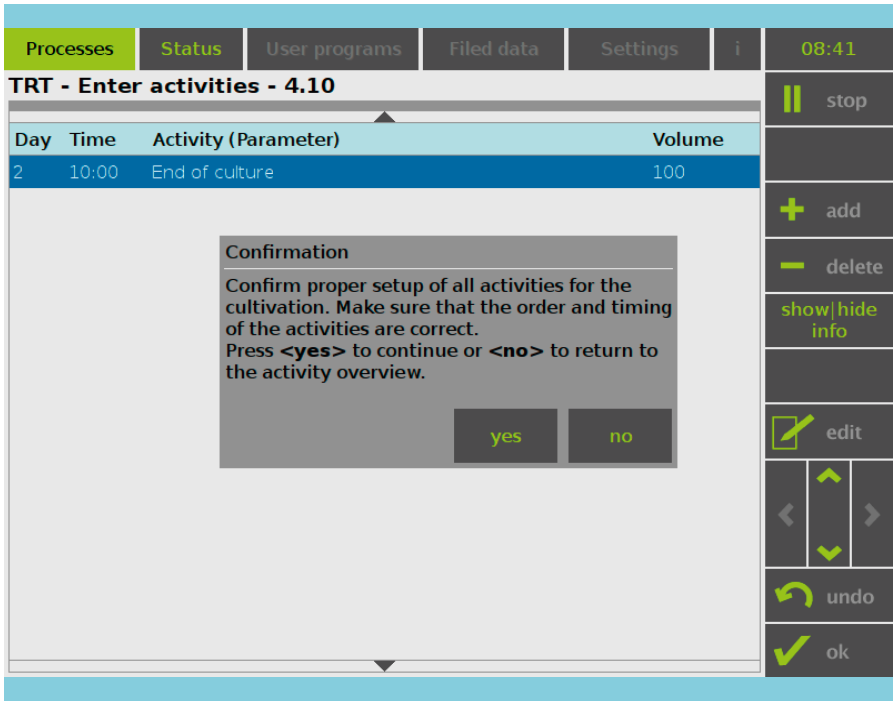
Screen 4.10: Editing end of stimulation culture

Enter the desired day and time when the stimulation should be finished. Consider that starting day of the culture is considered as day 0. If stimulation culture should be finished on the next day, enter Day 1.

A screen specific for that activity opens. Tap **edit** to change the parameters of the activity.

If necessary, change the day and time of the culture end. Tap **ok** to co confirm and to return to the matrix.

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



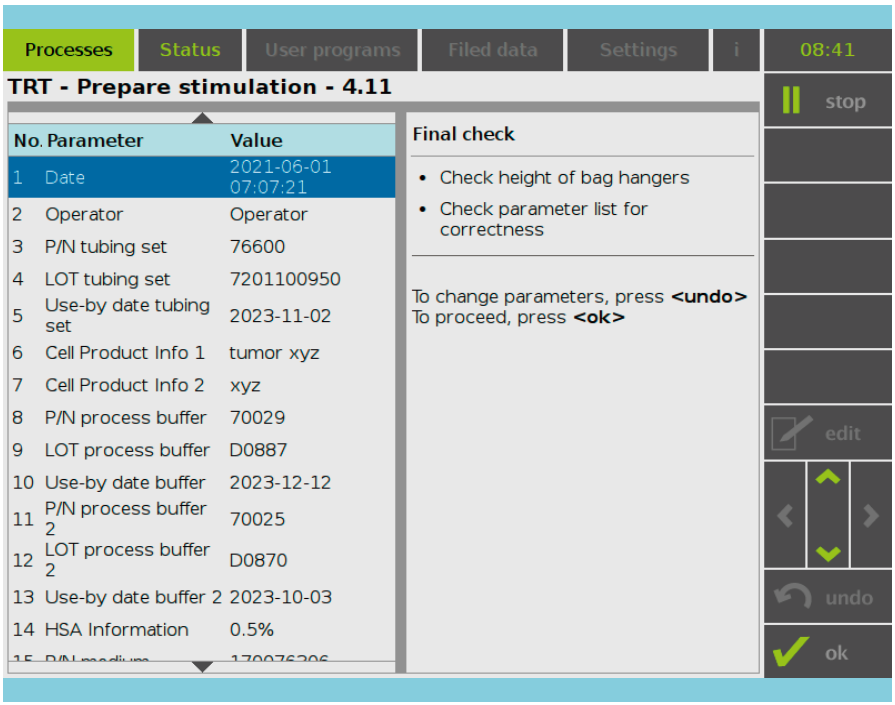
Screen 4.10: Pop-up – Set end of stimulation culture

Tap **ok** to proceed to the final setup check. Tap **yes** to confirm the matrix.

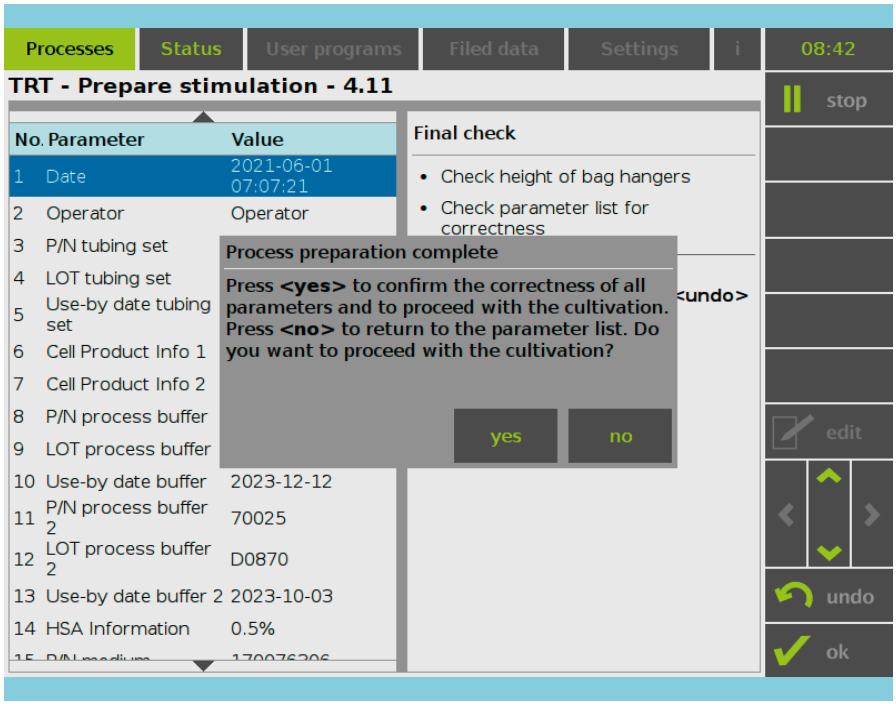
7.1.5 Final setup check

The user is asked to check the height of the bag hangers and the parameter list. Tap **undo** to change entered parameters. In that case the user has to enter all parameters again starting from Screen 4.1 in section 7.1.

Note: At this point, all preparative steps for the stimulation part are complete and the automated stimulation culture can begin.

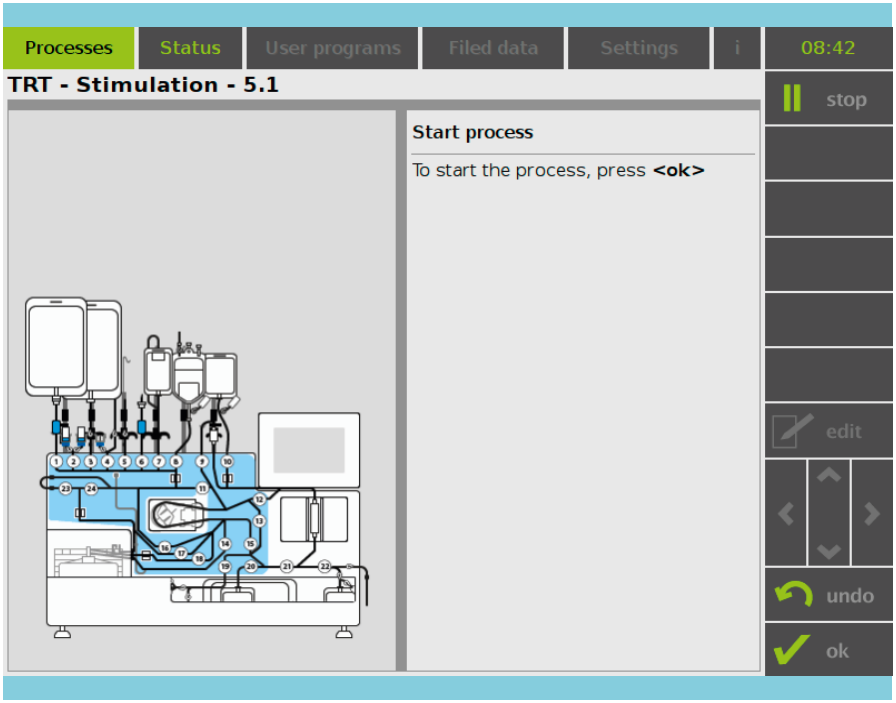


Screen 4.11: Final setup check for the stimulation culture



Screen 4.11: Pop-up – Final setup check for the stimulation culture

Tap **ok** to proceed. A pop-up window will appear. Tap **yes** to confirm the start of the stimulation culture. Tap **no** to check the parameter list again.



Screen 5.1: Start of the stimulation culture

Tap **ok** to proceed. A pop-up window will appear. Tap **yes** to confirm the start of the stimulation culture. Tap **no** to check the parameter list again.

7.2 Automated culture setup

The fully automated stimulation process is divided into several phases:

1. Transfer of the user-defined amount of cells from the Application Bag to the chamber.

2. Washing with process buffer and rebuffering with medium (different number of cycles for tumor- and leukapheresis-derived material).

3. Addition of supplements and/or peptide(s).

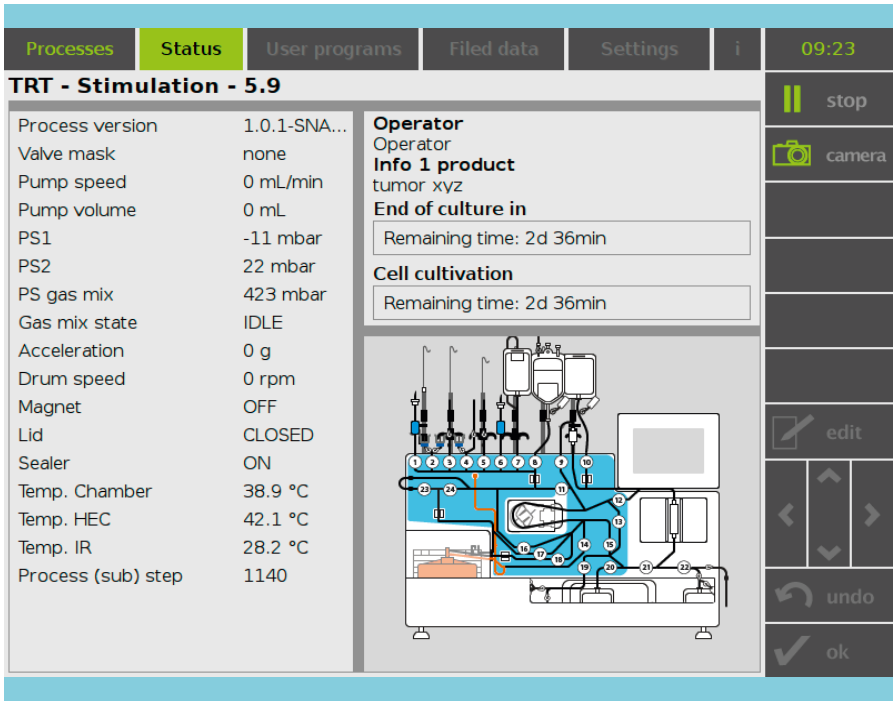
4. Setup of aeration and temperature.

7.3 Stimulation culture

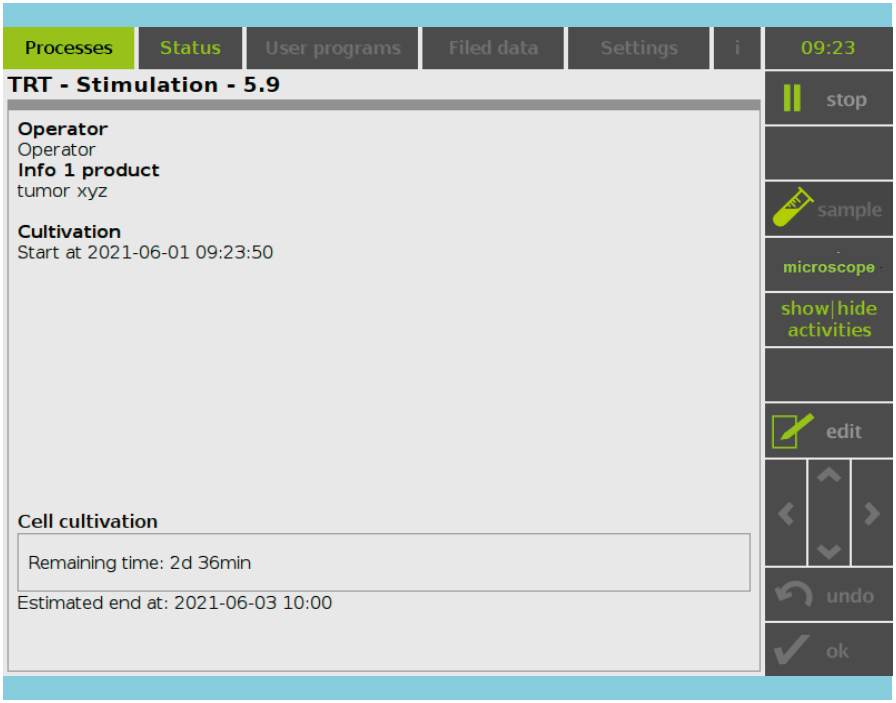
After initial setup of the stimulation culture, the monitor shows the current step and the remaining time of the cultivation process in the **Status** screen. Additionally, several sensor values can be monitored on the **Status** screen.

The pressure sensor "PS gas mix" is inside the gas mix unit and should show values between 0 mbar and 800 mbar during cultivation.

The cultivation will proceed as defined in the default matrix.



Screen 5.9: Stimulation culture **Status**



Screen 5.9: Stimulation culture **Processes**

Tap **edit** in the **Processes** screen to change the activity matrix during the stimulation culture. To show the current activity matrix, tap **show/hide** activities.

Tap **microscope** to make a photographic record of cells (see section 9.3.5 for detailed handling instructions).

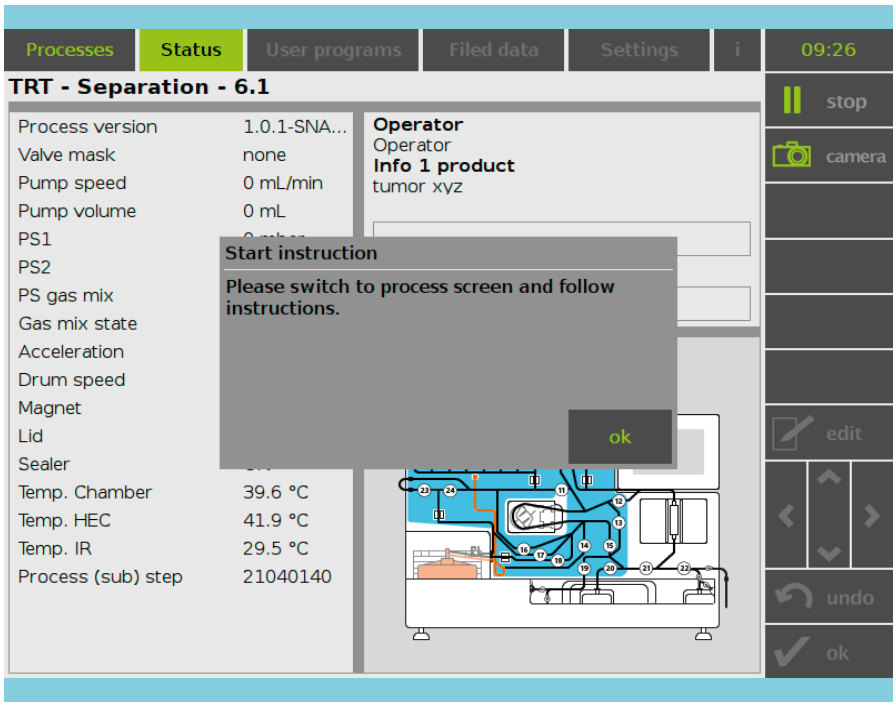
Tap **sample** to take a sample during stimulation culture (see section 8.2.2 for further handling instructions).

In the default matrix, the cells will be cultured in a volume of 100 mL.

7.4 End of stimulation culture

At the end of the stimulation culture, switch to the **Processes** screen.

The preparation of the separation part is started directly without any further confirmation.



Screen 6.1: Pop-up – End of stimulation culture

8

CD137 enrichment

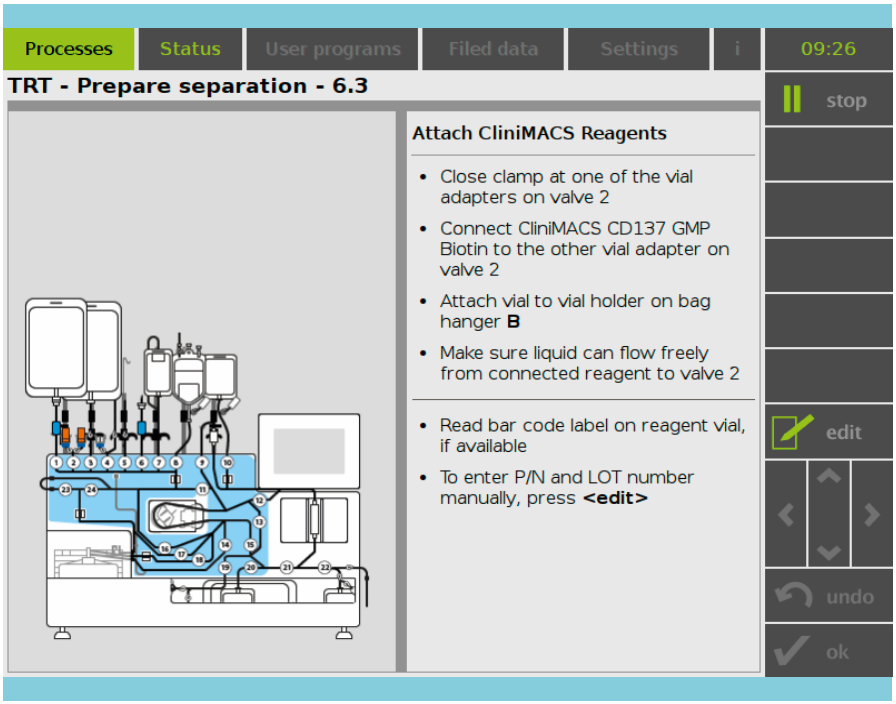
The following chapter describes the enrichment part of case 1. Differences to the enrichment part in case 2 are explained in section 10.1.

8.1 Preparation for the automated CD137 enrichment

This section guides step by step through the setup of the enrichment part of the Tumor Reactive T cell Process.

8.1.1 Connection of CliniMACS Reagents

The enrichment is performed in a two-step labeling by labeling the CD137^{+} cells with CliniMACS CD137 GMP Biotin followed by CliniMACS Anti-Biotin GMP MicroBeads/CliniMACS Anti-Biotin Reagent. Therefore, two different reagents have to be connected to the tubing set.



Screen 6.3: Connection of CliniMACS CD137 GMP Biotin

Connect one vial of the CliniMACS CD137 GMP Biotin to one of the vial adapters on valve 2:

- Close one of the clamps on the vial adapters 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 CD137 GMP Biotin vial.
- Carefully push the vial into the adapter while slightly turning until the spike penetrates the elastomer stopper and the adapter plastic ring snaps into place.
- Ensure that the septum is punctured, allowing free flow of liquid.
- In one smooth motion, turn the vial and vial adapter back again and insert the CliniMACS CD137 GMP Biotin vial into the vial holder on bag hanger B. For correct connection, see Figure 8.1.
- Adjust the height of the vial holder.

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

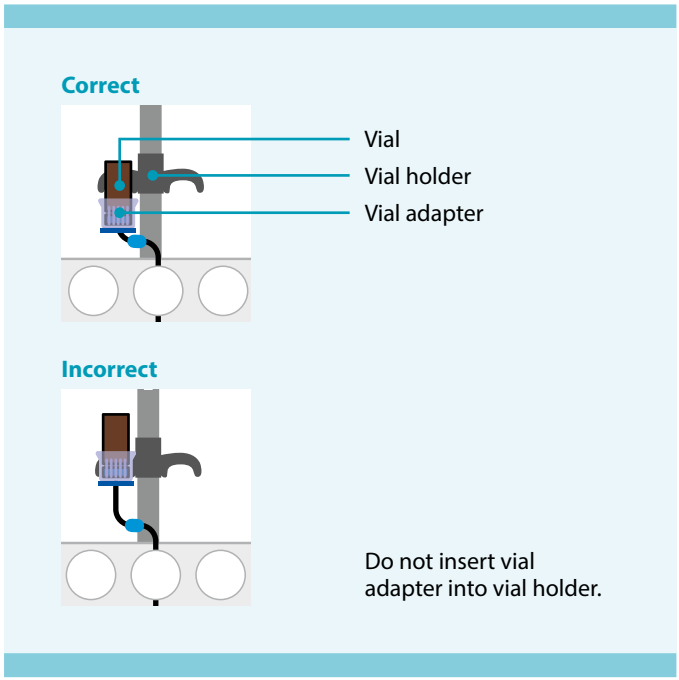
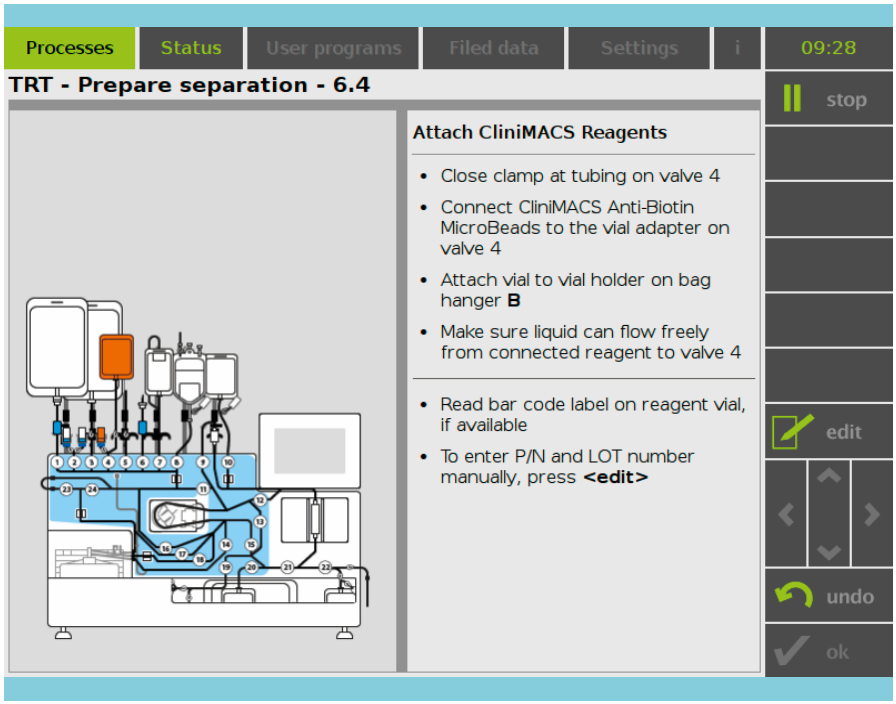


Figure 8.1: Insertion of vials into vial holder

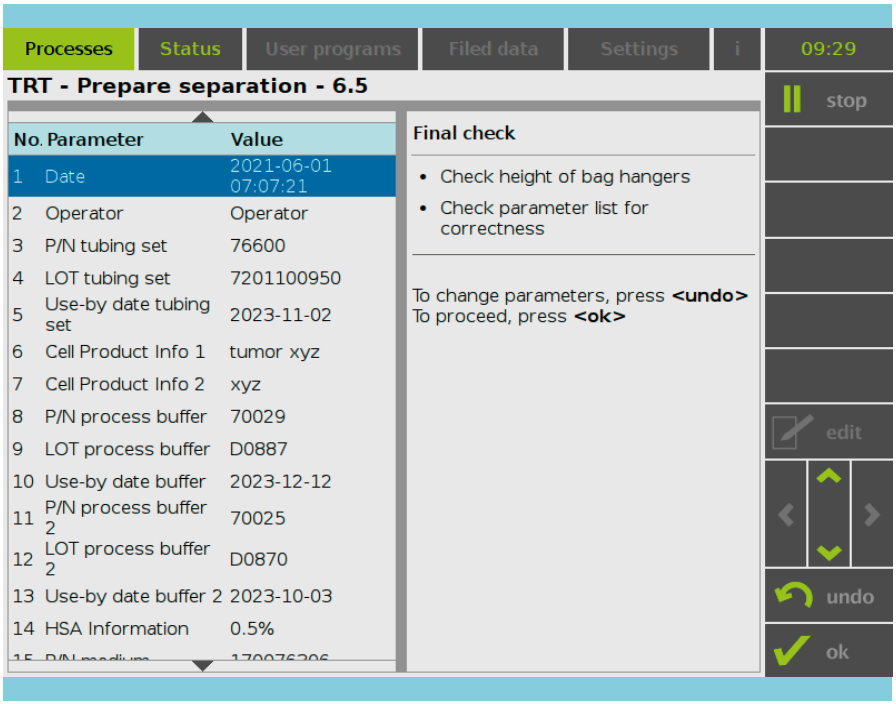


Screen 6.4: Connection of CliniMACS Anti-Biotin GMP MicroBeads/ CliniMACS Anti-Biotin ReagentAnti-Biotin Reagent

Connect one vial of the CliniMACS Anti-Biotin GMP MicroBeads/CliniMACS Anti-Biotin Reagent to the vial adapters on valve 4 in a similar fashion as described above. Make sure to close the clamp on the tubing of valve 4.

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

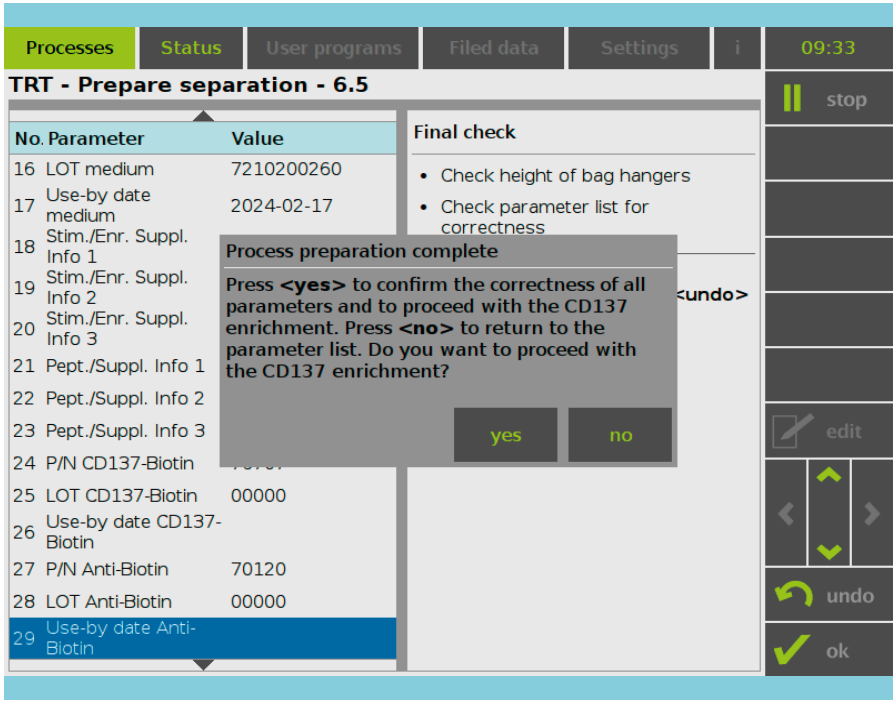
8.1.2 Final check



Screen 6.5: Final check for the enrichment

Check the height of the bag hangers and the parameter list. Tap **undo** to change entered parameters. In that case the user has to enter all parameters again starting from Screen 6.3 in section 8.1.1.

Note: At this point, all preparative steps for the enrichment part of the process are complete and the automated enrichment process can begin.

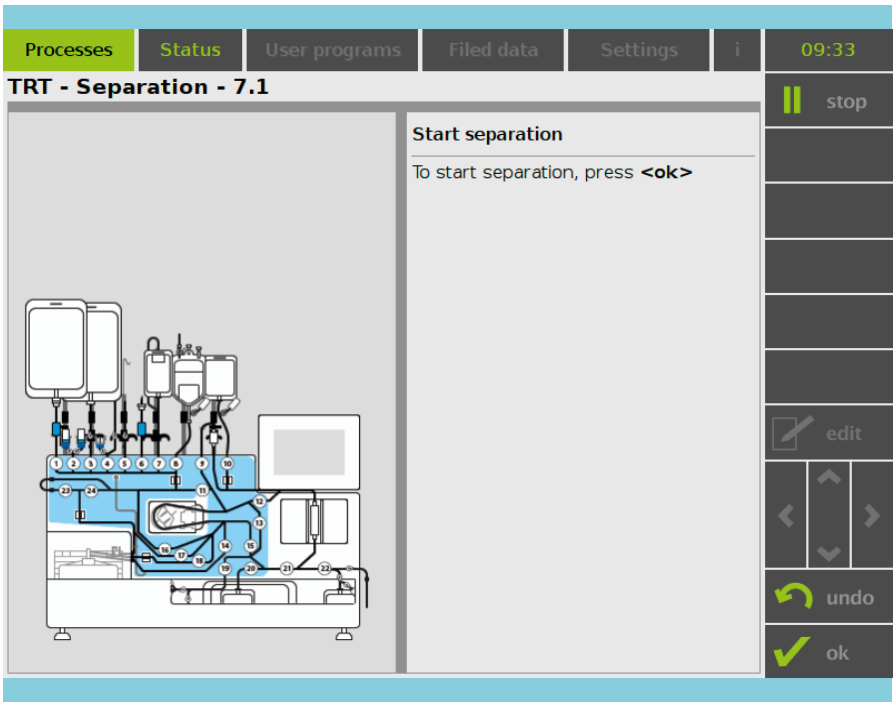


Screen 6.5 – Pop-up – Final check for the enrichment

Tap **ok** to proceed. A pop-up window will appear. Tap **yes** to confirm the start of the enrichment process. Tap **no** to check the parameter list again.

8.2 Automated enrichment process

8.2.1 Start of enrichment process

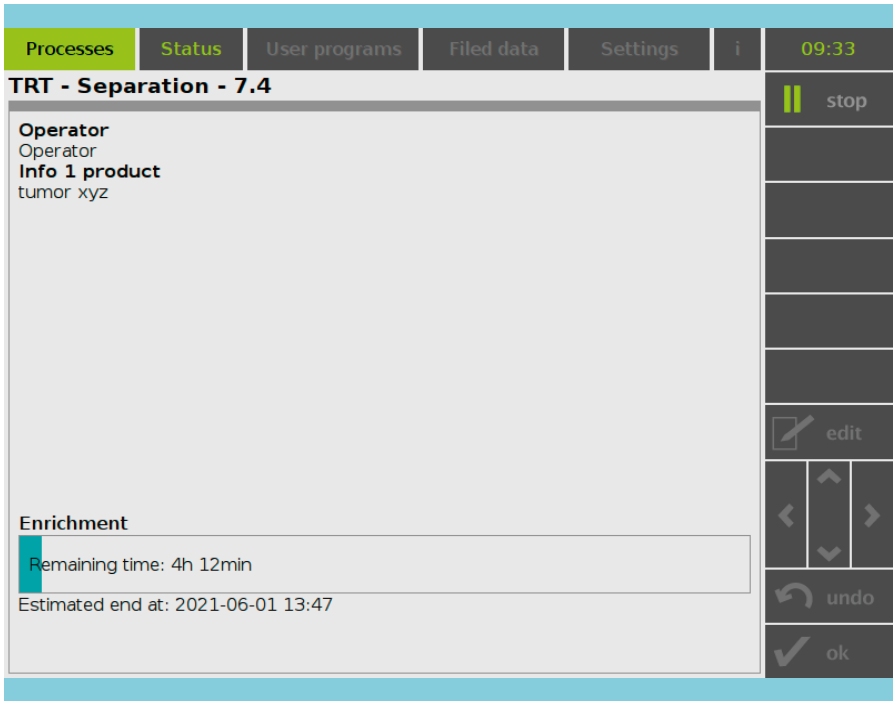


Screen 7.1: Start of automated enrichment process

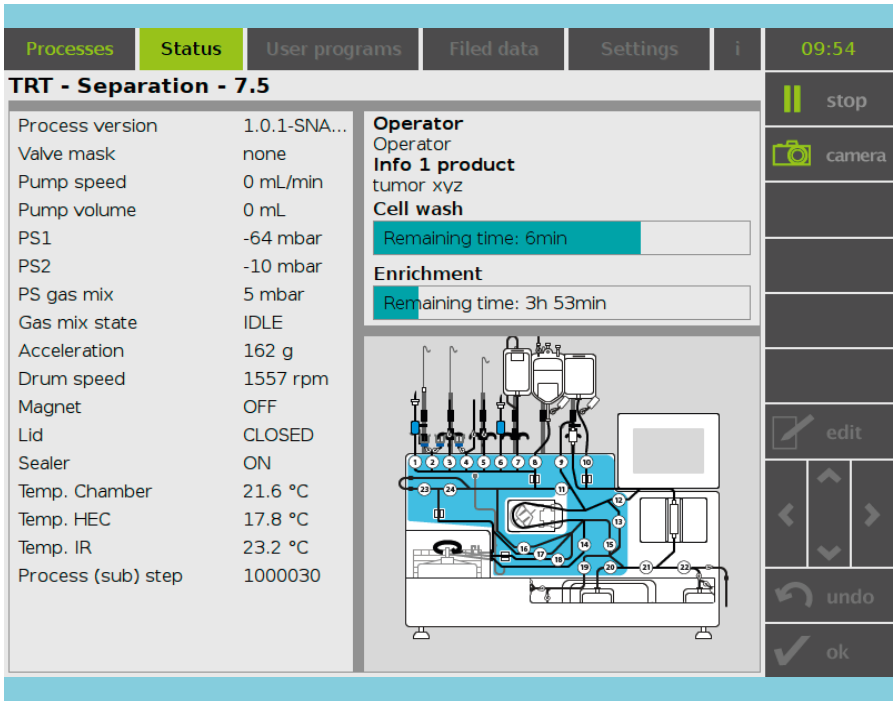
Tap **ok**. A pop-up window will appear. Tap **yes** to confirm the start of the enrichment.

The fully automated enrichment process takes about 4 hours and is divided into several phases:

1. Rebuffering the cells and the tubing set with process buffer.
2. Labeling with CliniMACS CD137 GMP Biotin.
3. Removal of excess reagent.
4. Labeling with CliniMACS Anti-Biotin GMP MicroBeads/CliniMACS Anti-Biotin Reagent.
5. Removal of excess reagent.
6. Cell filtration.
7. Magnetic separation.
8. Elution of the positive fraction into Reapplication Bag.



Screen 7.4: Generic information screen **Processes**



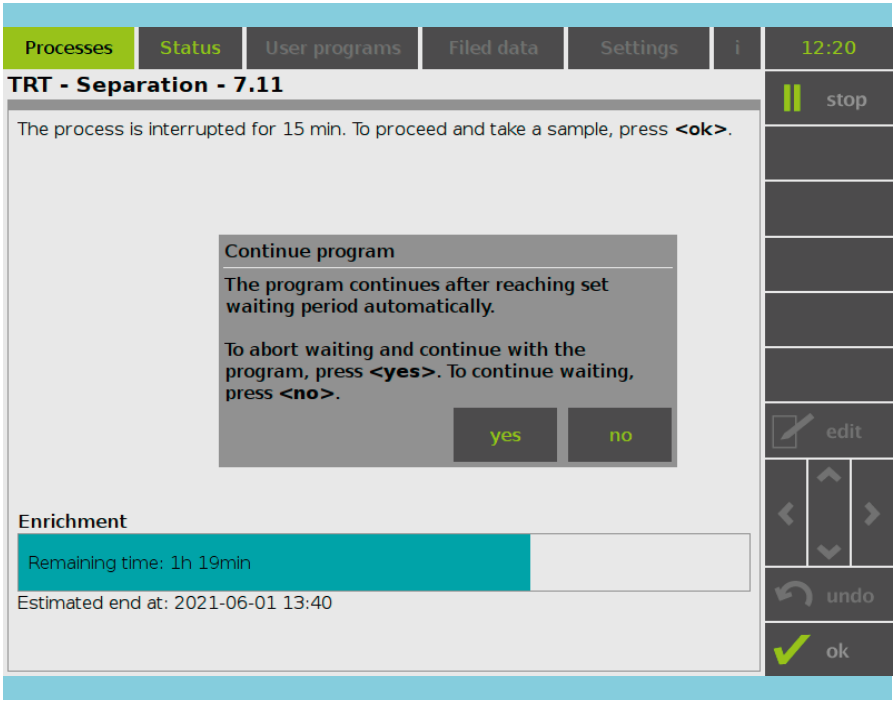
Screen 7.5: Generic status screen displaying real time process info **Status**

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

8.2.2 Optional sampling of the original fraction

Before magnetic separation, an optional sample of the original fraction can be taken in which the cells can be analyzed for appropriate labeling. The process is interrupted for 15 minutes after cell filtration (approximately 2 h and 50 min after start of the enrichment part). If no user interaction takes place during the 15 minutes time-out, the process will continue automatically.

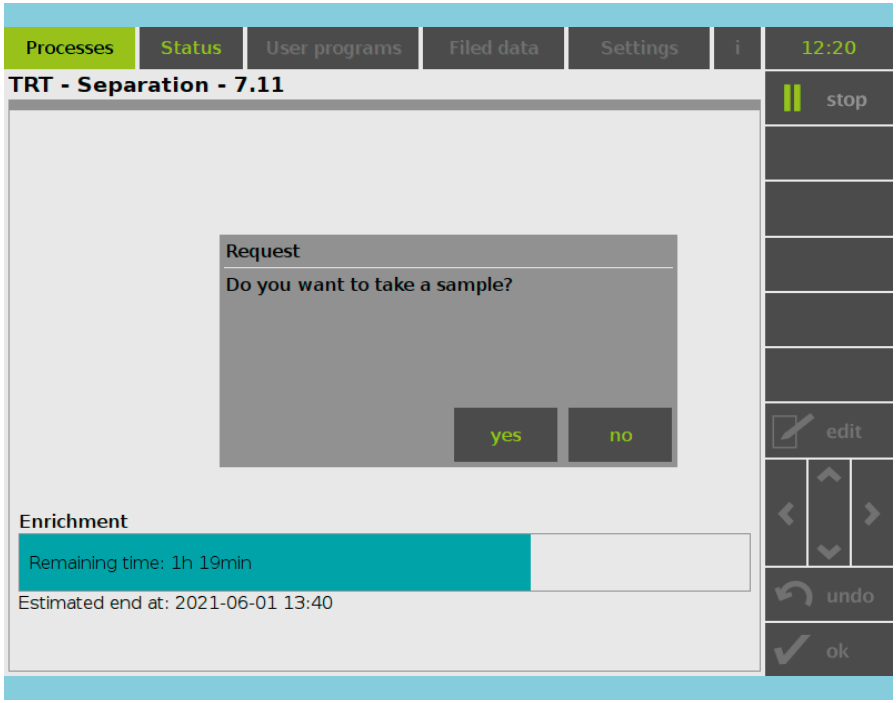
Tap **ok** to start the user interaction.



Screen 7.11: Pop-up 1 – Interruption of the enrichment process for sampling

Tap **no** to go back to the 'wait for sampling' screen. The magnetic separation will start after the 15 minutes time-out.

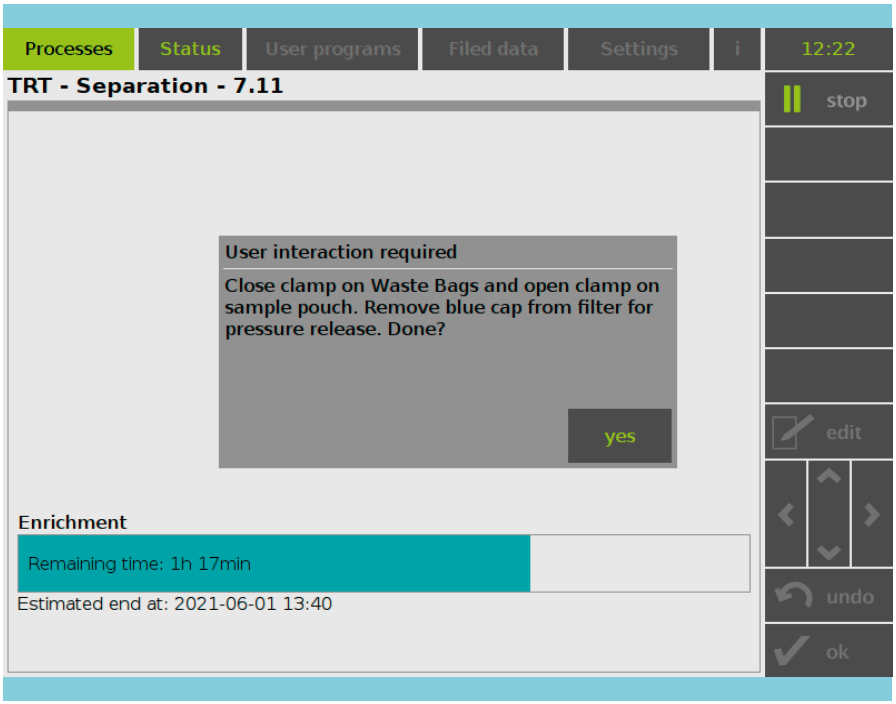
Tap **yes** to continue with the process without waiting time. The following screen is shown.



Screen 7.11: Pop-up 2 – Do you want to take a sample

Tap **yes** to take a sample, tap **no** to continue directly with the separation.

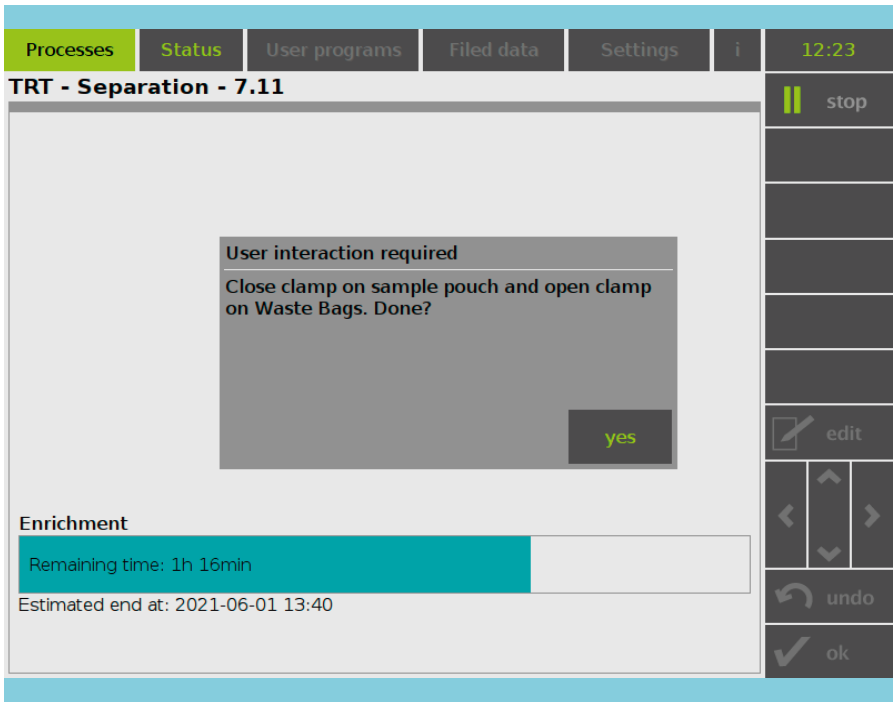
To take a sample, follow the instructions in the pop-ups and the procedure described below.



Screen 7.11: Pop-up 3 – User interaction before sampling

Close the clamp on the Waste Bags. Make sure to open both the clamp at the QC Pouch assembly/Triple Sampling Adapter AND the clamp on the single sample pouch. Remove the blue cap of filter at the sample pouch.

Hold the sampling pouch with the sterile filter facing up to prevent filter clogging. Tap **yes**. Wait until the sample pouch is filled and the next pop-up appears.



Screen 7.11: Pop-up 4 – User interaction after taking a sample

Close the clamp on the used sample pouch and open the clamp on the Waste Bags.

After sampling, there is residual sample liquid within the tubing, which has to be removed for future sampling. Open the clamp of an empty sample pouch, squeeze the sample pouch to push the residual liquid into the Waste Bag and close the clamp of the pouch. Release the sample pouch again, remove the blue cap from the filter of this pouch so that sterile air can enter the pouch. Re-attach the blue cap to the filter. Close the clamps at the QC Pouch assembly/Triple Sampling Adapter. Tap **yes**.

After the sampling is completed, weld off the pouch and collect the sample by unscrewing the sterile filter (under a hood to preserve sterility, if desired).

The magnetic separation will start after the sample was taken.

8.3 Completion of enrichment process



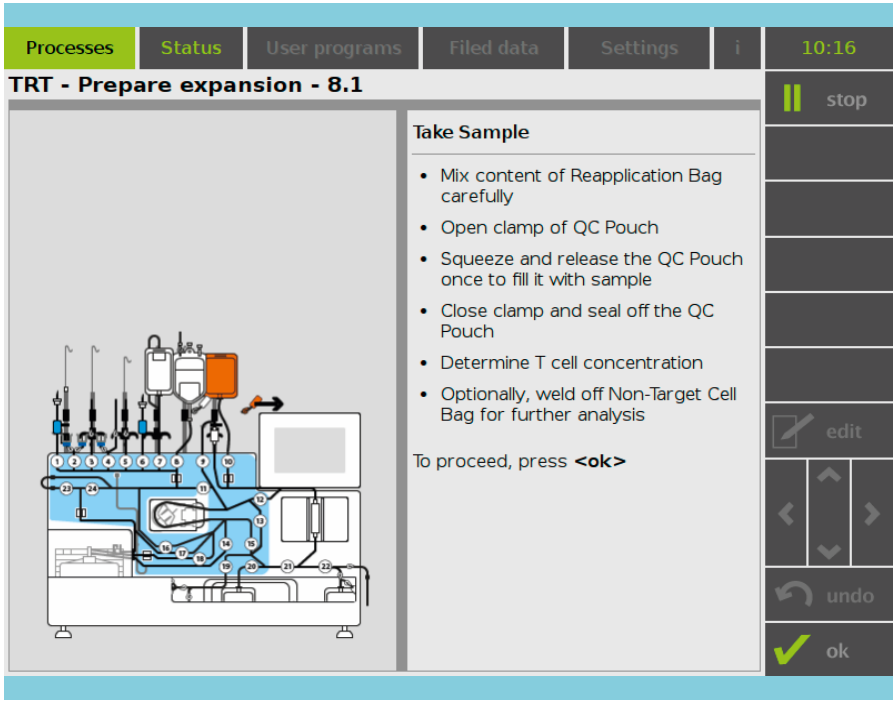
Screen 7.12: Pop-up – End of enrichment process

After completion of the enrichment process, a pop-up window will ask if the process should continue with the expansion.

If no cultivation is required tap **no** and proceed with the final handling (see section 9.5).

To perform the expansion culture, tap **yes** and proceed with the analysis of the enriched target cell fraction in the Reapplication Bag (section 8.3.1).

8.3.1 Analysis of enriched target cell fraction



Screen 8.1: Taking of sample from target cell fraction in the Reapplication Bag

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

- Open the clamp of the QC Pouch.
- Squeeze and release the QC pouch in order to fill it with sample. Multiple squeezes and releases will increase the 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.

Note: It is recommended to analyze the enriched target cell fraction and proceed with the expansion part within one hour after completion of the enrichment process as the cells are in suboptimal conditions in the Reapplication Bag (no CO_2 supply and only room temperature which may lead to quality loss).

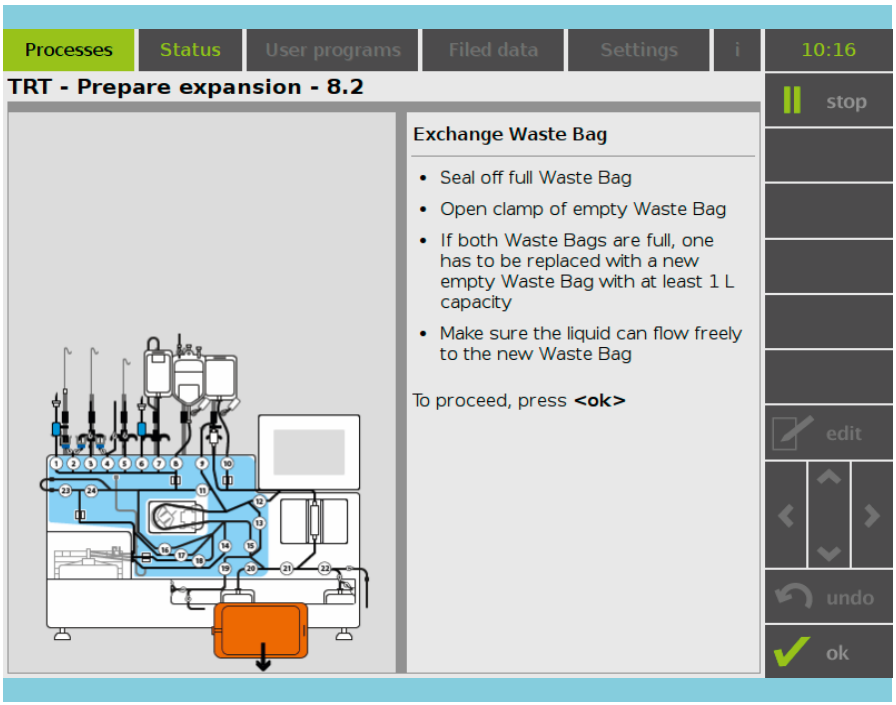
Store all QC samples at +4 °C to +8 °C (+39 °F to +46 °F).

⚠ CAUTION

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

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

Make sure that the Waste Bags are not overfilled. Seal off one Waste Bag if it is full and open the clamp of the empty Waste Bag. If necessary, connect a new Waste Bag via sterile welding.



Screen 8.2: Exchange of Waste Bag after enrichment

9 Expansion

9.1 General remarks

The following chapter describes the expansion cultivation (similar to the Rapid Expansion Protocol or REP) of case 1 and 2. Differences in the setup of the expansion cultivation of case 3 are explained in section 10.2.

During expansion, the following steps are performed:

- Transfer of T cells to the chamber and washing of T cells.
- CO_2 and culture temperature setup.
- Execution of the user-defined activities (including feeder cell addition, activation and optionally transduction).
- At the end of the expansion the cells are harvested.

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

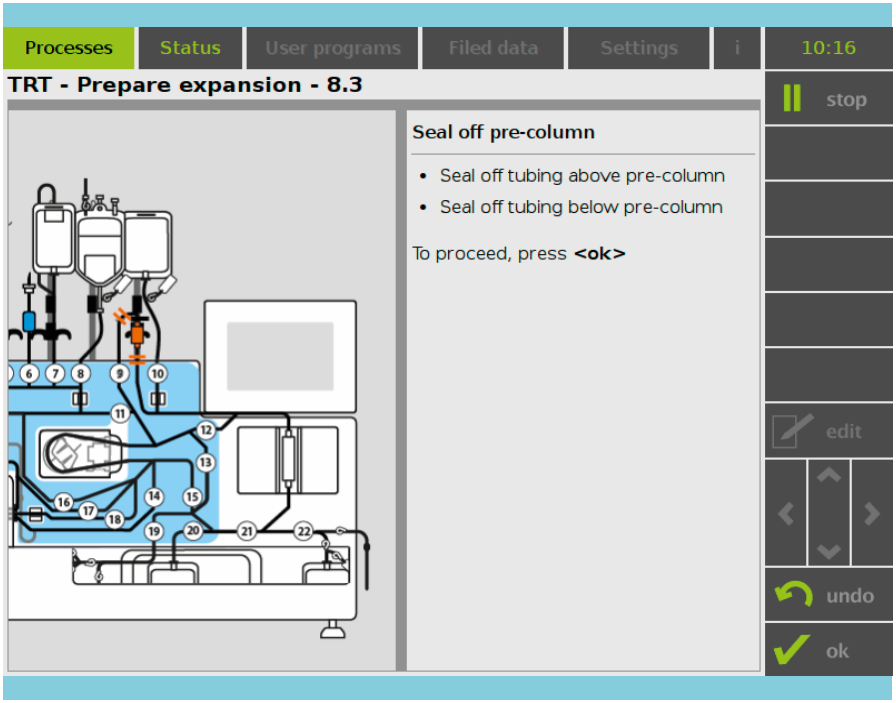
- *Every 10 hours, the tubing set is rinsed with 10 mL medium connected to valve 3. Therefore, always connect sufficient medium to the tubing on valve 3 (e.g., if the device is running for 72 hours, a minimum of 80 mL medium has to be connected).*
- *Each Waste Bag connected to the CliniMACS Prodigy TS 520 has a capacity of five liters. If a long process with many media exchanges is performed, regularly check that the Waste Bags are not overfilled during expansion culture. If necessary, connect a new 5 L Collection Bag via sterile welding. Optional: The activity 'Waste Bag exchange' can be programmed in the activity matrix (see section 9.3.9) to get instructions about the handling.*

- *Every feed, media exchange and sampling during the expansion culture requires an additional 60 mL medium for rinsing purposes. Take this into account when calculating the required volume of medium for prolonged periods of user absence.*

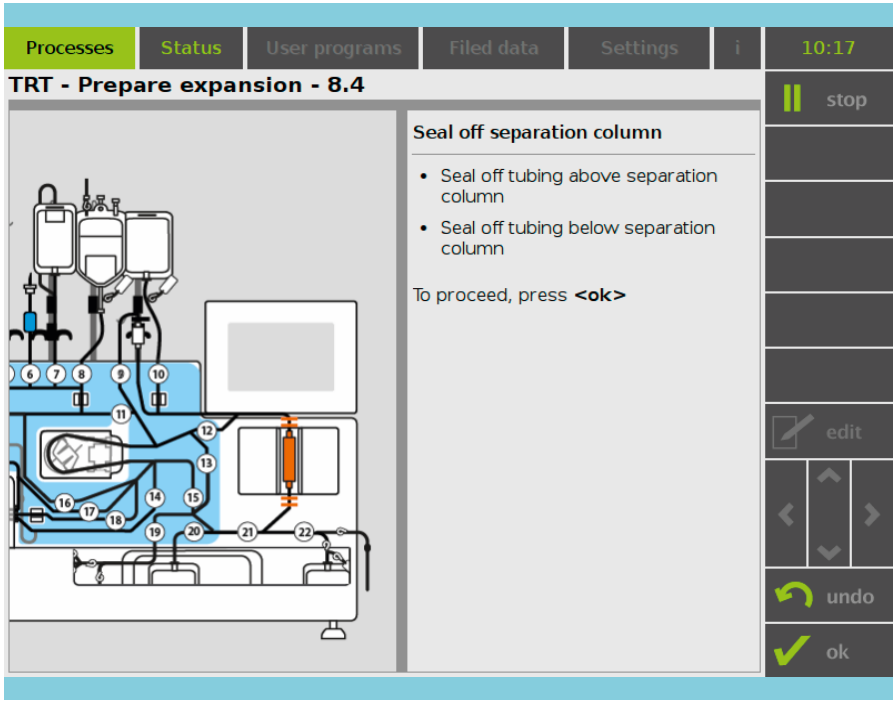
9.2 Preparation for the expansion culture

This section guides step by step through the setup of the expansion culture of the Tumor Reactive T cell Process.

9.2.1 Sealing off the pre-column and separation column



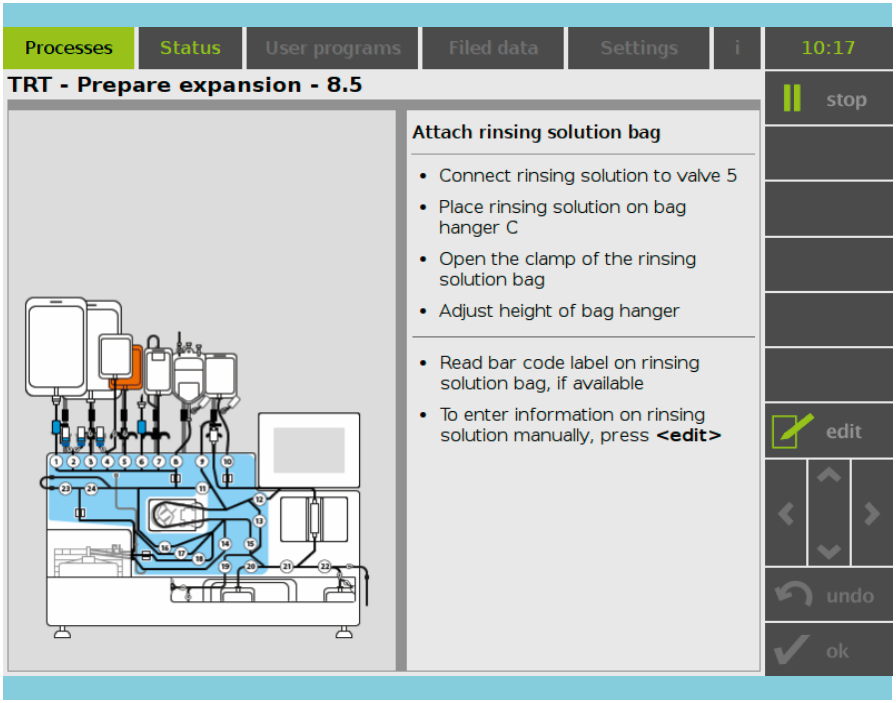
Screen 8.3: Sealing off the pre-column



Screen 8.4: Sealing off the separation column

To avoid diffusion of cells from the columns during the expansion culture, the pre-column and the separation column of the mounted tubing set should be sealed off or clamped off as shown on Screen 8.3 and Screen 8.4.

9.2.2 Rinsing of chamber and tubing set

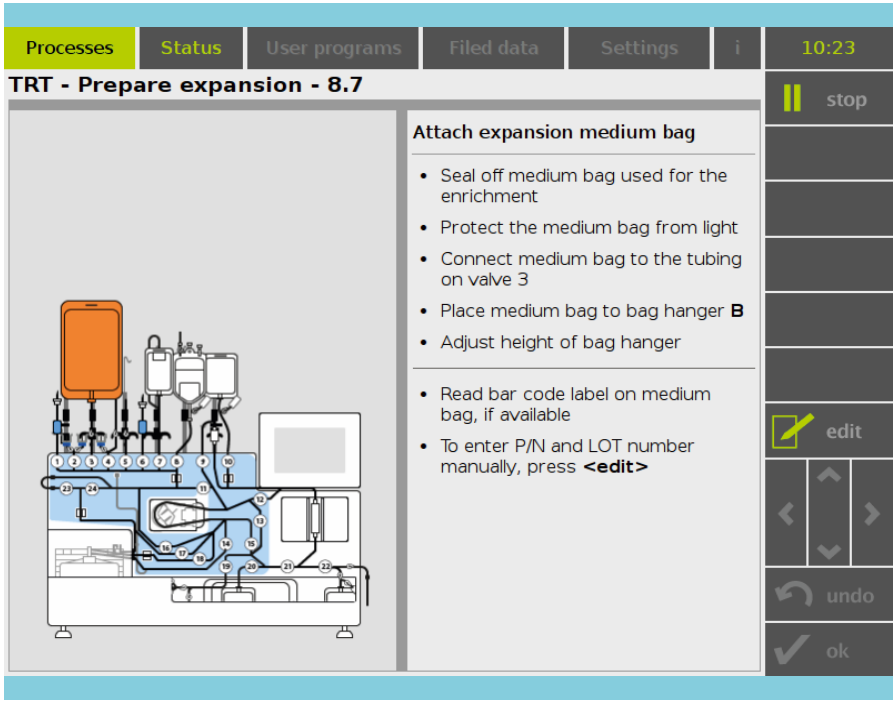


Screen 8.5: Attachment of the rinsing solution

Before setup of the expansion culture, the chamber and the tubing set are rinsed to remove all cells remaining in the culture chamber after the completed enrichment. Connect a bag with at least 300 mL rinsing solution to valve 5 and hang the bag on bag hanger C. It is highly recommended to use sterile water as rinsing solution to lyse and remove the remaining cells in the chamber.

Tap **edit** to enter information on the rinsing solution (e.g., sterile water).

9.2.3 Connection of medium



Screen 8.7: Connection of expansion medium bag and enter P/N, LOT number of the medium bag

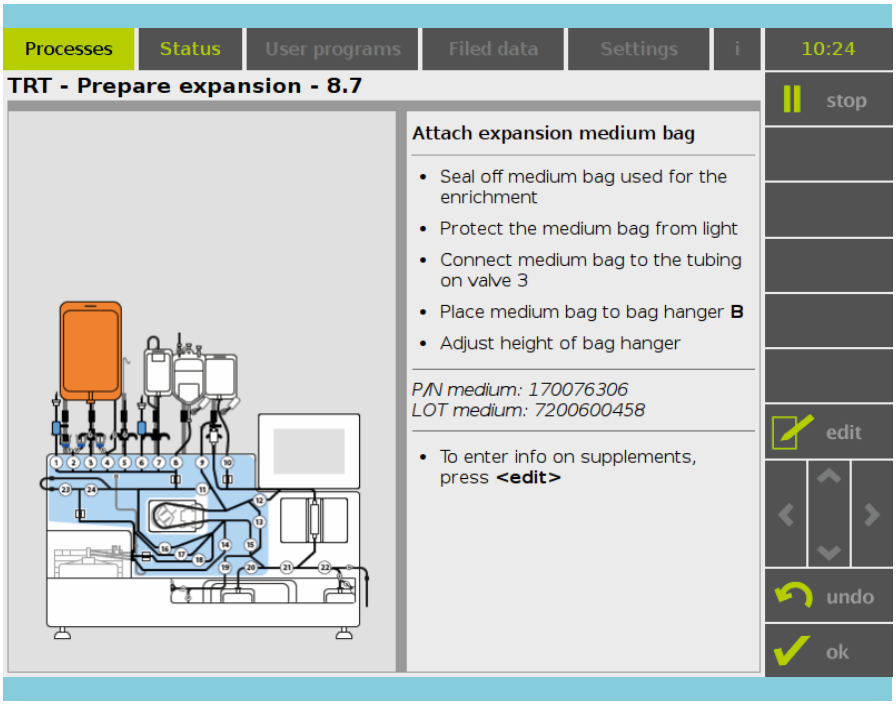
Prepare the expansion medium containing IL-2 (recommended) (see section 5.1.7). Exchange the medium bag on valve 3 by sterile welding and place the expansion medium bag on bag hanger B.

IMPORTANT

To protect the medium and IL-2 against light exposure, place the expansion medium bag in a yellow UV protection bag before welding it onto the tubing set.

Note: Make sure to remove the medium bag used for stimulation and/or enrichment. This medium does not contain IL-2 and would otherwise dilute the expansion medium.

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



Screen 8.7: Enter information on supplements of the medium

Tap **edit** to enter information on supplements (e.g., 3% human AB serum, 3,000 IU/mL IL-2, antibiotics). Up to 3 different texts on the supplements can be entered.

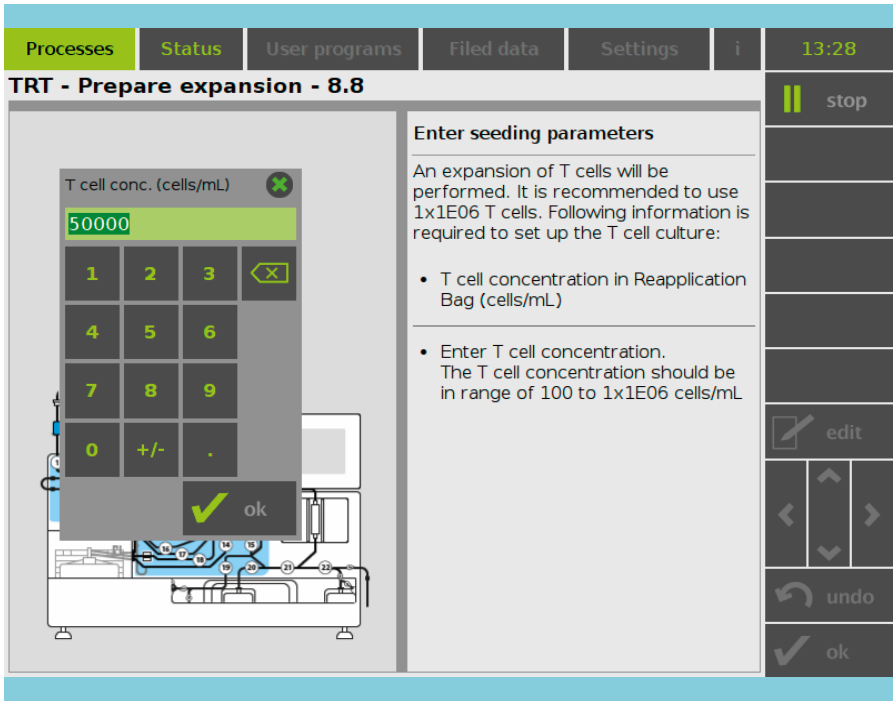
If necessary, adjust the height of the bag hanger to prevent severe bending or overstretching of the tubing.

9.2.4 Input of cell seeding parameters

After analyzing the enriched target cells (see section 8.3.1 and 5.1.5 to 5.1.6), enter the following values:

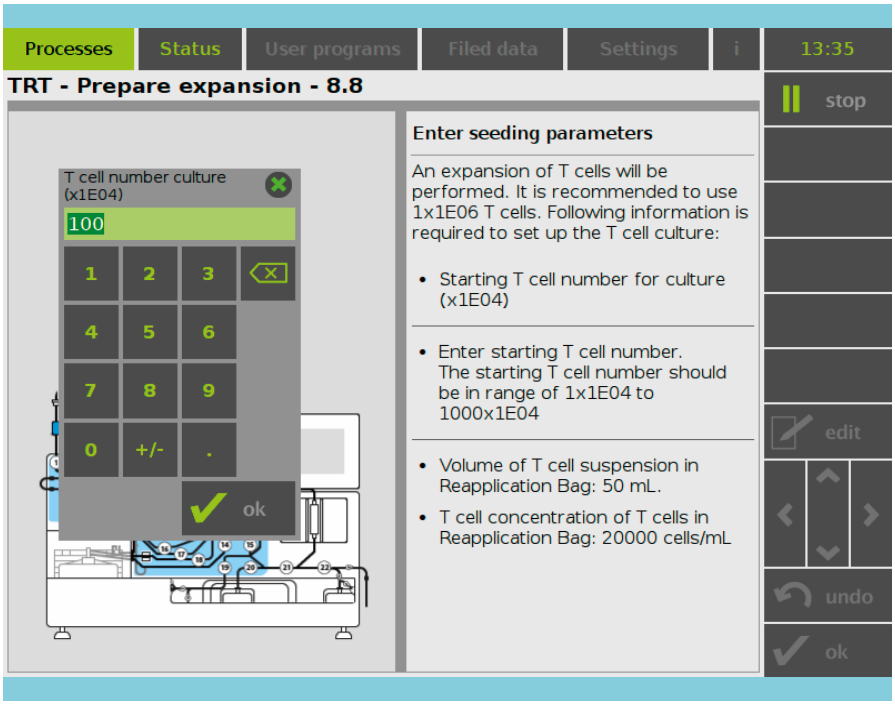
- T cell concentration that is present in the Reapplication bag (cells/mL).
- Desired starting T cell number (x1E04) for the expansion culture.

For culture setup, 10-40 mL cells can be transferred from the Reapplication Bag to the chamber.



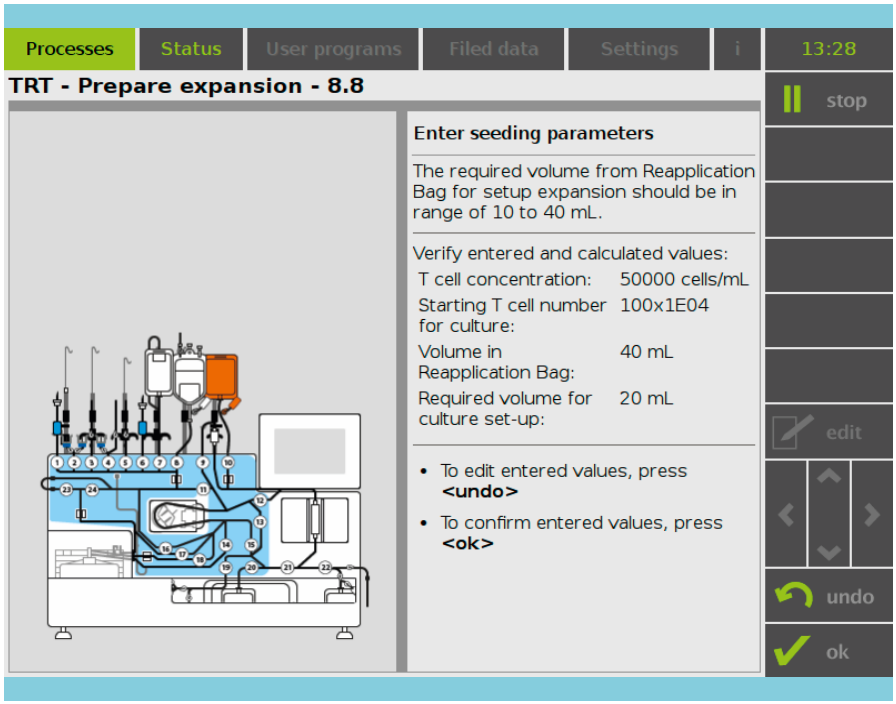
Screen 8.8: Input of the seeding parameters for the expansion culture – T cell concentration

The T cell concentration should be in the range of 100 to 1×10^6 viable T cells/mL.



Screen 8.8: Input of the seeding parameters for the expansion culture – Starting T cell number

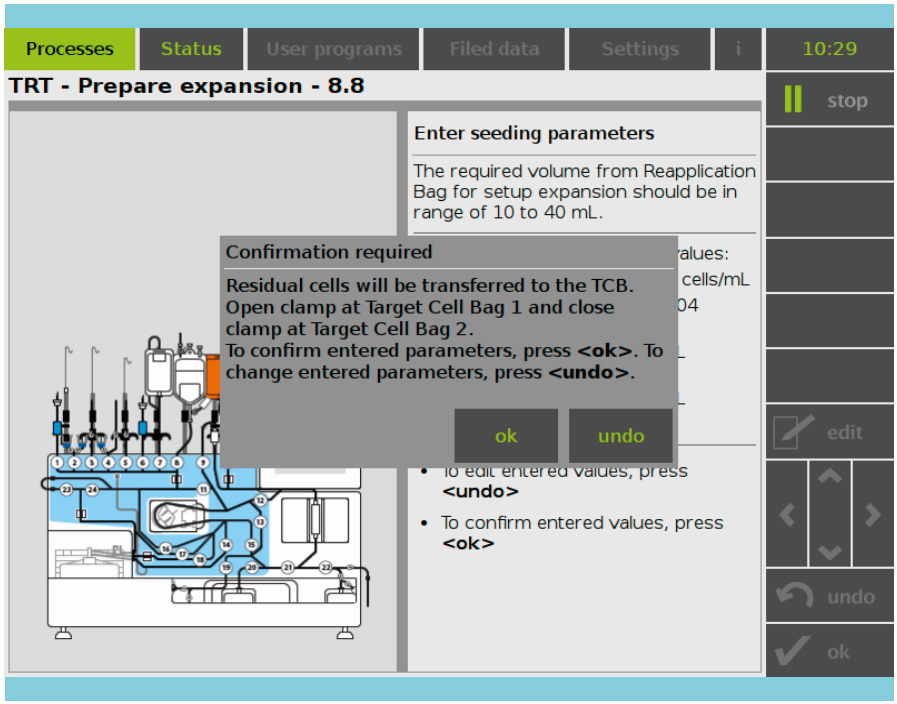
The recommended starting T cell number for culture is 1×10^6 viable T cells.



Screen 8.8: Calculation of the required volume for culture setup of the expansion culture

The volume of the cell suspension in the Reapplication Bag that is transferred for the expansion culture is calculated automatically.

Note: It is recommended to mix the cells in the Reapplication Bag carefully prior to the transfer to the chamber.



Screen 8.8: Pop-up – Transfer of residual cells to the Target Cell Bag

Note: If only a part of the volume in the Reapplication Bag will be transferred to the chamber for expansion, the remaining cells will be transferred to one of the Target Cell Bags on valve 22 during the culture setup.

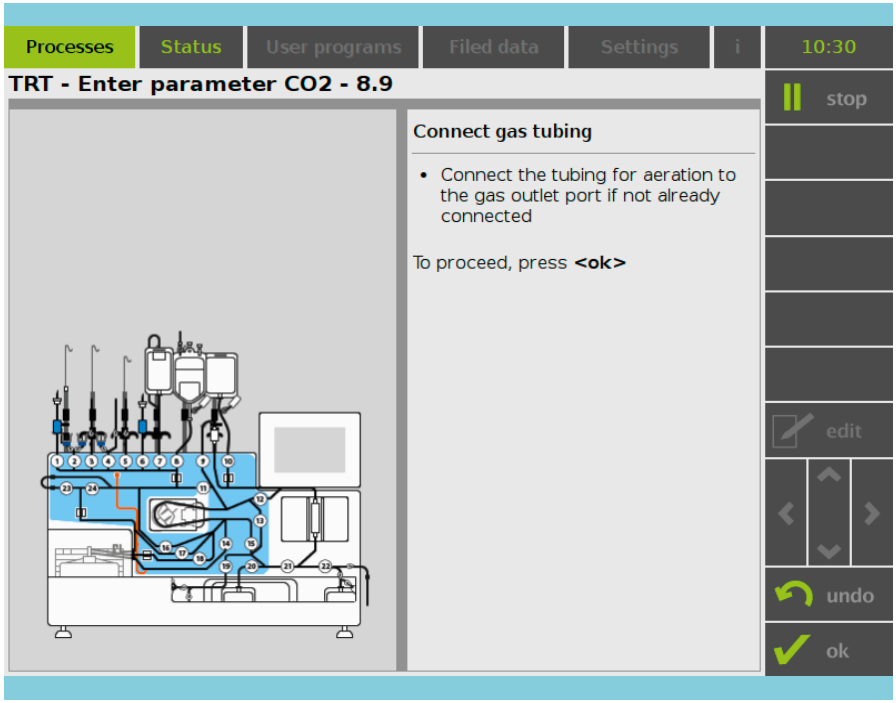
Make sure that only one of the two Target Cell Bags is opened and close the clamp of the second bag. Make sure that the clamp of the QC Pouch of the selected bag is closed. Make sure that liquid can flow freely to the used Target Cell Bag.

Note: If using the Target Cell Bag with the in-line filter, ensure that the filter is upright.

After transfer of the cells to the Target Cell Bag, the Reapplication Bag will be rinsed. This rinsing volume will be transferred into the Target Cell Bag as well. The used Target Cell Bag can be welded off for further use of the cells.

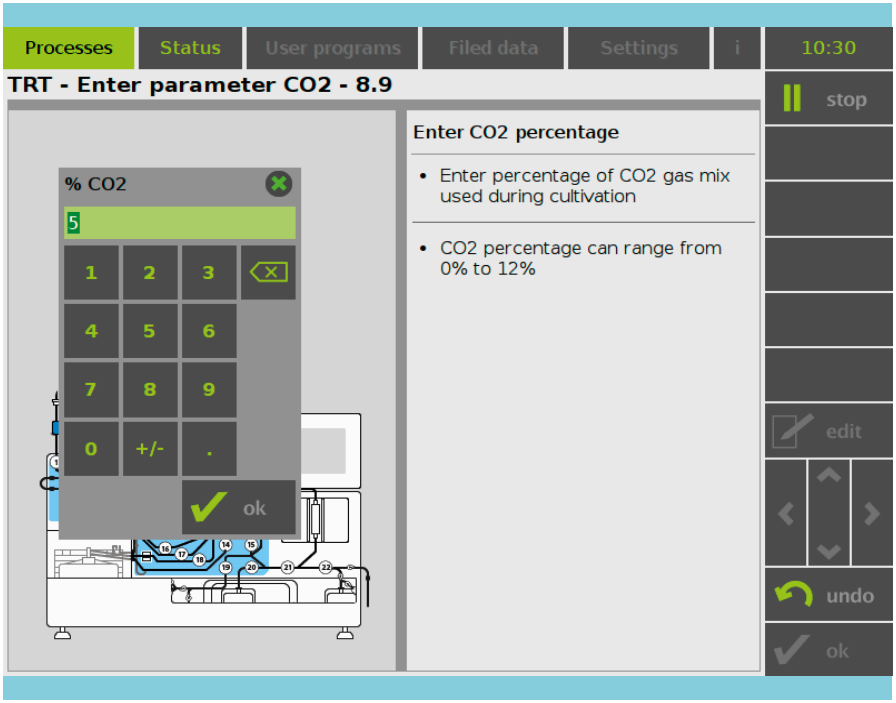
Note: Due to the rinsing procedure the concentration of cells in the Target Cell Bag will be reduced.

9.2.5 Input of CO_2 and temperature parameters



Screen 8.9: Connection of gas tubing

During tubing set installation, the gas outlet port was already connected (see Screen 2.18 in section 6.3.2). Double check if the tubing is connected tightly.



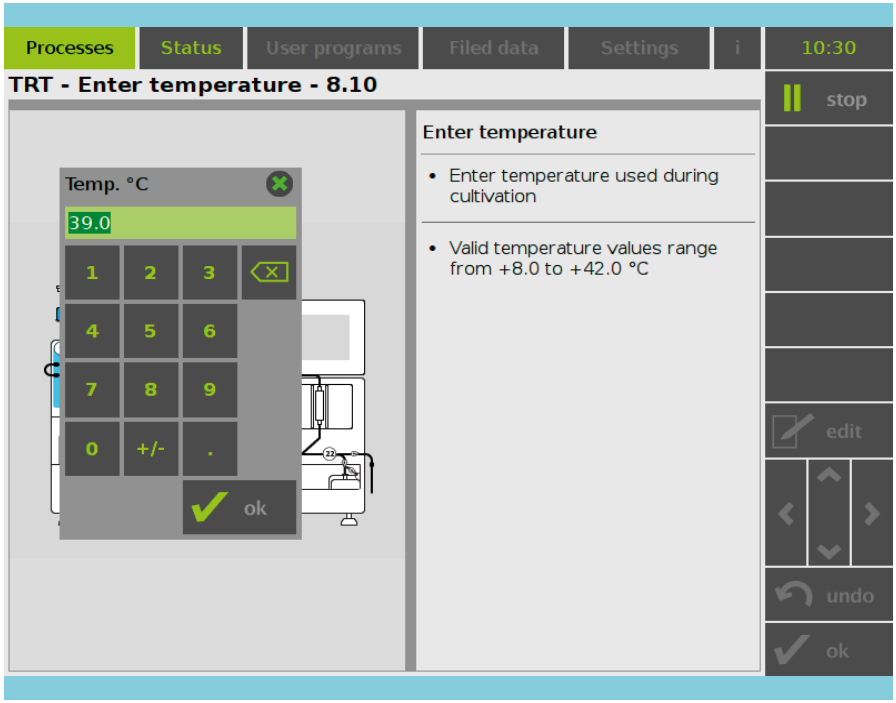
Screen 8.9: Input of CO_2 percentage

For the cultivation of the cells, the value for CO_2 percentage has to be entered. For TexMACS GMP Medium enter 5% CO_2.

The temperature parameter has to be entered manually only 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).

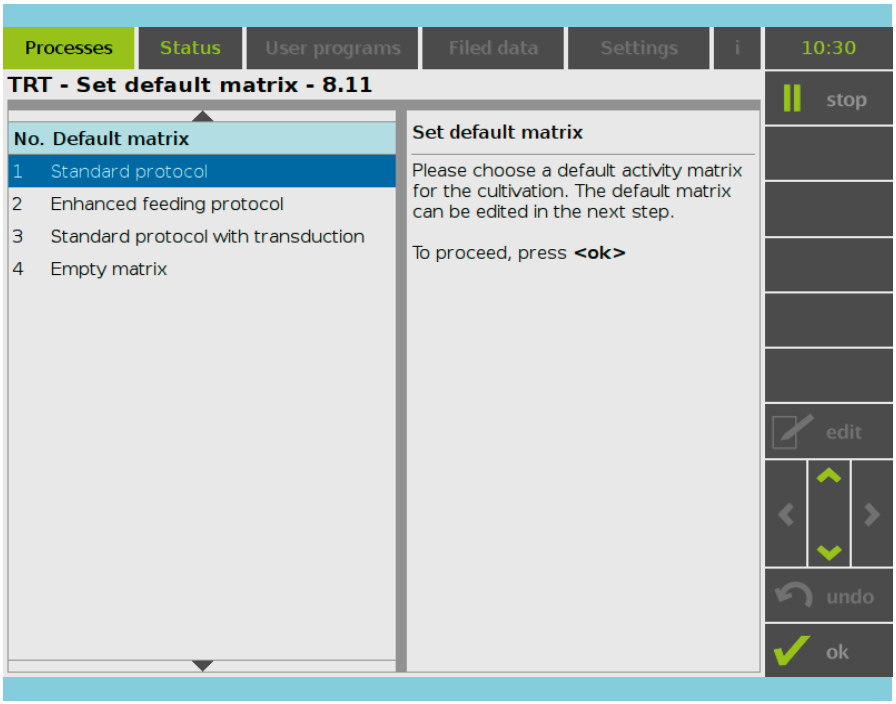


Screen 8.10: Input of temperature

9.2.6 Setup of the activity matrix

The activity matrix of the Tumor Reactive T cell Process allows customized programming of T cell cultivation steps.

Choose the default activity matrix most suitable for the needs of the application, which can later be adapted accordingly.



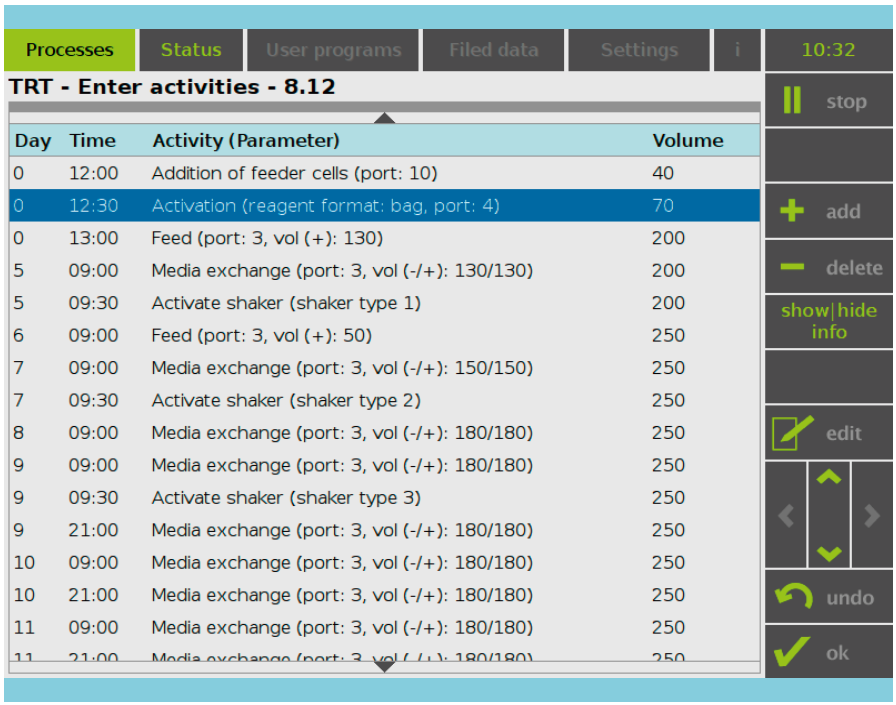
Screen 8.11: Set default matrix

For starting responder cell numbers up to 2×10^6 viable T cells it is recommended to use the standard protocol (default matrix 1) and change it according to the requirements. It is recommended to initiate this culture in a starting volume of 200 mL. If necessary, add medium using the activity 'feed' after feeder cells and the activation reagent are added to the T cells.

In case of higher starting responder cell numbers ($\geq 2\times10^6$ viable T cells; up to 1×10^7 viable T cells has been tested), it is recommended to use the enhanced feeding protocol (default matrix 2). In case of good expansion, the chamber capacity might be reached already around day 8–10. The culture will yield lower total fold-expansion due to the maximum chamber capacity of ~4×10^9 cells.

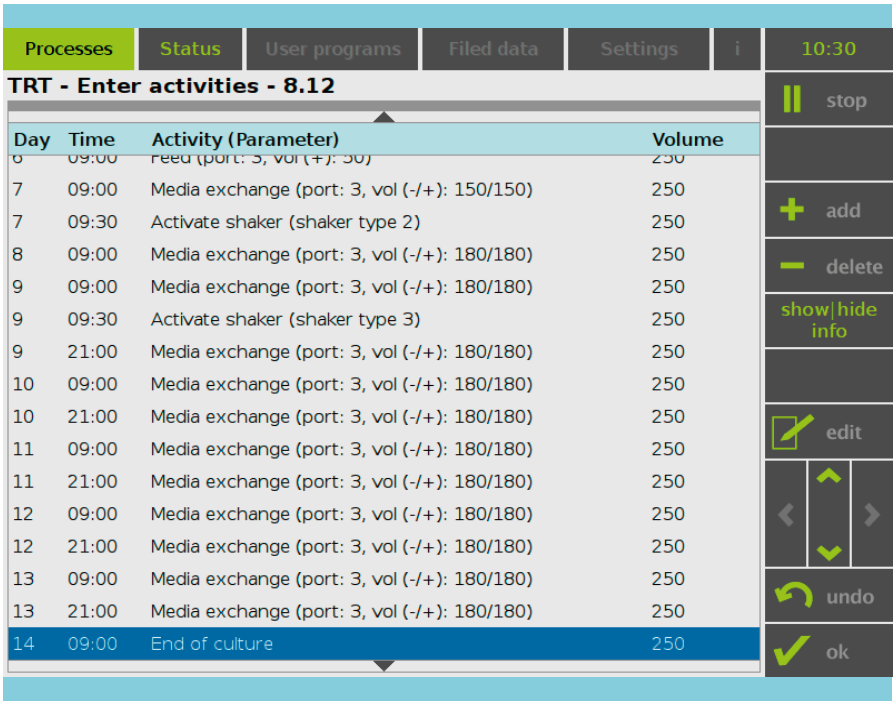
If it is planned to transduce the T cells, use the standard protocol with transduction (default matrix 3). This activity matrix includes the pre-programmed activities 'transduction' and 'culture wash' to remove excess viral vector particles two days after transduction. The cells are initially cultured in 70 mL as opposed to 200 mL in the other default matrices in order to have a higher concentration of viral particles during transduction.

To enter all activities manually use the empty matrix (default matrix 4).



Day	Time	Activity (Parameter)	Volume
0	12:00	Addition of feeder cells (port: 10)	40
0	12:30	Activation (reagent format: bag, port: 4)	70
0	13:00	Feed (port: 3, vol (+): 130)	200
5	09:00	Media exchange (port: 3, vol (-/+): 130/130)	200
5	09:30	Activate shaker (shaker type 1)	200
6	09:00	Feed (port: 3, vol (+): 50)	250
7	09:00	Media exchange (port: 3, vol (-/+): 150/150)	250
7	09:30	Activate shaker (shaker type 2)	250
8	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
9	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
9	09:30	Activate shaker (shaker type 3)	250
9	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250
10	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
10	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250
11	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
11	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250

Screen 8.12: Default activity matrix – Standard protocol



Day	Time	Activity (Parameter)	Volume
6	09:00	Feed (port: 3, vol (+): 50)	250
7	09:00	Media exchange (port: 3, vol (-/+): 150/150)	250
7	09:30	Activate shaker (shaker type 2)	250
8	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
9	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
9	09:30	Activate shaker (shaker type 3)	250
9	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250
10	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
10	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250
11	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
11	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250
12	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
12	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250
13	09:00	Media exchange (port: 3, vol (-/+): 180/180)	250
13	21:00	Media exchange (port: 3, vol (-/+): 180/180)	250
14	09:00	End of culture	250

Screen 8.12: Default activity matrix – Standard protocol

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

Tap **show/hide info**, to show information on the entered parameters and instructions to edit the setup of the activity matrix.

IMPORTANT

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

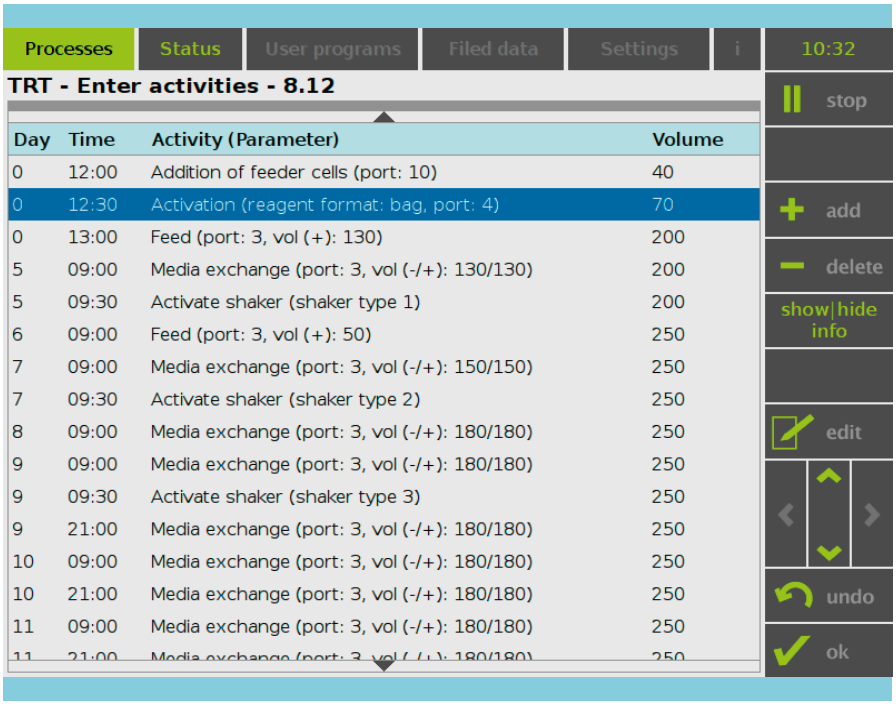
During the automated culture setup the T cells are transferred to the chamber and the volume in the chamber will be approximately 70 mL.

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 is recommended to be maintained between 70 mL and 250 mL (except after the activity 'Feeder addition' – there the final volume is 40 mL). If these values are exceeded during the setup of the activity matrix, a warning will be shown at the final confirmation step.

Note: When planning the process, consider that user interaction is required prior to the execution of the activities 'Addition of feeder cells', 'Activation', 'Transduction', 'Medium bag exchange', 'Waste Bag exchange', and 'End of culture' (see Table 9.1).

Activity	Description	Remark
Addition of feeder cells	Add feeder cells and volume reduction to 40 mL	User interaction upon execution required
Activation	Add activation reagent	User interaction upon execution required
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
Transduction	Transfer of viral vector to chamber	User interaction upon execution required
Spin	Centrifugation step including the option for a temperature change to be used after addition of viral vector for spinoculation protocol	Automatic execution
Culture wash	Wash of culture to remove excess viral vector particles	Automatic execution
Activate shaker	Start a continuous shaking of the chamber for suspension culture	Continuous in background
Deactivate shaker	Switch off a previously activated shaker	Automatic execution
Medium bag exchange	User guidance to exchange medium bag	User interaction upon execution required
Waste Bag exchange	User guidance to exchange Waste Bag	User interaction upon execution required
End of culture	Wash and re-suspend the cells in final formulation solution	User interaction upon execution required

Table 9.1: Available activities for cultivation

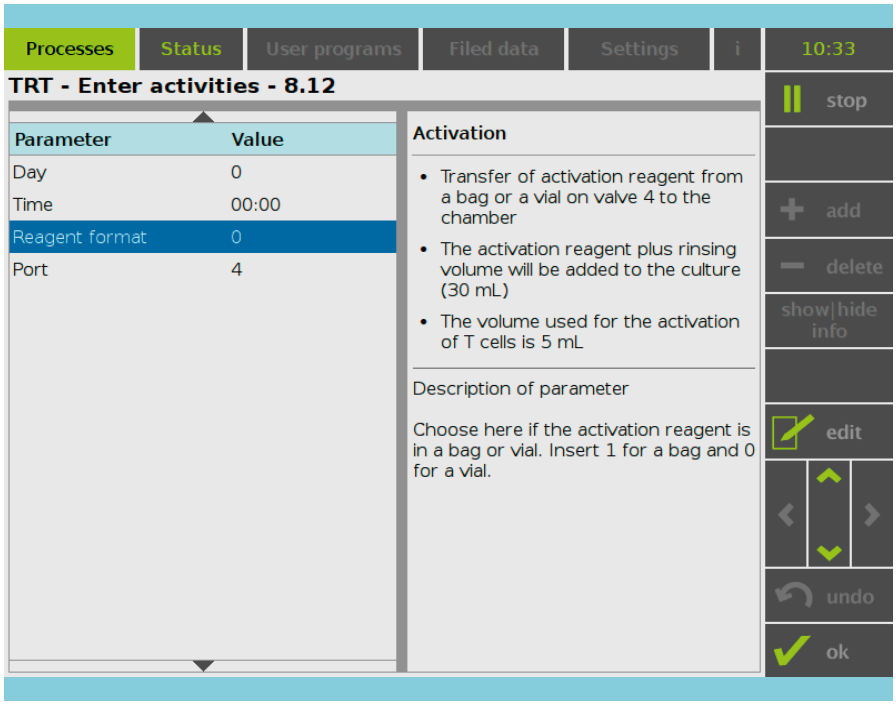


Screen 8.12: Addition of an activity

To add an activity, tap **add** and select the activity from the pop-up list.
Tap **ok** to confirm the activity.

IMPORTANT

If the activity 'Activation' or 'Transduction' are programmed directly before 'Addition of feeder cells', these reagents will be washed out. Make sure to program the 'Addition of feeder cells' before 'Activation'.



Screen 8.12: Editing of entered activity 'Activation'

A screen specific for the activity opens. Tap **edit** to change the parameters of this activity. Tap **ok** to confirm and to return to the matrix.

To edit an activity already listed in the matrix, select the activity of choice and tap **edit**. A screen specific for that activity opens. Tap **edit** to change the parameters of the activity. Tap **ok** to confirm and to return to the matrix.

To delete an activity already listed in the matrix, select the activity of choice and tap **delete**. Tap **ok** to confirm and to return to the matrix.

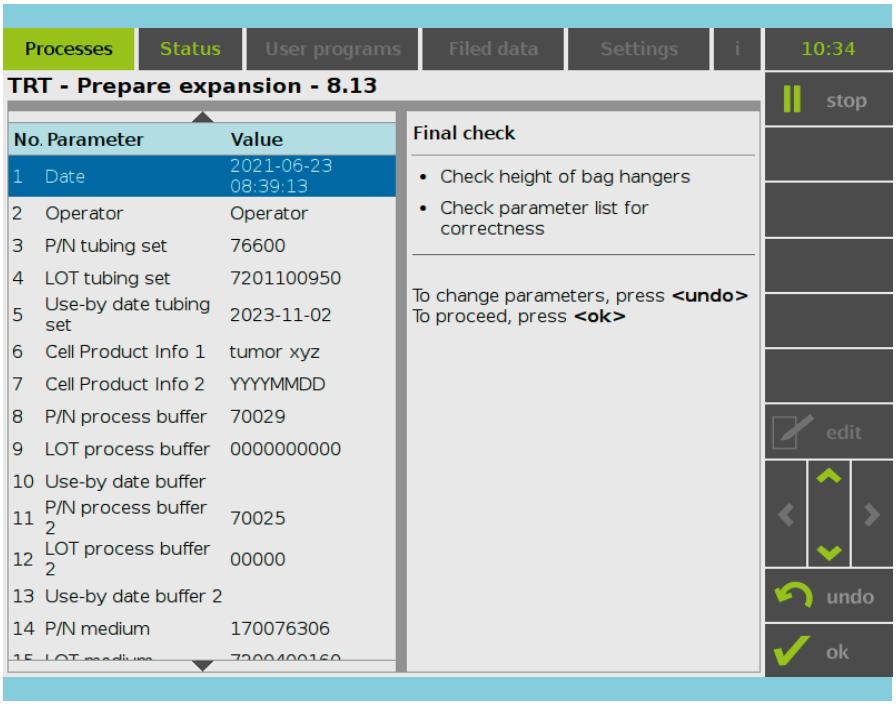
Note: The replacement of the expansion medium bag should be planned according to the activity matrix and the corresponding usage of medium for feeds and media exchanges. Keep in mind that additional medium is required for tubing set rinsing, sampling as well as every feed and medium exchange (see section 9.1). The exchange of the Waste Bag should be planned depending on the activity matrix and the corresponding filling of the Waste Bag. If the two Waste Bags are used one after another, it will be necessary at some time point to switch from the full one to the empty one by sealing off the full one and opening the clamp of the empty one.

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

IMPORTANT

Make sure to include the activity 'Addition of feeder cells' prior to 'Activation', in the activity matrix; otherwise the expansion will be dramatically impaired.

9.2.7 Final setup check

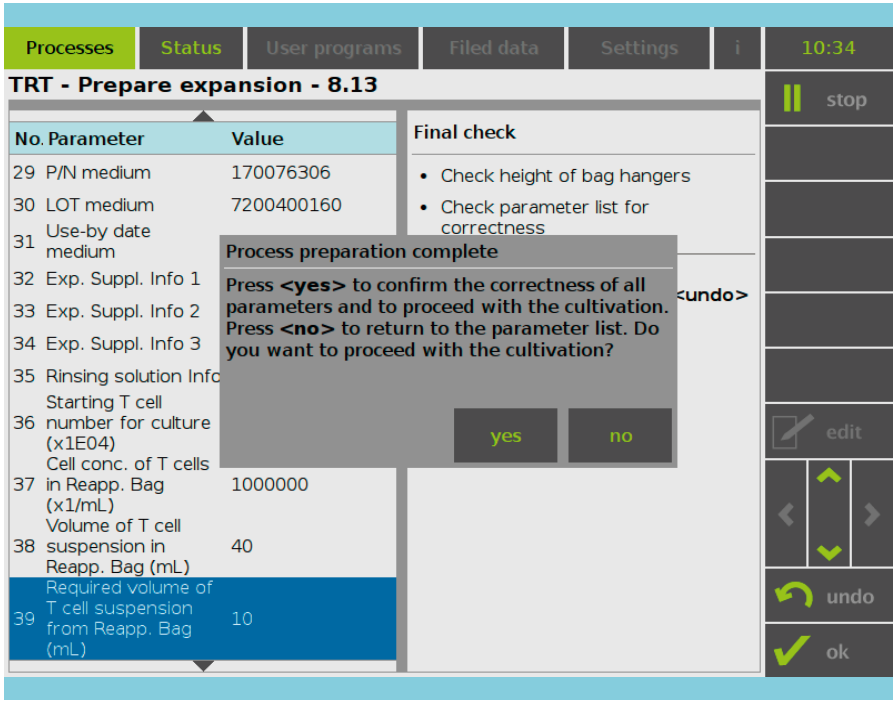


Screen 8.13: Final setup check for the expansion culture

Check the height of the bag hangers and the parameter list. Tap **undo** to change entered parameters. In that case the user has to enter all parameters again starting from Screen 8.7 in section 9.2.3.

Note: At this point all preparative steps are complete and the automated expansion culture can begin.

Note: It is recommended to mix the cells in the Reapplication Bag carefully prior to the transfer to the chamber.



Screen 8.13: Pop-up – Final setup check for the expansion culture

Tap **ok** to proceed. A pop-up window will appear. Tap **yes** to confirm the start of the expansion culture. Tap **no** to check the parameter list again.

9.2.8 Automated culture setup

The automated expansion process is divided into several phases. In order to have a highly flexible expansion process (including transduction option), the transfer of feeder cells and activation reagent are not part of the initial culture setup but are activities within the user-defined activity matrix.

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

1. Transfer of the user-defined amount of T cells from the Reapplication Bag to the chamber.

2. Washing of the cells with medium.

3. Setup of aeration and temperature.

9.3 Expansion culture

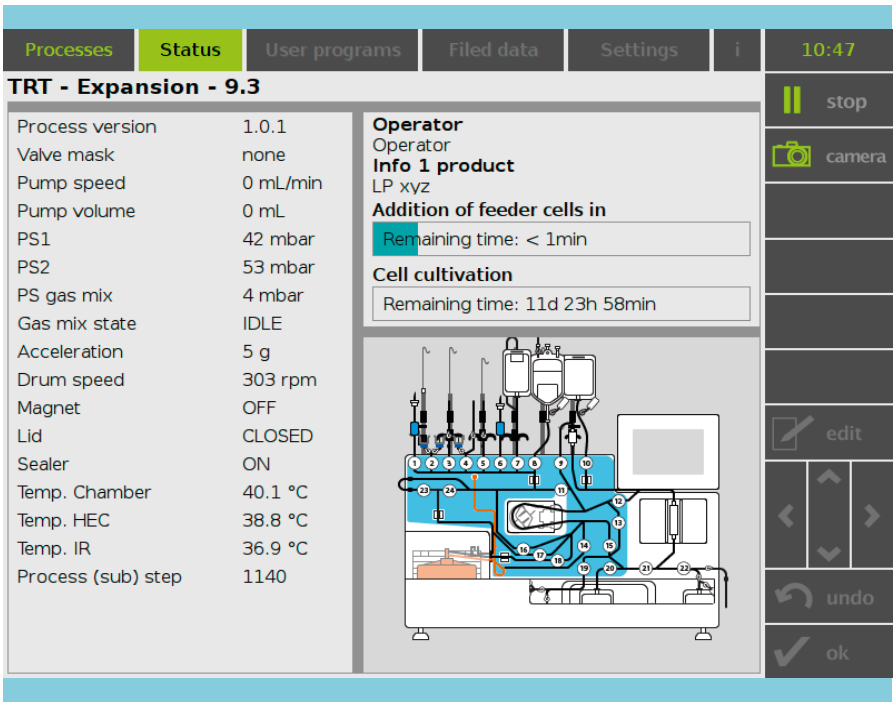
After initial setup of the expansion culture, the monitor shows the current step and the remaining time of the cultivation process in the **Status** screen (see Screen 9.3). Additionally, several sensor values can be monitored on the **Status** screen.

The pressure sensor "PS gas mix" is inside the gas mix unit and should show values between 0 mbar and 800 mbar during cultivation.

The remaining enriched target cells can now be retrieved for further use of the cells by sealing off the used Target Cell Bag.

Note: Due to the rinsing procedure the concentration of cells in the Target Cell Bag will be reduced.

The cultivation will proceed as defined in the default matrix.



Screen 9.3: Expansion **Status**



Screen 9.3: Expansion Processes

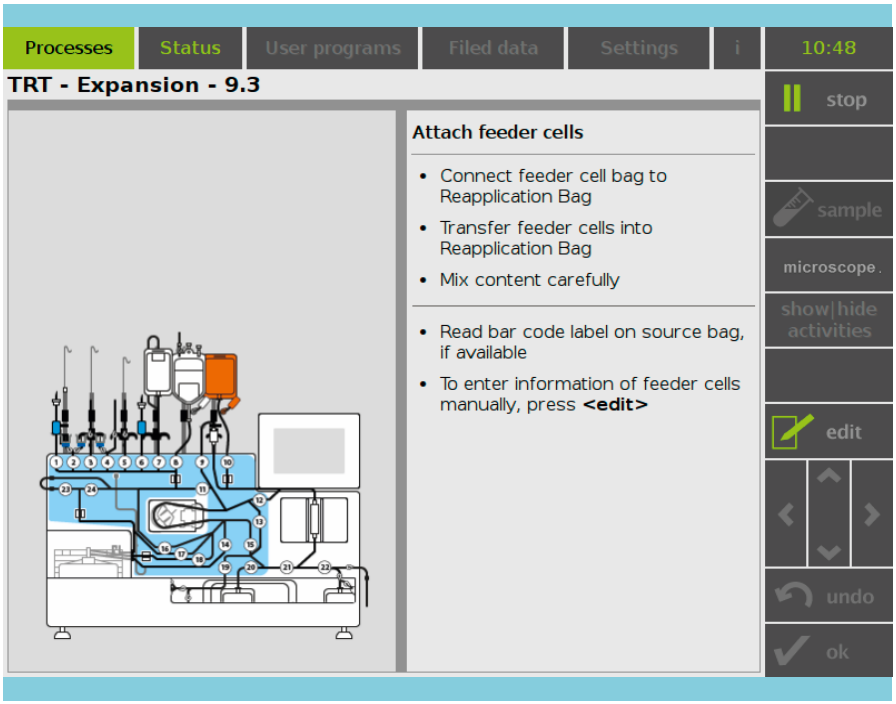
Tap **edit** to change the activity matrix during the expansion culture. To show the current activity matrix, tap **show/hide** activities.

Tap **microscope** to make a photographic record of cells (see section 9.3.5 for detailed handling instruction).
Tap **sample** to take a sample during expansion culture (see section 8.2.2 for a detailed handling instruction).

9.3.1 Addition of feeder cells

Note: For the activity 'Addition of feeder cells' user interaction is required upon execution.

Change to the **Processes** screen for user interaction.



Screen 9.3: Connection of the feeder cells

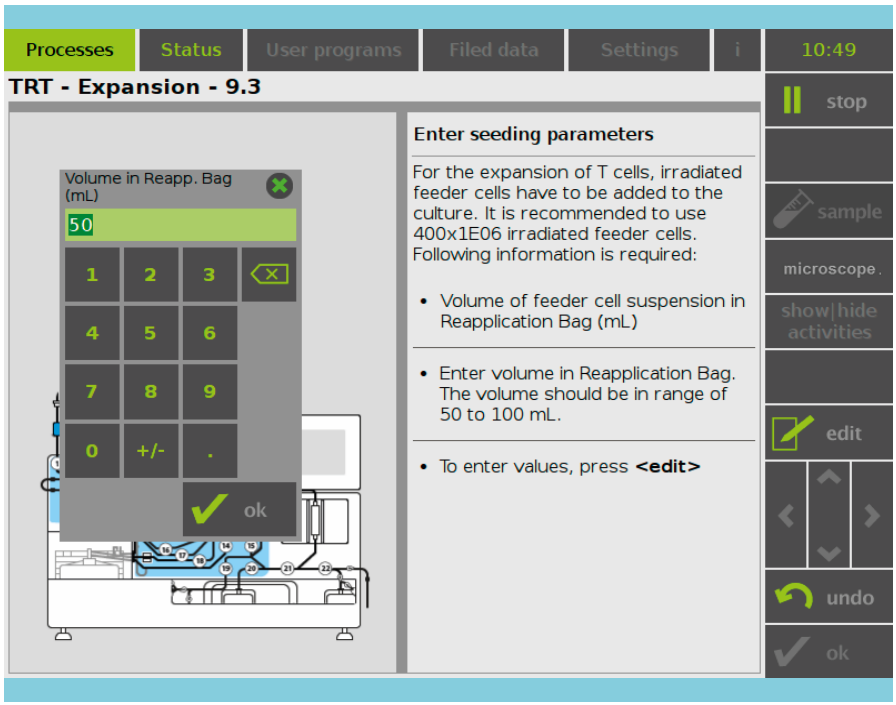
Connect the bag containing the irradiated feeder cells (see section 5.1.8) to the Reapplication Bag by sterile welding. Transfer the feeder cells into the Reapplication Bag and seal off the empty bag using the heat sealer.

Tap **edit** to enter information on the feeder cells (see Screen 9.3). Up to two different texts on the feeder cells can be entered.

Enter the following values:

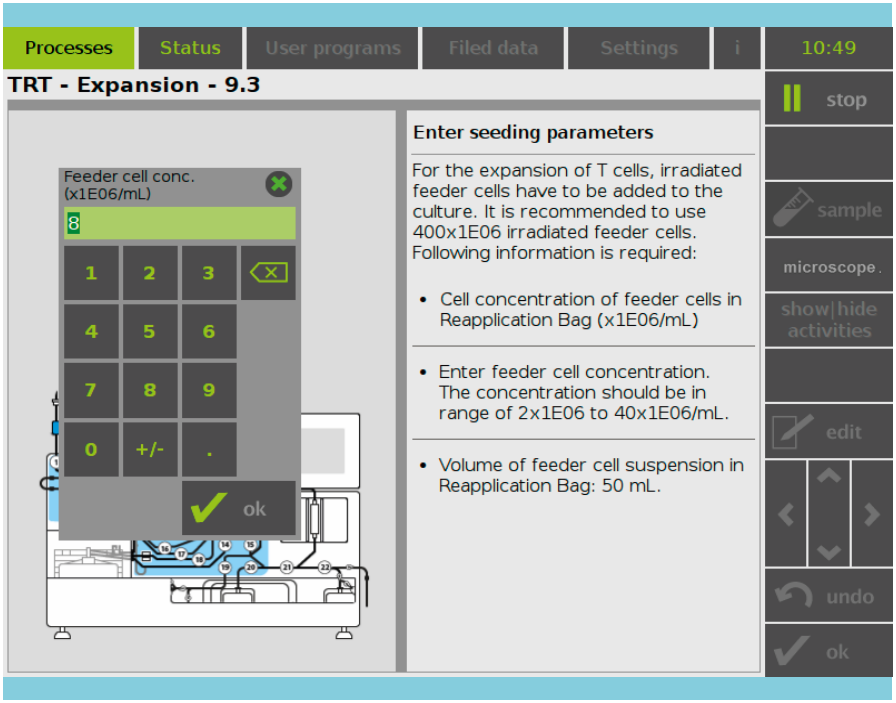
- Volume of the feeder cells in the Reapplication Bag (mL).
- Cell concentration of the feeder cells in the Reapplication Bag (×1E06/mL).
- Desired feeder cell number (×1E06) for culture.

For feeder cell addition, 50–100 mL cell suspension can be transferred from the Reapplication Bag to the chamber.



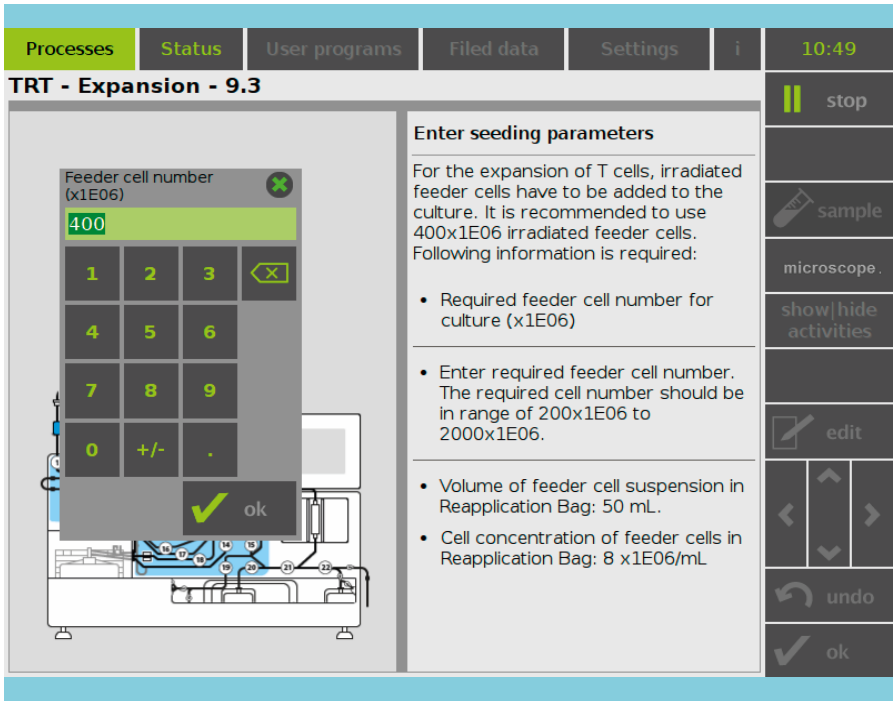
Screen 9.3: Input seeding parameters of the feeder cells – Volume in Reapplication Bag

The volume should be in the range of 50–100 mL.



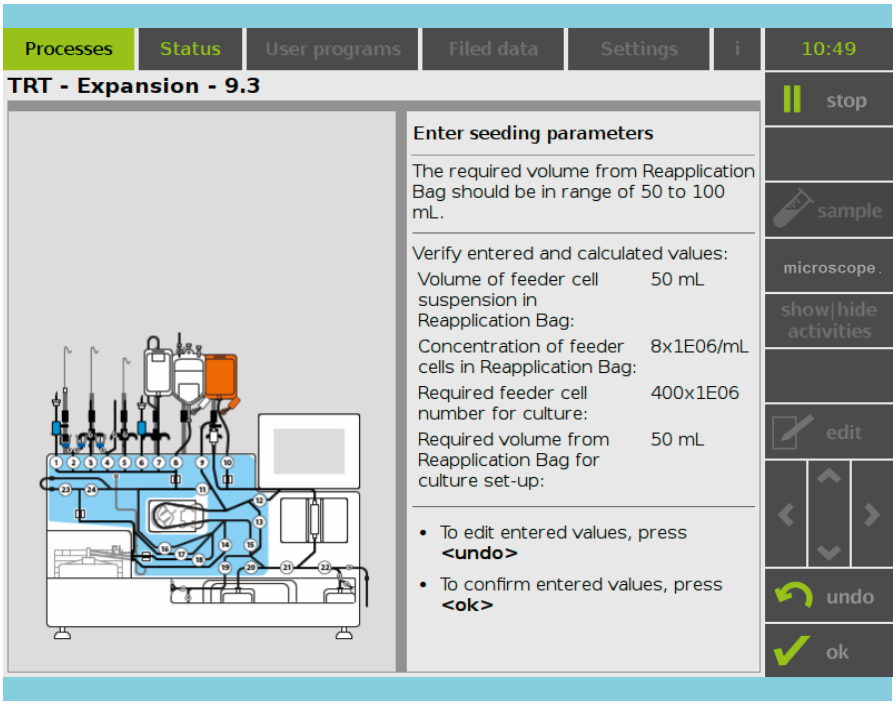
Screen 9.3: Input seeding parameters of the feeder cells – Feeder cell concentration

The feeder cell concentration should be in the range of 2×10^6 to 4×10^7 viable white blood cells /mL.



Screen 9.3: Input seeding parameters of the feeder cells – Required feeder cell number

The recommended required feeder cell number for culture is 4×10^8 viable white blood cells in order to confluently cover the chamber surface.



Screen 9.3: Calculation of the required volume for feeder addition

The volume of the cell suspension in the Reapplication Bag that is transferred for the expansion culture is calculated automatically.

Feeder cells will be added to the culture and a volume reduction to 40 mL will be performed automatically.

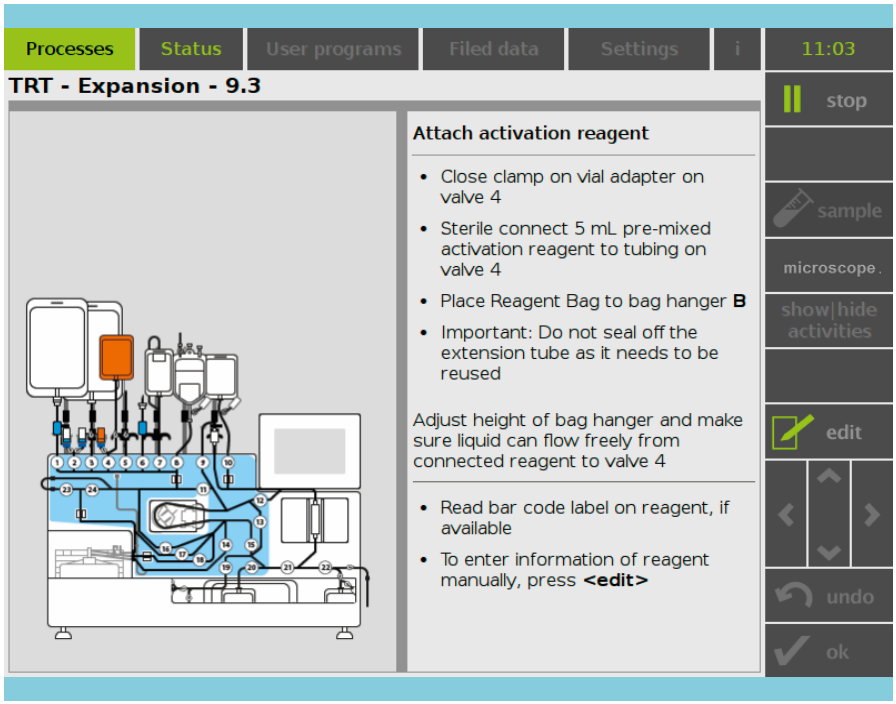
IMPORTANT

If the activity 'Activation' or 'Transduction' are programmed directly before 'Addition of feeder cells', these reagents will be washed out. Make sure to program the 'Addition of feeder cells' before 'Activation'.

9.3.2 Activation

Note: For the activity 'Activation' user interaction is required upon execution.

Change to the **Processes** screen for user interaction.



Screen 9.3: Connection of the activation reagent (option 'bag' was selected)

It is recommended to use a bag for the transfer of the activation reagent. Make sure to close the clamp at the vial adapter on valve 4. Connect the bag with the pre-diluted 5 mL activation reagent (MACS GMP CD3 pure) (see section 5.1.9) to the tubing of valve 4 by sterile welding. If there is already a bag present on valve 4 used for a previous addition (e.g., for 'Supplements and/or peptides'), exchange the bags by sterile welding. Hang the bag on bag hanger B and adjust the height of the bag hanger.

Alternatively, if using a vial, make sure to close the clamp at the tubing of valve 4. Connect the vial to the vial adapter of valve 4 as described in section 8.1.1.

Note: If the vial port has already been used (for the CliniMACS Anti-Biotin GMP MicroBeads/CliniMACS Anti-Biotin Reagent in case 1 or case 2), this option is not feasible. This process has been developed with MACS GMP CD3 pure – therefore, any use of a vial (e.g., MACS GMP T Cell TransAct) has to be further investigated.

Make sure liquid can flow freely from the connected reagent to valve 4.

Read the bar code label on the activation reagent. Alternatively, tap **edit** to enter information on the activation reagent, part number (P/N) and batch code (LOT) manually.

The activation reagent plus rinsing volume will be added to the culture (approximately 30 mL total volume added).

IMPORTANT
Do not seal off the extension tube after the transfer of the activation reagent is performed as the tubing may need to be reused. Alternatively, if it is desired to seal off the Reagent Bag after reagent transfer, make sure to keep the tubing long enough to be able to weld on another bag at a later time point.

IMPORTANT
If the activity 'Activation' or 'Transduction' are programmed directly before 'Addition of feeder cells', these reagents will be washed out. Make sure to program the 'Addition of feeder cells' before 'Activation'.

9.3.3 Feed

Note: The activity 'Feed' will be executed automatically. When the programmed time for the activity 'Feed' is reached, the activity will start automatically. No user interaction is required.

Ensure that sufficient medium is present in the expansion medium bag connected to valve 3. Consider the additional medium consumption of 60 mL for the activity 'Feed' (see section 9.1). It is recommended to use the activity 'Medium bag exchange' to attach a new expansion medium bag.

9.3.4 Sampling to monitor the culture

It is highly recommended to monitor the culture by taking a sample regularly. Measure the glucose and lactate in the sample and count the cells as patient-derived T cells can be highly variable in their expansion. These values give important information about the cell growth and help determining a suitable activity matrix.

IMPORTANT
Take the sample before a feed or medium exchange is performed. Otherwise the glucose and lactate values are not representative.



Screen 9.3: Sampling during expansion culture

To take a sample from the chamber during cultivation, change to the **Processes** screen and tap **sample**. Follow the instructions in the pop-ups and the procedure as described in section 8.2.2.

Consider the additional medium consumption of 60 mL for the sampling. If more than three samplings are required, then weld on a new Triple Sampling Adapter by sterile welding.

It is recommended to adapt the default matrices depending on the glucose and lactate measured in the samples as well as on the growth of the cells. The default matrices are a general guideline but may need adjustments for optimal growth. The optimal matrix is dependent on the individual cell growth and can vary even if starting the expansion with the same T cell number.

Program additional daily media exchanges if the glucose tends to drop or is below 2 g/L and/or lactate is high (>15 mmol/L).

In case of higher starting responder T cell numbers (≥ 2×10^6 viable T cells; up to 1×10^7 viable T cells has been tested) or if the cells expand well, up to three media exchanges can be performed daily at an interval of 8 hours each. The media exchange and shaking can already commence on day 3 in case of higher starting responder T cell numbers - this correlates with the enhanced feeding

protocol (default matrix 2) (see also 9.2.6). Additionally, if starting with a high responder T cell number the chamber capacity might be reached already around day 8–10 in case of good expansion. The culture will yield lower total fold-expansion due to the maximum chamber capacity of ~4×10^9 cells.

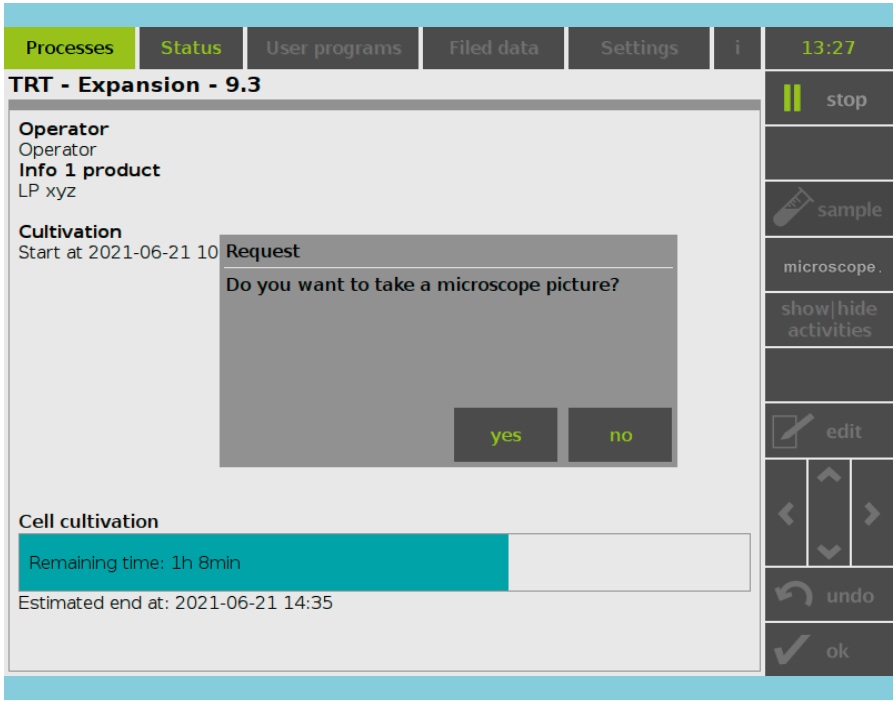
If starting the expansion culture with less cells than 1×10^6 viable T cells (>1×10^4 viable T cells has been tested) or if cells are growing slower than expected, the activity matrix may need to be adapted accordingly. For example, the activation of the shaker types 1, 2 and 3 as well as the feed on day 6 could be postponed to later time points (e.g., 1–2 days later). Media exchanges on later days can be postponed or only performed once per day as long as the lactate and glucose values are in a good range.

Note: Check the Waste Bag and the expansion medium bag regularly and exchange when required.

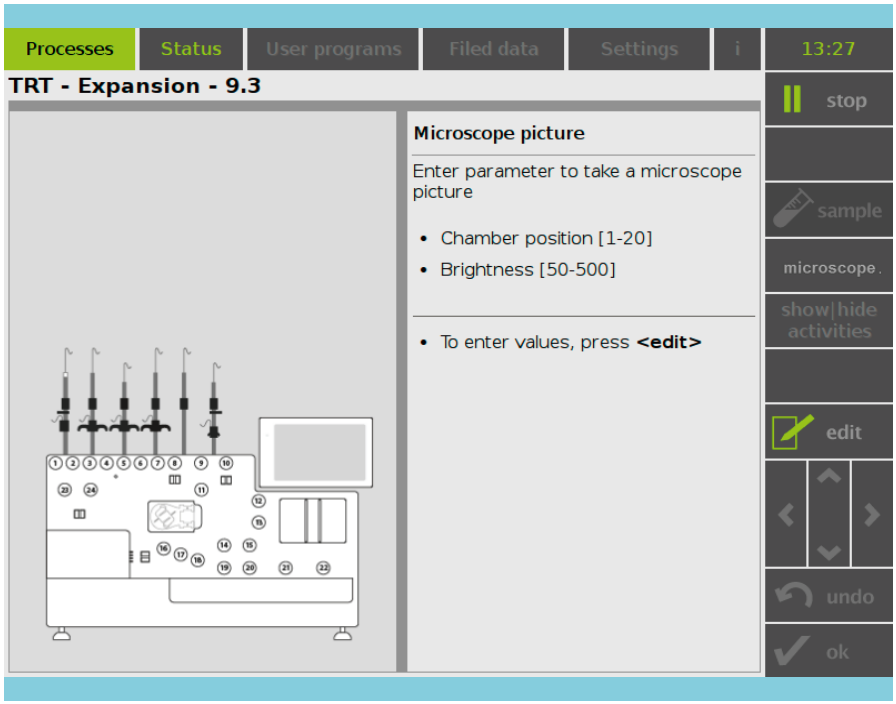
9.3.5 Taking microscope pictures during cultivation

It is possible to make a photographic record of the cells during cultivation. Follow the procedure described:

- Change to the **Processes** screen.
- Tap **microscope**.
- Tap **yes** to confirm taking a microscope picture.
- Tap **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.
- Change to the **Status** screen and tap **camera** to show the camera screen. The picture will be shown seconds later. To leave the camera view, tap **ok**.
- Tap **yes** to take another picture. Otherwise, tap **no** to return to the cultivation.



Screen 9.3: Pop-up – Take a microscope picture



Screen 9.3: Enter parameters for the microscope picture

Note: During cultivation, the gas mixing unit is refilled with gases in regular intervals. During this short time interval, no pictures can be taken. In this case microscope is not visible.

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

9.3.6 Media exchange

Note: The activity 'Media exchange' will be executed automatically. When the programmed time for the activity 'Media exchange' is reached, the activity will start automatically. No user interaction is required.

Ensure that sufficient medium is present in the expansion medium bag connected to valve 3. Consider the additional medium consumption of each 60 mL for the activity 'Media exchange' (see section 9.1). It is recommended to use the activity 'Medium bag exchange' to attach a new expansion medium bag.

9.3.7 Activate/Deactivate shaker

Note: The activity 'Activate shaker' and 'Deactivate shaker' will be executed automatically. The active shaker continuously shakes the suspension culture.

There are three different shaker types which can be set (see Table 9.2).
For higher cell densities, a more intense shaker regime is recommended.
The different shaker types can be adjusted depending on the starting cell number and the cell growth after an initial phase of static culture.

Shaker type	Description of shaker type
1 slow	Shaking for 2 s at 100 rpm with 30 s pause
2 medium	Shaking for 2 s at 300 rpm with 30 s pause
3 fast	Shaking for 2 s at 300 rpm with 30 s pause and change of direction

Table 9.2: Available shaker types

It is recommended to activate shaking depending on the starting cell number. Start shaking on day 5 if starting with up to 2×10^6 viable T cells (standard protocol) or already on day 3 if starting with more than 2×10^6 viable T cells (enhanced feeding protocol).

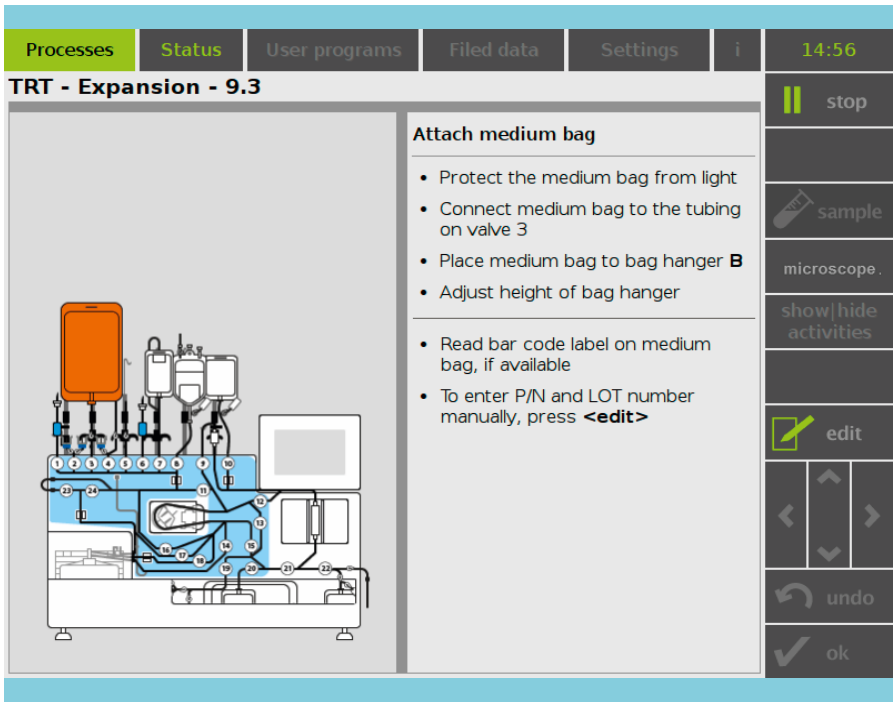
When the culture volume reaches 250 mL, it is recommended to start shaking irrespective of the cell count to ensure proper gas exchange.

Note: The optimal shaker types may be dependent on the quality and growth potential of the cells and should be further defined.

9.3.8 Medium Bag exchange

Note: For the activity 'Medium bag exchange' user interaction is required upon execution.

It is recommended to use the activity 'Medium bag exchange' to document the information on the new bag of medium and its supplements.



Screen 9.3: Connection of expansion medium bag

When the programmed time for the activity 'Medium bag exchange' is reached, change to the **Processes** screen for user interaction. Follow the instructions on the screen to connect a new expansion medium bag. See section 5.1.7 for details on the preparation of the medium.

Note: The subsequent expansion medium bags do not require supplementation of human AB serum in order to obtain optimal expansion (see section 5.1.7).

IMPORTANT
To protect the medium and IL-2 against light exposure, place the expansion medium bag in a yellow UV protection bag before welding on the tubing set.

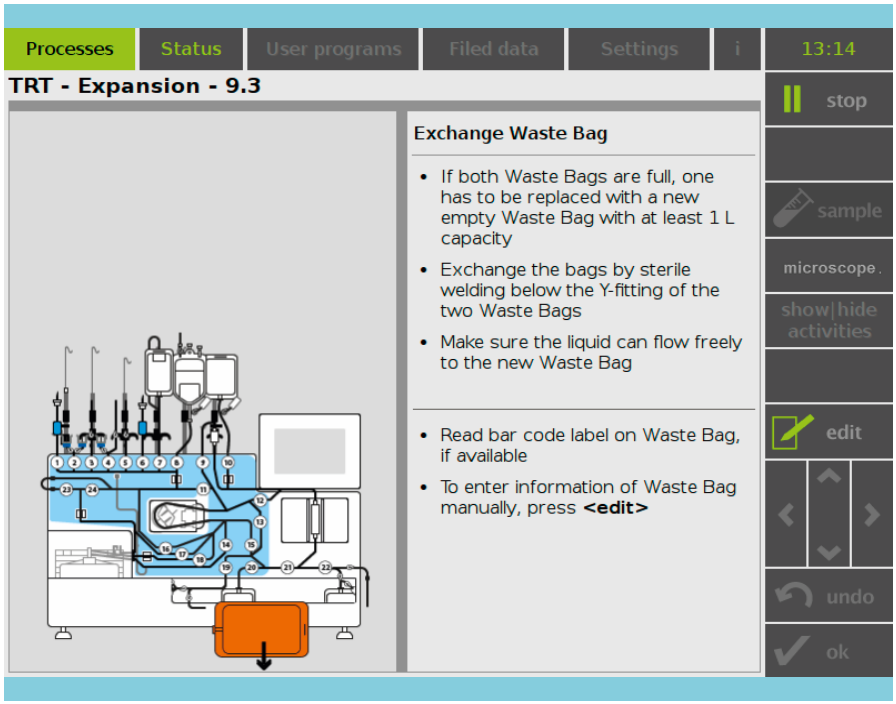
Read the bar code label on the medium bag. Alternatively, tap **edit** to manually enter the information. Tap **ok** to confirm. Tap **edit** to enter information regarding supplements. To complete the activity and return to cultivation confirm the entered values with **ok**.
It is strongly recommended that the user monitors the filling level of the medium bag to prevent any errors during expansion (see section 9.1).

9.3.9 Waste Bag exchange

Optional: The activity 'Waste Bag exchange' can be programmed in the activity matrix.

Note: For the activity 'Waste Bag exchange' user interaction is required upon execution.

When the original Waste Bags of the tubing set are full, change to the **Processes** screen and connect a new Waste Bag (e.g., a 5 L Collection Bag) via sterile welding.



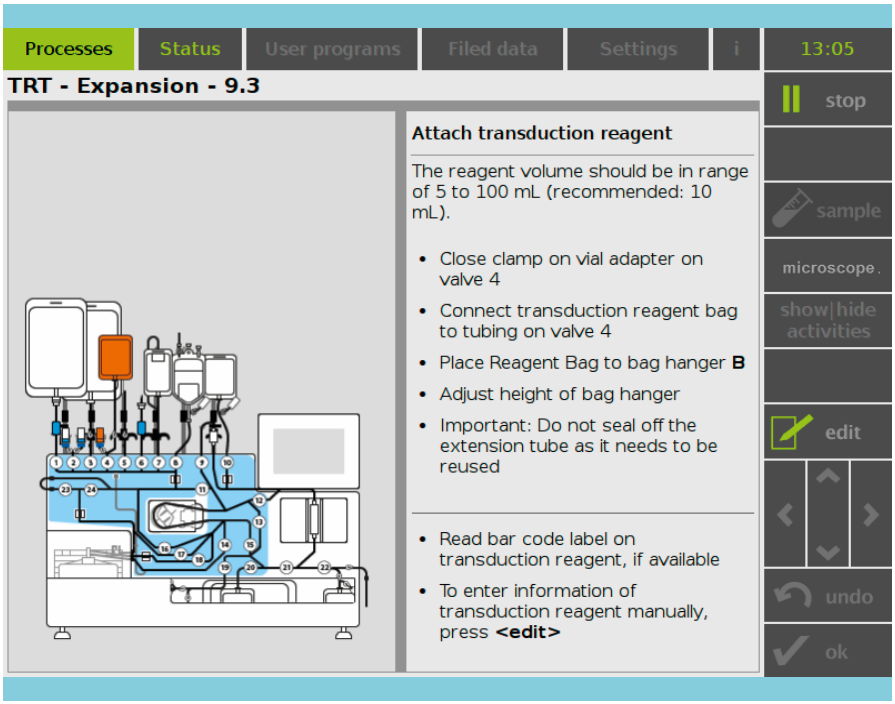
Screen 9.3: Waste Bag exchange

Read the bar code label on the Waste Bag. Alternatively, tap **edit** to enter optional information manually. Tap **ok** to confirm the entered values and return to cultivation.

9.3.10 Transduction

Note: For the activity 'Transduction' user interaction is required upon execution.

Change to the **Processes** screen for user interaction.



Screen 9.3: Connection of the transduction reagent

Make sure to close the clamp at the vial adapter on valve 4. Connect the bag with transduction reagent (see section 5.1.10) to the tubing of valve 4 by sterile welding. If there is already a bag present on valve 4 used for a previous activity (e.g., 'Activation'), exchange the bags by sterile welding. Hang the bag on bag hanger B and adjust the height of the bag hanger.

Make sure liquid can flow freely from the connected reagent to valve 4.

Read the bar code label on the transduction reagent. Alternatively, tap **edit** to enter information on the transduction reagent part number (P/N) and batch code (LOT) manually.

The transduction reagent (5–100 mL; recommended: 10 mL) plus 20 mL rinsing volume will be added to the culture.

IMPORTANT

Do not seal off the extension tube after the reagent transfer of the transduction reagent is performed as the tubing may need to be reused. Alternatively, if it is desired to seal off the Reagent Bag after reagent transfer, make sure to keep the tubing long enough to be able to weld on another bag at a later time point.

IMPORTANT

If the transduction reagent is transferred directly before addition of feeder cells, then this reagent will be washed out. Make sure to program enough time between 'Transduction' and 'Addition of feeder cells' in case the transduction is performed before feeder cells are added.

9.3.11 Spin

Note: The activity 'Spin' will be executed automatically.

The activity 'Spin' is a centrifugation step at 400 g with the option to change the temperature and duration of the spin to be used after addition of viral vector for a spinoculation protocol.

9.3.12 Culture wash

Note: The activity 'Culture wash' will be executed automatically.

It is recommended to perform one culture wash cycle two days after transduction (e.g., culture wash on day 3, if transduction was performed on day 1) to remove excess viral vector particles.

Make sure there is sufficient medium in the expansion medium bag connected to valve 3. The amount of medium needed for the culture wash depends on the cycles programmed in the activity matrix. For 1, 2, and 3 cycles a volume of 600, 860, and 1120 mL shall be available in the expansion medium bag on valve 3. If necessary, use the activity 'Medium bag exchange' to attach a new medium bag (see section 9.3.8).

9.3.13 Volume reduction

Note: The activity 'Volume reduction' will be executed automatically.

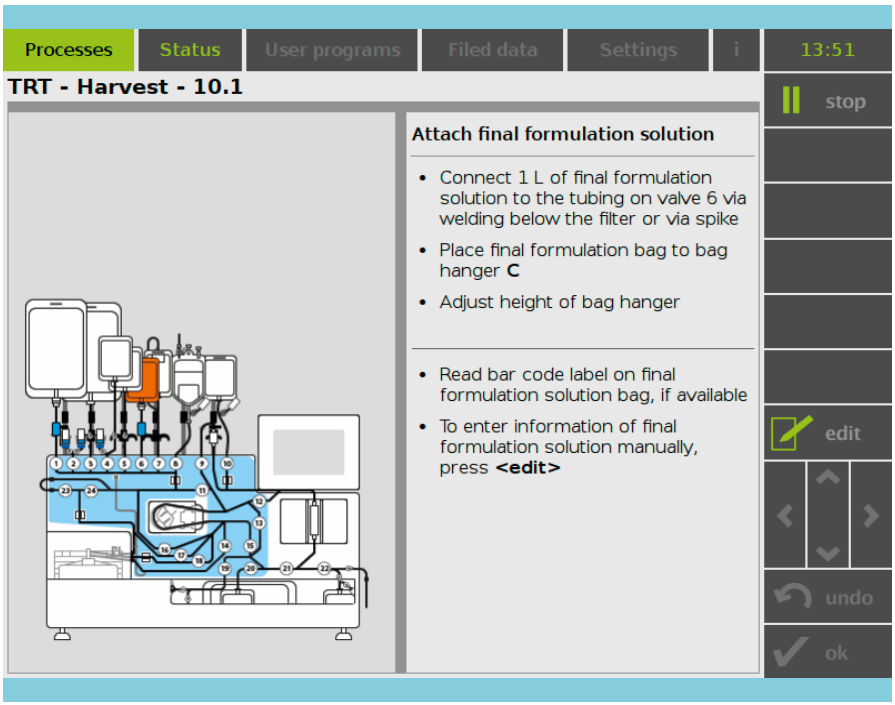
In case the culture volume needs to be reduced (e.g., prior to transduction), use the activity 'Volume reduction'. The cells get centrifuged and the supernatant is removed until the desired culture volume is reached.

Make sure there is sufficient medium in the expansion medium bag connected to valve 3. The activity 'Volume reduction' requires 90 mL of medium. If necessary, use the activity 'Medium bag exchange' to attach a new medium bag (see section 9.3.8).

9.4 Final formulation and harvest

Note: For the activity 'End of culture' user interaction is required upon execution. The harvest will not start without confirmation by the user. Change to the **Processes** screen for user interaction.

Prepare 1 L of final formulation solution (e.g., CliniMACS Formulation Solution or sodium chloride solution for injection with 0.5% HSA) and follow the instructions on the screen.

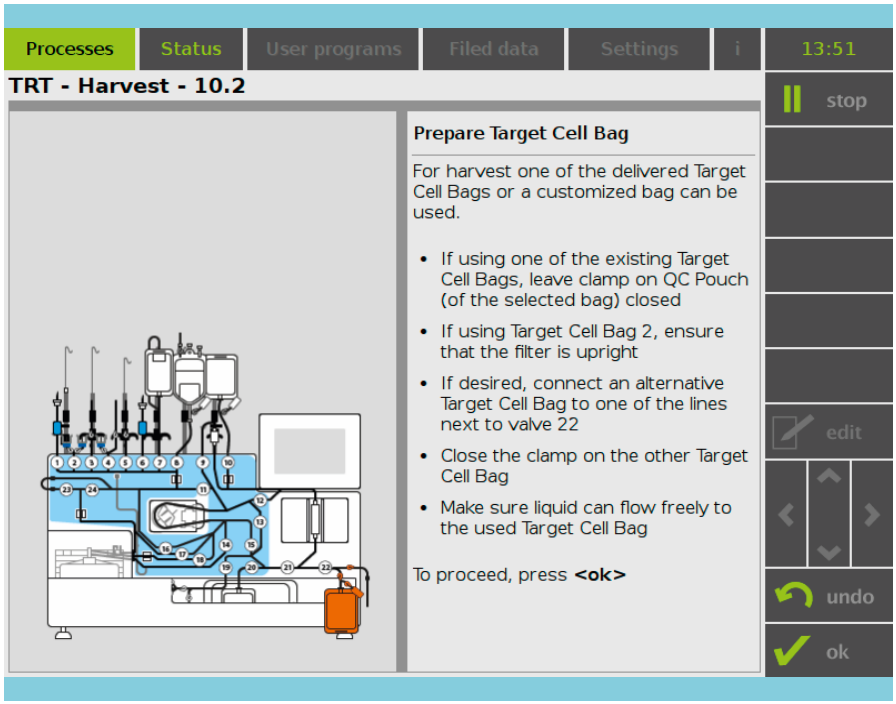


Screen 10.1: Connection of final formulation solution

Connect the final formulation solution to the spike port on valve 6 or weld the bag below the sterile filter on valve 6. Hang the bag on bag hanger C and adjust the height of the bag hanger.

Read the bar code label on the final formulation solution bag (see Screen 10.1). Alternatively, tap **edit** to enter the information manually. Tap **ok** to continue.

Note: Solutions containing AB serum should not be spiked as the sterile filter on port 6 can clog. Connect sterile solutions with AB serum by sterile welding below the sterile filter.



Screen 10.2: Preparation of Target Cell Bag

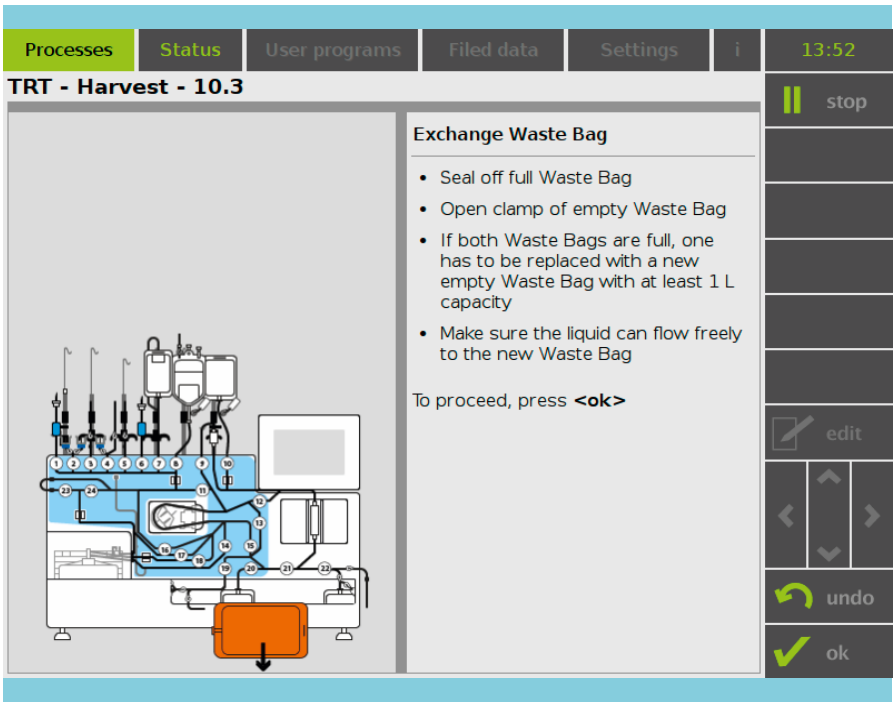
Note: One of the Target Cell Bags could have been used already for remaining cells after setup of the expansion culture.

During harvest, target cells can optionally be passed through the pre-assembled in-line filter connected to the designated Target Cell Bag. Make sure that the clamp to the relevant Target Cell Bag is opened. Close the clamp of the Target Cell Bag that will not be used (if still available).

Make sure that the clamp of the QC Pouch (of the selected bag) is closed.

Note: If using the Target Cell Bag with the in-line filter, ensure that the filter is upright e.g., by replacing the sealed off separation column from the magnet unit with the in-line filter.

If an alternative Target Cell Bag is required, connect an alternative Target Cell Bag between valve 22 and the in-line filter by sterile welding. Make sure that liquid can flow freely to the used Target Cell Bag.



Screen 10.3: Exchange of Waste Bag

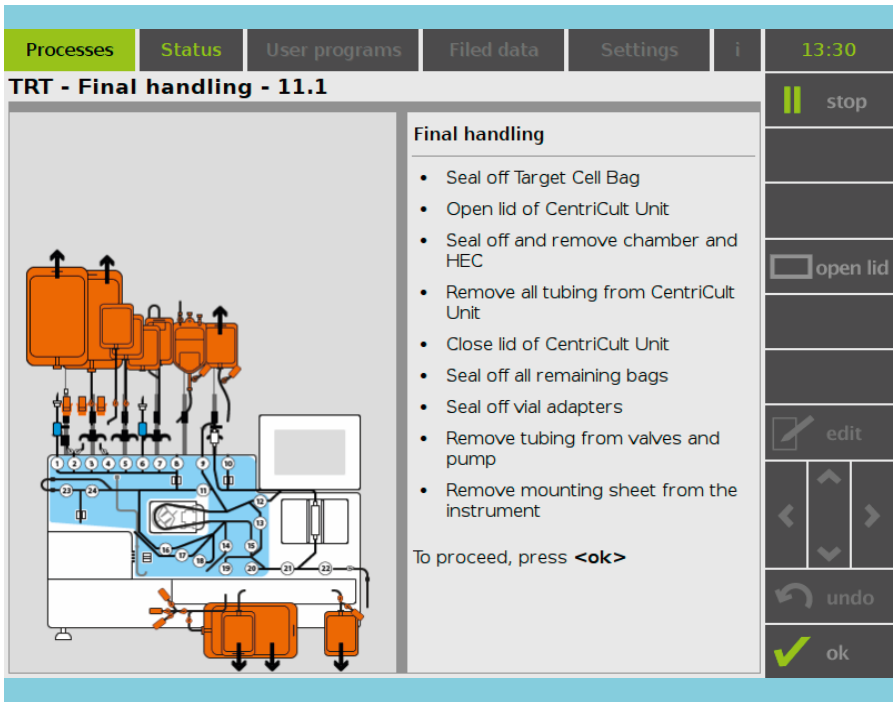
With the next screen the user is asked to exchange the Waste Bag (see Screen 10.3). This is only necessary if the capacity of the current bag is nearly reached and additional waste liquid capacity is required. In this case, connect a new empty Waste Bag by sterile welding.

Tap **ok** to continue. If not required, directly tap **ok**.

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

The volume of the final product will be 100 mL.

9.5 Final handling



Screen 11.1: Final handling after expansion

After termination of the process, the program guides through the final handling steps.

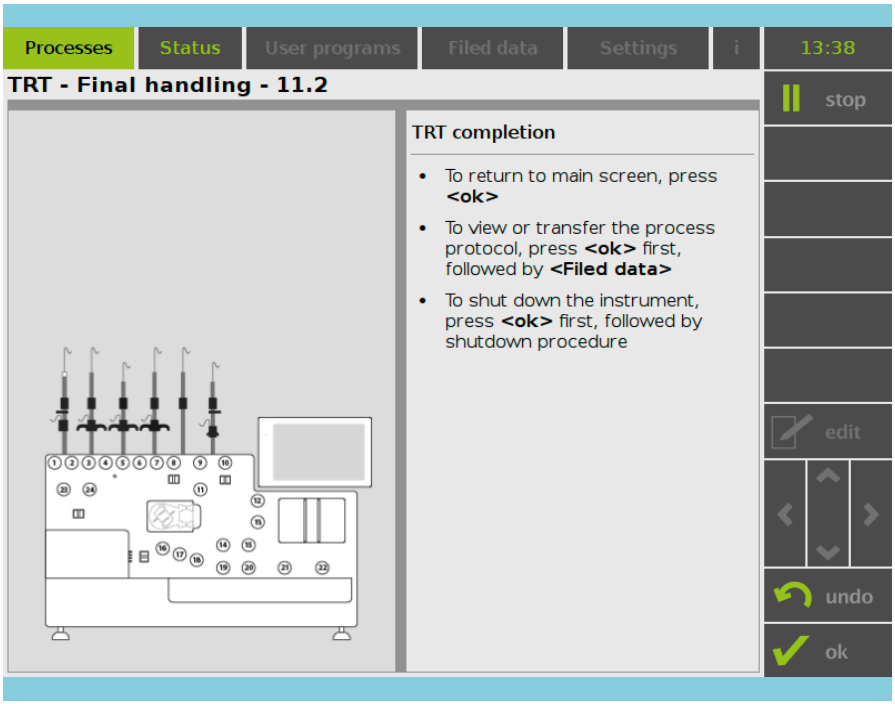
Seal off the Target Cell Bag containing the expanded cells below valve 22. Alternatively, if no expansion culture was performed (see section 8.3), seal off the Reapplication Bag containing the enriched target cells above valve 10 and if desired the Non-Target Cell Bag below valve 20.

Take a sample 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, tap **open lid** on the menu bar. In case the lid cannot be opened, contact Miltenyi Biotec Technical Support.

Note: Dispose of the tubing set as biohazardous material according to the institution's standard operating procedures. If applicable, 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 11.2: Process completion

The process protocol is stored in **Filed data**. Transfer the process protocol to other media with a standard USB storage device. To do so, plug an USB storage device into the USB port located at the right side of the touchscreen. Tap **Filed data** on the menu bar and select the requested process. Then tap **ok** to open the protocol. Finally tap **save** to transfer the process protocol to the USB storage device.

Clean and disinfect the CliniMACS Prodigy after each procedure. Refer to the CliniMACS Prodigy User Manual (Instrument) regarding the cleaning and disinfection of the device. It is recommended to follow the standard procedures for the treatment of infectious materials.

⚠ CAUTION

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

For the manufacture and use of the generated T cell product, national and international legislation and regulations must be followed.

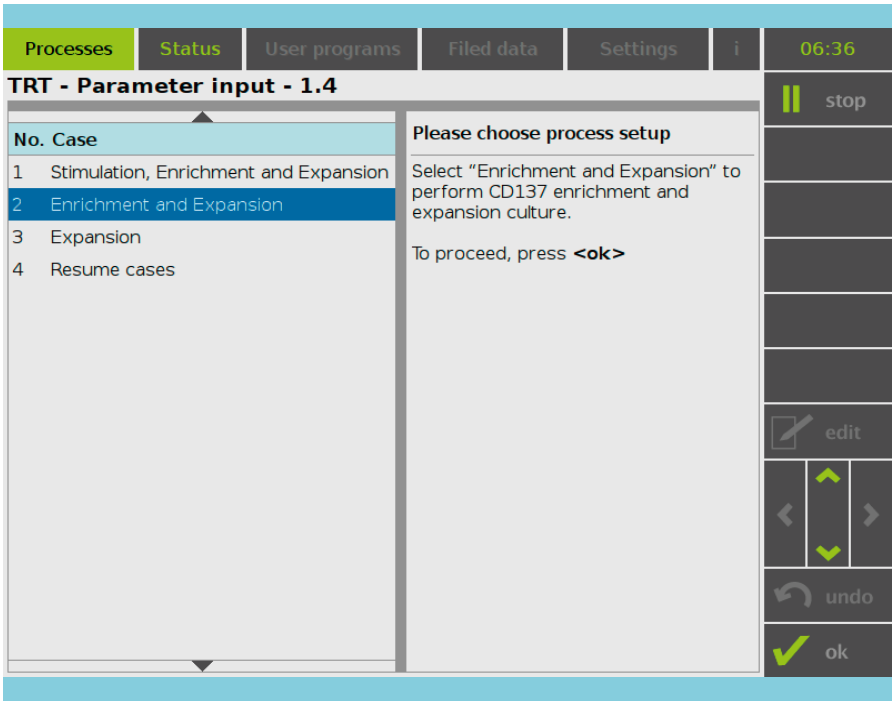
Miltenyi Biotec as the provider of the Tumor Reactive T cell Process does not provide any recommendations regarding the use of the manufactured cells for therapeutic purposes and does not make any claim regarding a clinical benefit.

10

Cases 2 + 3

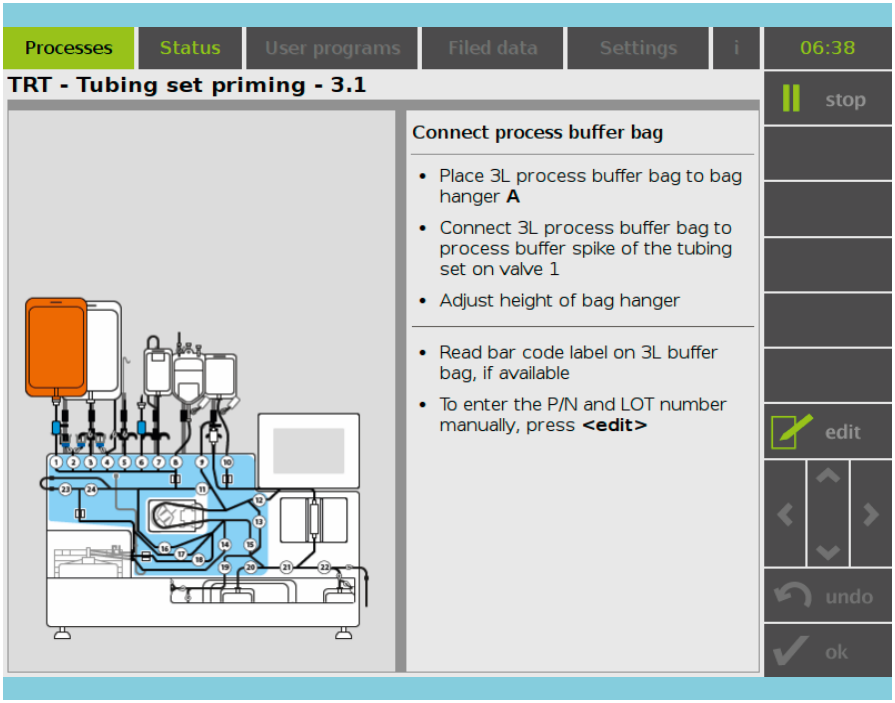
10.1 Case 2 (Enrichment and Expansion)

10.1.1 Process setup and differences to case 1



Screen 1.4: Process setup of case 2 'Enrichment and Expansion'

After initialization of the TRT process (see section 6.1), entry of the operator and input of the tubing set parameter (see section 6.2), select case 2 'Enrichment and Expansion'.



Screen 3.1: Connection of process buffer bag and enter P/N and LOT number of the 3 L process buffer bag

First, perform the tubing set installation (see section 6.3) and priming with process buffer (see section 6.4). In contrast to case 1, case 2 only requires a 3 L buffer bag. See sections 5.2.2 and 5.2.3 for details on the preparation of the process buffer and medium for enrichment.

Place the 3 L process buffer bag on bag hanger A. In order to connect the process buffer bag to the tubing set on valve 1, remove the capping of the spike of the tube connected to valve 1 and spike it into the 3 L process buffer bag.

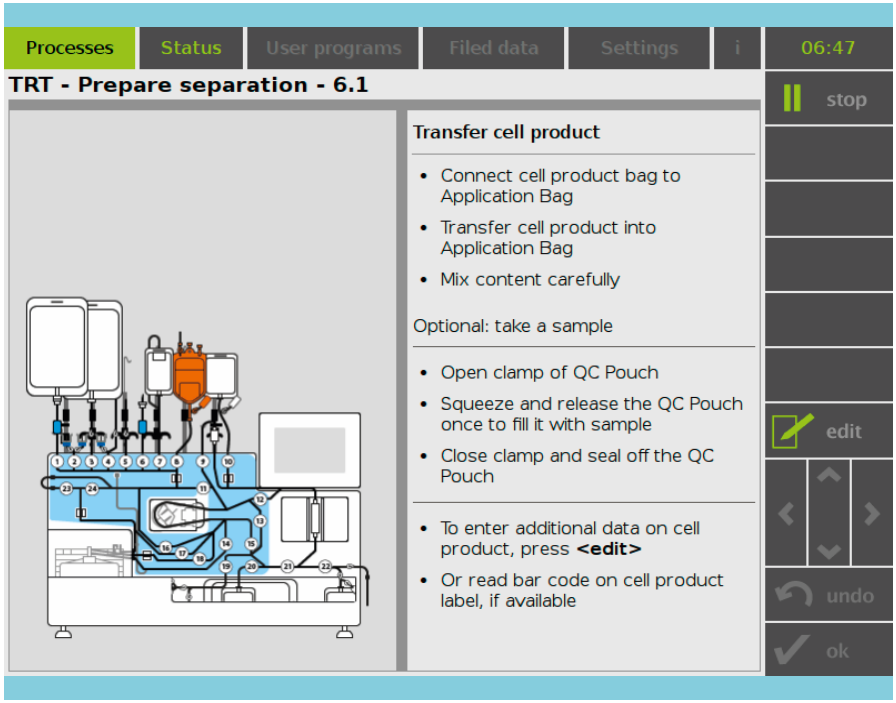
If necessary, raise or lower the bag hanger to adjust the height of the hanger. Ensure that the bags are positioned at a suitable height to prevent severe bending or overstretching of the tubing.

IMPORTANT

Only hang bags with a maximal capacity of 4 L on one bag hanger.

Read the bar code label on the 3 L process buffer bag using the bar code reader. Alternatively, tap **edit** to enter the part number (P/N) and batch code (LOT) manually with the keyboard shown on the screen.

10.1.2 Transfer of cellular starting product



Screen 6.1: Connection and transfer of cellular starting product to Application Bag

See section 5.2.1 for more details on the preparation of the starting material and e.g., volume requirements.
Connect the bag containing the cells to the Application Bag by sterile welding. Transfer the cellular starting product into the Application Bag and seal off the empty bag using the heat sealer.

Optional: take a sample of the filtered starting product from the Application Bag with the QC Pouch:

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

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

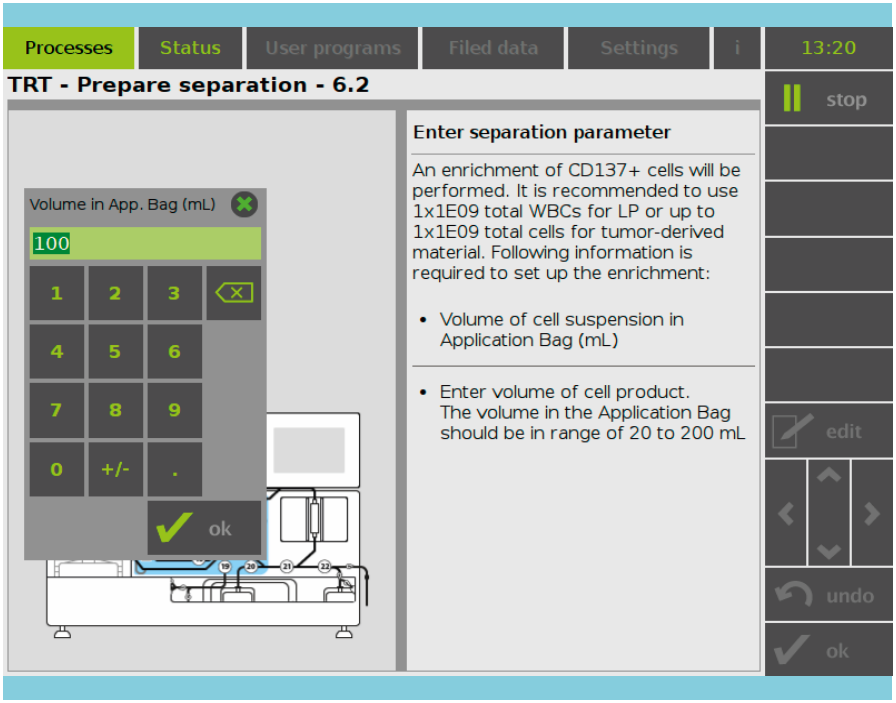
Determine the cell concentration (x1E6/mL) of the filtered cell suspension within the Application Bag.

Tap **edit** to enter information on the cellular starting product (see Screen 6.1). Up to two different texts can be entered.

Enter the following values:

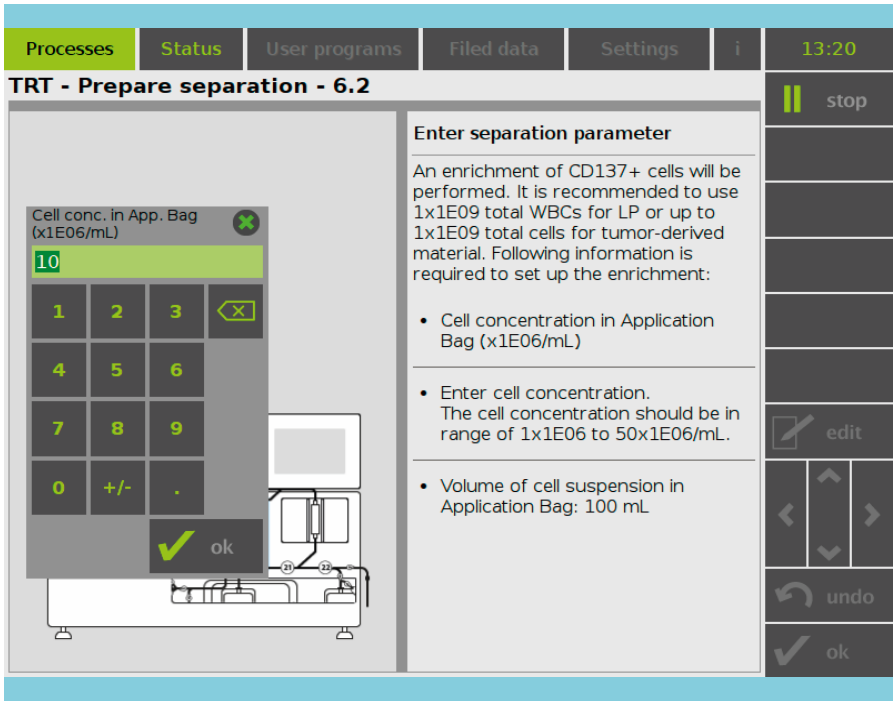
- Volume of the cell suspension in the Application Bag (mL).
- Cell concentration that is present in the Application Bag (x1E06/mL).
- Desired starting cell number (x1E06 total cells) for the enrichment.

For enrichment, 20–200 mL cells can be transferred from the Application Bag to the chamber.



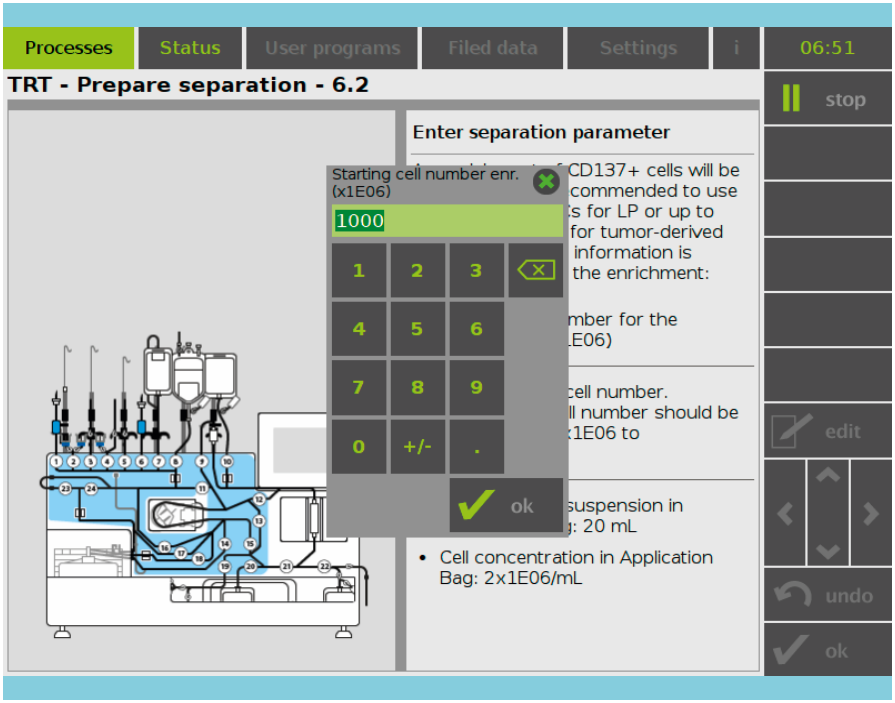
Screen 6.2: Input of parameters for the separation – Volume in Application Bag

The volume of the cell suspension should be in the range of 20–200 mL.



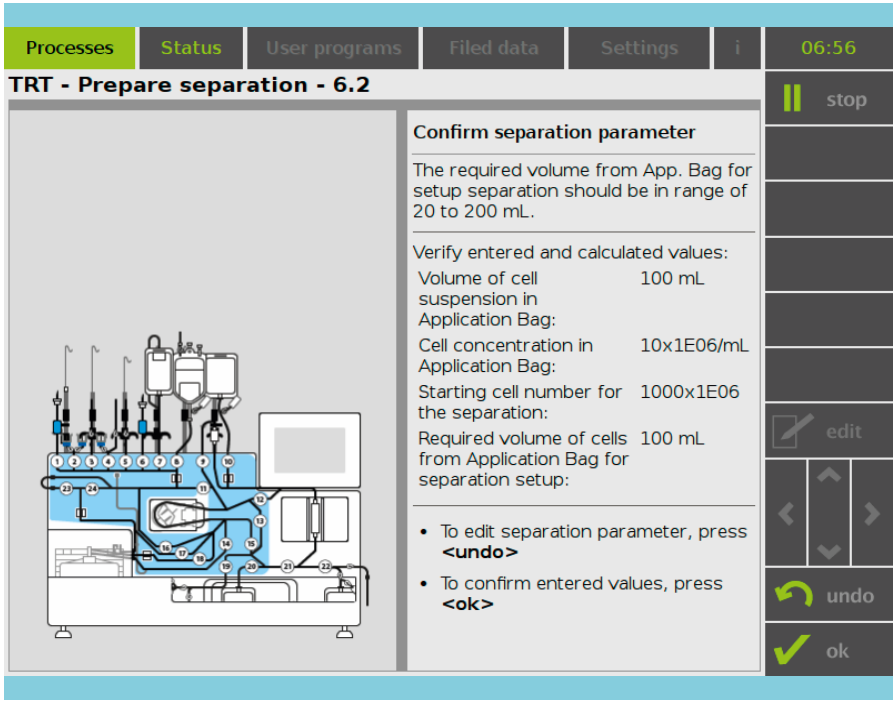
Screen 6.2: Input of parameters for the separation – Cell concentration

The cell concentration should be in the range of 1×10^6 to 5×10^7 total cells/mL.



Screen 6.2: Input of parameters for the separation – Starting cell number

The recommended starting cell number for enrichment is up to 1×10^9 total cells for tumor-derived material or 1×10^9 total white blood cells for leukapheresis-derived material.



Screen 6.2: Calculation of the required volume for the separation

The volume of the cellular starting product in the Application Bag that is transferred for the enrichment is calculated automatically.

In case the volume in the Application Bag is higher than the volume that is calculated to be transferred into the chamber, tap **ok** to confirm the entered parameters or tap **undo** to change the entered parameters.

10.1.3 CD137 enrichment (case 2)

After transfer of the starting material into the Application Bag and input of the cell parameters, the rest of the process is identical to the complete process in case 1. First the CliniMACS Reagents are connected (see section 8.1.1), followed by the final check (see section 8.1.2) before the separation is performed (see section 8.2) and completed (see section 8.3) automatically.

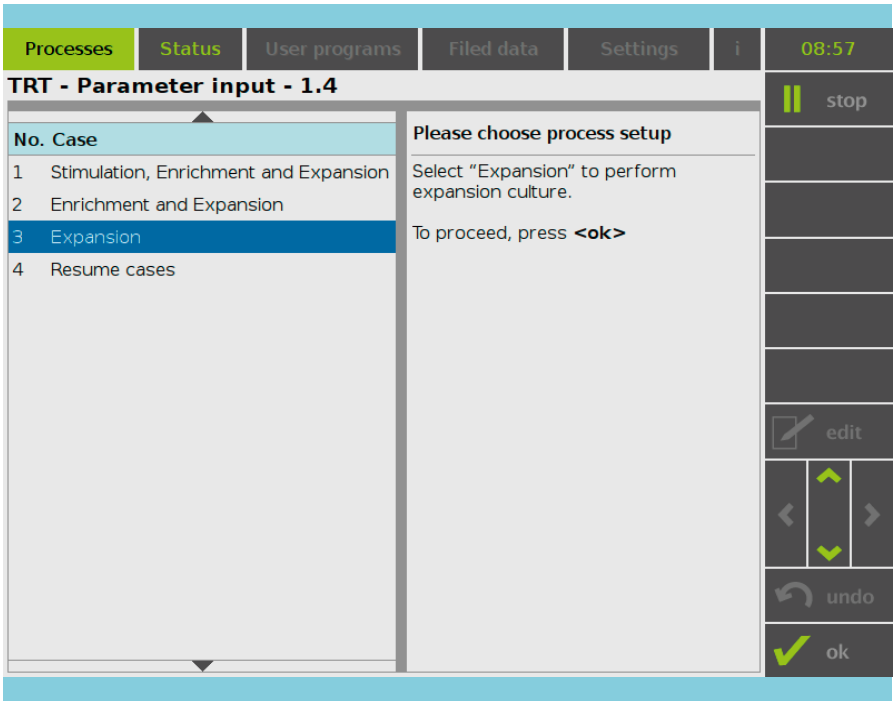
Note: As in case 1, an optional sample of the original fraction (ori) can be taken before magnetic separation takes place. The cells can be analyzed for appropriate labeling. The process is interrupted for 15 minutes after cell filtration (approximately 2 h and 50 min after the start of the enrichment part).

10.1.4 Expansion (case 2)

If it is desired to expand the enriched target cells, see section 5.2.4 for preparation of the expansion. Perform the expansion as it is described in section 9.

10.2 Case 3 (Expansion)

10.2.1 Process setup and differences to case 1

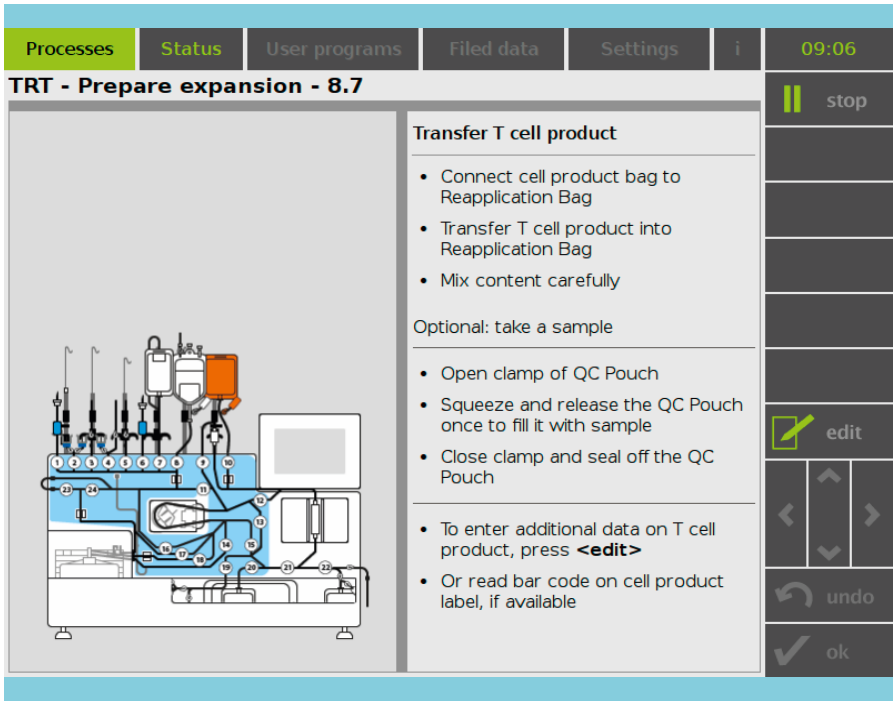


Screen 1.4: Process setup of case 3 'Expansion'

After initialization of the TRT process (see section 6.1), entry of the operator and input of the tubing set parameter (see section 6.2) select case 3 'Expansion'.

In case 3 no process buffer or CliniMACS Reagents are required as only expansion of T cells with feeder cells will be performed (e.g., tumor digest, total outgrown tumor-infiltrating lymphocytes, or isolated T cells of interest via other means, e.g., MACSQuant Tyto sorting). Following the connection of expansion medium and tubing set installation (see section 6.3), the tubing set will be primed with medium (similar to section 6.4). Seal off the columns (see section 9.2.1). See section 5.1.7 for preparation of the expansion medium.

10.2.2 Transfer of cellular starting product



Screen 8.7: Connection and transfer of T cells to Reapplication Bag

See section 5.3.1 for details on the preparation of the starting material and e.g., volume requirements. Connect the bag containing the T cells to the Reapplication Bag by sterile welding. Transfer the cells into the Reapplication Bag and seal off the empty bag using the heat sealer.

Optional: take a sample of the filtered starting product from the Application Bag with the QC Pouch:

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

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

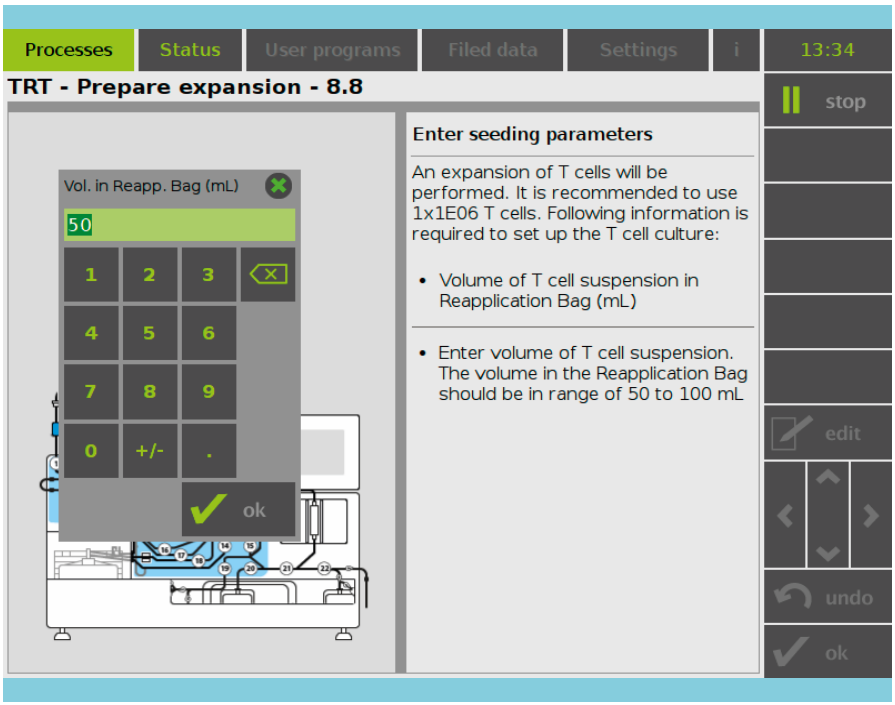
Determine the cell concentration (x1E6/mL) of the filtered cell suspension within the Application Bag.

Tap **edit** to enter information on the cellular starting product (see Screen 8.7). Up to two different texts can be entered.

Enter the following values:

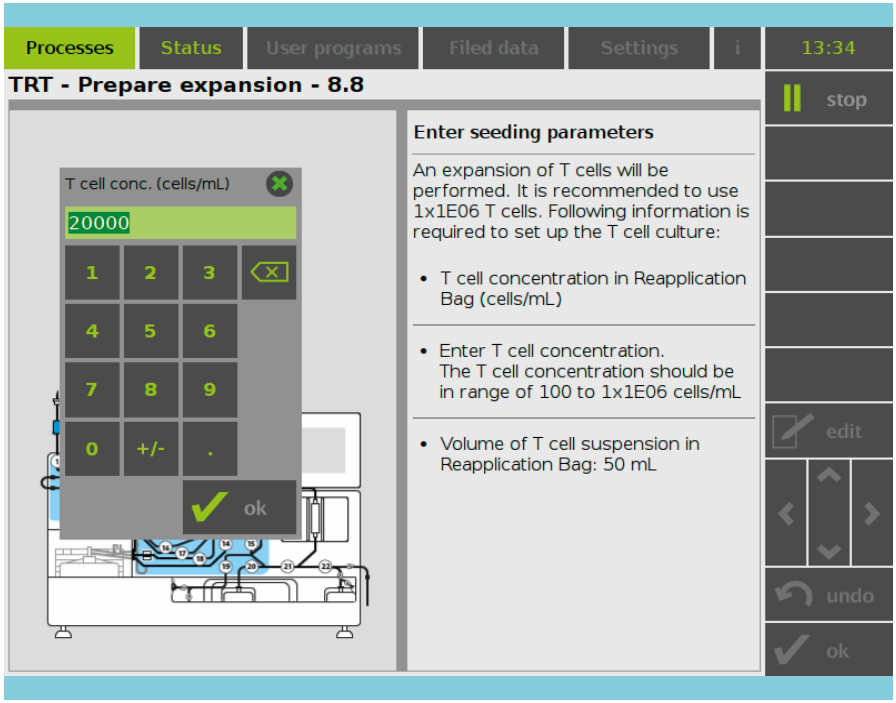
- Volume of the T cell suspension in the Reapplication Bag (mL).
- T cell concentration that is present in the Reapplication Bag (cells/mL).
- Desired starting T cell number (x1E04) for the expansion culture.

For culture setup, 50-100 mL cells can be transferred from the Reapplication Bag to the chamber.



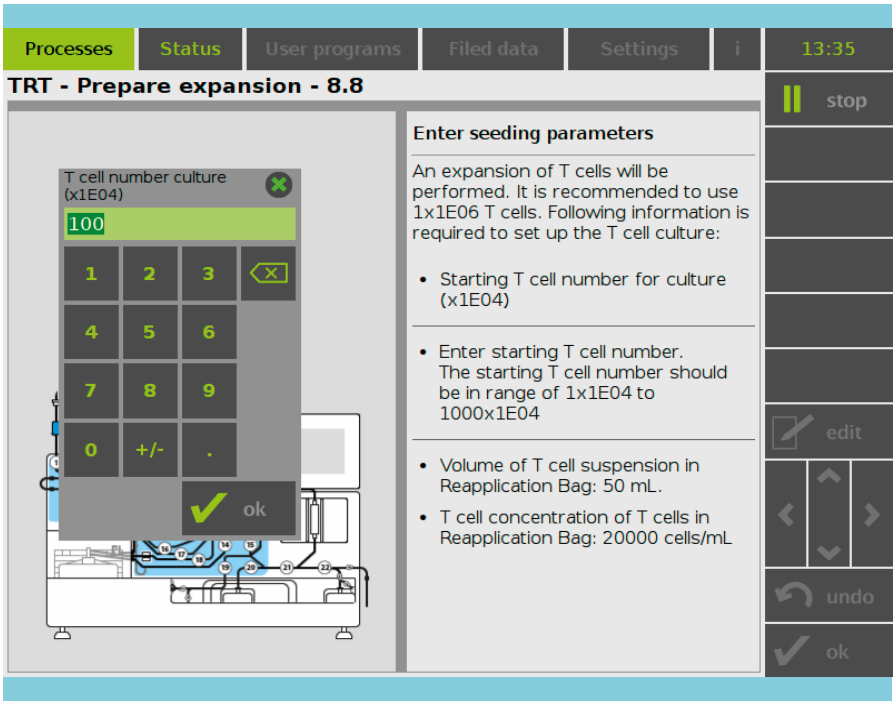
Screen 8.8: Input of the seeding parameters for the expansion culture – Volume in Reapplication Bag

The volume of the T cell suspension should be in a range of 50-100 mL.



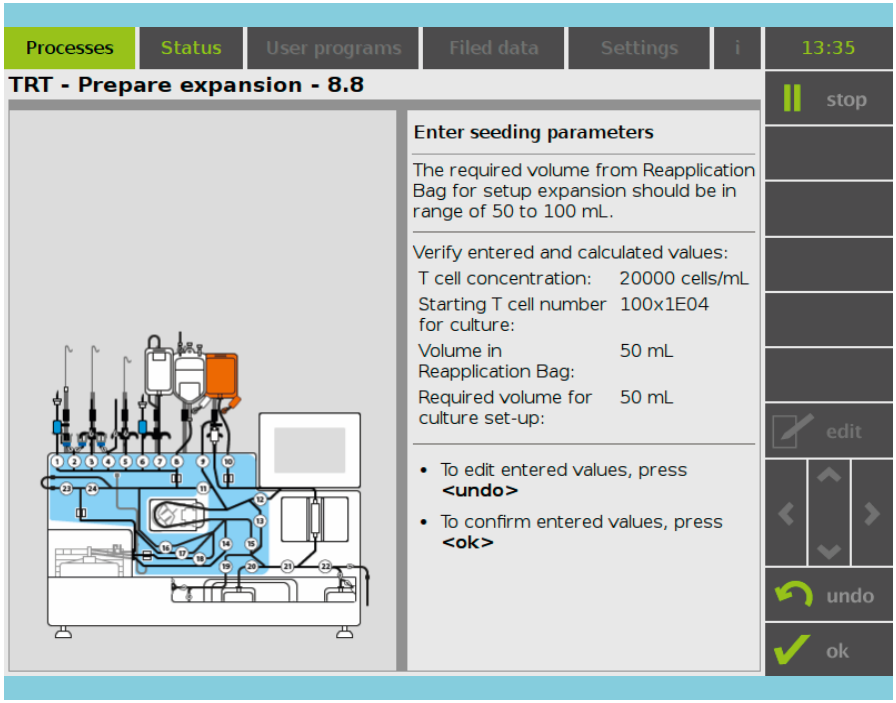
Screen 8.8: Input of the seeding parameters for the expansion culture – T cell concentration

The T cell concentration should be in the range of 100 to $1x10^6$ viable T cells/mL.



Screen 8.8: Input of the seeding parameters for the expansion culture – Starting T cell number

The recommended starting T cell number for culture is 1×10^6 viable T cells.



Screen 8.8: Calculation of the required volume for culture setup of the expansion culture

The volume of the cellular starting material in the Reapplication Bag that is transferred for the expansion culture is calculated automatically.

Note: If only a part of the volume in the Reapplication Bag will be transferred to the chamber for expansion, then the remaining cells will be transferred to one of the Target Cell Bags on valve 22 during the culture setup.

Make sure that only one of the two Target Cell Bags is opened. Close the clamp of the second bag. Make sure that the clamp of the QC Pouch of the selected bag is closed. Make sure that liquid can flow freely to the used Target Cell Bag.

Note: If using the Target Cell Bag with the in-line filter, ensure that the filter is upright.

After transfer of the cells to the Target Cell Bag, the Reapplication Bag will be rinsed. This rinsing volume will be transferred into the Target Cell Bag as well. The used Target Cell Bag can be welded off for further use of the cells.

Note: Due to the rinsing procedure the concentration of cells in the Target Cell Bag will be reduced.

10.2.3 Expansion (case 3)

After transfer of the starting material into the Reapplication Bag and input of the cell seeding parameters, the rest of the process is identical to the complete process in case 1. First the CO_2 and temperature parameters are defined (see section 9.2.5), followed by setup of the activity matrix (see section 9.2.6) and final setup check (section 9.2.7). Finally, the culture is automatically set up (section 9.2.8).

Cultivation of the cells is performed as described in section 9.3. See sections 5.1.8 to 5.1.10 for preparation of the feeder cells, the activation reagent and optionally the transduction reagent.

Final formulation, harvest and final handling are described in section 9.4 and 9.5.

11

Resume cases 4.1–4.3

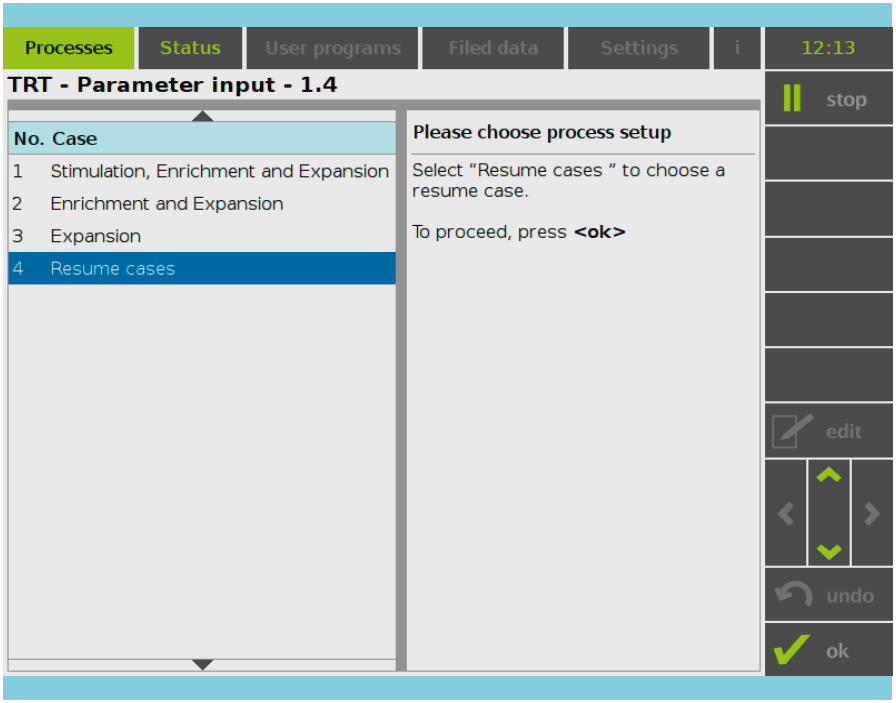
The following chapter describes the resume cases that can be used if the Tumor Reactive T cell Process was interrupted (e.g., due to a power failure). These special cases allow the user to continue at different steps within the process without prior tubing set installation and priming.

There are 3 different resume cases available:

- Resume the stimulation culture of case 1 (case 4.1, see section 11.2).
- Resume after enrichment in case 1 or case 2 (case 4.2, see section 11.3).
- Resume the expansion culture in case 1, case 2 or case 3 (case 4.3, see section 11.4).

11.1 General remarks

In case a TRT process was interrupted, re-start the TRT process (see section 6.1). Enter operator-related information as well as the tubing set parameter (see section 6.32).



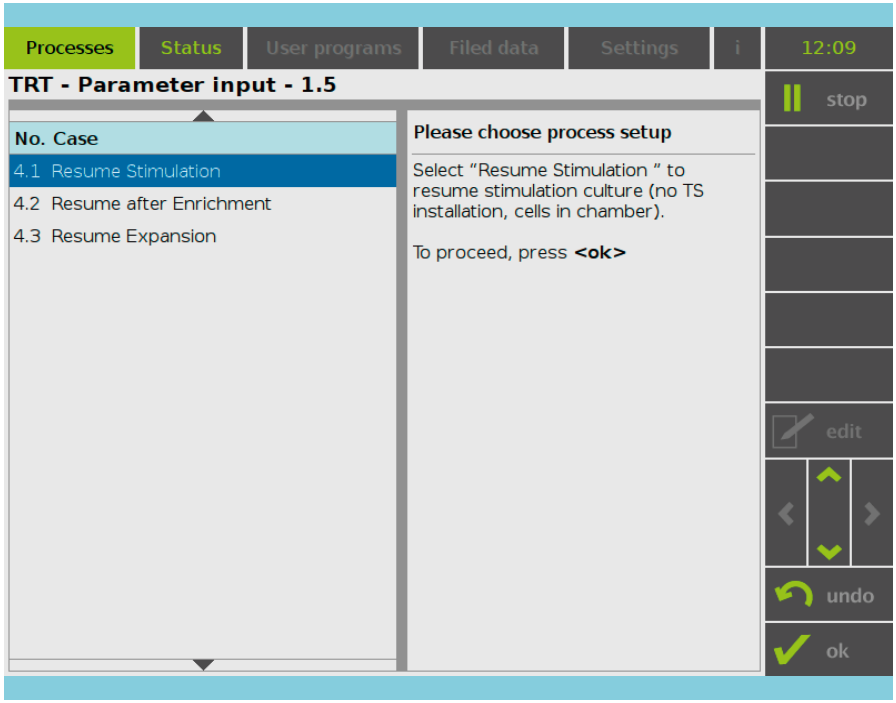
Screen 1.4: Process setup of case 4 'Resume cases'

To resume the process select case 4 'Resume cases' (see Screen 1.4).

Continue with the desired resume case (see Screen 1.5):

- To resume the stimulation culture of case 1, select case 4.1 (see section 11.2).
- To resume after enrichment in case 1 or case 2, select case 4.2 (see section 11.3).
- To resume the expansion culture in case 1, case 2 or case 3, select case 4.3 (see section 11.4).

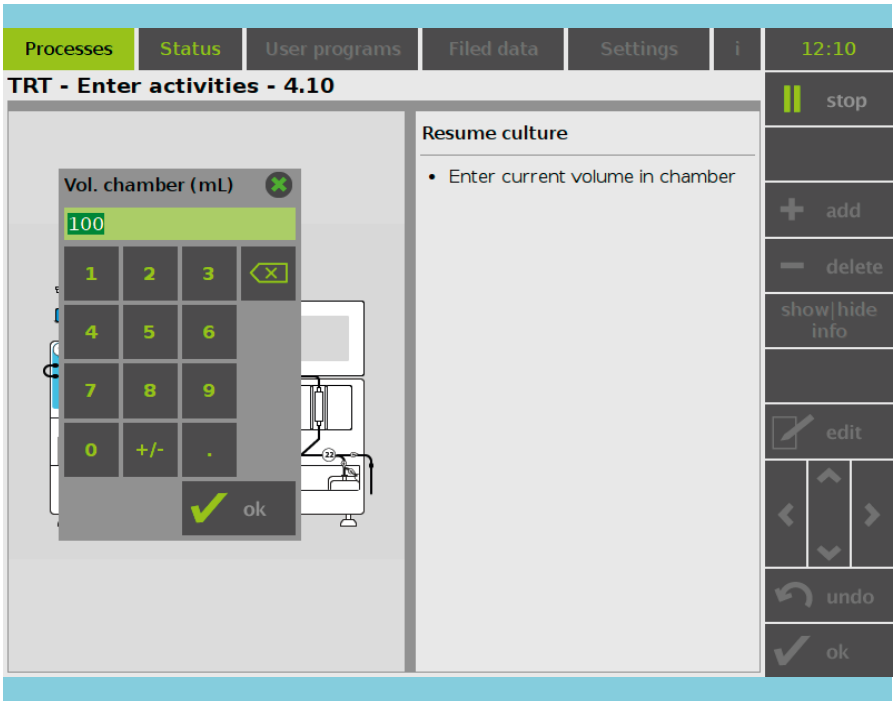
11.2 Case 4.1 (Resume Stimulation)



Screen 1.5: Process setup of resume case 4.1 'Resume Stimulation'

Select case 4.1 'Resume Stimulation' to resume the stimulation culture. Only proceed with this resume case, if the cells are present in the chamber for the stimulation culture.

Double check that the tubing for aeration is connected tightly to the gas outlet port. Enter CO_2 and temperature parameters (see section 7.1.3).

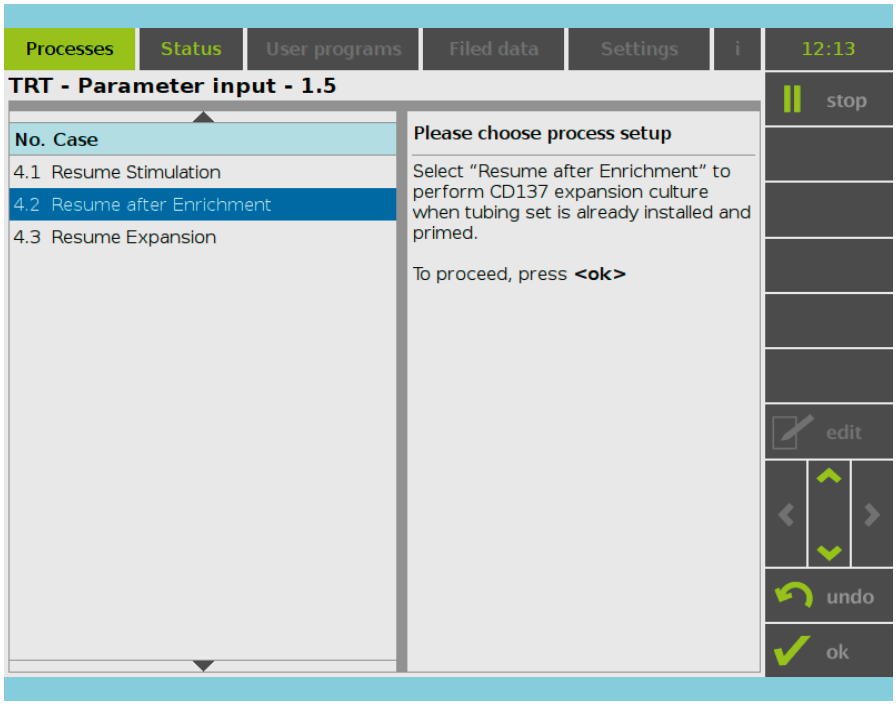


Screen 4.10: Input of current culture volume in chamber to resume culture

The activity matrix must be set up starting from the current day which is called day 0 (see section 7.1.4). To start the culture again (see Screen 4.10) enter the current culture volume in the chamber – 100 mL in case the default activity matrix was used – and confirm the activity matrix of the stimulation culture.

From now on, continue with the process as described starting from section 7.3.

11.3 Case 4.2 (Resume after Enrichment)

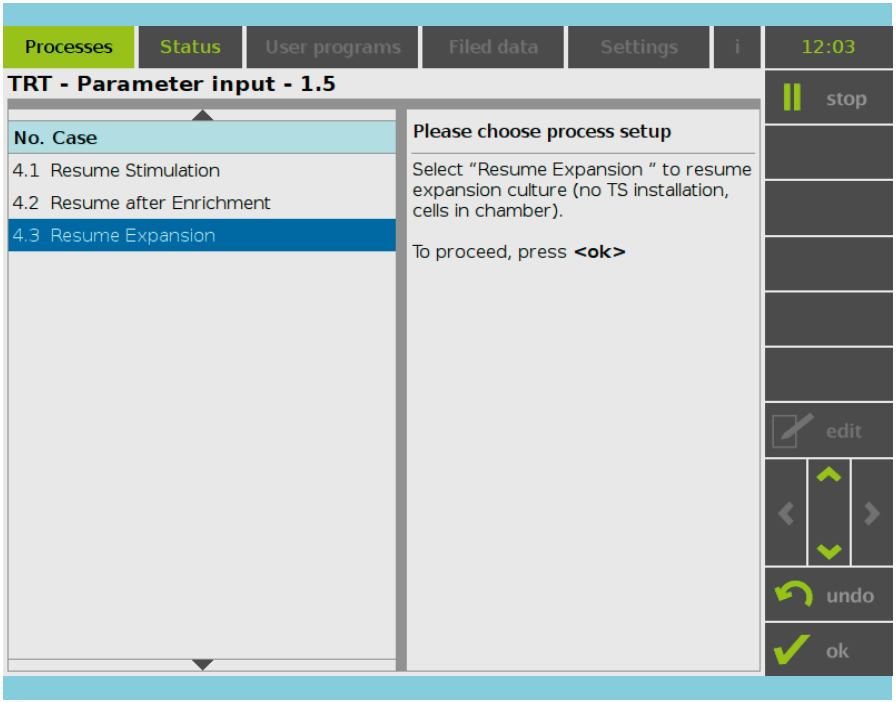


Screen 1.5: Process setup of resume case 4.2 'Resume after Enrichment'

Select case 4.2 'Resume after Enrichment' to resume the process after enrichment. Only proceed with this resume case, if the enriched cells are present in the Reapplication Bag and shall be expanded in an expansion culture.

From now on, continue with the process as described starting from section 8.3.1.

11.4 Case 4.3 (Resume Expansion)



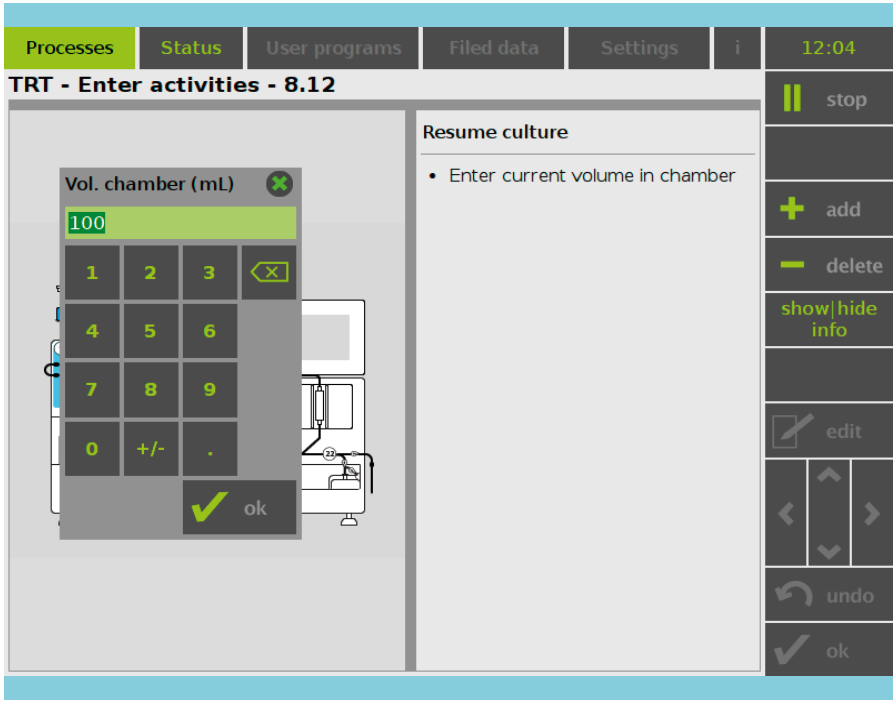
Screen 1.5: Process setup of resume case 4.3 'Resume Expansion'

Select case 4.3 'Resume Expansion' in order to resume the expansion culture. Only proceed with this resume case, if the cells are present in the chamber for the expansion culture.

Double check that the tubing for aeration is connected tightly to the gas outlet port and enter CO_2 and temperature parameters (see section 9.2.5).

The activity matrix must be set up starting from the current day which is called day 0, including all activities that should be performed from today until the end of the culture (see section 9.2.5).

Note: To set up the gas and temperature parameters of the culture as quickly as possible after process abort, it is recommended to continue with the empty matrix (default matrix 4). Apply the changes after the culture setup has been performed.



Screen 8.12: Input of current culture volume in chamber to resume culture

Finally, enter the current culture volume in the chamber and confirm the input parameters (see Screen 8.12). The chamber volume depends on the time of process interruption.

From now on, continue with the process as described starting from section 9.3.

12 Troubleshooting

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

+49 2204 8306-3803
technicalsupport@miltenyi.com
www.miltenyibiotec.com/support

13
Legal notices

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