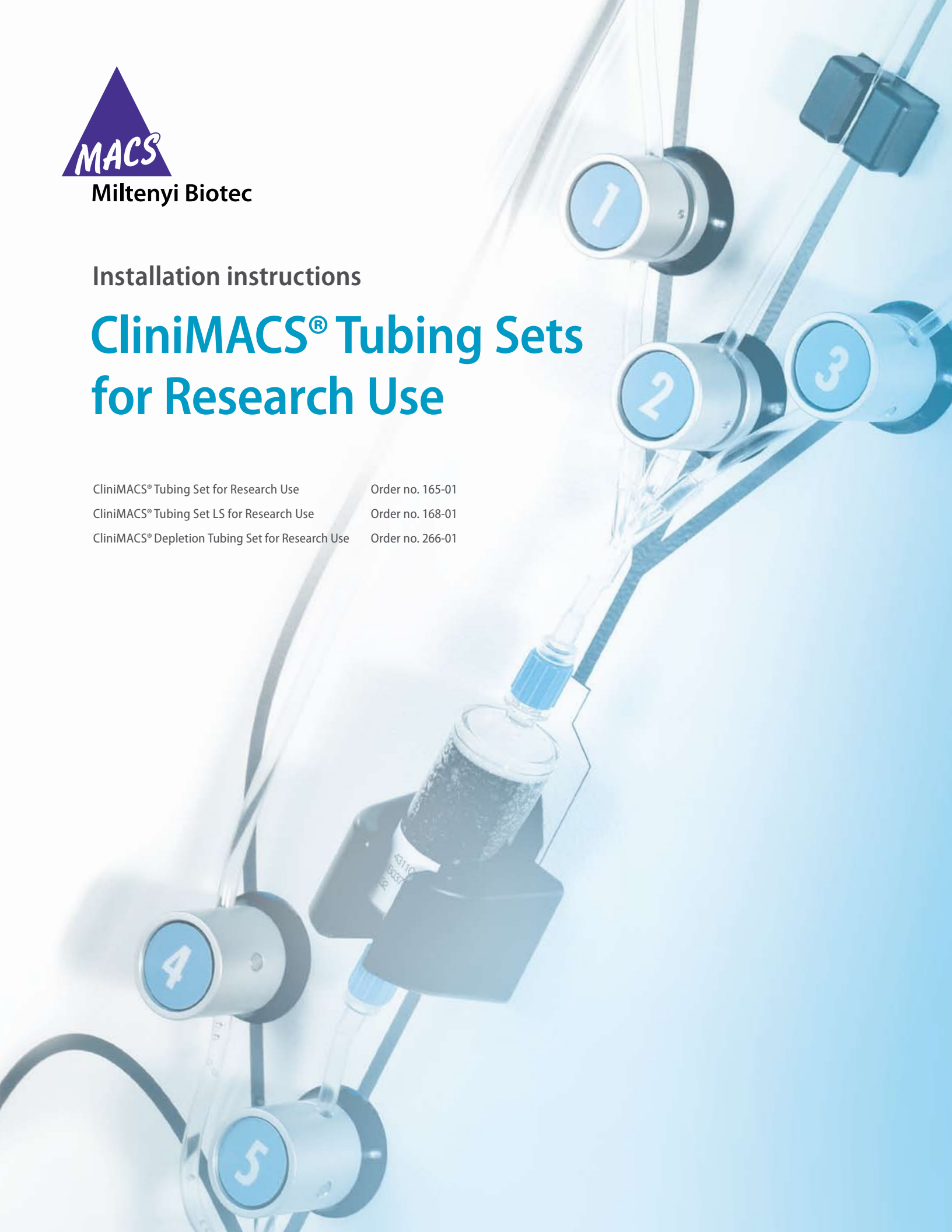
Miltenyi Biotec

Installation instructions

CliniMACS® Tubing Sets for Research Use

CliniMACS® Tubing Set for Research Use	Order no. 165-01
CliniMACS® Tubing Set LS for Research Use	Order no. 168-01
CliniMACS® Depletion Tubing Set for Research Use	Order no. 266-01



Contents

Important note

The CliniMACS® Tubing Sets for Research Use are for research use only and not for diagnostic or therapeutic use.

1. Background information

1.1 CliniMACS® Technology

The CliniMACS® System for research use comprises the CliniMACS Plus Instrument, CliniMACS Tubing Sets for Research Use , CliniMACS MicroBeads, and CliniMACS PBS/EDTA Buffer for Research Use. The system enables the operator to perform large scale magnetic enrichment of target cells, or depletion of unwanted cells, in a closed and sterile system.

The CliniMACS System for research use combines manual and automated processes based on MACS® Technology. The CliniMACS MicroBeads use selective monoclonal antibodies conjugated to superparamagnetic particles, which are only 50 nanometers in size. They are composed of a biodegradable matrix, and therefore do not need to be removed from cells after the separation process. In general, CliniMACS MicroBeads have been developed for the enrichment or depletion of specific human cell types from heterogenous hematologic cell populations in combination with all other parts of the CliniMACS System for research use.

The CliniMACS PBS/EDTA Buffer for Research Use is used for sample preparation and serves as buffer for the automated processes together with a CliniMACS Tubing Set for Research Use and the CliniMACS Plus Instrument.

The CliniMACS Tubing Sets for Research Use have been designed to process a specific amount of cells in a closed and sterile fluid path, and to allow for an optimized magnetic separation of target cells due to a highly developed separation column integrated into the tubing.

The operator can select from a range of protocols developed for different applications and sample requirements to allow for an optimal end result.

For availability in your country, please contact your local country representative.

1.2 Key components of the CliniMACS® Plus Instrument with installed CliniMACS® Tubing Set

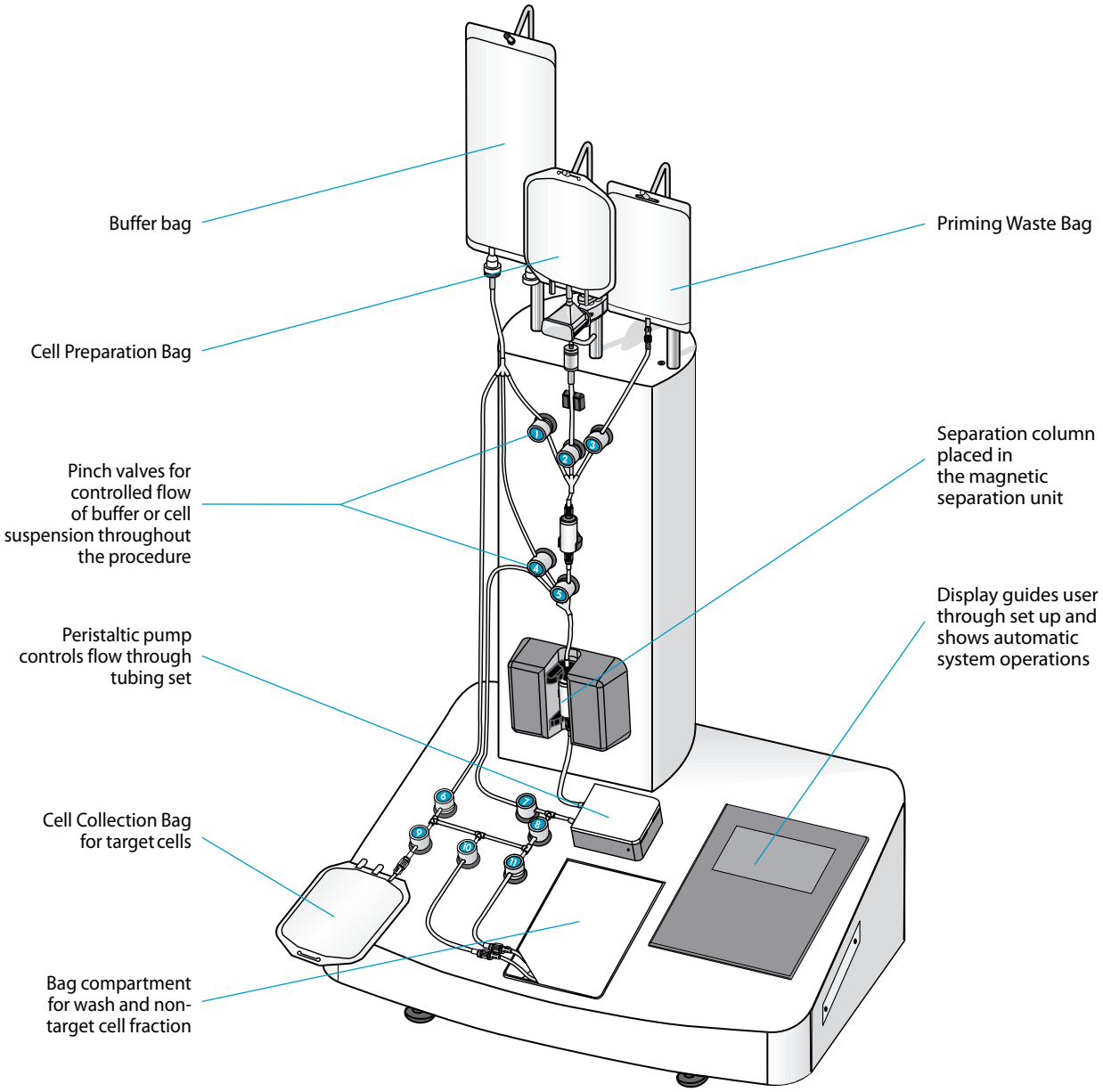


Figure 1: Key components of the CliniMACS® Plus Instrument with installed CliniMACS® Tubing Set for Research Use.

1.3 CliniMACS® Tubing Sets for Research Use

1.3.1 Description

CliniMACS® Tubing Sets for Research Use and CliniMACS Tubing Sets LS for Research Use consist of pre-assembled tubing including a pre-column and a separation column.

CliniMACS Depletion Tubing Sets contain tubing with a pre-assembled Cell Collection Bag, Reapplication Bag, Non-Target Cell Bag, and Buffer Waste Bag.

The fluid path of the tubing sets is sterile and non-pyrogenic.

One package unit contains one sealed tubing set for single use.

The performance of the CliniMACS Tubing Sets depends on the individual CliniMACS Separation strategy. For information on respective capacities, refer to the CliniMACS Tubing Sets for Research Use data sheet or contact your local representative.

Please inquire about required CliniMACS System for research use components and accessories.

1.3.2 Applications

The CliniMACS Tubing Sets for Research Use and CliniMACS Tubing Sets LS for Research Use are designed for *in vitro* selection of human cells from heterogeneous hematologic cell populations in combination with the CliniMACS System for research use.

The CliniMACS Depletion Tubing Set for Research Use has been specifically designed for depletion of large quantities of cells from human heterogeneous hematologic cell populations in combination with the CliniMACS System for research use.

It is also designed for a faster depletion of more cells as compared to other CliniMACS Tubing Sets for Research Use.[1, 2]

References

1. Fritsch *et al.* (2004) Bone Marrow Transplant. 33 (Suppl.1): 595.
2. Fritsch *et al.* (2004) Cytotherapy 6(4): 432.

1.4 Installation instructions

These installation instructions are a supplement to the CliniMACS Tubing Sets for Research Use data sheet and give a detailed description of how to install the various tubing sets on the CliniMACS Plus Instrument.

For all other information please refer to the CliniMACS Tubing Sets for Research Use data sheet.

For further information, please contact the Technical Support at +49-2204-8306-8484 or the US Technical Support at +1 530 888 8871.

2. Installation of the CliniMACS® Tubing Set for Research Use (# 165-01) and the CliniMACS® Tubing Set LS for Research Use (# 168-01)

2.1 Preparation for tubing set installation

The window will display screen no. 1 as shown in figure 2.

The instruction is on the right, and a diagram corresponding to the instruction is displayed on the left. The blinking features on the screen indicate the areas of attention.

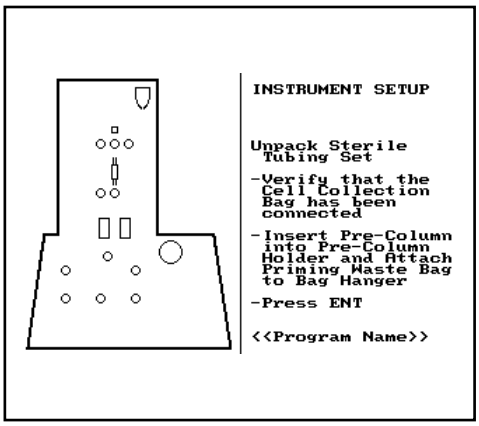


Figure 2: Screen no. 1.

Note

- The CliniMACS Plus Instrument shows the chosen program name, e.g., CD34 SELECTION 1, in the bottom line of the instrument screen.
- At any step during the tubing set installation the Undo Key can be pushed to return to the previous step.

The CliniMACS® Tubing Set for Research Use and the CliniMACS Tubing Set LS for Research Use are provided in a sealed, sterilized package. Each tubing set contains pre-assembled tubing and columns for one cell separation (see fig. 4). When the packaging is intact, a sterile fluid path is provided.

1. When required, record the lot number and use-by date of the tubing set. Unpack the sterile tubing set under sterile conditions (e.g., in a laminar flow hood).
2. Check luer lock connections to bags. Luer lock must be closed tightly.

2.2 Attach Cell Collection Bag

1. Note the weight of the empty Cell Collection Bag.
2. In an aseptic environment, remove caps and attach the sterile Cell Collection Bag to the luer connector on the tubing set before loading the tubing set onto the CliniMACS Plus Instrument.
3. Make sure that unrestricted flow to the Cell Collection Bag is possible.

2.3 Attach Priming Waste Bag and insert pre-column

The window will display screen no. 1 as shown in figure 2.

1. Attach the Priming Waste Bag (1) to the right hand bag hanger on the instrument as shown (see fig. 3).

Note

- The bag hangers are made for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

2. Place the pre-column into the holder as shown (see fig. 9).

Note

- When the pre-column is placed into the pre-column holder, ensure that the plastic projections found at the bottom of the column are facing you.

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the Priming Waste Bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow, and that it is low enough to avoid the tubing or connections being stretched.

To proceed, press ENT

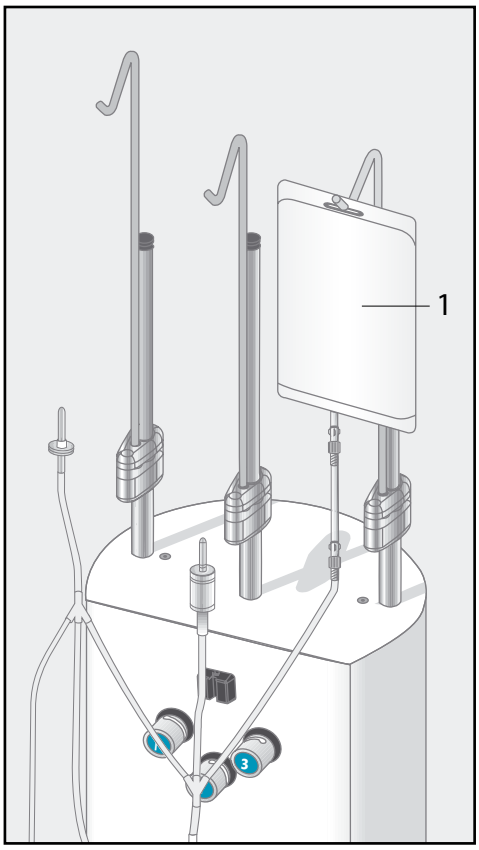


Figure 3: Attach Priming Waste Bag (1) to bag hanger.

1 Priming Waste Bag
2 Buffer spike
3 Bubble trap spike
4 Bubble trap
5 4-way fitting
6 Pre-column
7 Separation column
8 Upper retaining ring
9 Pump tubing
10 Lower retaining ring
11 Negative Fraction Bag
12 Buffer Waste Bag
13 T-fitting
14 Luer connector

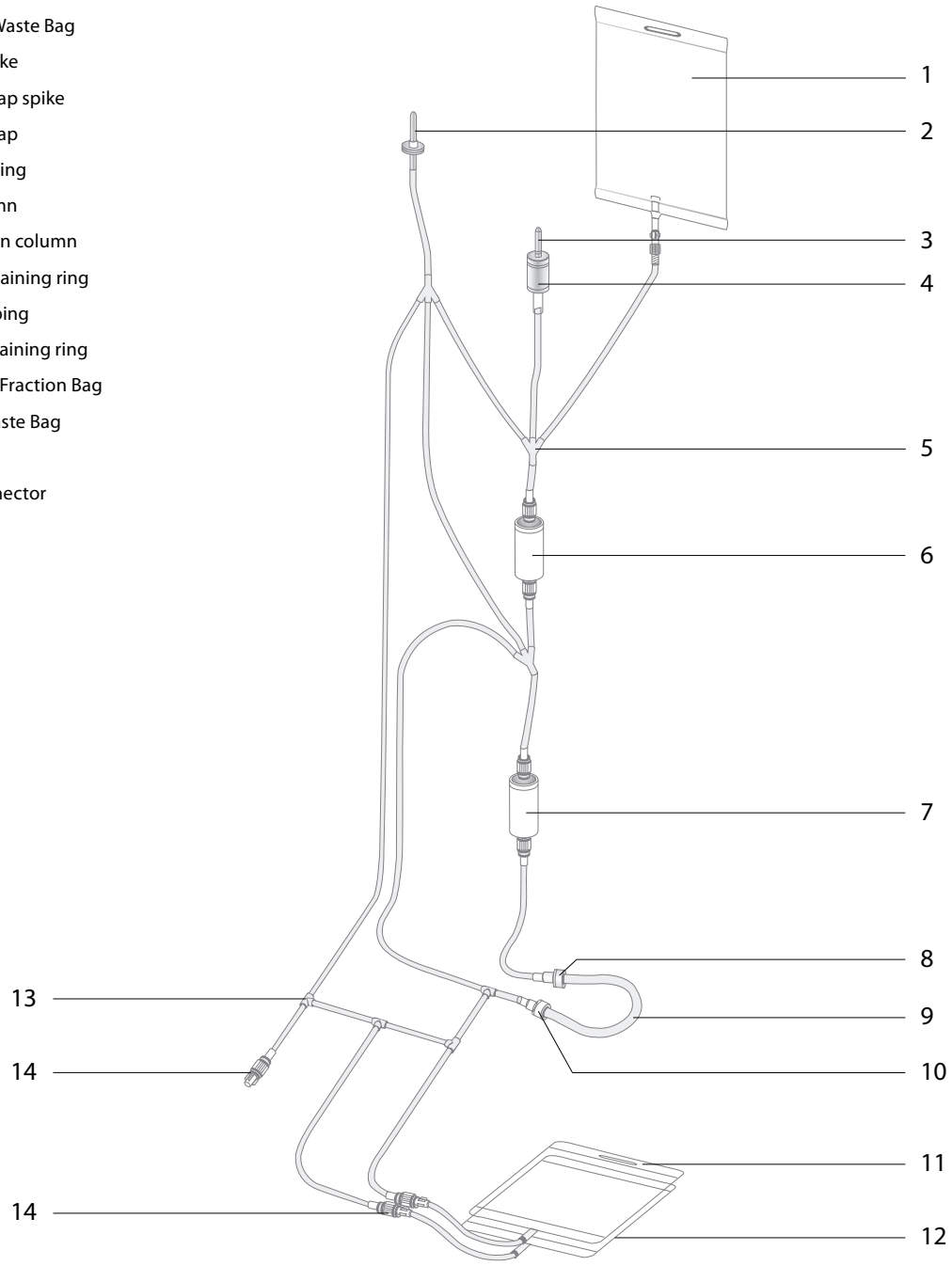


Figure 4: General construction of a CliniMACS® Tubing Set for Research Use (# 165-01).

2.4 Insert separation column and load valve no. 5

The window will display screen no. 2 as shown in figure 5.

The valves shown on the screen will be opened automatically.

1. Insert the separation column into the separation column holder as shown (see fig. 6).

Note

- To avoid possible pinch injury, insert the separation column as follows: Hold the top and bottom of the column between thumb and index finger, then carefully insert the separation column into the separation column holder.

2. Load the tubing into valve no. 5.

Note

- As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly.
- Only insert the tubing set into open valves (when button is pushed inwards). The tubing will not fit correctly if inserted into a closed valve.
- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve (see fig. 7).

To proceed, press ENT

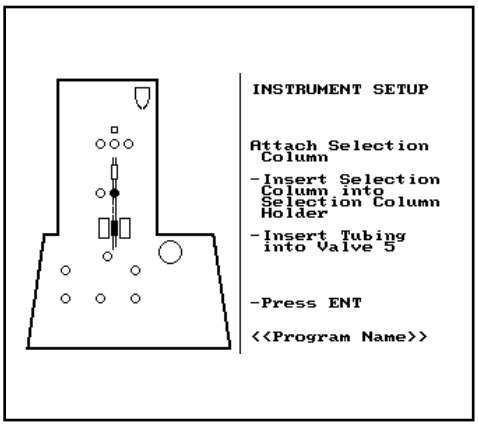


Figure 5: Screen no. 2.

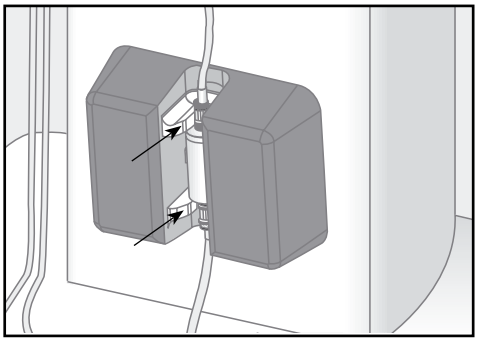


Figure 6: Separation column in separation column holder.

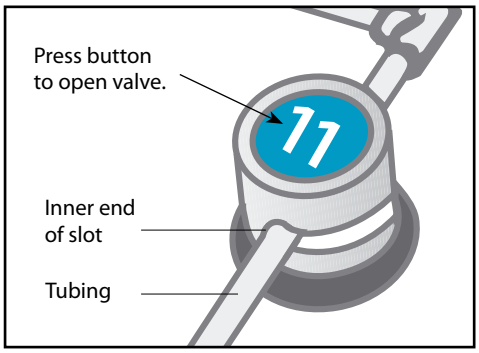


Figure 7: Correctly inserted tubing.

2.5 Load valves nos. 1, 2, 3, and 4

The window will display screen no. 3 as shown in figure 8.

1. Load the tubing into valve no. 4. Confirm that the tubing is placed securely in the valve opening (see fig. 7). Pay particular attention to the area between valves nos. 4 and 5 (16, fig. 9).
2. Insert the tubing into valve no. 1.
3. Position the 4-way fitting just below valve no. 2. Pay particular attention to the area below valve no. 2 (16, fig. 9).
4. Insert the tubing into valve nos. 2 and 3.
5. Mount the tubing between valve no. 2 and the bubble trap into the liquid sensor (15, fig. 9). Confirm that the tubing is placed correctly into the sensor fitting.

Note

- To assure proper operation, both the liquid sensor and the tubing being inserted MUST BE DRY. Carefully inspect both. If any liquid is present, dry the area with a soft, lint-free cloth.

To proceed, press ENT

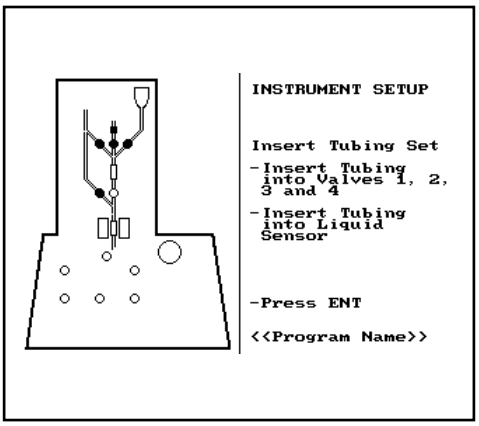


Figure 8: Screen no. 3.

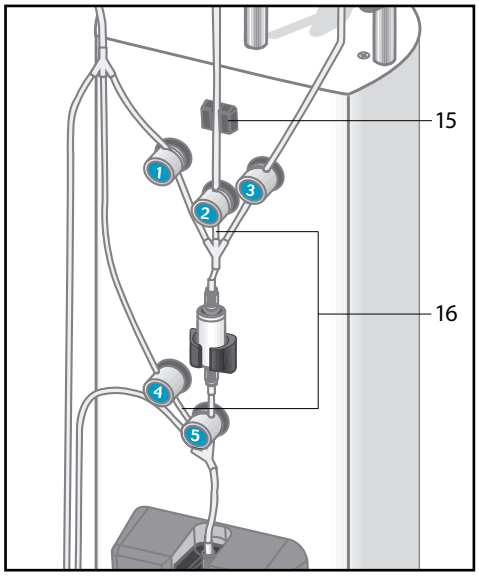


Figure 9: Tubing in valves.
15: Liquid sensor
16: Critical areas between valves nos. 4 and 5 and below valve no. 2.

2.6 Load pump tubing

The window will display screen no. 4 as shown in figure 10.

1. Open the pump door by lifting up at the left hand edge.
2. Insert the upper retaining ring on the pump tubing into the retaining ring groove (17, fig. 11) on the pump housing.
3. Rotate the pump roller clockwise (18, Fig. 11) until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins. If adjustment of the tubing inside the pump is necessary, the tubing can be unloaded by lifting the lower end and turning the pump roller anti-clockwise.
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove (19, fig. 11) on the pump housing.
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely.
6. Close the pump door.

Note

- During the cell separation program the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

To proceed, press ENT

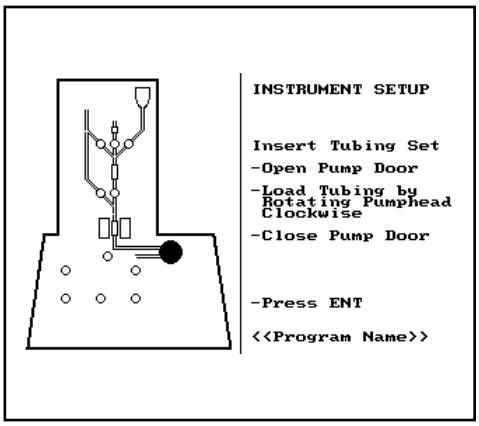


Figure 10: Screen no. 4.

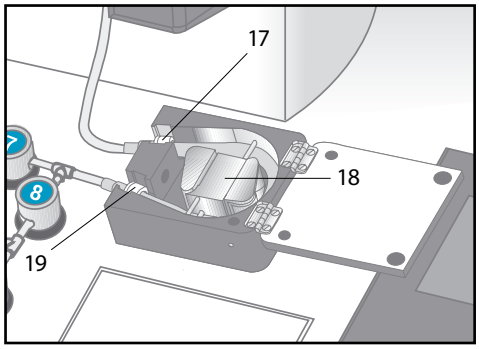


Figure 11: Loading of pump tubing.
17: Upper retaining ring
18: Pump roller
19: Lower retaining ring

2.7 Load valves nos. 7 and 8

The window will display screen no. 5 as shown in figure 12.

1. Load the tubing into valve no. 7.
2. Load the tubing into valve no. 8.

To proceed, press ENT

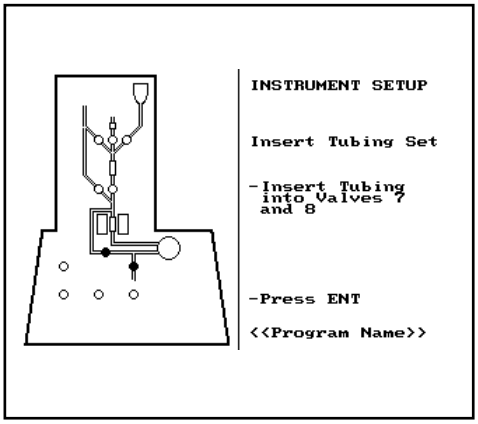


Figure 12: Screen no. 5.

2.8 Load valves nos. 6, 9, 10, and 11

The window will display screen no. 6 as shown in figure 13.

1. Load the tubing into valves nos. 6, 9, 10, and 11.
2. Place the Negative Fraction Bag and the Buffer Waste Bag in the bag compartment. Make sure the tubing is not compressed under the bag compartment lid.

To proceed, press ENT

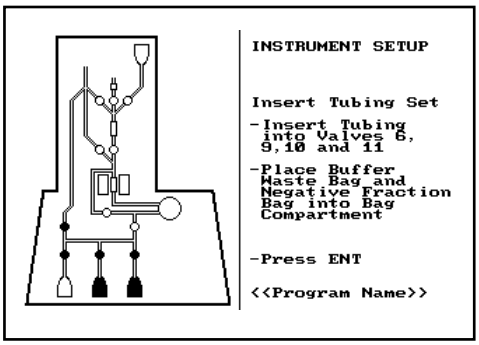


Figure 13: Screen no. 6.

2.9 Recheck all tubing and attachments

The window will display screen no. 7 as shown in figure 14.

Before beginning the run, recheck all tubing and attachments.

Note

- Check all valves for proper tubing insertion. Make sure that the tubing is spaced uniformly, and that there are no kinks or stretched areas in the tubing. Pay particular attention to the pre-column area, as well as the area between the pump and valves nos. 7 and 8 (20, fig. 15), and between valves nos. 4 and 5 (16, fig. 9).
- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve. If a tubing has been adjusted, it is absolutely necessary to press the corresponding valves firmly two times.

To proceed, press ENT

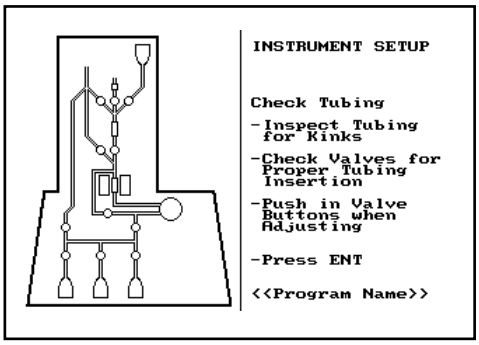


Figure 14: Screen no. 7.

2.10 Seating of valves

The window will display screen no. 8 as shown in figure 16.

In order to ensure the proper fit of tubing in the valves, the CliniMACS Plus Instrument will operate all of the valves in sequence, twice. Watch and listen to make sure all valves are working properly. This step can be repeated by using the Undo Key followed by the Enter Key. If any valve does not operate correctly, refer to the troubleshooting section in chapter 5.

The magnet drive will also be tested during this check sequence.

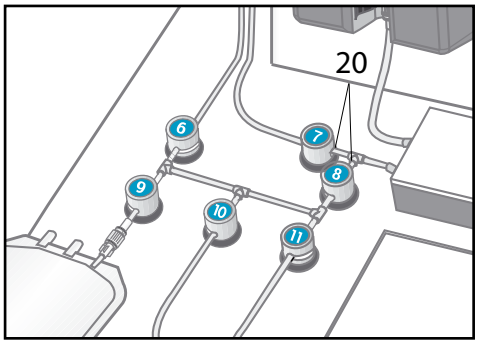


Figure 15: Tubing in valves.
20: Critical area between valve nos. 7 and 8

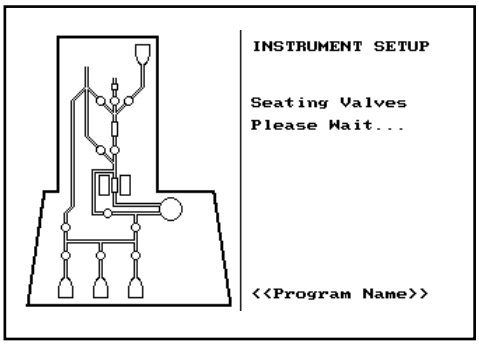


Figure 16: Screen no. 8.

2.11 Attach CliniMACS® PBS/EDTA Buffer for Research Use

The window will display screen no. 9 as shown in figure 17.

The buffer for CliniMACS® Research Separations is CliniMACS PBS/EDTA Buffer for Research Use supplemented with HSA to a final concentration of 0.5% (w/v).

1. Using aseptic techniques, remove the cap from the buffer spike on the tubing set (2, fig. 4) and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag. If more than one liter buffer is necessary for a separation, connect the buffer bags using a plasma transfer set.

2. Attach the buffer bag (23) to the buffer bag hook (22) on the bag hanger (21, fig. 18).

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow, and that it is low enough to avoid the tubing or connections being stretched (see fig. 18).

To proceed, press ENT

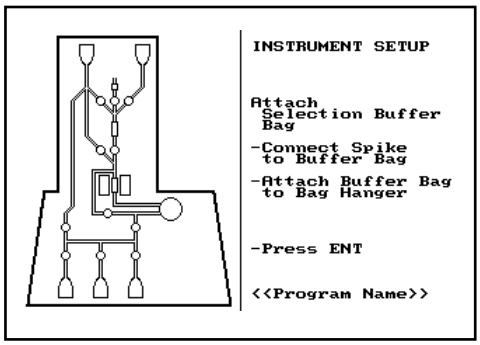


Figure 17: Screen no. 9.

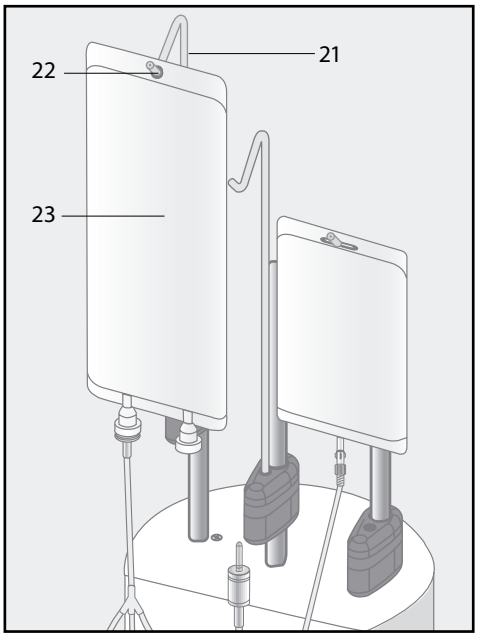


Figure 18: Attach CliniMACS PBS/EDTA Buffer for Research Use to bag hanger.
21: Bag hanger for buffer bag
22: Buffer bag hook
23: CliniMACS PBS/EDTA Buffer for Research Use

2.12 Start priming

The window will display screen no. 10 as shown in figure 19.

To start priming, press RUN

The window will display screen no. 11 as shown in figure 20. During the priming phase the tubing set is filled with buffer. The buffer will be circulated through the tubing set including both the pre-column and the separation column. Priming waste is collected in the Priming Waste Bag (1) and the Buffer Waste Bag (12, fig. 4). The priming cycles will continue, repeating a series of steps. The priming phase will take approximately 1 minute. Priming status will be updated on the display.

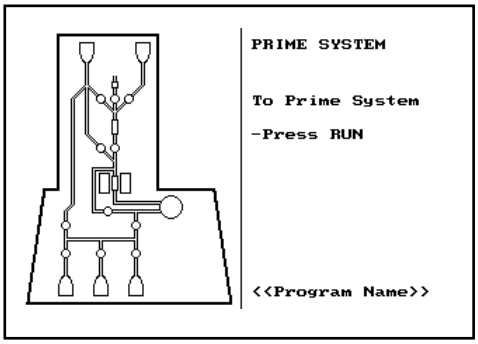


Figure 19: Screen no. 10.

2.13 Check during the priming

During the priming phase, check all tubing, fittings, valves, and columns for the appearance of any leaks or the presence of any folds that may block fluid flow.

If leaks or malfunctions are observed, stop run by pressing 'STOP'. You will have 600 seconds to resolve the problem. Restart the process by pressing 'RUN'.

After 600 seconds, the separation will be aborted. If you cannot resolve the problem or if the tubing set is defective remove the tubing set and replace it with a new one.

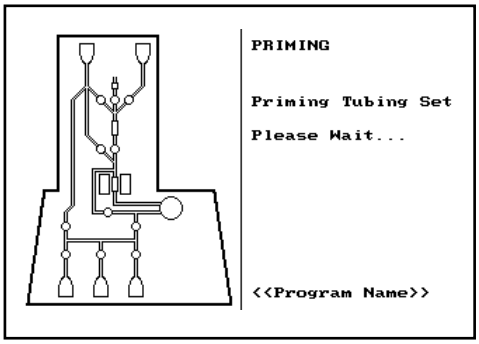


Figure 20: Screen no. 11.

Note

- Once priming has started, it is not possible to return to the instrument set-up procedure.

2.14 Final check of all tubing and attachments

The window will display screen no. 12 as shown in figure 21.

Before beginning the run, check the following:

- fluid in all parts of tubing set,
- **no** excess air in tubing set,
- fluid in the Priming Waste Bag and the Buffer Waste Bag,
- **no** fluid in the Negative Fraction Bag or in the Cell Collection Bag.

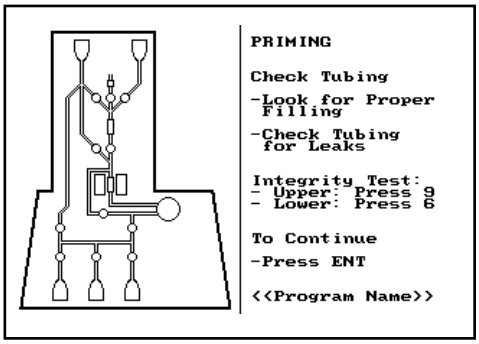


Figure 21: Screen no. 12.

2.15 Integrity test

For additional safety, an integrity test must be performed, where the tubing set is tested for leakages. The test sequence consists of two automated sequence parts, which allows the upper and the lower part of the tubing set to be tested separately.

2.15.1 Integrity test for the upper part of the tubing set

1. When the operator performs "Final check of all tubing and attachments" the window displays screen no. 12 as shown in figure 21.
2. After finalizing "Final check of all tubing and attachments", DO NOT press 'ENT'.
3. To enter the integrity test for the upper part, press 9
4. The window will display screen no. 13 as shown in figure 22.
5. To start the test sequence, press RUN

   To go back to screen no. 12, press ◀
6. Once the RUN button has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

   The window will display screen no. 14 as shown in figure 23.

   Overpressure will be created and held for 2 minutes. During this time the operator should watch the connections above and under the pre-column and separation column, and the upper pump tube connection.

   At each point the test sequence can be finished by pressing 'ENT'.
7. After 2 minutes the pressure is automatically released, and the window displays screen no. 12 as shown in figure 21.

   Using tissue the operator should now check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Send the defective tubing set back to your Miltenyi Biotec office or to your CliniMACS® distributor.
8. If no leaks are observed, continue with the integrity test of the lower part of the tubing set.

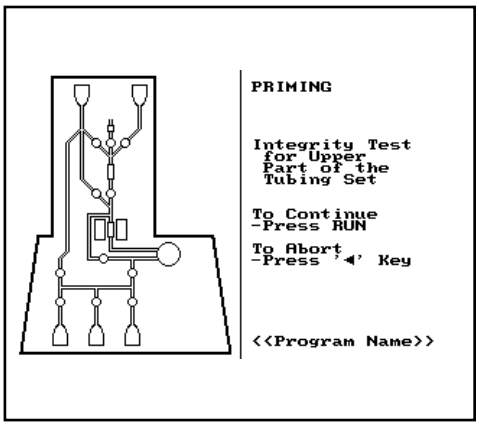


Figure 22: Screen no. 13.

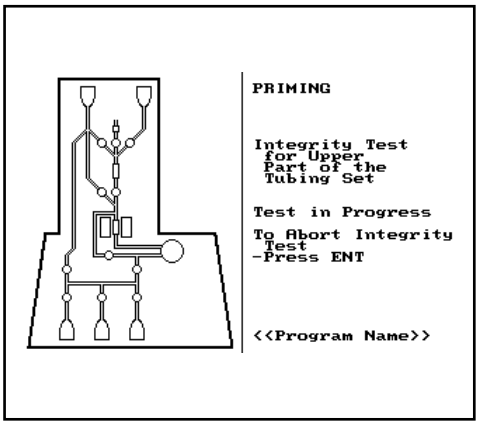


Figure 23: Screen no. 14.

2.15.2 Integrity test for the lower part of the tubing set

1. The window displays screen no. 12 as shown in figure 21.
2. To enter the integrity test for the lower part, press 6

   DO NOT press 'ENT'.
3. The window will display screen 15 as shown in figure 24.
4. To start the test sequence, press RUN

   To go back to screen no. 12, press ◀
5. Once the 'RUN' button has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set.

   The window will display screen no. 16 as shown in figure 25.

   Overpressure will be created and held for 30 seconds. During this time the operator should watch the lower pump tube connection and the T-fittings between valves nos. 6, 8, 9, 10, and 11.

   At each point the test sequence can be finished by pressing 'ENT'.
6. After 30 seconds the pressure is automatically released, and the window displays screen no. 12 as shown in figure 21.

   Using tissue the operator should now check if any leaks have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Send the defective tubing set back to your Miltenyi Biotec office or to your CliniMACS® distributor.
7. If no leaks are observed the operator can now continue with the next step by pressing ENT

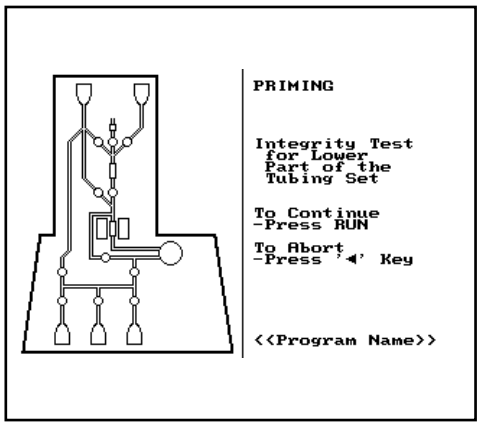


Figure 24: Screen no. 15.

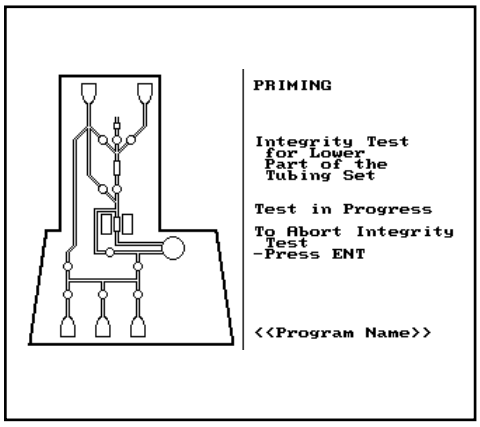


Figure 25: Screen no. 16.

2.16 Connect Cell Preparation Bag

The window will display screen no. 17 as shown in figure 26.

After the priming phase has been completed and no leaks or malfunctions are observed, the Cell Preparation Bag can be attached (see fig. 28). Use aseptic techniques for all steps.

Connect the Cell Preparation Bag containing the magnetically labeled and washed cells with the pre-system filter:

1. Remove the cap from the bubble trap spike (3) of the bubble trap (4, fig. 4).
2. Remove the cap from the lower opening of the pre-system filter (28, fig. 28). **Firmly** insert the spike into the pre-system filter. DO NOT remove the top cap of the pre-system filter.
3. Remove the cap from the pre-system filter spike (29, fig. 28).
4. Spike the Cell Preparation Bag (26) with the pre-system filter (28) ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to confirm that the spike has penetrated the bag.
5. Check the connection between the pre-system filter and the tubing set to confirm that the connection is secure.
6. Hang the Cell Preparation Bag on the bag hanger (14, fig. 28).
7. Adjust the bag hanger for the Cell Preparation Bag (14, fig. 28) to hold the Cell Preparation Bag in an upright position.

To proceed, press ENT

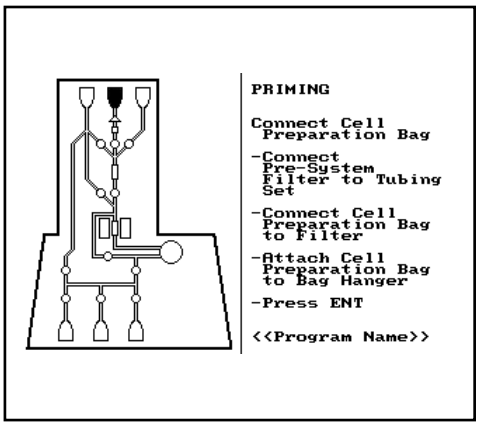


Figure 26: Screen no. 17.

2.17 Final check of the liquid sensor

The window will display screen no. 18 as shown in figure 27.

1. Check the liquid sensor tubing. Ensure the tubing has been properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure.
2. Make sure that unrestricted flow to the Cell Collection Bag is possible.

To proceed, press ENT

Proceed to CliniMACS Cell Separation.

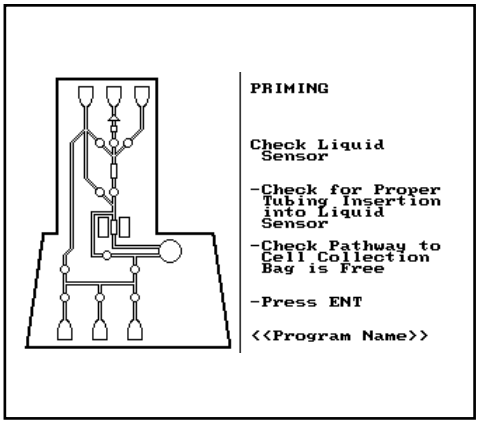
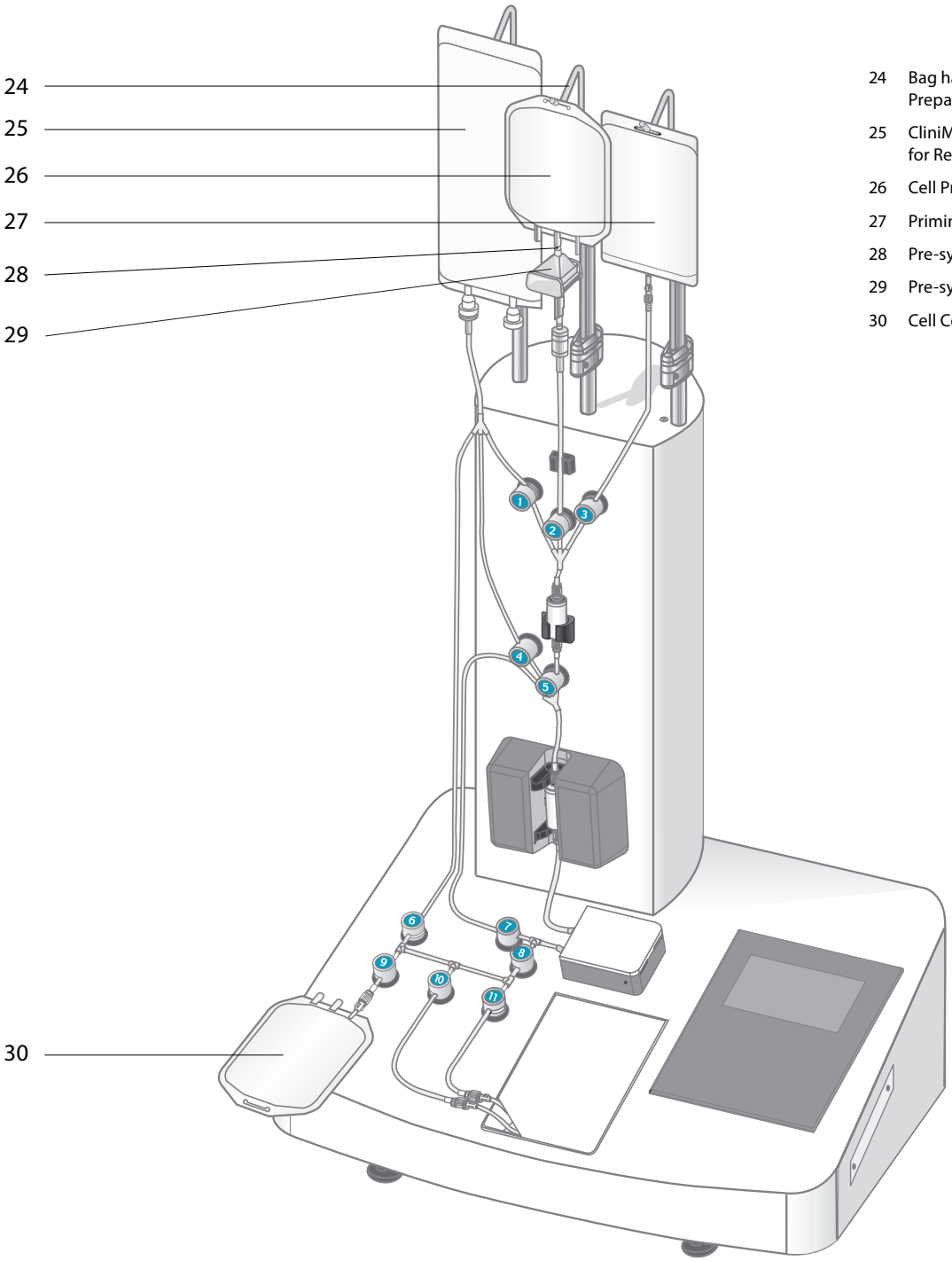


Figure 27: Screen no. 18.



24 Bag hanger for Cell Preparation Bag
25 CliniMACS PBS/EDTA Buffer for Research Use
26 Cell Preparation Bag
27 Priming Waste Bag
28 Pre-system filter spike
29 Pre-system filter
30 Cell Collection Bag

Figure 28: CliniMACS® Plus Instrument with CliniMACS Tubing Set for Research Use, CliniMACS PBS/EDTA Buffer for Research Use, Cell Preparation Bag, and Cell Collection Bag.

3. Installation of the CliniMACS® Depletion Tubing Set for Research Use (# 266-01)

3.1 Preparation for tubing set installation

The window will display screen no. 19 as shown in figure 29.

The instruction is on the right and a diagram corresponding to the instruction is displayed on the left. The blinking features on the screen indicate the areas of attention.

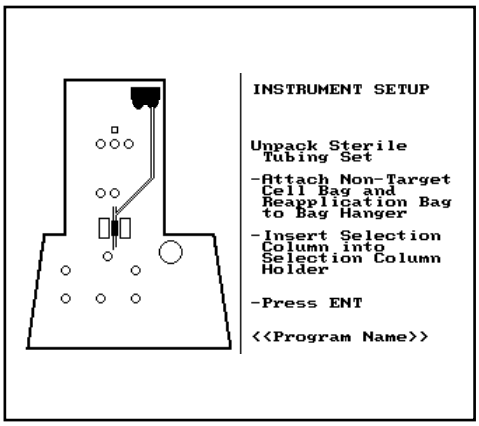


Figure 29: Screen no. 19.

Note

- The CliniMACS Plus Instrument shows the chosen program name, e.g., DEPLETION 3.1, in the bottom line.
- At any step during the tubing set installation the Undo Key can be pushed to return to the previous step.

The CliniMACS® Depletion Tubing Set for Research Use is provided in a sealed, sterilized package. Each tubing set contains pre-assembled tubings, column, and bags for one cell separation (see fig. 32). When the packaging is intact, a sterile fluid path is provided.

1. Record the lot number and use-by date of the tubing set. Unpack the sterile tubing set under sterile conditions (e.g., in a laminar flow hood).
2. Check luer lock connections to bags. Luer lock must be closed tightly.

3.2 Cell Collection Bag

The CliniMACS Depletion Tubing Set for Research Use is provided with an attached Cell Collection Bag.

The weight of the empty Cell Collection Bag attached is 32 g. In case of bag replacement, determine and note the weight of the new empty bag.

3.3 Attach Non-Target Cell Bag, Reapplication Bag, and insert separation column

1. Attach the Non-Target Cell Bag (4) and the Reapplication Bag (5) to the right hand bag hanger on the instrument as shown (see fig. 30).

Note

- The bag hangers are made for a maximum load of 3 kg. Overloading the bag hangers can cause damage to the instrument.

2. Adjust the height of the bag hangers. Raise or lower the hangers to accommodate the height to the size of the Non-Target Cell Bag and Reapplication Bag. Ensure that they are positioned high enough to prevent severe bending of the tubing that could restrict the flow and that it is low enough to avoid the tubing or connections being stretched.

3. Insert the separation column into the separation column holder as shown (see fig. 31).

Note

- To avoid possible pinch injury, insert the separation column as follows: Hold the top and bottom of the column between thumb and index finger, then carefully insert the separation column into the separation column holder.

To proceed, press ENT

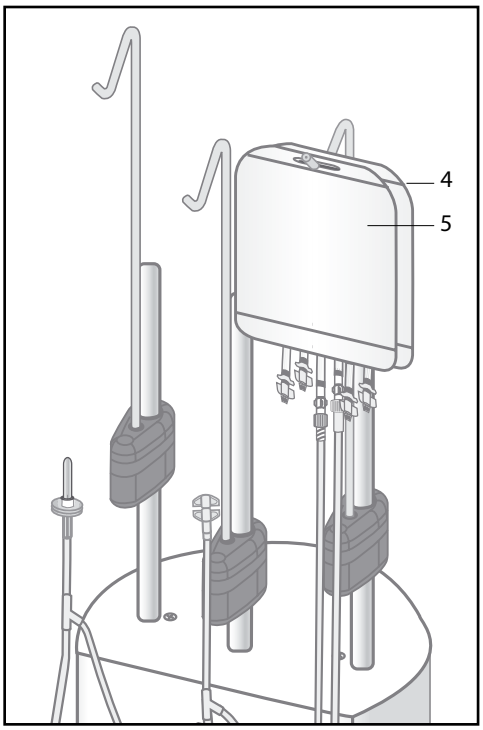


Figure 30: Attach Non-Target Cell Bag (4) and Reapplication Bag (5) to bag hanger.

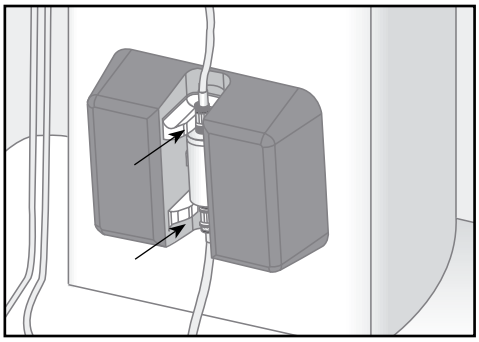


Figure 31: Separation column in separation column holder.

1 Cell Preparation Bag
2 Spike connector
3 Pre-system filter
4 Non-Target Cell Bag
5 Reapplication Bag
6 Check valve
7 Twist-off cap
8 Y-connector
9 Separation column
10 Upper retaining ring
11 Pump tubing
12 Lower retaining ring
13 Buffer Waste Bag
14 Buffer spike
15 T-connector
16 Luer connector
17 Cell Collection Bag

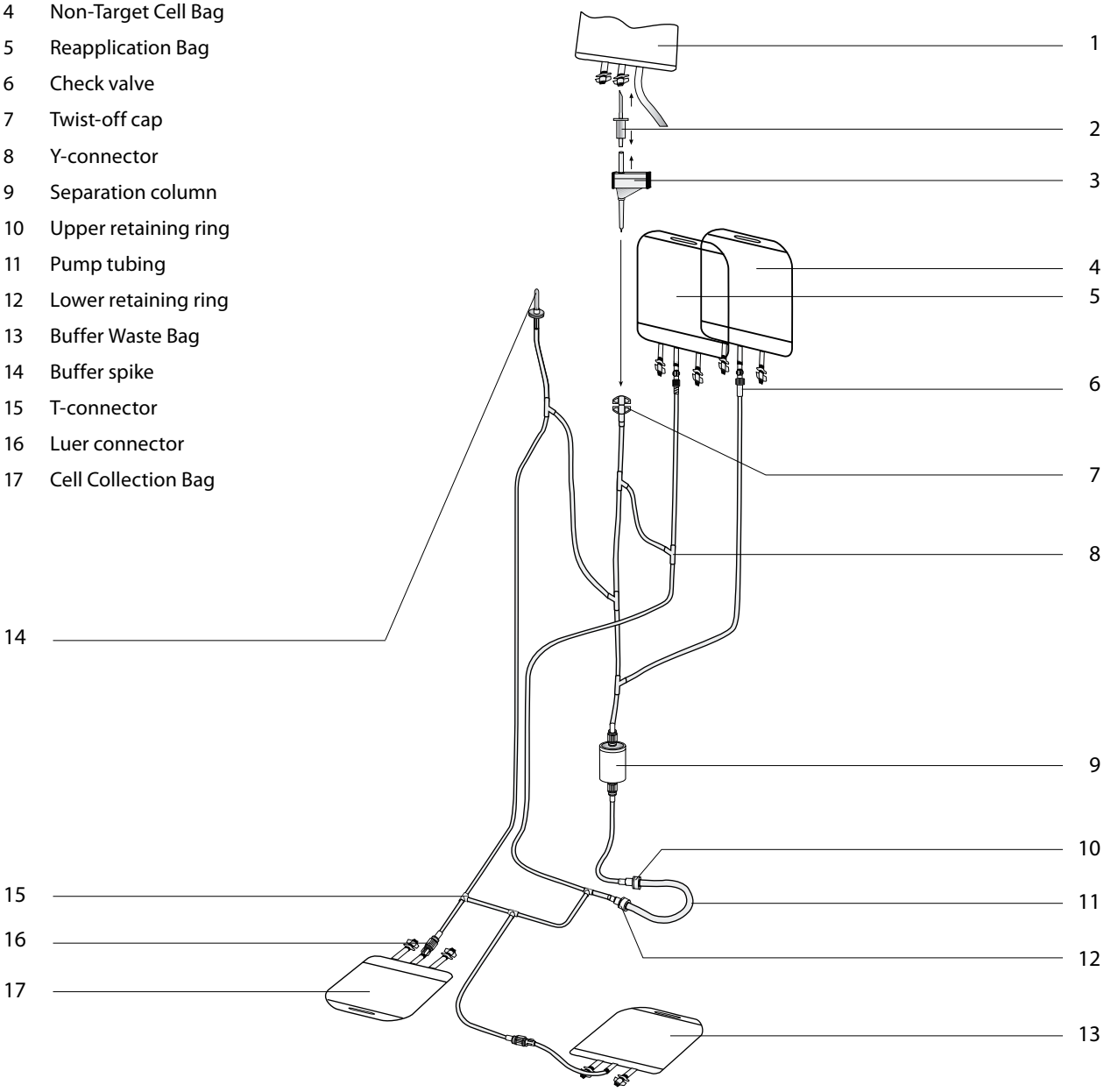


Figure 32: General construction of a CliniMACS® Depletion Tubing Set for Research Use (# 266-01).

3.4 Load valves nos. 1, 2, 3, 4, and 5

The window will display screen no. 20 as shown in figure 33.

The valves shown on the screen will be opened automatically.

1. Load the tubing into valves nos. 1, 2, 3, 4, and 5.
2. Mount the tubing between valve no. 2 and the twist-off cap into the liquid sensor (18, fig. 34). Ascertain that the tubing is placed correctly into the sensor fitting.

Note

- To ensure proper operation, both the liquid sensor and the tubing being inserted MUST BE DRY. Carefully inspect both. If any liquid is present, dry the area with a soft, lint-free cloth.

To proceed, press ENT

Note

- As each step is performed, check all tubing and attachments for any kinks or severe bending that could restrict the flow of liquid through the tubing. Check all valves to ensure the tubing fits snugly.
- Only insert the tubing set into open valves (when button is pushed inwards). The tubing will not fit correctly if inserted into a closed valve.
- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve (see fig. 35).

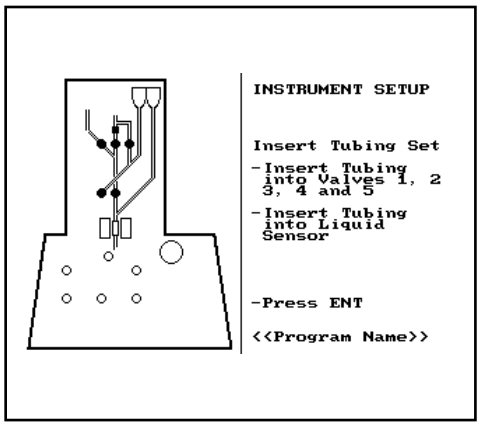


Figure 33: Screen no. 20

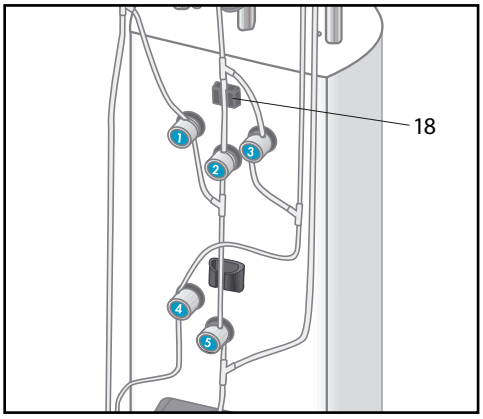


Figure 34: Tubing in valves
18: Liquid sensor

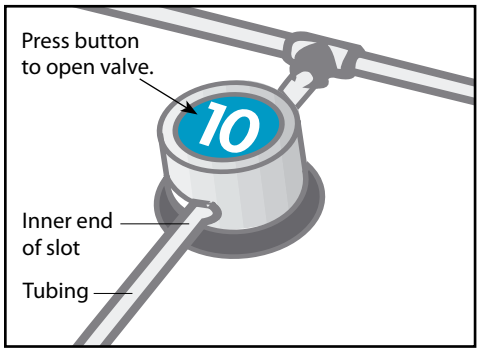


Figure 35: Correctly inserted tubing.

3.5 Load pump tubing

The window will display screen no. 21 as shown in figure 36.

1. Open the pump door by lifting up at the left hand edge.
2. Insert the upper retaining ring on the pump tubing into the retaining ring groove (19) on the pump housing (see fig. 37).
3. Rotate the pump roller clockwise (20, fig. 37) until the tubing is threaded between both sets of the tubing guide pins and the tubing fits snugly around the pump roller. Ensure the tubing is not pinched at the end of the guide pins (If adjustment of the tubing inside the pump is neccessary, the tubing can be unloaded by lifting the lower ending and turning the pump roller anti-clockwise.).
4. Insert the lower retaining ring on the pump tubing into the retaining ring groove (21, fig. 37) on the pump housing.
5. Repeat clockwise rotation of the pump roller, to be certain that the pump roller moves freely.
6. Close the pump door.

Note

- During the cell separation the pump will immediately stop the run whenever the pump housing is opened. If left open for more than 600 seconds the instrument will abort the run in progress.

To proceed, press ENT

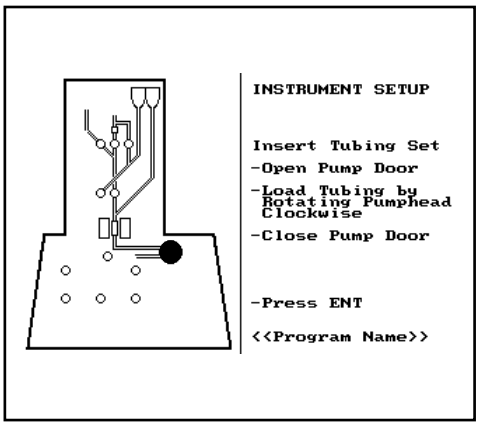


Figure 36: Screen no. 21.

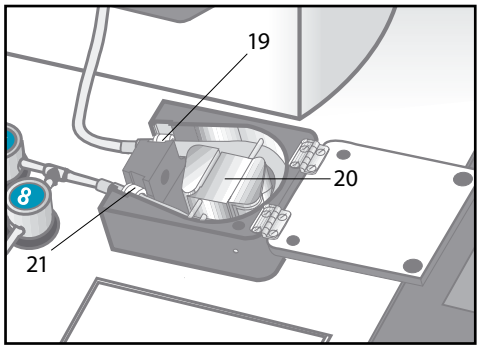


Figure 37: Loading of pump tubing.
19: Upper retaining ring
20: Pump roller
21: Lower retaining ring

3.6 Load valves nos. 6, 7, 8, 9, and 10

The window will display screen no. 22 as shown in figure 38.

1. Load the tubing into valve nos. 6, 7, 8, 9, and 10. Ascertain that the tubing is placed securely in the valve opening.
2. Place the Buffer Waste Bag in the bag compartment. Make sure the tubing is not compressed under the bag compartment lid.

To proceed, press ENT

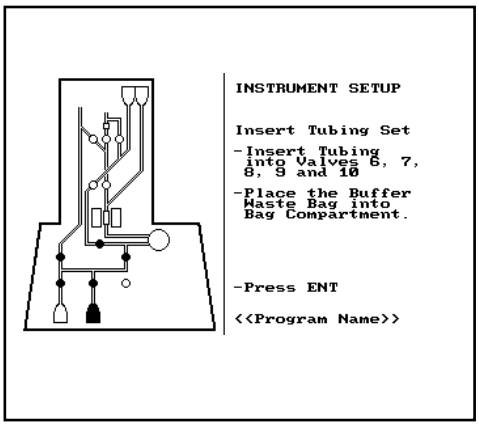


Figure 38: Screen no. 22.

3.7 Recheck all tubing and attachments

The window will display screen no. 23 as shown in figure 39.

1. Beginning with valve no. 1, verify that the tubing fits properly and is positioned in each valve correctly.
2. Re-inspect the tubing in each valve. Be certain that the tubing enters and leaves each valve through the enlargement at the inner end of the slot and is positioned in the center of the jaws of the valve (see fig. 35). Check that the tubing is not kinked or twisted and does not show any tendency to move away from the center of the pinch valve.

Note

- If the tubing has to be adjusted after a valve has been closed, do not pull the tubing without pressing the valve button to open the valve.

To proceed, press ENT

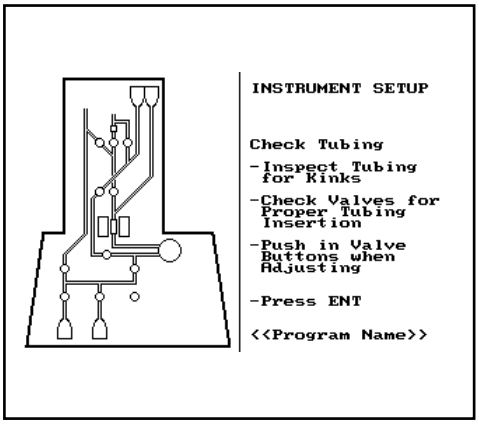


Figure 39: Screen no. 23.

3.8 Seating of valves

The window will display screen no. 24 as shown in figure 40.

In order to ensure the proper fitting of tubing in the valves, the instrument will operate all of the valves in sequence, twice. Watch and listen to make sure all valves are working properly. This step can be repeated by using the Undo Key followed by the Enter Key. If any valve does not operate correctly, refer to troubleshooting section in chapter 5.

The magnet drive will also be tested during this check sequence.

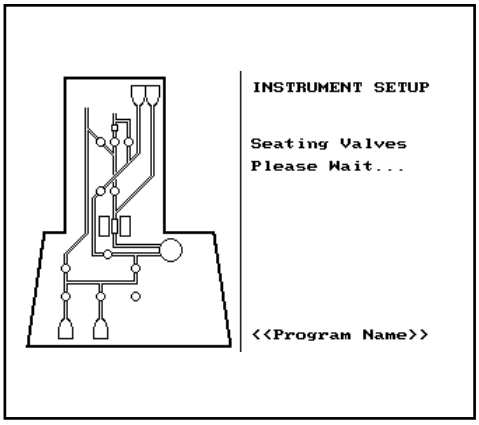


Figure 40: Screen no. 24.

3.9 Attach CliniMACS® PBS/EDTA Buffer for Research Use

The window will display screen no. 25 as shown in figure 41.

The buffer for CliniMACS® Research Separations is CliniMACS PBS/EDTA Buffer for Research Use supplemented with HSA to a final concentration of 0.5% (w/v).

1. Using aseptic techniques, remove the cap from the buffer spike (14, fig. 32) on the tubing set and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag. If more than one liter buffer is necessary for a separation, connect the buffer bags using a plasma transfer set.

2. Attach the buffer bag (24) to the buffer bag hook (23) on the bag hanger (22, fig. 42).

3. Adjust the height of the buffer bag hanger. Raise or lower the hanger to accommodate the size of the buffer bag. Ensure that it is positioned high enough to prevent severe bending of the tubing that could restrict the flow and that it is low enough to avoid the tubing or connections being stretched (see fig. 42).

To proceed, press ENT

Important

- Due to the gravimetric rinsing steps, it is important that the buffer bag is positioned higher than the Reapplication Bag and the Non-Target Cell Bag (see fig. 54).

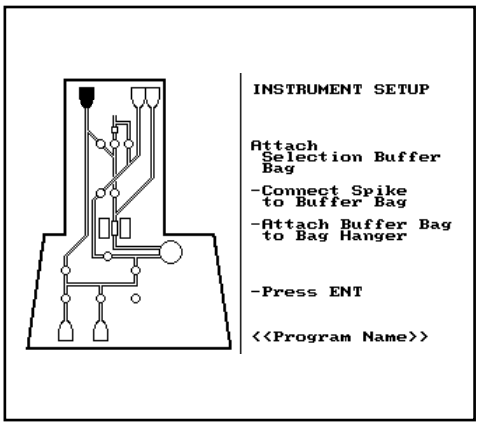


Figure 41: Screen no. 25.

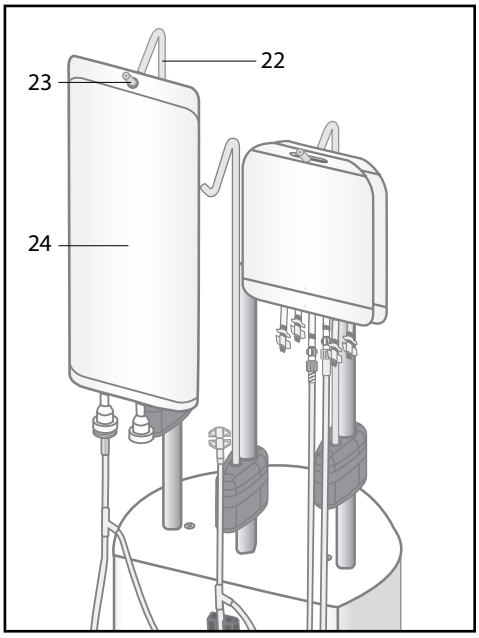


Figure 42: Attach CliniMACS PBS/EDTA Buffer for Research Use to bag hanger.
22: Bag hanger for buffer bag
23: Buffer bag hook
24: CliniMACS PBS/EDTA Buffer for Research Use

3.10 Start priming

The window will display screen no. 26 as shown in figure 43.

To start priming, press RUN

The window will display screen no. 27 as shown in figure 44.

During the priming phase the tubing set is filled with CliniMACS® PBS/EDTA Buffer for Research Use. The buffer will be circulated through the tubing set including the separation column. Priming waste is collected in the Buffer Waste Bag, Reapplication Bag, and the Non-Target Cell Bag (13, 5, and 4, fig. 32). The priming cycles will continue, repeating a series of steps. The priming phase will take approximately 2.5 minutes. Priming status will be updated on the display.

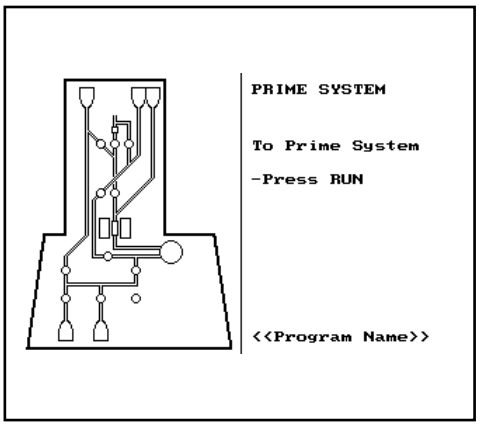


Figure 43: Screen no. 26.

3.11 Check during the priming

During the priming phase, check all tubing, fittings, valves and the separation column for the appearance of any leaks or the presence of any folds that may block fluid flow.

If leaks or malfunctions are observed, stop run by pressing 'STOP'. You will have 600 seconds to resolve the problem. Restart the process by pressing the 'RUN'.

If you cannot resolve the problem or if the CliniMACS Depletion Tubing Set for Research Use is defective, remove the tubing set and replace it with a new one.

Note

- Once priming has started, it is not possible to return to the instrument set-up procedure.

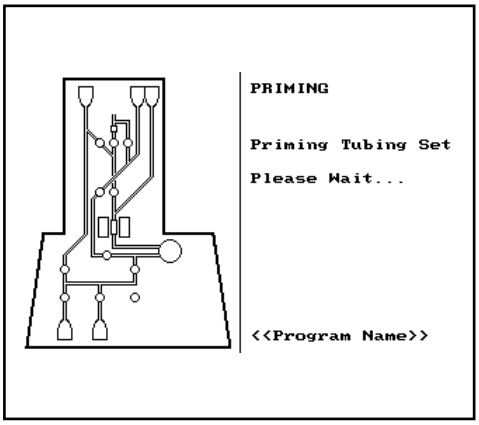


Figure 44: Screen no. 27.

3.12 Final check of all tubing and attachments

The window will display screen no. 28 as shown in figure 45.

Before beginning the run, check the following:

- fluid in all parts of tubing set except for tubing above valves 2 and 3,
- **no** excess air in tubing set,
- fluid in Reapplication Bag, Buffer Waste Bag and Non-Target Cell Bag,
- **no** fluid in the Cell Collection Bag.

DO NOT press 'ENT' yet.

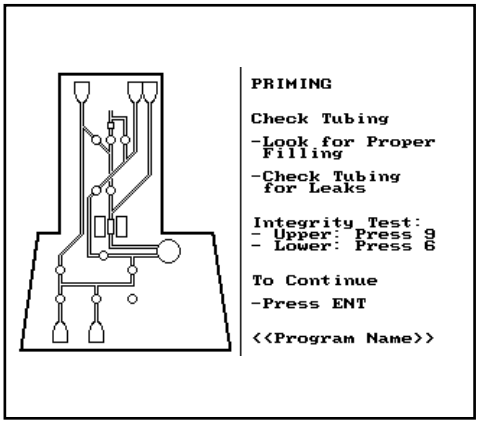


Figure 45: Screen no. 28.

3.13 Integrity test

For additional safety, an integrity test must be performed to test for leakages. The test sequence consists of two automated sequence parts, which allows the upper and the lower part of the tubing set to be tested separately.

3.13.1 Integrity test for the upper part of the tubing set

1. When the operator performs "Final check of all tubing and attachments" the window displays screen no. 28 as shown in figure 45.
2. After finalizing "Final check of all tubing and attachments", DO NOT press 'ENT'.
3. To enter the integrity test for the upper part, press 9
4. The window will display screen no. 29 as shown in figure 46.

Important

- **Clamp the tubing underneath the check valve of the Non-Target Cell Bag.**

5. To start the test sequence, press RUN

   To go back to screen no. 28, press ◀
6. Once the RUN button has been pressed, the instrument starts the automated test sequence for the upper part of the tubing set.

   The window will display screen no. 30 as shown in figure 47.

   Overpressure will be created and held for 2 minutes. During this time the operator should watch the connections above and under the separation column and the upper pump tube connection.

   At each point the test sequence

   can be finished by pressing ENT
7. After 2 minutes the pressure is automatically released and the window displays screen no. 28.

   Using a tissue the operator should now check if any leakages have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Send the defective tubing set back to your Miltenyi Biotec office or to your CliniMACS distributor.
8. If no leakages are observed, continue with the integrity test of the lower part of the tubing set.

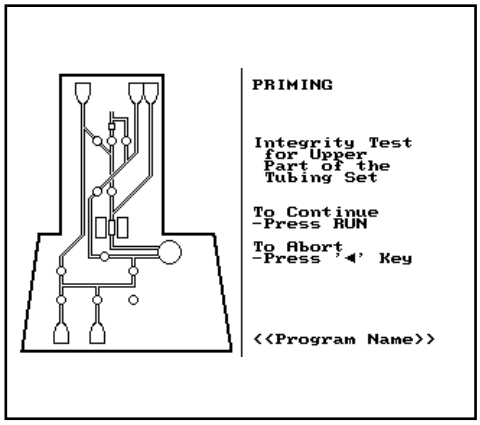


Figure 46: Screen no. 29.

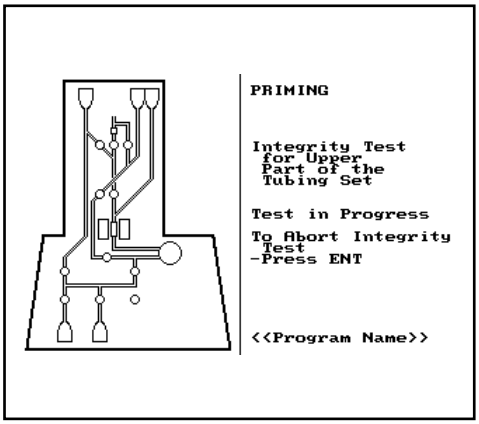


Figure 47: Screen no. 30.

3.13.2 Integrity test for the lower part of the tubing set

1. The window displays screen no. 28 as shown in figure 45.
2. To enter the integrity test for the lower part, press [6]

   DO NOT press 'ENT'.
3. The window will display screen no. 31 as shown in figure 48.
4. To start the test sequence, press [RUN]

   To go back to screen no. 28, press [◀]
5. Once the RUN button has been pressed, the instrument starts the automated test sequence for the lower part of the tubing set.

   The window will display screen no. 32 as shown in figure 49.

   Overpressure will be created and held for 30 seconds. During this time the operator should watch the lower pump tube connection and the T-fittings between valves nos. 6, 8, 9, and 10.

   At each point the test sequence can be finished by pressing [ENT]
6. After 30 seconds the pressure is automatically released and the window displays screen no. 28 as shown in figure 45.

   Using a tissue the operator should now check if any leakages have occurred during the test sequence. If leakage is observed at any connection of the tubing set, the tubing set must be removed and be replaced by a new one. Send the defective tubing set back to your Miltenyi Biotec office or to your CliniMACS® distributor.
7. **Open the pathway to the Non-Target Cell Bag by removing the clamp underneath the check valve.**
8. If no leakages are observed, the operator can now continue with the next step by pressing [ENT]

Important

- Remove the clamp when the integrity test is finished.

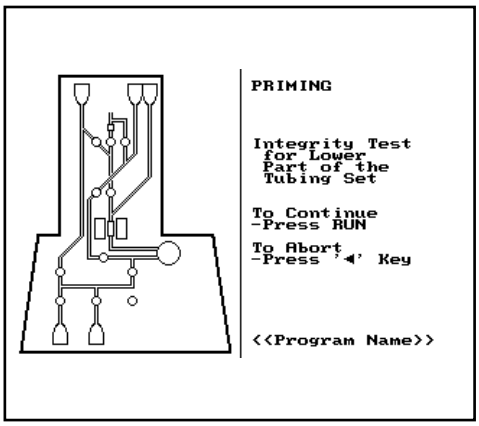


Figure 48: Screen no. 31.

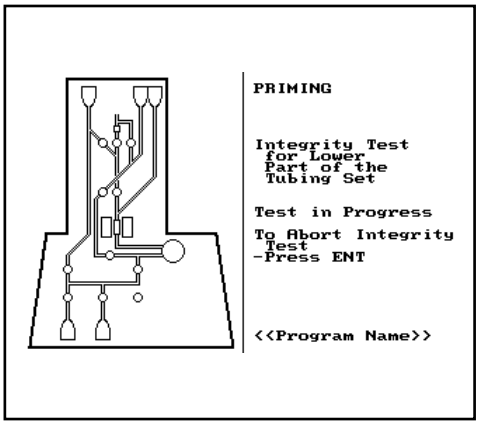


Figure 49: Screen no. 32.

3.14 Connect Cell Preparation Bag

The window will display screen no. 33 as shown in figure 50.

After the priming phase has been completed and no leaks or malfunctions are observed, the Cell Preparation Bag can be attached (see fig. 53). Use aseptic techniques for all steps.

Connect the Cell Preparation Bag containing the magnetically labeled and washed cells with the tubing set:

1. Remove the twist-off cap from the tubing set (7, fig. 32).
2. Remove the cap from the spike of the pre-system filter (3, fig. 32). **Firmly** insert the spike of the pre-system filter into the tubing set, ensuring the septum is punctured.
3. Remove the caps from the pre-system filter (3, fig. 32) and the blunt end of the spike connector (2) and connect both parts.
4. Remove the other cap from the spike connector and connect the spike to the Cell Preparation Bag (1) ensuring that the septum is punctured, allowing free flow of liquid (see fig. 32). Gently squeeze the bag to ascertain that the spike has penetrated the bag.
5. Check the connection between the Cell Preparation Bag, spike connector, the pre-system filter and the tubing set to confirm that the connection is secure.
6. Hang the Cell Preparation Bag on the bag hanger (26, fig. 53).
7. Make sure the bags and tubings attached to the bag hanger are neither stretched nor bent.

   Due to the gravimetric rinsing steps performed by the instrument during the automated separation it is VERY IMPORTANT that the bags are leveled correctly (see fig. 51):

   - **Highest position**: buffer bag,
   - **Middle position**: Reapplication Bag and Non-Target Cell Bag,
   - **Lowest position**: Cell Preparation Bag.

To proceed, press ENT

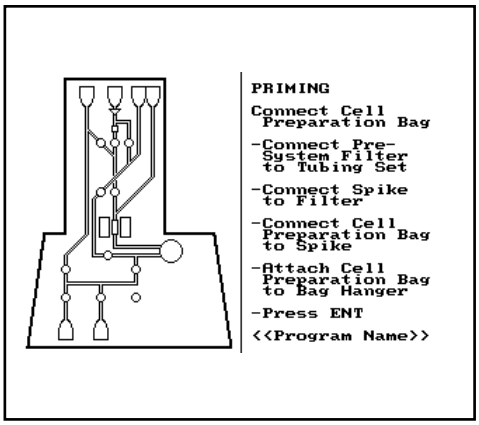


Figure 50: Screen no. 33.

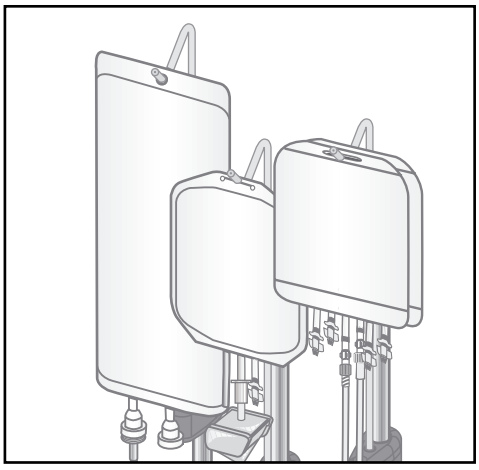


Figure 51: Correctly leveled bags.

3.15 Final check of the liquid sensor

The window will display screen no. 34 as shown in figure 52.

1. Check the liquid sensor tubing. Ensure the tubing has been properly inserted, that it is free of any external liquid and has not been dislodged during the loading procedure.
2. Make sure that unrestricted flow to all bags is possible.

To proceed, press ENT

Proceed to CliniMACS Cell Separation.

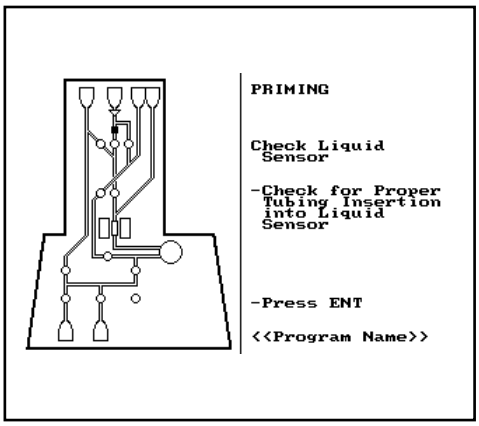
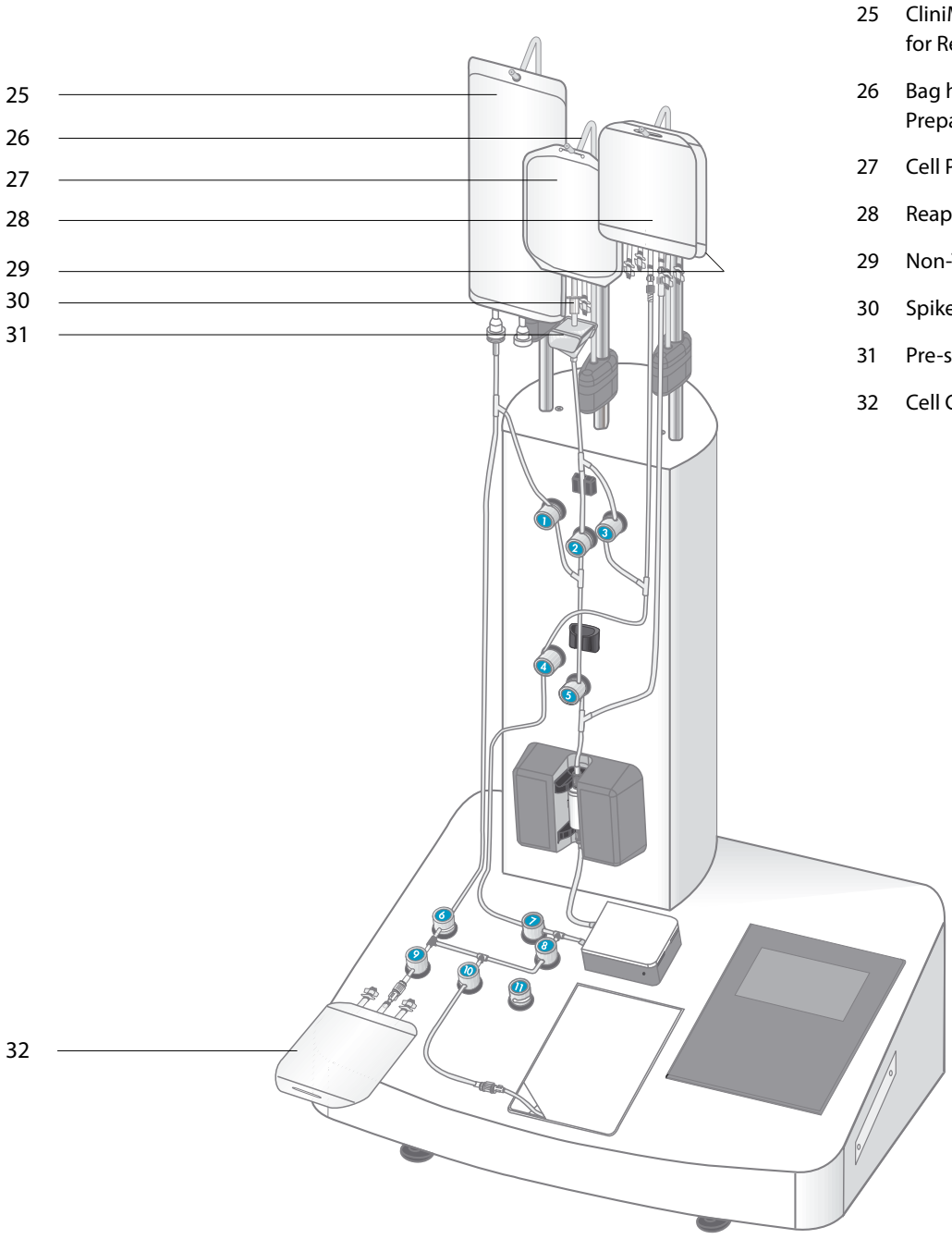


Figure 52: Screen no. 34.



25 CliniMACS PBS/EDTA Buffer for Research Use

26 Bag hanger for Cell Preparation Bag

27 Cell Preparation Bag

28 Reapplication Bag

29 Non-Target Cell Bag

30 Spike connector

31 Pre-system filter

32 Cell Collection Bag

Figure 53: CliniMACS® Plus Instrument with CliniMACS Depletion Tubing Set for Research Use, CliniMACS PBS/EDTA Buffer for Research Use, Cell Preparation Bag, and Cell Collection Bag.

4. Alternative installation of CliniMACS® Tubing Sets for Research Use

The instructions in chapter 2 and the screens displayed by the CliniMACS® Plus Instrument describe the installation of the CliniMACS Tubing Sets for Research Use under sterile conditions (clean room).

The CliniMACS System for research use itself is a closed system which does not necessarily need to be operated in a clean room. However, if operated outside a clean room, the installation procedure of the tubing set needs to be adapted, in order to ensure that the sterility of the cell separation process is guaranteed.

The sterility of the cell separation process is jeopardized during the attachment of the Cell Collection Bag*, the CliniMACS PBS/EDTA Buffer for Research Use, the pre-system filter**, and the Cell Preparation Bag. In order to ensure that the system remains sterile, these components need to be attached to the tubing set under sterile conditions (e.g., in a laminar flow hood). When the components are attached to the tubing set before installation onto the CliniMACS Plus Instrument, the order of the instructions provided by the instrument and the instructions in chapter 2 needs to be changed and further actions taken.

When operating the CliniMACS Plus Instrument outside a clean room, follow the following additional instructions, altering the instructions in chapter 2.

* In contrast, the Depletion Tubing Set is supplied with a pre-connected Cell Collection Bag.

** Please note the different installation directions of the pre-system filter between CliniMACS Tubing Set TS/LS and CliniMACS Depletion Tubing Set, which is triggered by the absence of a bubble trap in the CliniMACS Depletion Tubing Set.

4.1 Preparation for tubing set installation

The window will display screen no. 35 as shown in figure 54.

As described, the Cell Collection Bag, the CliniMACS® PBS/EDTA Buffer for Research Use, the pre-system filter and the Cell Preparation Bag need to be attached to the CliniMACS Tubing Sets for Research Use before installing the tubing set onto the instrument under sterile conditions.

Unpack the tubing set under sterile conditions and attach the following components under sterile conditions:

1. Attachment of Cell Collection Bag

Follow the instructions:

- Attach Cell Collection Bag*

2. Attachment of CliniMACS PBS/EDTA Buffer for Research Use

Clamp the tubing just below the buffer spike with a clamp in order to prevent the buffer from flowing into the tubing set during its installation (see (1), fig. 57). Using aseptic techniques remove the cap from the buffer spike on the tubing set and connect it to the buffer bag. Ensure that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

3. Attachment of pre-system filter

Remove the cap from the spike of the bubble trap. Remove the cap from the lower opening of the pre-system filter.** Firmly insert the spike into the pre-system filter. DO NOT remove the top cap of the pre-system filter. Close the tubing just below the bubble spike using a clamp (see (2), fig. 57). This prevents the prepared cell suspension in the Cell Preparation Bag from entering the pre-system filter.

4. Attachment of Cell Preparation Bag

Connect Cell Preparation Bag containing the magnetically labeled and washed cells to the tubing set. Spike the Cell Preparation Bag with the pre-system filter ensuring that the septum is punctured, allowing free flow of liquid. Gently squeeze the bag to ascertain that the spike has penetrated the bag.

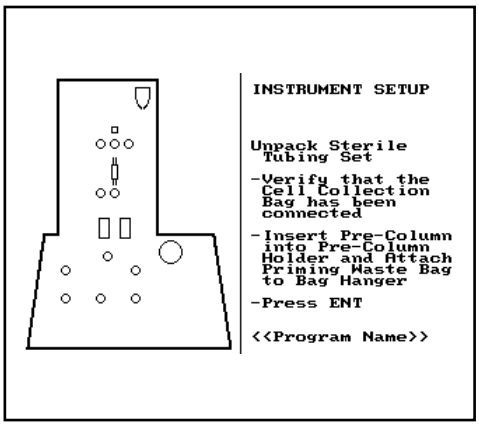


Figure 54: Screen no. 35.

* In contrast, the Depletion Tubing Set is supplied with a pre-connected Cell Collection Bag.

** Please note the different installation directions of the pre-system filter between CliniMACS Tubing Set TS/LS and CliniMACS Depletion Tubing Set, which is triggered by the absence of a bubble trap in the CliniMACS Depletion Tubing Set.

5. Installation on the CliniMACS Plus Instrument

Attach the buffer bag to the left bag hanger, the Cell Preparation Bag to the middle bag hanger and the Priming Waste Bag to the right bag hanger on the instrument.

To proceed, press ENT

Follow the instructions:

- Attach Priming Waste Bag and insert pre-column
- Insert separation column and load valve no. 5
- Load valves nos. 1, 2, 3, and 4
- Load pump tubing
- Load valves nos. 7 and 8
- Load valves nos. 6, 9, 10, and 11
- Recheck all tubing and attachments
- Seating of valves

4.2 Attach CliniMACS® PBS/EDTA Buffer for Research Use

The window will display screen no. 36 as shown in figure 55.

1. The buffer bag has been attached during „Preparation for tubing set installation“. Therefore, only the height of the buffer bag hanger has to be adjusted. Raise or lower the hanger to accomodate the size of the buffer bag, ensuring that the height allotted is high enough to prevent the tubing from severe bending that could restrict the flow, and low enough to avoid streching the tubing or connections.
2. Remove the clamp from the tubing just below the buffer spike.

To proceed, press ENT

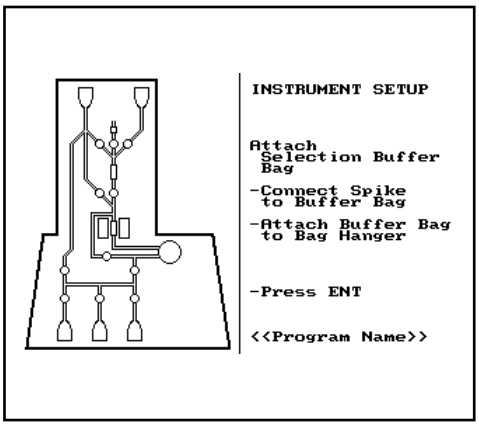


Figure 55: Screen no. 36

Follow the instructions:

- Start priming
- Check during priming
- Final check of all tubing and attachments
- Integrity test

4.3 Connect Cell Preparation Bag (and pre-system filter)

The window will display screen no. 37 as shown in figure 56.

1. The Cell Preparation Bag and the pre-system filter have been attached during „Preparation for tubing set installation“.
2. Remove the clamp below the bubble spike.

To proceed, press ENT

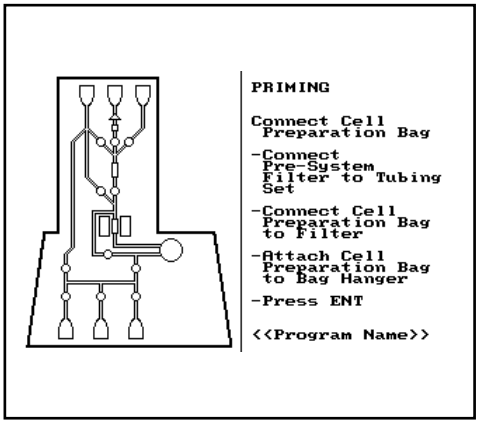


Figure 56: Screen no. 38.

Follow the instructions:

- Final check of the liquid sensor

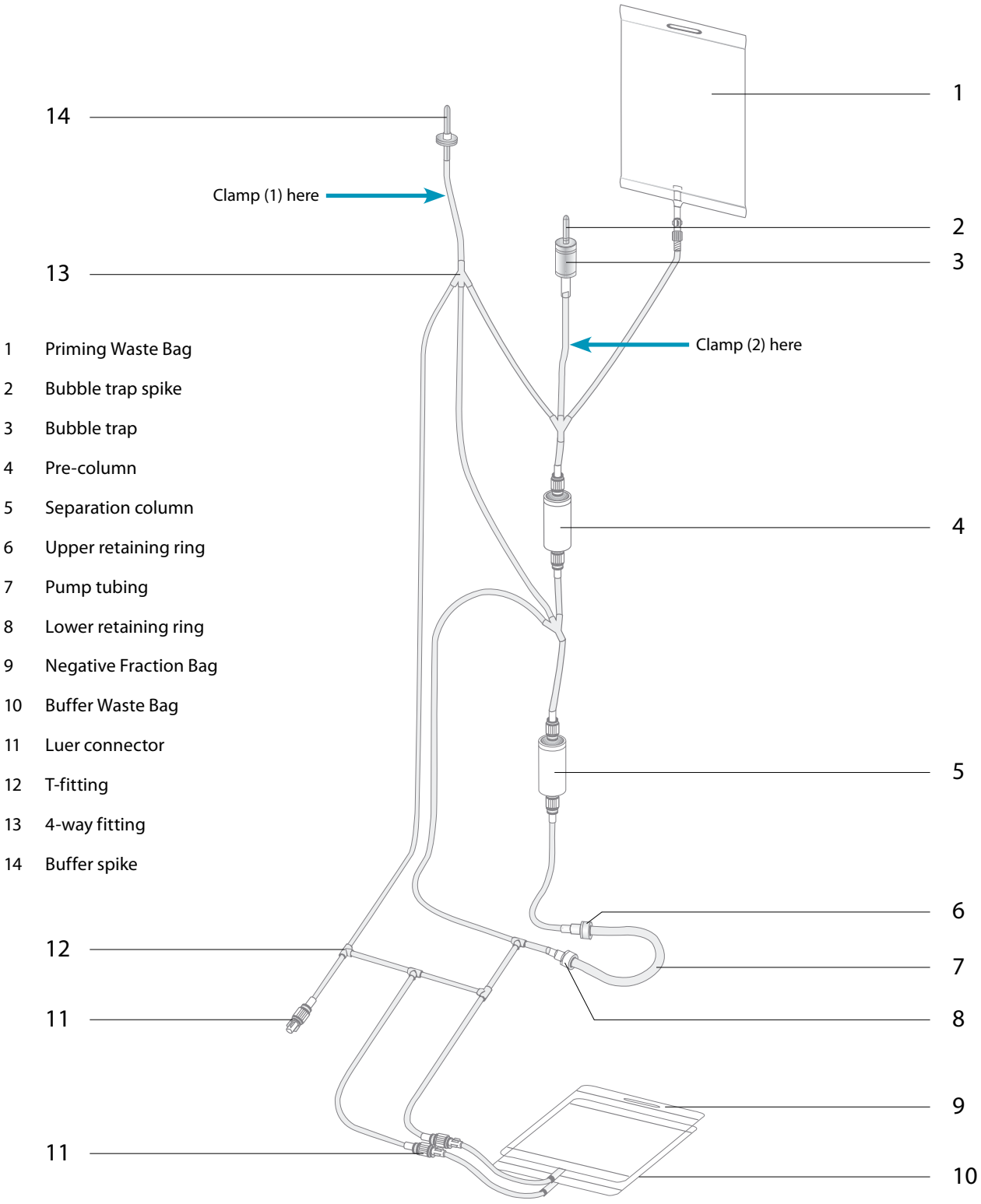


Figure 57: General construction of a CliniMACS® Tubing Set for Research Use (e.g., CliniMACS Tubing Set for Research Use, # 165-01).

5. Troubleshooting

This section is intended as a reference to provide information about possible unexpected events that might occur during loading and priming of the tubing set and to suggest appropriate corrective action. For information not covered in the following section, please contact our Clinical Technical Support as soon as possible.

Valve does not open when operator is instructed to insert tubing into a particular valve.	The valves are designed to work properly once the tubing has been inserted. Press the valve manually to open it. Watch the valve carefully during the valve exercise sequence. If the valve does not depress during the valve exercise sequence, refer to section „Valve does not depress during valve exercise sequence.“
Valve does not depress during valve exercise sequence.	Make sure that tubing is correctly inserted. Check whether valves have been cleaned thoroughly. Any valve that has been contaminated by fluid has to be exchanged. Please contact the Clinical Technical Service.
Buffer is leaking from tubing set after priming.	Tubing set is defective. Turn off the CliniMACS Plus Instrument and restart priming with a new tubing set installed and sufficient new buffer.
Excessive air occurs in tubing set after priming.	Buffer bag is not properly spiked. Use a new tubing set and sufficient new buffer and restart the CliniMACS run. Make sure that the septum of the buffer bag is properly punctured.
Unexpected volume of buffer in bags after priming. After priming, liquid should only be in the Priming Waste Bag and Buffer Waste Bag.	Tubing set is not mounted correctly. Liquid can leak behind the valves if the tubing set is not installed correctly or the valves are not functioning properly. Remove the tubing set and replace it with a new one. Restart the priming procedure with sufficient new buffer. Poor CliniMACS Separation performance may result if the tubing set is not inserted properly.
Pump motor stalls during priming.	Pump tubing has not been inserted correctly. Press “STOP“ to interrupt the priming and turn the power “OFF“ and then “ON“ again. Clamp the buffer line with a hemostat during the installation procedure and remove the clamp before restarting the priming sequence.

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CliniMACS® CD34 Reagent System

Rx only

For *in vitro* use only.

HUMANITARIAN DEVICE:

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 use has not been demonstrated.

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INDICATIONS FOR USE

The CliniMACS® CD34 Reagent System is indicated for processing hematopoietic progenitor cells collected by apheresis (HPC, Apheresis) from an allogeneic, HLA-identical, sibling donor to obtain a $CD34^+$ cell-enriched population for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft versus host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first morphologic complete remission.

CONTRAINDICATIONS

Do not use $CD34^+$ cells prepared with CliniMACS® CD34 Reagent System in patients with known hypersensitivity to murine (mouse) proteins or iron-dextran.

DESCRIPTION

The CliniMACS® CD34 Reagent System is a medical device system that consists of the following components:

- CliniMACS® CD34 Reagent - dark amber, non-viscous, colloidal solution containing an antibody conjugate in buffer. The conjugate consists of a murine IgG_1 monoclonal antibody directed against the Class II epitope of the human CD34 antigen, which is chemically conjugated to dextran beads having an iron oxide/hydroxide core. (See the CliniMACS® CD34 Reagent Package Insert for more information.)
- CliniMACS® plus Instrument - software-controlled instrument that processes the HPC, Apheresis. (See the CliniMACS® User Manual for the CD34 Reagent System for more information.)
- CliniMACS® Tubing Set TS or Tubing Set LS - a single-use, sterile, disposable tubing set with two proprietary cell separation columns. The CliniMACS® Tubing Set TS is for processing HPC, Apheresis preparations containing up to 0.6×10^9 $CD34^+$ cells out of a total cell number not exceeding 60×10^9 white blood cells. The CliniMACS® Tubing Set LS is for larger scale preparations containing up to 1.2×10^9 $CD34^+$ cells out of a total cell number not exceeding 120×10^9 white blood cells. (See the CliniMACS® Tubing Sets Package Insert and the CliniMACS® User Manual for the CD34 Reagent System for more information.)
- CliniMACS® PBS/EDTA Buffer - a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the *in vitro* processing of HPC, Apheresis. (See the CliniMACS® PBS/EDTA Buffer Package Insert and the CliniMACS® User Manual for the CD34 Reagent System for more information.)

PRINCIPLES OF OPERATION

The CliniMACS® CD34 Reagent System is an *in vitro* medical device system used to select and enrich $CD34^+$ cells from HPC, Apheresis while passively depleting other cells, such as $CD3^+$ T cells, which cause graft versus host disease. The system is based on "magnetically-activated cell sorting" (MACS) employing antibodies conjugated to iron-containing particles that can be attracted to a magnetic field (referred to as "magnetic labeling"). Using the specificity of anti-CD34 antibody interaction with cell surface CD34 antigen found on hematopoietic progenitor cells, the system enriches $CD34^+$ cells from HPC, Apheresis by passing the antibody-labeled cell suspension through a separation column with a strong magnetic gradient. The separation column retains the magnetically labeled $CD34^+$ target cells while unlabeled cells flow through and are collected in the Negative Fraction Bag. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically-selected $CD34^+$ cells are released from the separation column when the magnet is disengaged, removing the magnetic field, and the target $CD34^+$ cells are eluted into the Cell Collection Bag.

WARNINGS

- Do not infuse the CliniMACS® CD34 Reagent or the CliniMACS® PBS/EDTA Buffer into patients directly.
- Hypersensitivity Reactions

  Hypersensitivity reactions, including anaphylaxis, have been observed during infusion of $CD34^+$ cells from the CliniMACS® CD34 Reagent System. Monitor the patient for hypersensitivity reactions, including anaphylaxis, during infusion of $CD34^+$ cells from the CliniMACS® CD34 Reagent System.
- Engraftment failure

  Failure to infuse an adequate number of functioning $CD34^+$ cells can result in engraftment failure. Collect sufficient HPC, Apheresis to yield at least 2.4×10^6 $CD34^+$ cells per kg of patient body weight after system processing (see Device Performance below). The clinical trial (see Clinical Performance below) using the CliniMACS® CD34 Reagent System to process HPC, Apheresis did not test allografts with less than 2.4×10^6 $CD34^+$ cells per kg of recipient body weight. Monitor patients for laboratory evidence of hematopoietic recovery after transplantation.
- Acute and chronic graft versus host disease (GVHD)

  GVHD can occur in patients who receive HPC, Apheresis processed using the CliniMACS® CD34 Reagent System. Use pharmacologic prophylaxis if more than 1×10^5 $CD3^+$ cells per kilogram of recipient body weight are infused.
- Delayed immune reconstitution after transplantation

  Removal of T cells from the HPC, Apheresis can delay immune reconstitution after transplantation. Patients who receive the $CD34^+$ cell-enriched population prepared using the CliniMACS® CD34 Reagent System are at risk for serious opportunistic viral infections, including post-transplant lymphoproliferative disorder caused by Epstein-Barr virus (EBV) and cytomegalovirus (CMV). Monitor for EBV and CMV in the peripheral blood of patients after transplantation and initiate appropriate treatment promptly.

PRECAUTIONS

- Safety and probable benefit in children under the age of 17 years have not been established.
- Drugs may be incompatible with the CliniMACS® PBS/EDTA Buffer. Do not add drugs to the buffer other than Human Serum Albumin as specified in the CliniMACS® User Manual for the CD34 Reagent System.
- Do not use cryopreserved and thawed HPC, Apheresis because cryopreservation promotes cell clumping, which may lead to device performance issues. Process HPC, Apheresis as soon as available, but not longer than 24 hours after collection.
- Use only HPC, Apheresis from an allogeneic, HLA-identical sibling donor with the CliniMACS® CD34 Reagent System.
- Collect HPC, Apheresis according to standard hospital or institutional leukapheresis procedures in standard leukapheresis collection bags. Do not include additional anticoagulants or blood additives, such as heparin, other than those normally used during leukapheresis. Keep the HPC, Apheresis at controlled room temperature (+19 °C to +25 °C (67° to 77° F)) if it has to be stored, e.g., overnight, before processing. Do not allow the concentration of leukocytes to exceed 0.2×10^9 cells per mL.
- Only trained operators should use the CliniMACS® CD34 Reagent System to prepare $CD34^+$ cells for infusion. Operator training is provided by Miltenyi Biotec authorized personnel.

PROCEDURES

Refer to the CliniMACS® User Manual for the CD34 Reagent System for complete instructions. An on-line version is available at www.cd34-aml.com/usermanual.

Do not connect the CliniMACS® CD34 Reagent System to the patient at any time.

Collect the following data on the leukapheresis product before starting the preparation of the HPC, Apheresis for enrichment:

- Total number of leukocytes
- Percentage of $CD34^+$ cells
- Total number of $CD34^+$ cells
- Percentage of $CD3^+$ cells
- Total number of $CD3^+$ cells
- Viability

The $CD34^+$ cells are enriched in the following four steps:

Step 1 - Immunomagnetic Labeling of $CD34^+$ Hematopoietic Progenitor Cells

Add the CliniMACS® CD34 Reagent to the HPC, Apheresis and allow the reagent to bind to the $CD34^+$ cells. After incubation, remove unbound reagent from the suspension. The cells are now ready for selection in an automated continuous flow selection process using the CliniMACS® plus Instrument.

NOTE: Exceeding the capacity for either total cell number or $CD34^+$ cell number may impact the performance of the device. The standard-scale capacity for the enrichment of $CD34^+$ cells using the CliniMACS® CD34 Reagent System with one vial of CD34 Reagent and the CliniMACS® Tubing Set TS is 0.6×10^9 $CD34^+$ cells out of a total cell number not exceeding 60×10^9 cells. Large-scale capacity for the enrichment of up to 1.2×10^9 $CD34^+$ cells out of a total cell number of 120×10^9 cells (large-scale application) requires two vials of the CliniMACS® CD34 Reagent and the CliniMACS® Tubing Set LS.

Step 2 - Choice of Program

Choose CD34 Selection 1/2 program on the CliniMACS® plus Instrument.

Step 3 - Installation of Tubing Set

- Install tubing set (TS or LS).
- Attach Cell Collection Bag to the tubing set.
- Follow the prompts provided on the CliniMACS® plus Instrument screen to complete the tubing set installation.
- Join the bag containing the cells from Step 1 to the sterile CliniMACS® Tubing Set.

Step 4 - Selection of CD34+ Cells

Process the cells through the CliniMACS® plus Instrument using the CD34 Selection program. The instrument selects cells by passing the immunomagnetically-labeled suspension through the separation column, in which strong magnetic gradients are generated. The separation column retains the immunomagnetically-labeled $CD34^+$ target cells while unlabeled cells flow through and are collected in the Negative Fraction Bag. Automated washing steps dispose of excess liquid into the Buffer Waste Bag. The retained $CD34^+$ cells are released from the separation column when the magnet is disengaged, and the target $CD34^+$ cells are eluted into the Cell Collection Bag.

Establish the suitability of the target $CD34^+$ cells before infusion. Examine the following parameters:

- Total number of leukocytes
- Total viability
- Total number of $CD34^+$ cells
- Total number $CD3^+$ cells
- Purity and recovery of $CD34^+$ cells
- CD3 log depletion

See Table 1 for performance values observed in the clinical trial.

Assessment of the non-target fraction for the total number and viability of leukocytes is recommended to assess the performance of the device and quality of the device output ($CD34^+$ cells).

DO NOT infuse the $CD34^+$ cells in CliniMACS® PBS/EDTA Buffer! Exchange the CliniMACS® PBS/EDTA Buffer contained in the $CD34^+$ target fraction to a clinical grade infusion solution appropriate for infusion into humans prior to infusion of the $CD34^+$ cells.

See the CliniMACS® User Manual for the CD34 Reagent System for full instructions. The CliniMACS® User Manual for the CD34 Reagent System includes instructions on the preparation of solutions and samples, as well as a detailed list of equipment and materials that are required for $CD34^+$ cell selection. (See Chapter 3 of the CliniMACS® User Manual for the CD34 Reagent System.)

PERFORMANCE CHARACTERISTICS

Adverse Reactions

The safety of the CliniMACS® CD34 Reagent System was evaluated in a clinical trial that included 44 subjects with AML undergoing HPC, Apheresis transplantation from an HLA-identical sibling donor.[1] There were 16 males and 28 females of median age 49 years (range, 21 to 60 years). The myeloablative preparative regimen included total body irradiation, thiotepa, cyclophosphamide, and rabbit antithymocyte globulin. The median number of $CD34^+$ cells infused was 7.9×10^6 per kg (range 2.4 to 30.4). The median number of $CD3^+$ cells infused was 0.07×10^5 per kg (range 0.01 to 0.83).

Among the 44 subjects, there were no grades 3 to 5 infusion reactions, no allergic reactions, and no graft failures. Testing for development of human anti-mouse antibodies (HAMA) was not performed. A severe or life-threatening infection was reported for 38% of the subjects. An infection by any virus was reported for 61% of the subjects, and the 1-year incidence of EBV infection in particular was 25%. One subject (2%) developed a fatal post-transplantation lymphoproliferative disorder.

Device Performance

The safety and feasibility of use of the CliniMACS® CD34 Reagent System was evaluated in a multicenter, single-arm, clinical trial. In this study, allogeneic donors were mobilized with daily subcutaneous granulocyte colony-stimulating factor (G-CSF) at a dose of 10 to 16 µg per kg per day. Leukapheresis was performed on a continuous flow cell separator commencing on Day 5 of G-CSF treatment, and $CD34^+$ cell enrichment of the HPC, Apheresis was performed using the CliniMACS® CD34 Reagent System. Most donors underwent at least two, but not more than three, apheresis to reach the post-selection enrichment target of greater than 5.0×10^6 $CD34^+$ cells per kg recipient body weight while maintaining less than 1.0×10^5 $CD3^+$ cells per kg recipient body weight.

Eighty-four selection procedures were performed on HPC, Apheresis collected from a total of 44 donors. The minimum number of $CD34^+$ cells required for transplantation, greater than 2×10^6 per kg recipient body weight, was achieved for 100% (44) of donors. This was attained with one apheresis for 93% (41) of the donors and two apheresis for an additional 7% (3). The target number of $CD34^+$ cells, greater than 5×10^6 per kg recipient body weight, was achieved for 84% (37) of the 44 donors. This target number was attained with one apheresis for 36% (16), with two apheresis for 45% (20), and with three apheresis for 2% (1) of the 44 donors. Device performance is shown in the table below.

Table 1: Device Performance Summary; N=84

Attributes Measured		Mean	Std Dev	Median	Min	Max
Starting TNC $\times 10^{10}$		7.46	3.26	6.95	2.1	18.0
Initial Viability (%)		97.60	2.74	99.0	86.9	100.0
$CD34^+$ Cells $\times 10^7$	Starting Count	59.71	41.09	47.85	7.3	208.0
	Final Count	36.90	25.05	29.80	6.1	119.0
Final $CD34^+$ Yield (%)		66.06	20.25	65.00	29.9	125.6
Final $CD34^+$ Purity (%)		93.03	8.31	96.65	61.5	99.8
$CD3^+$ T Cells $\times 10^8$	Starting Count	179.45	69.80	168.50	55.00	362.00
	Final Count	0.00652	0.01039	0.00217	0.00026	0.04971
Log_{10} $CD3^+$ T-Cell Depletion		4.78	0.55	4.90	3.2	5.9
Final Viability (%)		96.57	3.84	97.70	74.0	100.0
Total $CD34^+$ Cells Infused/kg $\times 10^6$		8.81	5.21	7.924	2.41	30.360
Total $CD3^+$ Cells Infused/kg $\times 10^6$		0.015	0.020	0.0066	0.0011	0.08328

Clinical Performance

The clinical trial included 37 subjects with AML in first complete remission (CR) undergoing transplantation. All donors were HLA-identical siblings. The study subjects included 14 (37.8%) males and 23 (62.2%) females of median age 48 years (range: 21 to 60 years). The cytogenetics risk group was intermediate for 68%, unfavorable for 27%, and unknown for 5% of subjects. The myeloablative preparative regimen included total body irradiation, thiotepa, cyclophosphamide, and rabbit antithymocyte globulin. The median number of $CD34^+$ cells infused was 7.4×10^6 per kg recipient body weight (range: 2.4 to 30.4). The median number of $CD3^+$ cells infused was 0.07×10^5 per kg recipient body weight (range: 0.01 to 0.63). No immunosuppressive drugs were administered for prevention of GVHD.

All subjects achieved an absolute neutrophil count that exceeded 0.5×10^9 per liter by Day 21 after transplantation. The platelet count recovered to greater than 20×10^9 per liter by Day 100 for 91.9% (95% CI, 82.4 to 100%). There was one late graft failure. At Day 100 after transplantation, the cumulative incidence of grades 2 to 4 acute GVHD was 27% (95% CI, 14 to 42%), and that for grades 3 to 4 acute GVHD was 5% (95% CI, 1 to 16%). The cumulative incidence of chronic GVHD at 2 years after transplantation was 19% (95% CI, 8 to 33%).

Additional Safety Assessment

The potential risks of using the CliniMACS® CD34 Reagent System were evaluated via a Data Analysis Protocol (DAP), which retrospectively compared the Day-100, 1-year, and 2-year endpoints in the clinical trial to the same endpoints as had been measured in a historical control group of patients that had used a conventional pharmacologic method of GVHD prophylaxis. Table 2 shows the results of the comparison.

Table 2: Comparison of the Single-Arm CliniMACS® CD34 Reagent System Study to historical controls using pharmacological immunosuppression

Endpoints	Single-Arm CliniMACS® CD34 Reagent System (n=37) % (95% CI)	Historical Controls Using pharmacological immunosuppression (n=65) % (95% CI)
% Neutrophil Engraftment at Day 30 (>500/µL)*	100	100+
% Platelet Engraftment at Day 30 (>20,000/µL)*	92 (82.4, 100)	84#(72.5, 91.4)
Acute GVHD at Day 100, Grades 2–4*	27 (13.9, 42.0)	35 (23.9, 47.0)
Acute GVHD at Day 100, Grades 3–4*	5 (1, 16.1)	9 (3.7, 17.8)
Chronic GVHD at 2 years*	19 (8.2, 33.0)	49 (36.5, 61.0)
Relapse Rate at 2 years*	16 (6.5, 29.9)	28 (17.7, 39.7)
Non-relapse Mortality at 2 years*	20 (8.5, 34.5)	14 (6.8, 23.4)
Overall Survival at 2 years	67 (48.8, 79.7)	67 (54.1, 77.2)
Disease-free Survival at 2 years	64 (46.0, 77.4)	58 (44.8, 68.9)
GVHD-free Survival at 2 years	46 (29.6, 60.9)	18 (9.6, 28.2)

* Cumulative Incidence
\+ neutrophil engraftment data missing for two patients
\# Platelet data missing for one patient

REFERENCES

1 Devine SM, Carter S, Soiffer RJ, Pasquini MC, Hari PN, Stein A, Lazarus HM, Linker C, Stadtmauer EA, Alyea EP 3rd, Keever-Taylor CA, O'Reilly RJ. Low Risk of Chronic Graft-versus-Host Disease and Relapse Associated with T Cell-Depleted Peripheral Blood Stem Cell Transplantation for Acute Myelogenous Leukemia in First Remission: Results of the Blood and Marrow Transplant Clinical Trials Network Protocol 0303. Biology of Blood and Marrow Transplantation 2011;17:1343-1351.