AutoMACS User Guide

Starting Up

1. Check that all bottles are filled with the appropriate solutions. Empty the waste bottle and add 100ml bleach to the bottom.

Bottle	Symbol	User action
Running buffer		Green: no action required Red: refill bottle Grey: connect bottle sensor
Washing Solution		Green: no action required Red: refill bottle Grey: connect bottle sensor
Storage Solution		Gray: No liquid detection possible, check visually
Waste		Green: no action required Red: refill bottle, Grey: connect bottle sensor

2. Switch on the autoMACS Pro Separator- the instrument will automatically initialize and prime.
3. After priming, the instrument will automatically display the Status menu. Confirm that the status is ready by ensuring components are green.

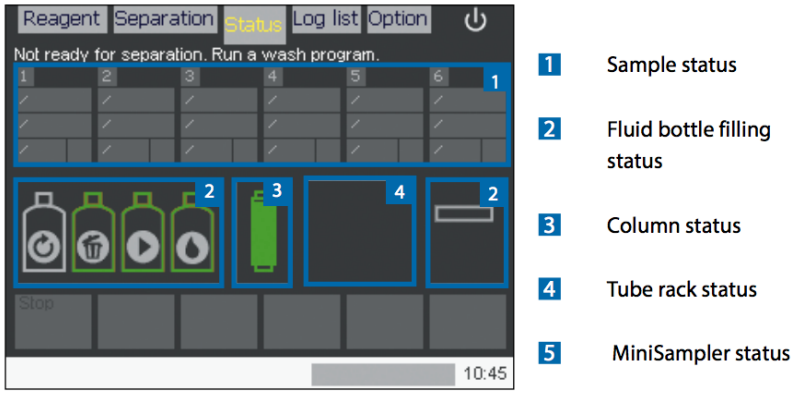


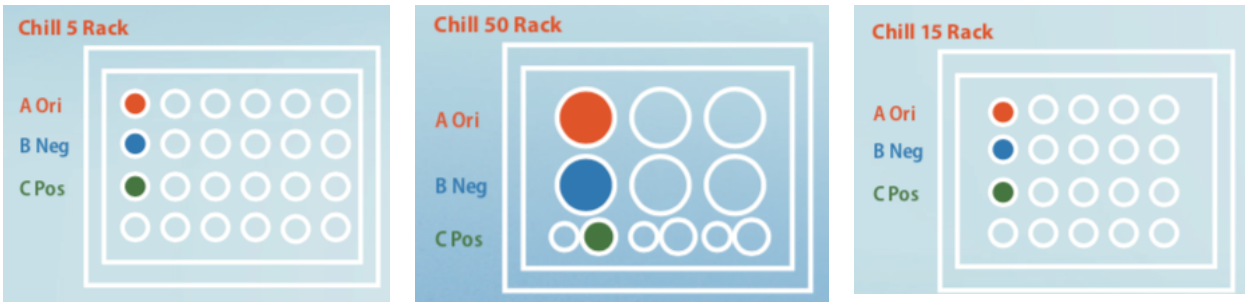
Figure 4.1: The status menu.

4. Confirm that the fluid container status is ready, the column status is ready, and the MiniSampler has been detected and is correctly installed.
5. Refill any fluid bottles that appear red.
6. Refer to pg. 6 under "autoMACS Short Instructions" to calculate approximately how much fluid will be needed depending on the number of samples being run.
7. If the column status appears red, refer to the "Column Exchange" section on pg. 4.
8. The instrument is now ready to perform an experiment.
9. Turn on the blower in the hood.

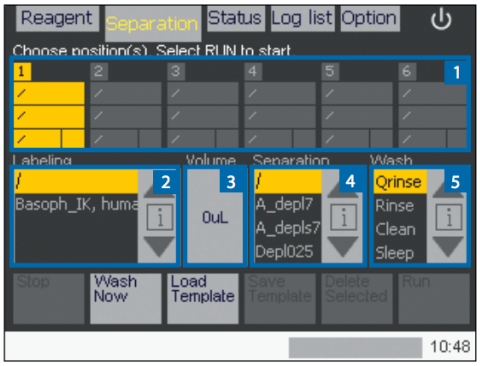
Performing Manual Labeling and Cell Separation

1. Ensure that the appropriate Chill Rack is pre-cooled to 4°C.

2. Dilute single-cell suspension according to the recommendations in the respective product data sheet. See info sheet below on pg. 6.
3. Place the sample tubes into row A of a pre-cooled Chill Rack. Load empty tubes into the corresponding positions along rows B and C. Row A is for the original sample fraction. Row B is for the negative (untouched) fraction. Row C is for the positive (enriched) fraction.



4. Place the sample rack onto the MACS MiniSampler and click on the **Separation** menu.



1 Sample Rack template
2 Sample labeling options
3 Sample processing volume
4 Separation program
5 Wash program

Figure 4.12: The **Separation** menu.

1 5. Define the sample rack template for cell separation. Select the desired positions in the sample separation template field. Assign a corresponding cell separation program using the Separation menu.
6. It is not mandatory to assign a volume for cell separation with manual labeling, however, the autoMACS Pro Separator requires this information to calculate and display the total sample processing time.
7. Use the Wash menu to assign a wash step between cell separation steps.
   Notice: It is recommended that a QRinse be run between samples if running more than one. Refer to pg. 3 under "Cleaning and Shutting Down" for more information.
8. Select "Run" to start the cell separation experiment and click "OK" to confirm that enough buffer is available for the experiment.
9. Monitor the cell separation experiment.

Performing Autolabeling and Cell Separation (If using Miltenyi antibody reagents)

1. Choose an appropriate Chill Rack and ensure it is pre-cooled to 4°C. Racks are automatically depleted by the AutoMACS Pro Separator.

2. Dilute single-cell suspension according to recommendations in the respective product data sheet. See info sheet below on pg. 6.
3. Place the sample tubes into row A of a pre-cooled Chill Rack. Load empty tubes into the corresponding positions along rows B and C. Row A is for the original sample fraction. Row B is for the negative (untouched) fraction. Row C is for the positive (enriched) fraction.
4. Insert the MACS Reagent Rack 4 into the MACS MiniSampler.
5. Scan reagent vials. On the "Reagent" menu, select "Read Reagent" and present a reagent vial in front of the reader.
6. After successfully scanning a reagent vial, the software will automatically highlight the next available reagent rack position. Insert the reagent vial into the correct rack position.
7. Place the sample rack onto the MACS MiniSampler and click on the Separation menu.
8. Define the sample rack template for cell separation. Highlight the desired position on the sample separation template and assign and autolabeling protocol from the Labeling menu. To assign a corresponding sample volume click on "Volume" and insert the sample volume. Select "Enter."
9. Select "Run" to start the cell separation experiment and click "OK" to confirm that enough buffer is available for the experiment. Check pg. 6 for recommended buffer volumes.
10. Monitor the cell separation experiment.

Cleaning and Shutting Down

1. It is possible to change the rinse mode between samples or to program the instrument to go into sleep mode after finishing the experiment.

Qrinse – Standard short wash program that only uses Running Buffer
It is recommended to use this program between separations of cells with normal frequency.

Rinse – An extensive rinsing program that uses Washing Solution and Running Buffer. It is recommended to use this program between separations of rare cells, e.g., stem cells, the separation of cells from different species, and is mandatory between whole blood separations.

Clean – An optional, very extensive rinsing program
It uses storage solution, Washing Solution, and Running Buffer. It may be used after whole blood and bone marrow applications. Clean has to be enabled first to appear in the **Wash** menu.

Sleep – Mandatory as the last wash program before overnight storage
Upon completion of this program, the fluidic system contains 70% ethanol.

2. The instrument must be set in Sleep mode for overnight storage. To do this from the main menu, Press the shutdown symbol. Alternatively, select the Sleep program as the last washing step. Upon completion, turn off the instrument.
3. Clean and replace the Chilled Rack in the fridge after shutdown.

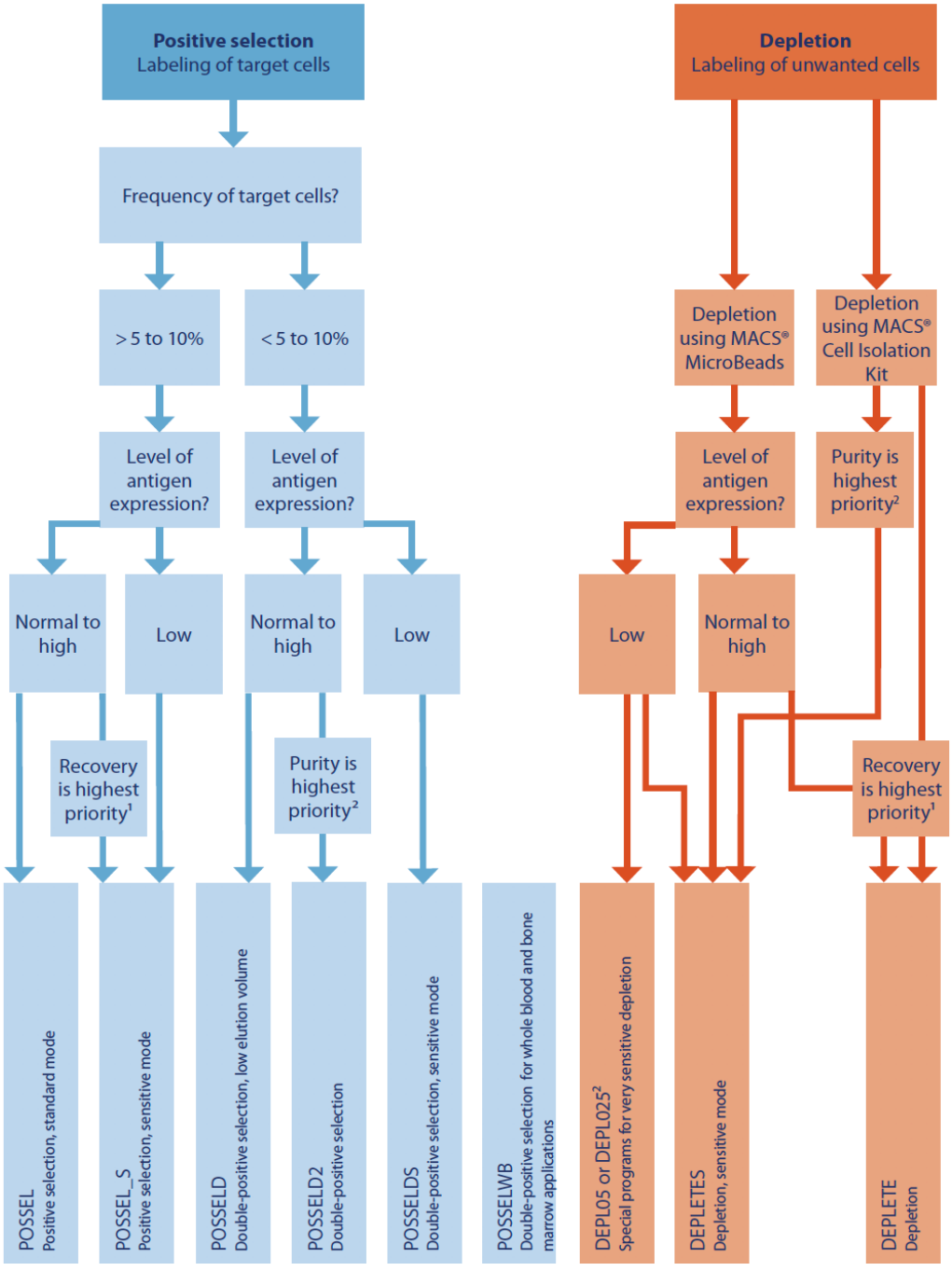
Column Exchange

1. Replace autoMACS columns every two weeks or after 100 runs.

2. Go to Option > Special.
3. Select **Col_ex** from the **Detail** panel.
4. Press Run.
5. When prompted, exchange the column.
6. Open front door and note the positions of the columns. Exchange one column at a time.
7. Remove column from slot, unscrew bottom column connector followed by the top column connector.
8. Dispose of the expired column in the Biohazard waste.
9. Point the bottom of the fresh column towards the autoMACS Pro Separator.
10. Insert bottom column connector. Screw in the column by turning it clockwise. Repeat the procedure for the top column connector.
11. Push column into the magnet housing, with the top column connector sitting on the guide in the column slot.
12. Repeat installation for the second column.

Creating a Template

1. Click on the **Separation** tab, and configure the sample template.
2. Press **Save Template.**
3. Allocate a name to the template.
4. Press **Ok.**



[1]Purity will slightly decrease

[2] Recovery will slightly decrease

Figure 6.1: Selecting the optimal separation program

autoMACS® Pro Separator

Short instructions

Chill rack specifications

Rack type and symbol	Slots	Maximal number of samples	Manual labeling	Autolabeling	
			Maximal sample volume	Minimal first incubation volume	Maximal final labeling volume
Chill 5 	24×5 mL	6 (5 mL tubes)	2.5 mL	0.2 mL 0.25 mL*	2.0 mL 1 mL*
Chill 15 	15×15 mL 5×5 mL	5 (15 mL tubes)	12.5 mL	0.2 mL 1 mL*	6.5 mL 4 mL*
Chill 50 	6×50 mL 3×15 mL 3×5 mL	3 (50 mL tubes)	50 mL	4 mL*	8 mL*

* Volumes refer to whole blood samles only.

Daily maintenance and rinsing programs

Program	Description	Recommended usage	Duration
Qrinse	Standard short rinse of separation columns and tubing system with Running Buffer	Between separations of frequent cells (>5 %)	1.5 min
Rinse	Rinse of separation columns and tubing system with Washing Solution and Running Buffer	Prior to first separation	4 min
Clean	Rinse of Rinse of separation columns and tubing system with storage solution, Washing Solution, and Running Buffer	After whole blood and bone marrow applications.	7 min
Sleep	Rinse with Washing Solution followed by filling with storage solution	Before switching off the autoMACS Pro Separator	5 min

Buffer consumption

Program	Washing Solution	Running Buffer	Storage solution	MACS Bleach Solution	Time
Qrinse	–	48 mL	–	–	1.5 min
Rinse	96 mL	48 mL	–	–	4 min
Clean	96 mL	48 mL	48 mL	–	7 min
Sleep	96 mL	–	48 mL	–	5 min
Safe	96 mL	96 mL		40	21 min
Store	96 mL	–	96 mL	–	8 min
Col_ex	96 mL	96 mL	–	–	6 min

Periodic maintenance

Action	Description	Recommended usage	Duration
Column exchange using (**Col_ex** program)	Replacement of separation columns	Every two weeks OR after 100 separations, whichever comes first	6 min
Running the **Safe** program	Decontamination procedure with MACS Bleach Solution	Every 3–6 months	21 min
Cleaning the pump syringe	Cleaning of pump syringe (refer to user manual)	Every 1–3 months	
Running the **Store** program	Rinse with Washing Solution, followed by storage solution; replacement of columns with substitutes	Before storing the instrument for a period longer than two weeks	

autoMACS® Pro Separator

Short instructions – sample dilution

Cell Separation Reagent	Strategy	No. of reagents	Dilution volume	Autolabeling			
				Minimal volume*	Minimal total cell number	Maximal volume	Maximal total cell number
Chill 5 Rack[1]							
Direct MicroBeads human, rat, non-human primate	Positive selection or depletion	1	10^7 cells per 80 µL	160 µL	$2×10^7$	1600 µL	$2×10^8$
Direct MicroBeads, mouse	Positive selection or depletion	1	10^7 cells per 90 µL	180 µL	$2×10^7$	1800 µL	$2×10^8$
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	0.25 mL		1 mL	
Cell Isolation Kits	Untouched isolation	2	10^7 cells per 40 µL	160 µL	$4×10^7$	800 µL	$2×10^8$
Cell Isolation Kits	Untouched isolation	3	10^7 cells per 30 µL	120 µL	$4×10^7$	600 µL	$2×10^8$
Chill 15 Rack[2]							
Direct MicroBeads human, rat, non-human primate	Positive selection or depletion	1	10^7 cells per 80 µL	160 µL	$2×10^7$	5200 µL	$6.5×10^8$
Direct MicroBeads, mouse	Positive selection or depletion	1	10^7 cells per 90 µL	180 µL	$2×10^7$	5850 µL	$6.5×10^8$
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	1 mL		4 mL	
Cell Isolation Kits	Untouched isolation	2	10^7 cells per 40 µL	160 µL	$4×10^7$	2600 µL	$6.5×10^8$
Cell Isolation Kits	Untouched isolation	3	10^7 cells per 30 µL	120 µL	$4×10^7$	1950 µL	$6.5×10^8$
Chill 50 Rack[3]							
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	4 mL		8 mL	

[1] Max. number of samples: 6; min. first incubation volume: 0,2 mL; max. final labeling volume: 2 mL
[2] Max. number of samples: 5; min. first incubation volume: 0,2 mL; max. final labeling volume: 6,5 mL
[3] Max. number of samples: 3; min. first incubation volume: 4 mL; max. final labeling volume: 8 mL.
* When working with fewer cells than the necessary minimal volume, resuspend cells in the stipulated minimal volume.