Miltenyi Biotec

autoMACS® Pro Separator

Short instructions

Version 02

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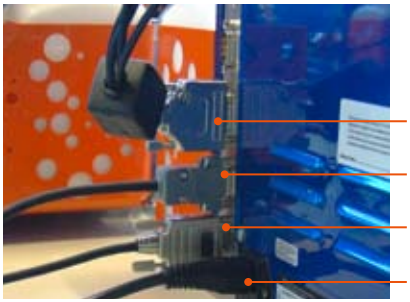
Version 02

1 Setting up and priming the autoMACS® Pro Separator

1.1 Check that all bottles are filled with the appropriate solutions. Empty the waste bottle.

1.2 Check that the MACS® MiniSampler, Code Reader, and Fluid Sensor cables are correctly attached to the back of the instrument.



1.3 Switch on the autoMACS Pro Separator—the instrument will automatically initialize.

Note: To prime the instrument select **Wash Now** from the **Separation** menu.

After priming, the instrument will automatically display the Status menu.

Figure 1.1: Location of the on/off switch.

1.4 Confirm that the instrument status is ready by viewing the Status menu.

Overview of the Status menu

1 Sample status
2 Fluid container filling status
3 Column status
4 Tube rack status
5 MiniSampler status

1.4.1 Confirm that the fluid container status is ready.

Left: Fluid containers are shown in green indicating ready.
Right: Fluid containers are shown in red and need replaced.

Status of fluid containers displayed in the Status menu

Container	Symbol	Symbol color and user action
Running Buffer		Green: no action required Red: refill container Gray: connect bottle sensor
Washing Solution		Green: no action required Red: refill container Gray: connect bottle sensor
Storage solution		Gray: no liquid detection; visually check volume
Waste		Green: no action required Red: empty waste or wrong sensor cable is connected Gray: connect bottle sensor

1.4.2 Confirm that the column status is ready.

Status of the columns:
Green: no action required
Red: exchange column
Grey: when no column has been installed

1.4.3 Confirm that the MiniSampler has been detected and is correctly installed.

Symbol		Features
	MACS MiniSampler detected	No action required
	No sampler detected	Connect MACS MiniSampler

1.5 The instrument is now ready to perform an experiment.

2 Performing autolabeling and cell separation

2.1 Choose an appropriate Chill Rack and ensure is it pre-cooled to 4 °C.

2.1.1 Racks are automatically detected by the autoMACS Pro Separator. Rack types and their features are tabulated below.

Rack type & symbol	Slots	Maximum number of samples	Minimum first incubation volume	Maximum final labeling volume	Maximum number of cells per tube
Chill 5 	24×5 mL	6 (5 mL tubes)	0.2 mL	2.0 mL	$2.0–4×10^8$ depends on cell labeling concentration and column capacity
			0.25 mL	1 mL	Whole blood only
Chill 15 	15×15 mL 5×5 mL	5 (15 mL tubes)	0.2 mL	6.5 mL	$6.5×10^8–1.3×10^9$ depends on cell labeling concentration and column capacity
			1 mL	4 mL	Whole blood only
Chill 50 	6×50 mL 3×15 mL 3×5 mL	3 (50 mL tubes)	4 mL	8 mL	Whole blood only

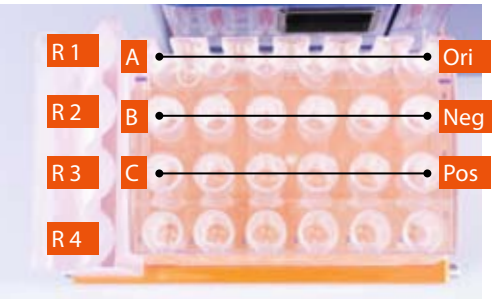
Note: Dilute cells in the volume required for the first labeling step.

2.2 Dilute single-cell suspension according to recommendations in the following table.

MACS Product	Strategy	Reagents	Cell concentration	Minimal volume*	Minimal absolute cell number
Direct MicroBeads - human - rat - non-human primate	Positive selection or depletion	1	10^7 cells per 80 µL	160 µL	2.0×10^7
Direct MicroBeads - mouse	Positive selection or depletion	1	10^7 cells per 90 µL	180 µL	2.0×10^7
Whole Blood MicroBeads	Whole blood or bone marrow	1	Original volume	0.25 mL – 8 mL	
Cell Isolation Kits	Untouched selection	2	10^7 cells per 40 µL	160 µL	4.0×10^7
Cell Isolation Kits	Untouched selection	3	10^7 cells per 30 µL	120 µL	4.0×10^7
MicroBead Kits	Positive selection or depletion	2	10^7 cells per 60 µL	120 µL	2.0×10^7

* When working with fewer cells than the necessary minimal volume, resuspend cells in the stipulated minimal volume.

2.3 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 i.e. “Ori”.
Row B is for the negative (“untouched“) fraction i.e. “Neg”.
Row C is for the positive (enriched) fraction i.e. “Pos”.
Reagent Rack positions are also shown (R1–R4).

2.4 Insert the MACS Reagent Rack 4 onto the MACS MiniSampler.

The MACS Reagent Rack snaps into position as illustrated above.

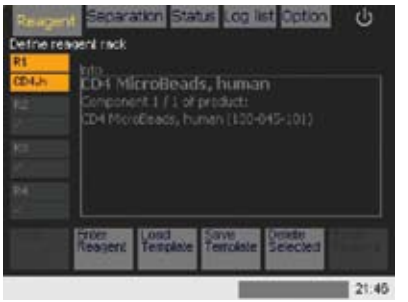
2.5 Scan reagent vials.

2.5.1 On the “Reagent” menu, select “Read Reagent” and present a reagent vial in front of the 2D code reader. Ensure the 2D code is facing the blinking code-reader light.

The optimal reading distance is 0.5–2.5 cm from the code reader cover, tilt the vial as shown above.

2.5.2 After successfully scanning a reagent vial, the software will automatically highlight the next available reagent rack position.

To view details about the scanned reagent vial, highlight the appropriate rack position. This is R1 in the example below.



2.5.3 Insert the reagent vial into the correct rack position.

2.6 (Optional) Manual entry of reagents

This is only recommended if the reagent cannot be identified by the barcode reader.

Please turn over.

2.6.1 Select “Reagent” menu and highlight the position where the vial will be placed on the reagent rack. Four positions are available: R1, R2, R3, and R4.

2.6.2 Select “Enter Reagent” from the lower navigation bar. Enter the reagent-specific product order number.

The order number is located on the product data sheet. In the event that the data sheet is misplaced, visit www.miltenyibiotec.com to download a printable PDF of the document.

2.6.3 If a correct product number is entered the software will immediately recognize and list the reagent or kit components.

To confirm your choice, touch the listed reagent (✓).
The reagent will be assigned to a rack position and the next available reagent rack position will be automatically highlighted. Repeat the procedure for any remaining reagents or kit components.
Finally, select “OK“ to complete reagent entry.

The NK Cell Isolation Kit, mouse was entered.

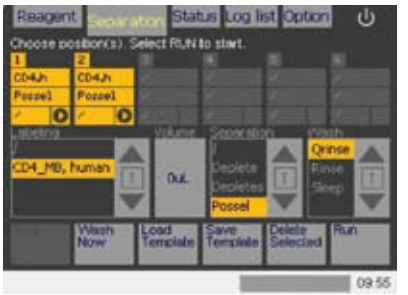
2.7 Place the sample rack onto the MACS MiniSampler and click on the Separation menu.

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

2.8 Define the sample rack template for cell separation.

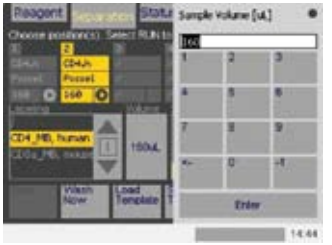
2.8.1 Highlight the desired position(s) on the sample separation template and assign an autolabeling protocol from the Labeling menu.

The recommended cell separation and wash program will be automatically displayed. To change these settings highlight the desired cell separation and wash options using the Separation and Wash menus, respectively.



2.8.2 To assign a corresponding sample volume click on “Volume” and insert the sample volume. Select “Enter”.

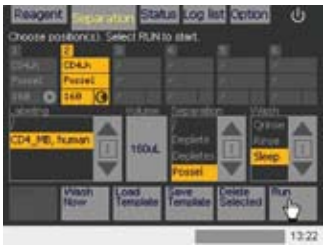
Refer to step 2.2 above for information about using the correct volume.



Tip: It is possible to change the rinse mode between samples or to instruct the instrument to go into sleep mode after finishing the last sample:

Qrinse - ; Rinse - ; Sleep - . Refer to section 3.5.4 for a more comprehensive explanation.

2.9 Select “Run” to start the cell separation experiment and click “OK” to confirm that enough buffer is available for the experiment.



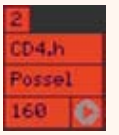
CD4 positive cell separation will be performed on sample positions 1 and 2. Clicking “Run” will start the experiment. The last wash step is Sleep, thus a full rinse will be performed before the machine goes into sleep mode.

2.10 Monitor the cell separation experiment

2.10.1 View the status of the fluid containers (refer to step 1.4.1).

2.10.2 Use the Status menu display to view the overall instrument status.

Sample processing statuses are shown as color-coded graphics. The table below shows an example of an autolabeling experiment performed using CD4 MicroBeads, human.

	Graphic	Definition	Graphic	Definition
1		Status: Waiting. Sample processing has not yet started.		Rinsing.
2		Sample autolabeling is underway.		Sample processing is completed.
3		Incubation of cells with labeling reagents.		Progress has been stopped or cancelled.
4		Sample is being processed, e.g., sample uptake.		

2.10.3 Check bottle illuminations, even from across the laboratory.

The autoMACS Pro Separator has a bottle illumination that facilitates monitoring of the instrument's status.

Code	Status	User action
Green	Ready for separation	No action required.
Blue	Instrument operating	No action required.
Yellow	Not ready for separation	Run wash program (Rinse or Qrinse) before starting a separation.
Red	Error	Check screen for error detection.
Purple	Program Sleep is completed	Switch off autoMACS Pro Separator.
Blinking	Action required	Check screen for required action.

2.11 (Optional) Switch the instrument off for long-term storage.

Note: To store the autoMACS Pro Separator for a period longer than two weeks run the Store program.

Note: For daily usage the instrument should not be switched-off but placed into SLEEP mode. See section 4.

1.11.1 Select “Option” and “Special”.

1.11.2 Select “Store” and press “Run”.

1.11.3 Replace the columns with column substitutes (refer to section 3.3.4 of the User Manual).

1.11.4 Select “Done”.

1.11.5 Switch off the autoMACS Pro Separator using the main power switch.

3 Performing manual labeling and cell separation

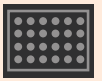
3.1 Ensure that the appropriate Chill Rack is pre-cooled to 4 °C.

3.1.1 Choose an appropriate Chill Rack

Racks are automatically detected by the autoMACS Pro Separator. Rack types and their features are tabulated below.

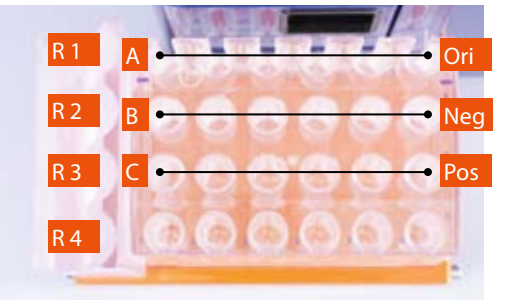
3.1.2 Dilute single-cell suspension according to the recommendations in the respective product data sheet.

Visit www.miltenyibiotec.com/protocols to download product data sheets and protocols.

Rack type & symbol	Slots	Maximum number of samples	Maximum sample volume	Maximum number of total cells per tube
Chill 5 	24×5 mL	6 (5 mL tubes)	2.5 mL	5.0×10^8
Chill 15 	15×15 mL 5×5 mL	5 (15 mL tubes)	12.5 mL	2.5×10^9
Chill 50 	6×50 mL 3×15 mL 3×5 mL	3 (50 mL tubes)	50 mL	4.0×10^9

3.3 Place the sample tubes into row A of the pre-cooled Chill Rack. Place empty tubes in the corresponding rows B and C; these are for the negative and positive cell fractions, respectively.

For example, a Chill 5 Rack has six available positions: A1–A6.



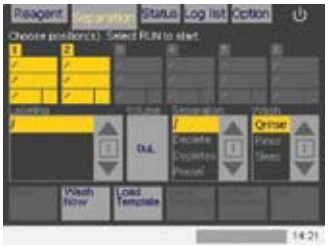
Row A is for the original sample fraction i.e. “Ori”.
Row B is for the negative (“untouched”) fraction i.e. “Neg”.
Row C is for the positive (enriched) fraction i.e. “Pos”.
Reagent Rack positions are also shown.

3.4 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 procedure

3.5 Define the sample rack template for cell separation

3.5.1 Select the desired position(s) in the sample separation template field.



Tip: Select multiple sample positions to program them simultaneously.

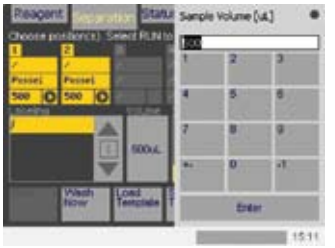
3.5.2 Assign a corresponding cell separation program using the Separation menu.

Positive selection programs:
Possel—Isolation of cells with normal antigen expression and frequencies higher than 5%; select if purity is the highest priority.
Possel_s—Isolation of cells with low antigen expression and frequencies higher than 5%; select if yield is the highest priority.
Posseld—For isolation of rare cells in low elution volume.
Posselds—For isolation of rare cells with low antigen expression.
Posseld2—For isolation of rare cells if purity is the highest priority.
Posselwb—For isolation of cell subsets from whole blood. Cell samples are automatically diluted with Running Buffer.

Depletion programs:
Deplete—For removal of cells with normal to high antigen expression and results in better target cell yield.
Depletes—Removal of cells with low antigen expression and results in better target cell purity.
Depl05—Removal of cells with low antigen expression and results in stringent depletion of cells.
Depl025—Removal of cells with low antigen expression and results in stringent depletion of cells.
A_Depl07—Removal of cells with normal to high antigen expression and results in a better target cell yield. This special program is disabled by default. To enable select “Option“, “User settings“, and “O_progs“.
A_Depls7—Removal of cells with low antigen expression and results in a better target cell purity. This special program is disabled by default. To enable select “Option“, “User settings“, and “O_progs“.

3.5.3 (Optional) 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.

To assign a corresponding sample volume click on “Volume“ and insert the sample volume. Select “Enter“.



3.5.4 Using the Wash menu assign a wash step between cell separation steps.

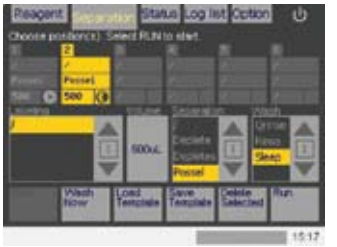
It is possible to change the rinse mode between samples or to program the instrument to go into sleep mode after finishing the experiment:

Qrinse - : Standard short wash program that only uses Running Buffer.

Rinse - : Extensive rinsing program that uses Washing Solution and Running Buffer.

Sleep - : It is mandatory to use Sleep as the last wash program before overnight storage. Upon completion of the Sleep program, the fluidic system contains 70% ethanol.

3.6 Select “Run“ to start the cell separation experiment and click “OK“ to confirm that enough buffer is available for the experiment.



Standard positive cell separation (Possel) will be performed on sample positions 1 and 2. Clicking “Run“ will start the experiment. As the last wash step is Sleep, a full rinse will be performed before the machine goes into sleep mode.

3.7 Monitor the cell separation experiment.

3.7.1 Use the Status menu display to view the overall instrument status. Refer to section 1.4 for more details.

3.7.2 Check bottle illuminations, even from across the laboratory.

The autoMACS Pro Separator has a bottle illumination that facilitates monitoring of the instrument’s status.

Code	Status	User action
Green	Ready for separation	No action required.
Blue	Instrument operating	No action required.
Yellow	Not ready for separation	Run wash program (Rinse or Qrinse) before starting a separation.
Red	Error	Check screen for error detection.
Purple	Program Sleep is completed	Switch off autoMACS Pro Separator.
Blinking	Action required	Check screen for required action.

Note: To store the autoMACS Pro Separator for a period longer than two weeks run the Store program.

Note: For daily usage the instrument should not be switched-off but placed into SLEEP mode. See section 4.

3.8 (Optional) Switch the instrument off for long-term storage.

3.8.1 Select “Option“ and “Special“.

3.8.2 Select “Store“ and press “Run“.

3.8.3 Replace the columns with column substitutes (refer to section 3.3.4 of the User Manual).

3.8.4 Select “Done“.

3.8.5 Switch off the autoMACS Pro Separator using the main power switch.

4 Setting the autoMACS Pro Separator in SLEEP mode

4.1 **- Press the shutdown symbol (upper right-hand corner of the display).**

4.2 **- Alternatively, select Sleep program as the last washing step.**

5 Maintenance of the autoMACS Pro Separator

5.1 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	Between and before separations of rare cells (< 5%)	4 min

5.2 Daily maintenance programs

Program	Description	Recommended usage	Duration
Rinse	Rinse of separation columns and tubing system with Washing Solution and Running Buffer	Prior to first separation	4 min
Sleep	Rinse with Washing Solution followed by filling with storage solution	Before switching OFF the autoMACS Pro Separator	5 min

5.3 Periodic maintenance

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

5.4 Column exchange

Replace autoMACS Columns every two weeks or after 100 separations, whichever comes first. Select menu Option

5.4.1 Select “Special“ and “Col_ex“.

5.4.2 Press “Run“.

5.4.3 When prompted, exchange the column.

Figure 5.1: “Col_ex” program.

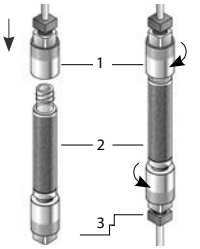
5.4.4 Open front door and note the positions of the columns (column 1: left; column 2: right). Exchange one column at a time.

5.4.5 Remove column from slot; unscrew top column connector followed by the bottom column connector as shown in figure 5.2.

5.4.6 Dispose of the expired column.

5.4.7 Point the bottom of the fresh column towards the autoMACS Pro Separator.

5.4.8 Insert bottom column connector. Screw in the column by turning it clockwise. Repeat the procedure for the top column connector.



1 Top Column Connector

2 autoMACS Pro Separation Column

3 Bottom Column Connector

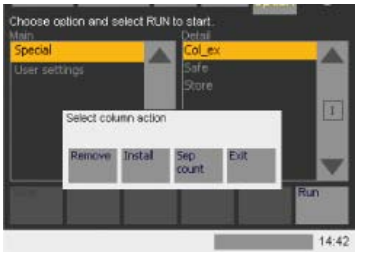


Figure 5.2: Left: Exchange of the column. Right: Starting the "Col_ex" program.

5.4.9 Push column into the magnet housing, with the top column connector sitting on the guide in the column slot.

5.4.10 Repeat installation for the second autoMACS Column.

After exchange of separation columns, select "Done". The autoMACS Pro Separator system will be automatically primed with Running Buffer and is then ready for cell separation.

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140-002-015.04

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