AutoMACS – User Guide

The autoMACS allows biomagnetic cell sorting of cells labelled with MACS microbeads. The MACS products are sold by *Miltenyi Biotec* (on-line catalogue at www.miltenyibiotec.com).

The Dunn School autoMACS is located in Rm. 41, where you can also find a booking sheet. If you want to use it inside the hood, do not forget to book the hood as well!

Prepare in advance:

Running buffer	PBS, 2mM EDTA, 0.5% BSA or FACSFlow, 0.5% BSA	Blue Bottle
Rinsing buffer	PBS, 2mM EDTA or FACSRinse	Yellow Bottle

For one sorting you will need 500 ml of each. The solutions should be filtered before use (use a 0.22μm bottle top filter) to prevent clogging.

The **cells** should be in a single cell suspension. For up to 10^8 cells, resuspend in 500μl PBS, 2mM EDTA, 0.5% BSA prior to sorting. For more cells increase the volume accordingly. Cells should have been previously labelled with the microbeads.

AutoMACS operation

1. Check that the black bottle has at least 200ml of 70% ethanol, and that the red bottle (waste) is empty. Change the caps of your buffer bottles, with the autoMACS caps (with probes) – check the colours (yellow – rinsing buffer; blue – running buffer). Clean the probes with ethanol before inserting in the medium.
2. Place empty 50ml Falcon tubes under each “pos” and “neg” port, and a 15ml tube in the sample uptake port.
3. Turn on the autoMACS.
4. After a while, the screen should display the main menu (fig.1). You can make your selection by touching the screen. In the first run of the day it is important to run **Clean**. The autoMACS will run ethanol through the tubes, columns and ports, followed by rinsing solution and leaves everything in running buffer, ready for sorting. It takes ~5min.

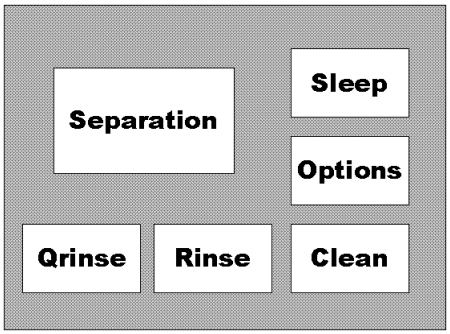

Fig.1 Main menu.

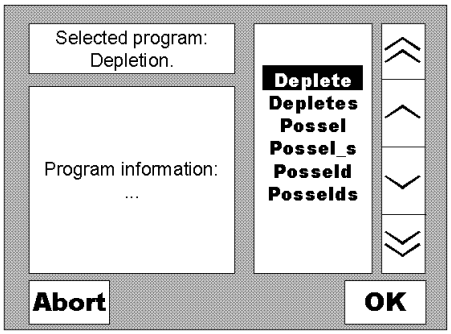

Fig.2 Separation menu.

5. The autoMACS is now ready for sorting. Place your cell sample in any tube with a U-bottom in the uptake port. Place 5ml collecting tubes under the “pos” and “neg” ports.
6. Choose **Separation** from the main menu (fig.1). Another menu is displayed (fig.2) allowing the selection of the separation program more suitable for your needs (see table 1). You can select the desired program by touching the arrows on the right. When that program is highlighted you can check on the boxes on the left side of the screen the characteristics of the program, if it is the right one you can press **OK** and the separation will proceed automatically.
7. The positive fraction of cells (the ones with beads attached) are recovered from the “pos1” port if a separation through a single column was selected; or from “pos2” if you decided to pass the cells through two columns (better purity, lower yield). The negative fraction (cells without beads) is always recovered from the “neg” port.
8. When the sort is completed the main menu is displayed again (fig.1). Place again the 50ml Falcon tubes in the collection ports and a 15 ml tube in the uptake port. If you have more samples to sort, you should run **Rinse** between the sorts. The Rinse program runs rinsing buffer through the system to remove any cells left behind. If there is another user in the booking sheet for the same day, run **Clean**. This program passes ethanol through all the tubes, before filling the system with running buffer. If you are the last user of the day select **Sleep**. This program washes everything with ethanol, and leaves all the tubes and columns filled with it. You just have to switch off the main switch when the screen prompts you to do so. Do not forget to empty the “waste” bottle and to store your buffers!

Table 1. How to select the most suitable program.

Goal		Obtain a cell population expressing a particular cell surface antigen		Eliminate cell population(s) from your sample
Strategy		Positive selection		Depletion
		Normal/high frequency cells	Rare cells or high purity (lower yield)	
Program	Normal/high antigen expression	**Possel** Positive selection	**Posseld** Positive selection through two columns	**Deplete** Depletion
	Low antigen expression (slow programs: more sensitive, but lower purity)	**Possel_s** Positive selection at a slower rate	**Posselds** Positive selection through two columns at a slow rate	**Depletes** Depletion at a slower rate (lower yield, better depletion)

For additional information contact Luis Graca in Rm.41 (75606), or consult the autoMACS manual in Rm.41.