Maintenance of the AutoMACS Cell Sorter

AfCS Procedure Protocol ID PP0000001700
Version 1, 02/14/02

This protocol has been established to maintain the efficient use of the AutoMACS cell sorter during periods of heavy use (e.g., three preparations of splenocytes from 16 spleens per week). The SAFE Clean Program (programmed into the AutoMACS by Miltenyi Biotec) should be performed on the AutoMACS sorter at least once per week or after 2.4E+09 cells have been passed through a single sorting column, whichever occurs first. Although the AutoMACS can carry two columns, negative selection only requires one; therefore, only one column is attached and used in these procedures. Before running the SAFE Clean Program, inspect the sorter to ensure that at least 200 ml of cleaning solution (70% ethanol) is available. Refill the bottle when necessary. The AutoMACS will display a warning if the running buffer or rinsing solution is low, or if the waste bottle is full. Be sure to check the levels of each bottle before beginning the program to avoid delays while running the SAFE Clean Program. Generally, it is advisable to open the cover of the AutoMACS during the cleaning program so that tubing and connections can be observed. If any leaks are noticed, consult the AutoMACS User Manual for repair/replacement instructions. AutoMACS separation columns cannot be used after exposure to Coulter Clenz; therefore, column exchange MUST be performed as part of this program. If a cell separation is to be performed immediately after running the SAFE Clean Program, run a Rinse Program before sorting to ensure that no disinfectant remains in the autoMACS.

Running the SAFE Clean Program

1. Load the appropriate solution (2 bottles of rinsing solution—magnetic cell sorting cleaning solution [MACS cleaning solution]; 1 bottle of cleaning solution—70% ethanol) at the rear of the AutoMACS. Turn on the sorter.
2. Select “Options” from the main onscreen menu. Then select “Safe.”
3. The first “Safe” screen outlines the procedure and verifies your choice. Press “Cont” to start the SAFE Clean Program. (Press “Abort” to return to the “Options” menu.)
4. Following the on-screen prompts, place 30 ml of Coulter Clenz in a 50-ml conical tube and load tube onto the sorter uptake port. (Turn the sample support arm to one side. Place the 30-ml aliquot of Coulter Clenz at the uptake port and return the lever to the middle position.)
5. Press “Cont” to confirm that Coulter Clenz is supplied. The AutoMACS will automatically fill the fluid system and clean the machine.
6. After the decontamination portion of the program, the AutoMACS will prompt you to exchange the separation columns. After the columns have been exchanged, press “Cont.”
7. The program will automatically complete itself, and the AutoMACS will display a completion screen. Press “OK” to return to the main AutoMACS menu.

Clearing Debris

8. Debris can accumulate at specific joints that will result in pump errors during the isolation process. To clear the debris from a key joint that is most susceptible, perform the following steps after the program is complete.
9. Remove the screw from the bottom of each glass pump cylinder, and unscrew the glass pump cylinder (with piston inside) from the cylinder head. Remove the piston from the cylinder, and clean both parts with a Coulter Clenz-saturated Kimwipe. Clean all open connectors. Wipe dry. Return the plunger and cylinder to the machine and tighten all components.
10. Close the front cover of the machine.

Reagents and Materials

Magnetic cell sorting cleaning solution (MACS cleaning solution): AfCS Solution Protocol ID PS0000003600

Ethanol, 70% (70% ethanol): AfCS Solution Protocol ID PS0000001100

AutoMACS: Miltenyi Biotec; catalog no. 201-01

Coulter Clenz: Beckman Coulter; catalog no. 8546930

Conical tubes, 50 ml: Greiner; catalog no. 4943

AutoMACS separation columns: Miltenyi Biotec; catalog no. 130-021-101

Kimwipe: Kimberly-Clark; catalog no. 34120

Author: Robert Hsueh

Date: 02/15/02

Approved: Paul Sternweis