Miltenyi Biotec

D Columns

5 D Columns

Order no. 130-041-201

Contents

5 MACS D Columns for SuperMACS and SuperMACS II plus accessories. Sterile packed. Capacity: up to 10^9 magnetically labeled cells.

Applications

- Depletion of large numbers of cells labeled with MACS MicroBeads.
- Magnetic separation of biological material labeled with MACS MicroBeads.

Storage of MACS Separation Columns

Store columns dry, protected from light. Do not use after expiry date.

Instrument and Reagent Requirement

Magnetic cell separator SuperMACS or SuperMACS II.
Column Adapter for CS and D Columns in combination with SuperMACS II.
MACS MicroBeads for magnetic labeling of the cells.
70 % ethanol in dd H_2O.
Buffer: phosphate buffered saline supplemented with 2 mM EDTA and 0.5 % bovine serum albumin.
50 ml syringe.

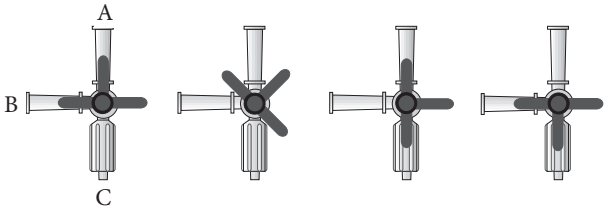
How to Use MACS Depletion Column Type D

D Columns have been developed for depletion of large amounts of magnetically labeled human and animal cells out of a heterogeneous suspension with SuperMACS or SuperMACS II. They can be used to separate different biological material including plant cells, bacteria, viruses, protozoa or cell organelles. Various running buffers may be used with the depletion column. The suitability of a specific buffer has to be tested experimentally. By passing the material through the column, the suspension is depleted for magnetically labeled material.

Protocol for Cell Separation Using D Columns

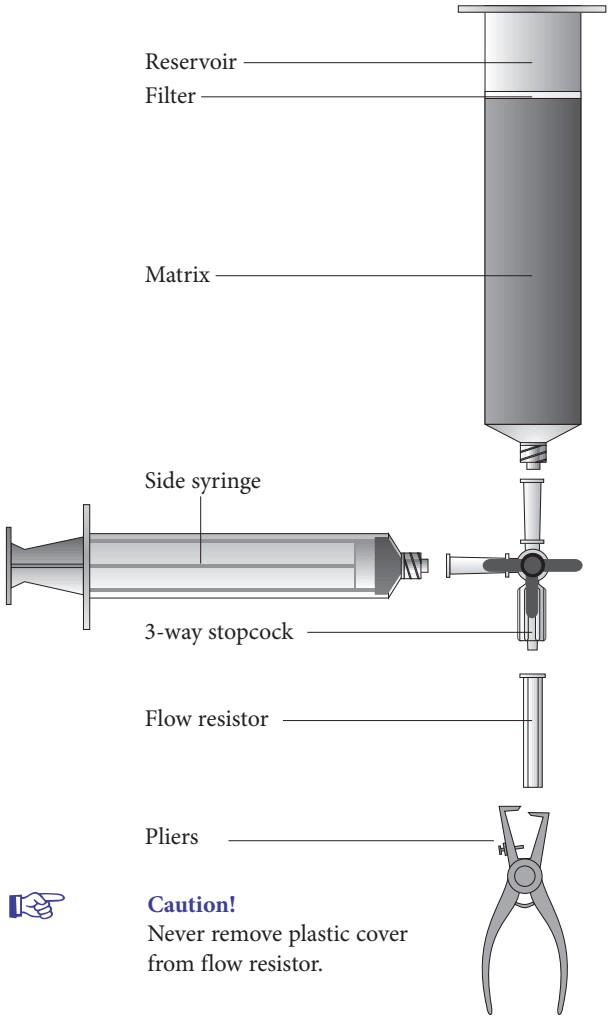
Assembly and Preparation of the Depletion Column

3-way stopcock positions



filling closed run rinse

Assembly of the D Column



Caution!
Never remove plastic cover from flow resistor.

- Remove yellow cap from separation column and attach 3-way stopcock to column at port A.
- Fill a syringe with **70 % ethanol** and attach to port B of the 3-way stopcock.
- Turn 3-way stopcock to position “fill”.
- Move the gap of the SuperMACS to approximately 3 cm width using the side wheel. Place assembled column in MACS separator with 3-way stopcock in adjustment and secure with the lever.
- When using in combination with SuperMACS II, insert column into the mounted Column Adapter for CS and D Columns and move Column Adapter into the magnetic field by turning the handle (for details, see SuperMACS II data sheet).

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- Fill the column upright from the bottom with 70 % ethanol from the syringe until the solution reaches the reservoir.
- Turn the 3-way stopcock to position "run" and rinse column by filling from the top with buffer. Allow 70 % ethanol to run into the column until it reaches the white filter device. Then add fresh buffer. Rinse with 500 ml of buffer. Do not let the column "run dry" at any time during procedure.
- Choose a flow resistor (22G; 21G or 20G, see table below) and cut off the tip of the plastic sheath with the pliers supplied with the SuperMACS Starting Kit. Leave the plastic sheath in place for safety and attach the flow resistor to port C of the 3-way stopcock.

  Caution: Never remove plastic sheath from flow resistor.

Flow resistor	Flow rates in ml/min for buffer
22G	3.5
21G	4.0
20G	8.0

Note: The lower the flow rate, the higher is the depletion efficiency of magnetically labeled cells.

- Fill the syringe with **buffer** and attach to port B of the 3-way stopcock. Leave the syringe attached during separation, except when refilling.
- Turn the stopcock to position "rinse" and flush the air from the flow resistor. Turn the stopcock in position "closed". The column is now ready for separation.

Depletion of Cells

- Apply magnetically labeled cell suspension in appropriate volume of buffer (up to 10^8 cells per 500 µl) onto the column with the flow resistor attached. Turn the stopcock to position "run" and allow cell suspension to penetrate the matrix.
- Wash the column with 200 ml of buffer. Collect effluent as negative fraction that is depleted of the magnetically labeled cells.

Important Notes

▲ Never use a flow resistor without plastic cover.

▲ Recommended buffer is PBS supplemented with 2 mM EDTA and 0.5 % BSA. Other buffers have to be tested for their flow conditions. Do not use buffers of too high viscosity.

▲ Fill column properly avoiding air bubbles. Small air bubbles underneath the filter can be neglected.

▲ Column must not "run dry" at any time during the procedure.

▲ Do not store column after filling.

▲ Columns can be cooled just prior to use by passing 2–3 column volumes of ice-cold buffer through it.

▲ The number of cells loaded onto the column can be up to 10^8 per 500 µl.

▲ When working with anti-coagulated blood or buffy coat, dilute before separation with buffer.

▲ Do not use separation columns in combination with magnetic beads other than MACS MicroBeads. Magnetic forces in the column are very high and may damage biological material, if other beads are used.

▲ If the buffer does not flow well, there may be an air bubble in the capillary of the flow resistor. Switch the stopcock to "rinse", flush the needle with buffer from the side syringe, switch the stopcock back to "run" and continue the run. The needle may also become blocked by cell clumps, which means the needle has to be replaced.

▲ If necessary, it is possible to pass your cells through the same column a second time.

Technical Specifications

- Matrix volume: 43 ml; reservoir volume: 20 ml.
- Typical capacity: e.g. 10^9 retained lymphocytes and up to 10^{11} total applied cells.
- Recommended sample size for leukocytes: 10^7-10^9 magnetically labeled cells in 10^9-10^{11} total cells. Sample concentration: up to 10^8 leukocytes/500 µl cell suspension.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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