Miltenyi Biotec

D Columns

Order no. 130-041-201

Contents

1. Description

This product is for research use only.
For use by professional laboratory personnel only.

Components	**5× D Columns,** sterile packed
	5× flow resistor (20G)
	5× flow resistor (21G)
	5× flow resistor (22G)
	5× 3-way stopcock
Storage	Store columns dry at +10 to +35 °C and protected from light. The expiration date is indicated on the box label. Do not use after this date.

1.1 Important safety information

⚠ **WARNING** **Contamination or infection could result in death or serious injury depending on the material used.**

All biological material must be considered potentially infectious

- Regulations for the treatment and disposal of infectious materials must be observed.

1.2 Background information

The patented MACS® Column Technology is based on the use of MACS MicroBeads, MACS Columns, and MACS Separators. The matrix of D Columns is composed of ferromagnetic fibers covered with a cell-friendly coating. When placed in the magnetic field of a MACS® Separator, the fibers amplify the magnetic field, thus inducing a high gradient within the column. This is crucial for isolation of cells which are only minimally labeled with MACS MicroBeads, leaving enough epitopes free for concurrent antibody staining. The space between the fibers is several times larger than primary and most cultured cells. This allows the cells to freely flow through the column. Magnetically labeled cells are held in suspension within the column and do not actually "bind" the column matrix. This suspension minimizes stress on the cells and allows for efficient sterile washing by avoiding cell aggregation.

1.3 Technical specifications

	Max. number of labeled cells	Max. number of total cells
Manual use	1×10^{9}	1×10^{11}

- 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.
- Void volume: 43 mL. Reservoir volume: 20 mL.
- Typical flow rates: refer to table on next page.
- D Columns are for single use only.
- Never remove plastic cover from flow resistor.

1.4 Applications

D Columns have been developed for depletion of large amounts of magnetically labeled human and animal cells out of a heterogeneous suspension using SuperMACS II Separator and MACS MicroBeads. They can be used to separate different biological material including plant cells, bacteria, viruses, protozoa, or cell organelles.

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

▲ To remove clumps and to prevent aggregates in the sample, resuspend material carefully and pass through 30 µm nylon mesh (Pre-Separation Filters (30 µm), # 130-041-407) before separation.

▲ Samples or buffers with high viscosity might cause reduced column flow or column clogging.

▲ Do not store column after filling.

▲ 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.

1.5 Reagent and instrument requirements

- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (+2 to +8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** The recommended buffer is PBS supplemented with EDTA and BSA. The suitability of other buffers has to be tested experimentally.

  ▲ **Note:** Use degassed buffer only! Degas buffer by applying vacuum, preferentially with buffer at room temperature. Excessive gas in running buffer will form bubbles in the matrix during separation. This may lead to clogging of the column and decrease the quality of separation.

- MACS MicroBeads for magnetic labeling of cells
- SuperMACS II Separator (# 130-044-104)

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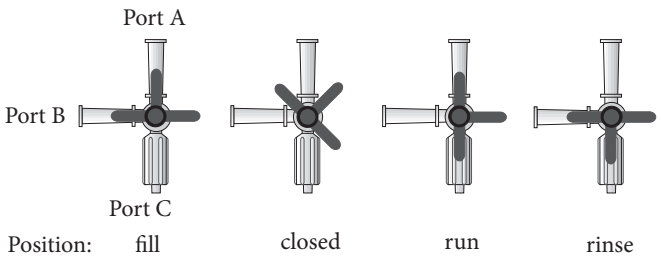
- Adapter for CS and D Columns for use with SuperMACS II Separator
- 70% ethanol in double-distilled water
- 50 mL syringe
- MACS Acrylic Tube Rack (# 130-041-406)
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

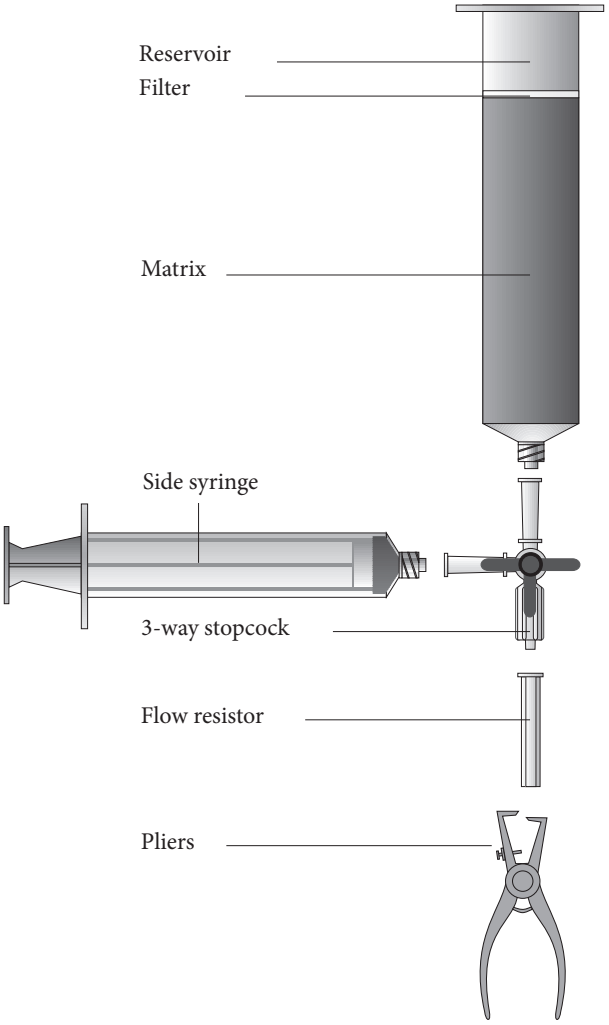
2. Use of D Columns

2.1 Preparation of D Columns

Positions of the 3-way stopcock



Assembly of the D Column



1. Remove yellow cap from separation column and attach 3-way stopcock to column at port A.
2. Fill a syringe with 70% ethanol and attach to port B of the 3-way stopcock.
3. Turn 3-way stopcock to position “fill”.
4. Insert column into the mounted Column Adapter for CS and D Columns and move Column Adapter into the magnetic field of the SuperMACS II Separator by turning the handle (for details refer to respective data sheet).
5. Fill the column upright from the bottom with 70% ethanol from the syringe until the solution reaches the reservoir.
6. 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.
7. Choose a flow resistor 22G, 21G, or 20G (refer to table below) and cut off the tip of the plastic sheath with the pliers supplied with the SuperMACS II Separator. Leave the plastic sheath in place for safety and attach the flow resistor to port C of the 3-way stopcock.

   ▲ **Note:** Do not remove plastic sheath from flow resistor.

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

Flow resistor	Buffer flow rates in mL/min
22G	3.5
21G	4.0
20G	8.0

8. Fill the syringe with buffer and attach to port B of the 3-way stopcock. Leave the syringe attached during separation, except when refilling.
9. 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.

2.2 Magnetic separation using D Columns

1. 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.
2. Wash the column with 200 mL of buffer. Collect effluent as negative fraction that is depleted of the magnetically labeled cells.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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