Miltenyi Biotec

XS Columns

Order no. 130-041-202

Contents

1. Description

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

Components	**5× XS Columns,** sterile packed
	5× 3-way stopcock
	5× syringe (20 mL)
	5× syringe (50 mL)
	5× tube with flow resistor
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 XS Columns is composed of ferromagnetic spheres, which are covered with a cell-friendly coating allowing fast and gentle separation of cells. When placed in a magnetic field of a MACS Separator, the spheres amplify the magnetic field by 10,000-fold, 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 spheres 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	2×10^{10}

- Recommended sample size for leukocytes: 10^6–10^9 magnetically labeled cells in 10^8–2×10^{10} total cells. Sample concentration: up to 10^9 leukocytes/5 mL cell suspension.
- Typical enrichment rate: 50-fold to up to 1,000-fold, depending on the strength and specificity of the magnetic labeling. Up to 10,000-fold enrichment can be achieved by separation over two sequential columns.
- Void volume: 6.2 mL. Reservoir volume: 50 mL.
- Typical flow rates with flow resistor: 20 mL/min; typical flow rates without flow resistor: 40 mL/min during wash and elution step.
- XS Columns are for single use only.
- Never remove plastic cover from flow resistor.

1.4 Applications

XS Columns have been developed for positive selection of high cell numbers out of heterogeneous suspensions in the magnetic field of the SuperMACS II with XS Column Adapter. They can be used to separate biological material including human and animal cells, plant cells, bacteria, cell organelles, or other bioparticles.

▲ Do not use XS 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 40 µm nylon mesh before separation.

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

▲ Do not store column after filling.

▲ XS Columns are not suitable for particles larger than 30 µm.

▲ 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.

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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.

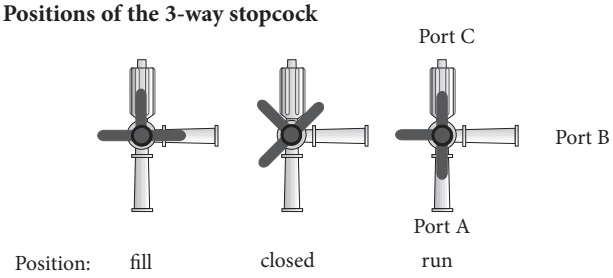
  ▲ **Note:** Use degassed buffer only! Degas buffer by applying vacuum, preferentially with buffer at room temperature. Excessive gas in running buffer will form bubbles in the matrix during separation. This may lead to clogging of the column and decrease the quality of separation.

- MACS MicroBeads for magnetic labeling of cells
- SuperMACS II Separator (# 130-044-104)
- Adapter for XS Columns for use with SuperMACS II Separator
- MACS Acrylic Tube Rack (# 130-041-406)
- 40 µm nylon mesh or filter to remove clumps

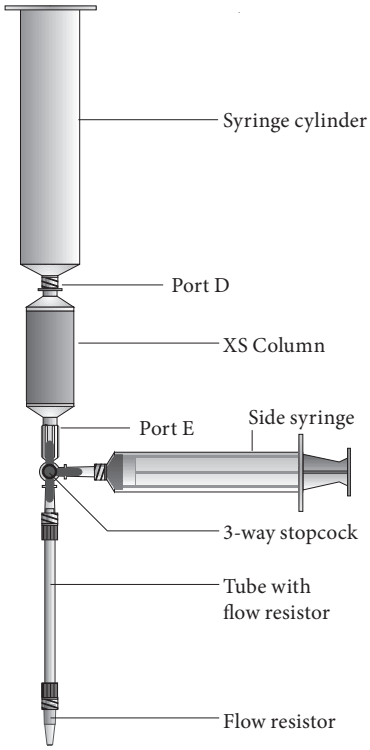
2. Use of XS Columns

2.1 Preparation of XS Columns

Positions of the 3-way stopcock



Assembly of the XS Columns



1. Attach the cylinder of the 50 mL syringe to port D of the column.
2. Attach the 3-way stopcock with port C to port E of the column.
3. Attach the tube with flow resistor to port A of the 3-way stopcock and remove the plastic sheath.
4. Fill the supplied 20 mL syringe (side syringe) with buffer and attach to port B of the 3-way stopcock.
5. Move the adapter holder out of the magnetic field of the SuperMACS II Separator by turning the handle, mount the XS Column Adapter, and insert the assembled column (for details refer to the respective data sheet).
6. Turn 3-way stopcock to position “fill”.
7. Carefully fill the column from the bottom with buffer from the syringe until the buffer reaches the syringe cylinder.
8. Turn the 3-way stopcock to position “run” and rinse column by filling from the top with buffer. Allow buffer to run into the column. Then, add more buffer. Rinse with 50 mL of buffer.
9. Close 3-way stopcock; leave the side syringe attached to port B of the 3-way stopcock during separation, except when refilling with buffer.
10. Move column in the magnetic field of the SuperMACS II Separator by turning the handle.

2.2 Magnetic separation using XS Columns

1. Pass cells through 40 µm nylon mesh or filter to remove cell clumps.
2. Apply up to 10^9 magnetically labeled cells in a maximum of 2×10^{10} total cells (up to 10^9 total cells per 5 mL) into the syringe cylinder that is set up on the XS Column and turn 3-way stopcock to position “run”. Allow the cells to pass through the column and rinse with 30 mL of buffer. Collect the flow through as negative fraction.
3. Close 3-way stopcock and remove flow resistor from the tube by attaching the plastic sheath and turning counterclockwise.
4. Wash column with 3 ×30 mL of buffer. Collect the flow through as wash fraction.
5. Remove column out of the magnetic field of the SuperMACS II Separator by turning the handle.
6. Detach syringe cylinder from port D of the XS Column.
7. Detach side syringe from port B of the 3-way stopcock, fill with buffer and attach side syringe to port D of the XS Column.
8. Elute retained cells with 20 mL buffer using the side syringe.
9. (Optional) Repeat magnetic separation step: apply the eluted cells to a new prefilled XS Column, wash, and elute retained cells in buffer.

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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