Miltenyi Biotec

LS Columns

LS Columns — Order no. 130-042-401
LS Columns plus tubes — Order no. 130-122-729

Contents

1. Description

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

Components

LS Columns (# 130-042-401):
25 LS Columns and plungers, sterile packed
or
LS Columns plus tubes (# 130-122-729):
25 LS Columns and plungers (# 130-042-401), sterile packed, and 75× 13 mL tubes for LS Columns (# 130-091-596), sterile packed as 3× 25 tubes

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. LS Columns have been developed for the gentle isolation of MicroBead-labeled cells. As MACS MicroBeads are extremely small, superparamagnetic particles, a high-gradient magnetic field is required to retain the labeled cells. LS Columns contain an optimized matrix to generate this strong magnetic field when placed in a permanent magnet, such as the MidiMACS™ Separator, QuadroMACS™ Separator, SuperMACS™ II Separator, or MultiMACS™ Cell24 Separator Plus.

1.3 Technical specifications

	Max. number of labeled cells	Max. number of total cells
Manual use	1×10^8	2×10^9
Use with MultiMACS Cell24 Separator Plus	1×10^8	1×10^9

▲ **Note:** Column capacity may decrease when separating cells larger than lymphocytes. Please refer to the respective MACS Cell Separation Reagent data sheet for column capacity of other cells than lymphocytes.

- Recommended sample size for leukocytes: 10^5–10^8 labeled cells in 10^7–2×10^9 total cells.
- 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.
- Columns are “flow stop” and do not run dry.
- Void volume: 400 µL. Reservoir volume: 8 mL.
- Typical flow rate for phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA): 1.2–2.1 mL/min.
- LS Columns are for single use only.

1.4 Applications

LS Columns have been developed for positive selection of human and animal cells, especially rare cells, out of a heterogeneous cell suspension in combination with a MACS Separator. LS Columns can also be used for depletion of cells which strongly express the magnetically labeled surface antigen. They can also be used to separate other biological material such as plant cells, bacteria, viruses, protozoa, cell organelles, etc.

▲ Do not use LS 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.

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

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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.

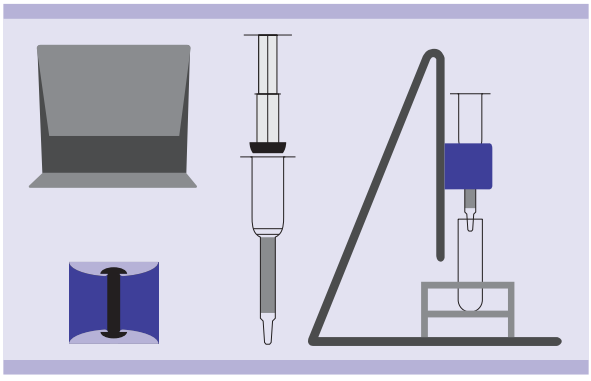
  ▲ **Note:** Use degassed buffer only! Degas buffer by applying vacuum, preferentially with buffer at room temperature. Excessive gas in running buffer will form bubbles in the matrix during separation. This may lead to clogging of the column and decrease the quality of separation.
- MACS MicroBeads for magnetic labeling of cells.
- MidiMACS Separator (# 130-042-302), QuadroMACS Separator (# 130-091-051), SuperMACS II Separator (# 130-044-104), MultiMACS Cell24 Separator Plus (# 130-098-637), or MultiMACS X Separator (# 130-118-515)
- MACS MultiStand (# 130-042-303) in combination with MidiMACS Separator or QuadroMACS Separator
- Adapter for MS, LS, and LD Columns for use with SuperMACS II Separator.
- Single-Column Adapter is needed for use of LS Columns with the MultiMACS Cell24 Separator Plus. The Single-Column Adapter is part of the MultiMACS Cell24 Separator Plus package.
- MACS Acrylic Tube Rack (# 130-041-406) or MACS 15 mL Tube Rack (# 130-091-052)
- (Optional) MS Columns (# 130-042-201)
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Use of LS Columns with manual separators

2.1 Preparation of LS Columns

1. Insert LS Column with the column wings to the front into a MACS Separator according to A) or B).

   A) Use with MidiMACS or QuadroMACS Separator



   Attach MidiMACS Separator or QuadroMACS Separator to the MACS MultiStand and place LS Column in the separator. Place a collection tube under the LS Column.

   ▲ **Note:** Check that the ejection blocks in the gap of the magnet are attached before placing the MACS Column into the magnetic field of the MidiMACS or QuadroMACS Separator.

   ▲ **Note:** Be careful when attaching the QuadroMACS Separator to the MultiStand to avoid trapping your fingers (for details refer to the QuadroMACS Starting Kit data sheet).

   B) Use with SuperMACS II Separator

   For use of LS Columns with the SuperMACS II Separator please refer to the respective data sheet.

2. Prepare LS Column by rinsing with buffer: apply 2 mL of degassed buffer on top of the column and let the buffer run through. LS Columns are “flow stop” and do not run dry.
3. Discard effluent and change collection tube. The LS Column is now ready for magnetic separation.

   ▲ **Note:** Use column immediately after filling to avoid formation of air bubbles caused by warming up. Do not store columns after filling.

   ▲ **Note:** The time for filling the column with buffer is dependent on the storage conditions, temperature, and humidity. Therefore, the time may vary from a few seconds to several minutes. This filling time has no influence on the quality of the separation.

2.2 Magnetic separation using LS Columns

▲ For details on magnetic labeling, refer to the MACS Cell Separation Reagent data sheets.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

1. Resuspend up to 10^8 total cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

   ▲ **Note:** When working with fresh anticoagulated blood or buffy coat, dilute before separation 1:2 with buffer. Alternatively, use the StraightFrom® Whole Blood MicroBeads or StraightFrom Buffy Coat MicroBead Kits in combination with Whole Blood Columns.

   ▲ **Note:** To remove clumps, pass cells through Pre-Separation Filters (30 µm).
2. Apply cell suspension onto the prepared LS Column. Collect flow-through containing unlabeled cells.
3. Wash LS Column with amount of degassed buffer as stated on respective MACS Cell Separation Reagent data sheet. Collect unlabeled cells that pass through and combine with the flow-through from step 2.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
4. Remove LS Column from the separator and place it on a new collection tube.
5. Pipette 5 mL buffer onto the LS Column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.
6. (Optional) To increase the purity of the magnetically labeled fraction, the eluted fraction can be enriched over a second MS Column (for up to 10^7 magnetically labeled cells) or LS Column (for up to 10^8 magnetically labeled cells). Repeat the magnetic separation procedure as described in steps 2 to 5 by using a new column.

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3. Use of LS Columns with the MultiMACS Cell24 Separator Plus

Up to 12 LS Columns can be used in a single run with the MultiMACS 12× Single Column Adapter LS (# 130-108-816).

For further details please refer to the MultiMACS Cell24 Separator Plus user manual.

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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