Miltenyi Biotec

LS Columns

LS Columns	Order no. 130-042-401
LS Columns plus tubes	Order no. 130-041-306

Contents

1. Description

Components

LS Columns (# 130-042-401):
25 LS Columns and plungers, sterile packed

or

LS Columns plus tubes (# 130-041-306):
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 and protected from light. The expiration date is indicated on the box label. Do not use after this date.

1.1 Background

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, VarioMACS™ Separator, SuperMACS™ II Separator, or MultiMACS™ Cell24 Separator Plus.

1.2 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

- 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 PBS (phosphate-buffered saline) containing 0.5% BSA (bovine serum albumin): 1.3–2.0 mL/min.
- LS Columns are for single use only.

1.3 Product 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, # 130-041-407) before separation.

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

1.4 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 (2–8 °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, QuadroMACS Separator, VarioMACS Separator, SuperMACS II Separator, or MultiMACS Cell24 Separator Plus.
- LS Column Adapter (# 130-090-544) for use with VarioMACS Separator or Adapter for MS, LS and LD Columns for use with SuperMACS II Separator.
- Single-Column Adapter is needed for use LS Columns with the MultiMACS Cell24 Separator Plus. The Single-Column Adapter

140-000-127.10

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

is part of the MultiMACS Cell24 Separator Plus package.

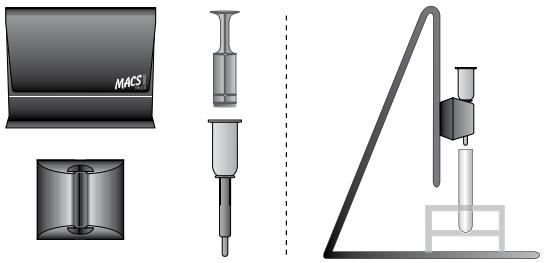
- MACS Acrylic Tube Rack (# 130-041-406) or MACS 15 mL Tube Rack (# 130-091-052).
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

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 an MACS Separator.

A) Use with MidiMACS™ or QuadroMACS™ Separator



Attach MidiMACS™ Separator or QuadroMACS™ Separator to the 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 see QuadroMACS Separator data sheet).

B) Use with the VarioMACS™ or SuperMACS™ II Separator

For use of LS Columns with the VarioMACS or 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.

2.2 Magnetic separation using LS Columns

▲ For details on magnetic labeling, see MACS Cell Separation Reagent data sheets.

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.

   ▲ **Note:** To remove clumps, pass cells through Pre-Separation Filters.
2. Apply cell suspension onto the prepared LS Column.
3. Collect unlabeled cells which pass through. Wash LS Column with amount of degassed buffer as stated on respective MACS Cell Separation Reagent data sheet, adding buffer each time once the column reservoir is empty. Collect total effluent. This is the unlabeled cell fraction.
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.

   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a new, freshly prepared MS Column (for up to 10^7 magnetically labeled cells) or LS Column (for up to 10^8 magnetically labeled cells).

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

Up to 9 LS Columns can be used in a single run with the Single-Column Adapter.
For further details please refer to the MultiMACS™ Cell24 Separator Plus user manual.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi BiotecGmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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