Miltenyi Biotec

LD Columns

Order no. 130-042-901

Contents

1. Description

This product is for research use only.

Components	25 LD Columns and plungers, sterile packed.
Storage	Store columns dry at 10–35 °C and protected from light. The expiration date is indicated on the box label. Do not use after this date.

1.1 Background information

The patented MACS® Column Technology is based on the use of MACS MicroBeads, MACS Columns and MACS Separators. LD Columns have been developed for the gentle depletion of MicroBead labeled cells. As MACS MicroBeads are extremely small, superparamagnetic particles, a high-gradient magnetic field is required to retain the labeled cells. LD 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.

1.2 Technical specifications

	Max. number of labeled cells	Max. number of total cells
Manual use	1×10^8	5×10^8
Use with MultiMACS Cell24 Separator Plus	1×10^8	5×10^8

- 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 minimal sample size for leukocytes: 10^6 labeled cells in 10^7 total cells.
- Columns are "flow stop" and do not run dry.
- Void volume: 1.35 mL. Reservoir volume: 7 mL.
- Typical flow rate for PBS (phosphate buffered saline) containing 0.5% BSA (bovine serum albumin): 0.17– 0.27 mL/min.
- LD Columns are for single use only.

1.3 Applications

LD Columns have been developed for the depletion of human and animal cells, out of a heterogeneous cell suspension in combination with a MACS Separator. LD Columns can also be used to separate other biological material such as plant cells, bacteria, viruses, protozoa, cell organelles etc.

LD Columns are recommended for depletion of cells with a low level of marker expression. They are also appropriate, if the purity of the unlabeled target cells is of highest priority, and the labeled, undesired cells express the marker at a normal to high level.

▲ Do not use LD 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.

▲ LD 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.

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 (# 130-042-302), QuadroMACS Separator (# 130-091-051), SuperMACS II Separator (# 130-044-104), MultiMACS Cell24 Separator Plus (# 130-098-637), or MultiMACS X (# 130-118-515).
- MACS MultiStand (# 130-042-303) in combination with MidiMACS Separator or QuadroMACS Separator.
- LS Column Adapter (# 130-090-544) for use with SuperMACS II Separator.
- MACS Acrylic Tube Rack (# 130-041-406) or MACS 15 mL Tube Rack (# 130-091-052).
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

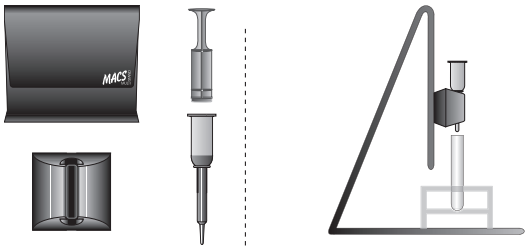
140-000-333.11

2. Use of LD Columns with manual separators

2.1 Preparation of LD Columns

1. Insert LD Column with the column wings to the front into a MACS® Separator.

A) Use with MidiMACS™ or QuadroMACS™ Separator



Attach MidiMACS Separator or QuadroMACS Separator to the MultiStand and place LD Column in the separator. Place a collection tube under the LD 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 Starting Kit data sheet).

B) Use with SuperMACS™ II Separator

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

2. Prepare LD Column by rinsing with buffer: apply 2 mL of degassed buffer on top of the column and let the buffer run through. LD Columns are "flow stop" and do not run dry.

3. Discard effluent and change collection tube. The LD 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 LD 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 1.25×10^8 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 LD Column. Collect flow-through containing unlabeled cells.

3. Wash LD Column with 2×1 mL degassed buffer. 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 LD Column from the separator and place it on a new collection tube.

5. Pipette 3 mL buffer onto the LD 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 an MS Column (for up to 10^7 magnetically labeled cells) or LS Column (for up to 10^8 magnetically labeled cells).

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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