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1. Description

Components	25 LD Columns and plungers, sterile packed.
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. 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, 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	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: 10⁶ labeled cells in 10⁷ 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): 0.2–0.25 mL/min.

- LD Columns are for single use only.

1.3 Product 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

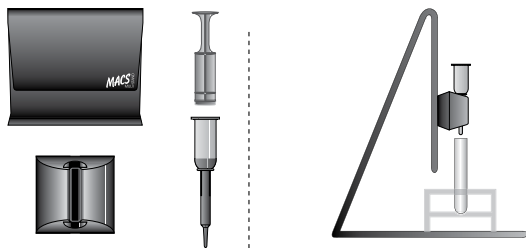
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C).
 - ▲ Note: The recommended buffer is PBS 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.
- 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.

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 an 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 VarioMACS™ or SuperMACS™ II Separator

For use of LD Columns with the VarioMACS™ or 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, see MACS Cell Separation Reagent data sheets.

1. Resuspend up to 1.25×10^8 total cells in 500 μ L 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.
3. Collect unlabeled cells which pass through. Wash LD Column with 2×1 mL degassed buffer, adding buffer each time once the column reservoir is empty. Collect total effluent. This is the unlabeled (depleted) cell fraction.

▲ **Note:** For the isolation of the magnetically labeled fraction, remove column from the separator and place it on a suitable collection tube. Apply 3 mL of buffer onto the column. Flush out fraction with the magnetically labeled cells by using the plunger supplied with the column. Optional, a second elution with 3 mL of buffer can be performed to increase the recovery. Avoid applying too much pressure onto the column. To increase the purity of the magnetically labeled fraction, it can be passed over an MS Column (for up to 10^7 magnetically labeled cells) or LS Column (for up to 10^8 magnetically labeled cells).

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

Up to 9 LD 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

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