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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™ Separator or SuperMACS™ II Separator.

LD Columns contain a hydrophilic coating which allows rapid filling. This coating is washed out by rinsing the LD Column with buffer before separation. After incubation with MACS MicroBeads, the cell suspension is loaded onto the LD Column. The unlabeled cells run through while the magnetically labeled cells are retained on the LD Column. The retained material is washed with buffer to collect the entire effluent as the wanted, unlabeled cell fraction. After removal of the LD Column from the magnetic field, the magnetically retained cells can be eluted, using the plunger supplied with the LD Column.

1.2 Technical specifications

- Column capacity: 1×10^8 magnetically labeled cells from up to 5×10^8 total cells.
 - ▲ **Note:** Column capacity may decrease when separating cells larger than lymphocytes.
- Recommended sample size for leukocytes: 10^6 – 10^8 labeled cells in 10^7 – 5×10^8 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 weak antigen expression level.
- if the antigen expression level of the to be depleted cells is normal to high, and highest purity of the unlabeled target cells is the priority.
- with some MACS Cell Isolation Kits for the depletion step prior to positive selection of the cell subset of interest.

▲ **Note:** Most MACS Cell Isolation Kits for the isolation of untouched T cells, B cells, monocytes, NK cells, or basophils are optimized for use with LS Columns and do not require the use of LD Columns.

Please refer to the column recommendations given in the data sheet of the particular MACS Cell Isolation Kit.

▲ 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, # 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 Separator, or SuperMACS II Separator.
- LS Column Adapter (# 130-090-544) for use with VarioMACS Separator or SuperMACS Separator, or Adapter for MS, LS, and LD Columns for use with SuperMACS II Separator.
- 13 mL tubes for LS Columns (# 130-091-596)
- 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.

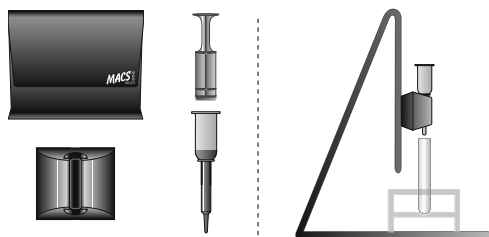


2. Use of LD Columns

2.1 Preparation of LD Columns

1. Insert LD Column with the column wings to the front into MACS Separator according to A), B), or C).

A) Use with MidiMACS™ or QuadroMACS™ Separator

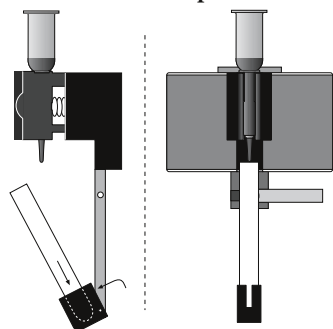


Attach MidiMACS™ Separator or QuadroMACS™ Separator to the MultiStand and place LD Column in the separator. Place a collection tube under the LD Column.

▲ **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.

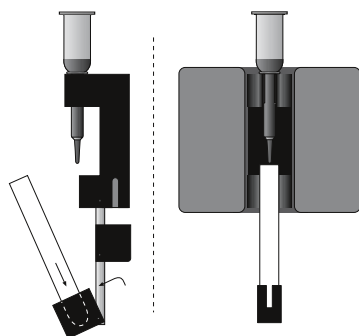
▲ **Note:** Be careful when attaching the QuadroMACS Separator to the MultiStand to avoid trapping your fingers (for details see QuadroMACS Starting Kit data sheet).

B) Use with VarioMACS™ or SuperMACS™ Separator



Insert LS Column Adapter in the magnetic field of the VarioMACS™ Separator or the SuperMACS™ Separator (for details, see LS Column Adapter Kit data sheet). Place the LD Column in the LS Column Adapter and a 13 mL collection tube in the tube holder.

C) Use with SuperMACS™ II Separator



Insert Adapter for MS, LS, and LD Columns in the magnetic field of the SuperMACS™ II Separator (for details, see SuperMACS II data sheets). Place the Column Adapter and a 13 mL collection tube in the lower tube holder.

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 minutes. This filling time has no influence on the quality of the separation.



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).

Warranty

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