Miltenyi Biotec

Large Cell Columns

Order no. 130-042-202

Contents

1. Description

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

Components	**25× Large Cell Column,** 25 Large Cell Columns and plungers, sterile packed
	25× flow resistor (23G)
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. Large Cell 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. Large Cell Columns contain an optimized matrix to generate this strong magnetic field when placed in a permanent magnet such as the MiniMACS™ Separator or OctoMACS™ Separator.

1.3 Technical specifications

	Max. number of labeled cells	Max. number of total cells
Manual use	1×10^7	2×10^8

▲ **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: 10^4–10^7 magnetically labeled cells in 10^6–2×10^8 total cells. Sample concentration: up to 10^8 leukocytes/500 µL cell suspension.
- Typical enrichment rate: 50-fold to up to 1,000-fold, depending on the strength and specificity of the magnetic labeling.
- Void volume: 80 µL. Reservoir volume: 3.5 mL.
- Typical flow rate without flow resistor for phosphate-buffered saline (PBS) containing 0.5 % bovine serum albumin (BSA): 1.2–2.0 mL/minute.
- Large Cell Columns are for single use only.
- Never remove plastic cover from flow resistor.

1.4 Applications

Large Cell Columns have been developed for positive selection of human and animal cells, especially large cells, out of heterogeneous cell suspensions in combination with the MiniMACS Separator and OctoMACS Separator. They can also be used to separate other biological material such as plant cells and protozoa.

▲ Positive selection of large cells, e.g. megakaryocytes, labeled with MACS MicroBeads from up to 2×10^8 **total** cells.

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
- MiniMACS Separator (# 130-042-102) or OctoMACS Separator (# 130-042-109) in combination with the MACS MultiStand (# 130-042-303)
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

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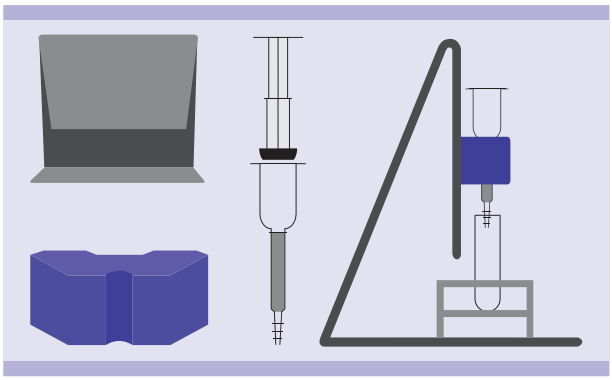
2. Use of Large Cell Columns

2.1 Preparation of Large Cell Columns

1. Attach the MACS Separator to the MACS MultiStand and place Large Cell Separation Column with the column wings to the front in the separator. Place a collection tube under the column.



2. Attach the flow resistor (23G needle) to the Large Cell Separation Column.
3. Apply 500 µL of degassed buffer on top of the column and let the buffer run through. Then discard effluent and change collection tube.

2.2 Magnetic separation using Large Cell 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 degassed buffer.

   ▲ **Note:** Use column immediately after filling to avoid formation of air bubbles caused by warming up of the buffer in the column.

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

   ▲ **Note:** To remove clumps, pass cells through Pre-Separation Filters (30 µm).
2. Apply cell suspension onto the prepared Large Cell Column. Collect flow-through containing unlabeled cells.
3. Wash Large Cell Column with 3×500 µL 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 Large Cell Column from the separator. Remove flow resistor and place it on a new collection tube.
5. Pipette 1 mL buffer onto the Large Cell 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 Large Cell Column. Repeat the magnetic separation procedure as described in steps 2 to 5 by using a new column.

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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