



Large Cell Separation Columns

Separation Columns

25 Large Cell Columns

Order No. 130-042-202

Contents

25 MACS High Gradient Magnetic Separation Columns for positive selection of large cells (up to 50 μm in diameter), flow resistors and plunger for the elution of positively selected material, sterile packed.

Capacity: max. 2×10^8 total cells and 1×10^7 magnetically labeled cells.

Applications

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

Storage of MACS Separation Columns

Store columns dry, protected from light. Do not use after expiry date.

Instrument and Reagent Requirement

Magnetic cell separator MiniMACS; MACS MicroBeads for magnetic labeling of cells.

Buffer: phosphate buffered saline (PBS) supplemented with 2 mM EDTA and 0.5 % bovine serum albumin.

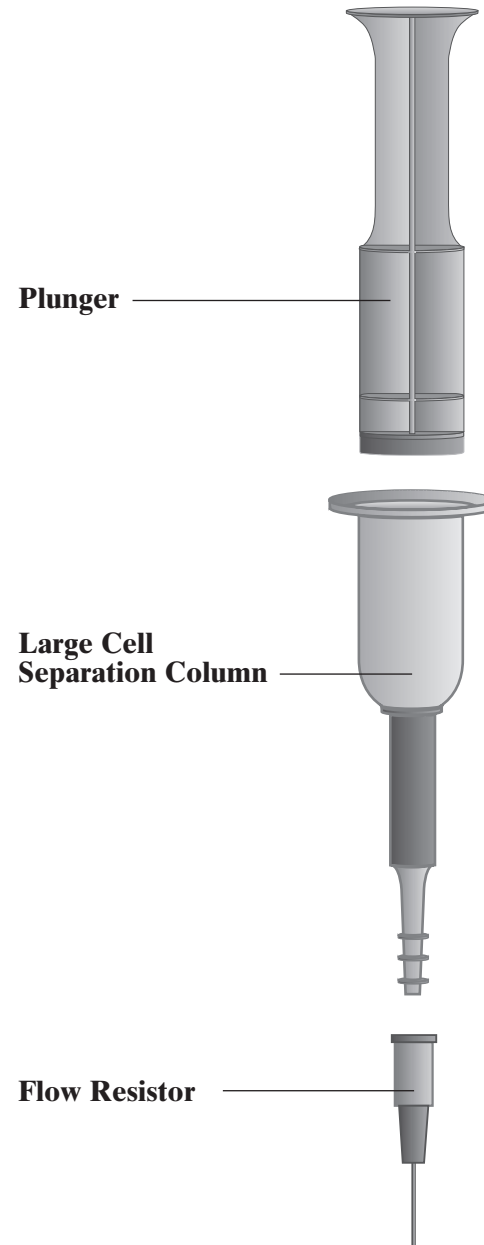
How to Use MACS Large Cells Separation Column

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

The column has a hydrophilic coating that allows rapid filling. The recommended buffer is PBS supplemented with EDTA and BSA, but also other running buffers may be used with the Large Cell Separation Column. The suitability of a specific buffer has to be tested experimentally.

The column is washed with buffer before separation. The magnetically labeled cells to be separated should be well suspended and should not contain clumps, aggregates or particles $>50 \mu\text{m}$. After applying the cells onto the column, the column is washed with buffer to remove non-labeled cells. To elute the retained cells, the column is removed from the magnet. After removal of the flow resistor, the cells are eluted in buffer using the plunger supplied with the columns.

Large Cell Separation Column



Protocol for Cell Separation Using Large Cell Separation Columns and MiniMACS

Preparation of the Large Cell Separation Column

- Place the Large Cell Separation Column in the MiniMACS Separation Unit.
- Attach the flow resistor (23G needle) to the Large Cell Separation Column.
- Apply 500 µl of degassed buffer on top of the column and let the buffer run through. Then discard effluent and change collection tube.

Cell Separation Using Large Cell Separation Column

- Pipette magnetically labeled cell suspension containing up to 10^7 positive cells in maximum 2×10^8 total cells onto the column (up to 10^8 cells per 500 µl of buffer). Allow the cell suspension to run through and wash with 500 µl buffer. Collect effluent as negative fraction.
- Wash column with 2–3x500 µl buffer and collect effluent as negative fraction.
- Remove column from separator. Remove flow resistor and place the column on a new collection tube.
- Apply 1 ml of buffer to the reservoir of the column and flush out cells using the plunger supplied.
- (Optional) Repeat separation on a new column to increase purity of the positive fraction.

Important Notes

- ▲ 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.
- ▲ The recommended buffer is PBS supplemented with 2 mM EDTA and 0.5 % BSA. Different buffers may be used, but have to be tested experimentally.
- ▲ The columns are for single use only. The columns contain a biocompatible hydrophilic coating. This coating is washed out during the filling process. Use column immediately after filling to avoid formation of air bubbles caused by warming up of the buffer in the column.
- ▲ Use a maximum cell concentration of 10^8 cells per 500 µl of buffer when applying cells to the separation column.

- ▲ 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.
- ▲ Do not use samples or buffers with too high a viscosity or with particles >50 µm.
- ▲ Large Cell Separation Columns are not suitable for particles larger than 50 µm. To remove clumps and prevent aggregate formation in the sample, resuspend cells carefully and pass through 50 µm nylon mesh or filter before separation.
- ▲ To increase purity, cells can be passed over a new column a second time.
- ▲ For details on magnetic labeling, see “MACS Reagent Data Sheets”.
- ▲ If the flow stops during separation, check if the buffer is properly degassed. Start flow again with a slight push of the plunger. Do not pass the cells through the column with the plunger.
- ▲ When working with fresh anticoagulated blood or buffy coat, dilute before separation 1:2 with buffer.
- ▲ Do not use Large Cell Separation Column in combination with magnetic particles other than MACS MicroBeads. Magnetic forces in the column are very high and may damage cells if other magnetic particles are used.

Technical Specifications

- Typical capacity: 2–10 µl of packed magnetically labeled material, e.g. 10^7 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 up to 1000fold, 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 PBS containing 0.5 % BSA: 1.6–2.3 ml/minute.

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 BIOTEC GmbH'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.