Miltenyi Biotec

XLS Columns

Order no. 130-136-286

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1. Description

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

Components	10 XLS Columns and plungers, sterile packed
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. XLS 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. XLS Columns contain an optimized matrix to generate this strong magnetic field when placed in a permanent magnet, such as the QuadroMACS XL Separator.

1.3 Technical specifications

Max. cell capacity per single column	
Max. number of labeled cells	5×10^8
Max. number of total cells	1×10^{10}
Max. volume per single column for the StraightFrom® Leukopak® MicroBead Kits	
Leukopak®	up to 25 mL
Max. volume per single column for the StraightFrom PBMC Isolation Kits	
Whole blood	up to 100 mL
Buffy coat	up to 40 mL
Leukopak®	up to 20 mL

▲ **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: 10^6–5×10^8 labeled cells in 10^7–10^{10} total cells.
- Typical enrichment rate: 50-fold to up to 1,000-fold, depending on the strength and specificity of the magnetic labeling. Up to 10,000-fold enrichment can be achieved by separation over two sequential columns.
- Columns are “flow stop” and do not run dry.
- Void volume: 1.85 mL. Reservoir volume: 27 mL.

  ▲ **Note:** Use a sufficiently large collection tube, and replace it as needed, to prevent any sample overflow.
- Typical flow rate for phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA: 3.6–8.4 mL/min.
- XLS Columns are for single use only.

1.4 Applications

XLS Columns have been developed for positive selection of human and animal cells, especially rare cells, out of a heterogeneous cell suspension in combination with a MACS Separator. XLS Columns can also be used for depletion of cells which strongly express the magnetically labeled surface antigen. They can also be used to separate other biological material such as plant cells, bacteria, viruses, protozoa, cell organelles, etc.

▲ Do not use XLS 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.

▲ XLS 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 before separation.

▲ Samples or buffers with high viscosity might cause reduced column flow or column clogging.

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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.

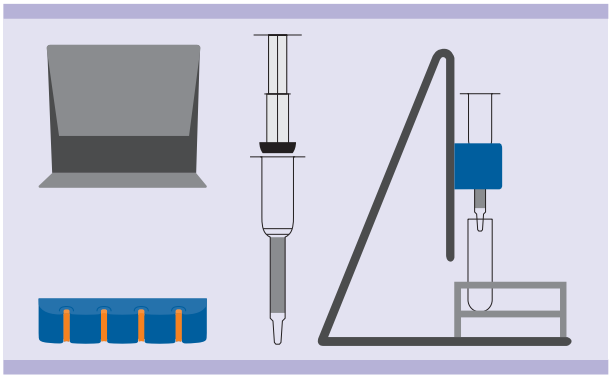
  ▲ **Note:** The recommended buffer is PBS supplemented with EDTA and BSA. The suitability of other buffers has to be tested experimentally.

  ▲ **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.
- QuadroMACS XL Separator (# 130-136-311)
- MACS MultiStand (# 130-042-303) in combination with QuadroMACS XL Separator
- (Optional) MACS XL Tube Rack (# 130-136-312) in combination with the QuadroMACS XL Separator. Refer to the data sheet of the MACS XL Tube Rack for detailed information.

2. Use of XLS Columns with the QuadroMACS XL Separator

2.1 Preparation of XLS Columns

1. Attach the QuadroMACS XL Separator to the MACS MultiStand. Place XLS Column in the separator. Place a collection tube under the XLS Column.



▲ **Note:** Check that the ejection blocks in the gap of the magnet are attached before placing the XLS Column into the magnetic field of the QuadroMACS XL Separator.

▲ **Note:** Be careful when attaching the QuadroMACS XL Separator to the MACS MultiStand and placing the XLS Column in the separator to avoid trapping your fingers (for details refer to the respective data sheet).

▲ **Note:** When using the MACS XL Tube Rack, refer to the data sheet for detailed information.

2. Prepare XLS Column by rinsing with buffer: apply 10 mL of degassed buffer on top of the column and let the buffer run through. XLS Columns are “flow stop” and do not run dry.
3. Discard effluent and change collection tube. The XLS 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 XLS Columns

▲ The workflow described below is applicable when PBMC is used as the starting material. For StraightFrom Technology applications, please refer to the respective StraightFrom Technology data sheet.

▲ 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 buffer.

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

   ▲ **Note:** To remove clumps, pass cells through MACS SmartStrainers (30 µm).
2. Apply cell suspension onto the prepared XLS Column. Collect flow-through containing unlabeled cells.
3. Wash XLS Column with amount of degassed buffer as stated on respective MACS Cell Separation Reagent data sheet. 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 XLS Column from the QuadroMACS XL Separator and place it on a new collection tube.
5. Pipette 10 mL buffer onto the XLS Column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger.

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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