XL Whole Blood Columns

Order no. 130-139-767

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1. Description

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

Components	**XL Whole Blood Columns (# 130-139-767):** 10 XL Whole Blood Columns with 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. XL Whole Blood 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. XL Whole Blood 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. volume of whole blood per single column	Max. buffy coat sample
XL Whole Blood column	100 mL	1× buffy coat

▲ **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.

- 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.
- Typical flow rate for phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) and 2 mM EDTA: 14.5–22.5 mL/min.
- XL Whole Blood Columns are for single use only.

1.4 Applications

XL Whole Blood Columns have been developed for positive selection of leukocyte subsets from anticoagulated whole blood or buffy coat sample labeled with MACSprep™ MicroBeads, StraightFrom® Whole Blood MicroBeads, or StraightFrom Buffy Coat MicroBeads in combination with the QuadroMACS XL Separator.

▲ Do not use XL Whole Blood 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.

▲ XL Whole Blood 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

- Separation 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). Alternatively, use autoMACS Running Buffer. Degas the buffer before use, as air bubbles could block the column.

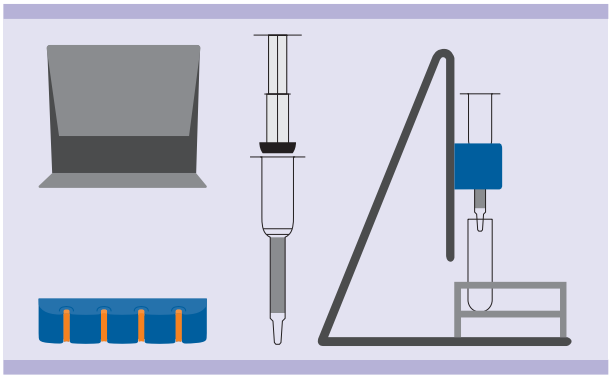
  ▲ **Note:** EDTA can be replaced with other supplements, such as anticoagulant citrate dextrose formula A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced with other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended.
- StraightFrom MicroBeads or MACSprep MicroBeads for magnetic labeling of cells.
- QuadroMACS XL Separator (# 130-136-311)
- MACS MultiStand (# 130-042-303) in combination with QuadroMACS XL Separator
- (Optional) MS Columns (# 130-042-201) or LS Columns (# 130-042-401).
- (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.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183).
- (Optional) Whole Blood Column Elution Buffer (# 130-131-889) to elute positive cell fraction

  ▲ **Note:** Allow the Whole Blood Column Elution Buffer to reach room temperature before use.
- (Optional) Pre-Separation Filters (30 µm) (# 130-041-407) to remove cell clumps.

2. Use of XL Whole Blood Columns with the QuadroMACS XL Separator

2.1 Preparation of XL Whole Blood Columns

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



▲ **Note:** Check that the ejection blocks in the gap of the magnet are attached before placing the XL Whole Blood 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 XL Whole Blood Column in the separator to avoid trapping your fingers (for details refer to the QuadroMACS XL data sheet).

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

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

▲ Adjust the degassed elution buffer to room temperature (+15 to +25 °C).

▲ 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. Apply magnetically labeled cell suspension onto the prepared XL Whole Blood Column. Collect flow-through containing unlabeled cells.
2. Wash XL Whole Blood 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 1.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
3. Remove XL Whole Blood Column from the QuadroMACS XL Separator and place it on a new collection tube.
4. Pipette 10 mL elution buffer onto the XL Whole Blood Column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the Manual Elution Plunger XL.

   ▲ **Note:** Alternatively, elution may be performed using Whole Blood Column Elution Buffer. For details, refer to the corresponding reagent data sheet.

   ▲ **Note:** The Manual Elution Plunger XL is intended for single-use only.
5. (Optional) To increase the purity of the magnetically labeled fraction, the eluted fraction can be enriched over a second MS Column (for up to 10^7 magnetically labeled cells) or LS Column (for up to 10^8 magnetically labeled cells). Repeat the magnetic separation procedure as described in steps 2 to 5 by using a new column. Remaining erythrocytes can be lysed using Red Blood Cell Lysis Solution (10×) (# 130-094-183).

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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