



## Separation Columns

### Contents

5 MACS High Gradient Magnetic Separation Columns of type XS to be used with SuperMACS or SuperMACS II, sterile packed. Capacity: max.  $2 \times 10^{10}$  total cells and  $10^9$  magnetically labeled cells. Five tubes with flow resistor, five 3-way stopcocks, five 20 ml syringes and five 50 ml syringes are included.

### Applications

- ▲ Positive selection of up to  $10^9$  **positive** cells labeled with MACS MicroBeads from up to  $2 \times 10^{10}$  **total** cells.
- ▲ Magnetic separation of biological material labeled with MACS MicroBeads such as bacteria, viruses, protozoa, cell organelles etc.

### Storage of MACS Separation Columns

Store columns dry. Do not use after expiry date.

### Instrument and Reagent Requirement

Magnetic cell separator SuperMACS or SuperMACS II with XS Column Adapter. MACS MicroBeads for magnetic labeling of cells or biological material.

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

### Additional Material Required

40  $\mu$ m nylon mesh or filter (e.g. Cell Strainer, Order No. 2340, from Becton Dickinson, San José, USA).

### How to Use MACS XS Separation Columns

XS Separation Columns have been developed for positive selection of high cell numbers out of heterogeneous suspensions in the magnetic field of the SuperMACS or SuperMACS II with XS Column Adapter. They can be used to separate biological material including human and animal cells, plant cells, bacteria, cell organelles, or other bioparticles.

The recommended buffer is PBS supplemented with EDTA and BSA, but also other running buffers may be used with the XS Separation Column. The suitability of a specific buffer has to be tested experimentally.

The column is washed with buffer before separation. The magnetically labeled material to be separated should be well suspended and should not contain clumps, aggregates or particles  $>30 \mu$ m. After applying the material onto the column, the column is washed with buffer to remove non-labeled material. To elute the retained material, e.g. bound cells, the column is removed from the magnet and the material is eluted in buffer.

# XS Separation Columns

5 XS Separation Columns

Order No. 130-041-202

### Technical Specifications

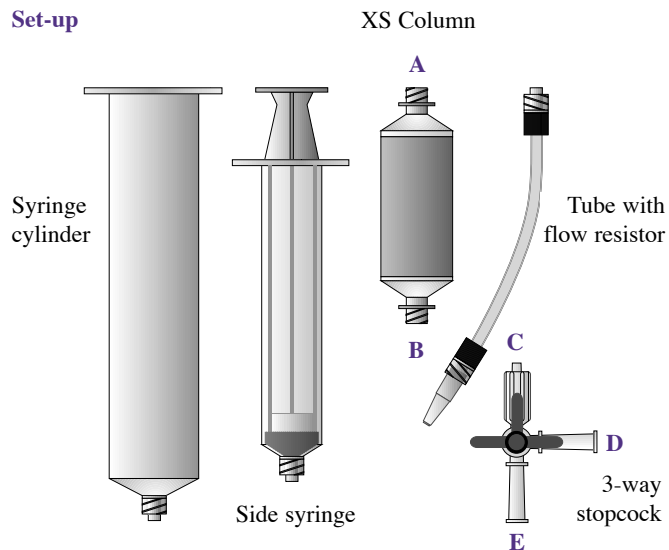
- Typical capacity: e.g.  $10^9$  lymphocytes.
- Recommended sample size for leukocytes:  $10^6$ – $10^9$  magnetically labeled cells in  $10^8$ – $2 \times 10^{10}$  total cells. Sample concentration: up to  $10^9$  leukocytes/5 ml cell suspension.
- Typical enrichment rate: 50 up to 1000fold, depending on the strength and specificity of the magnetic labeling. Up to 10,000fold enrichment can be achieved by separation over two sequential columns.
- Void volume: approx. 6.2 ml.
- Typical flow rate for PBS containing 0.5 % BSA: about 20 ml/min with flow resistor; about 40 ml/min without flow resistor during wash and elution step.



## Protocol for Cell Separation Using XS Separation Columns and SuperMACS or SuperMACS II

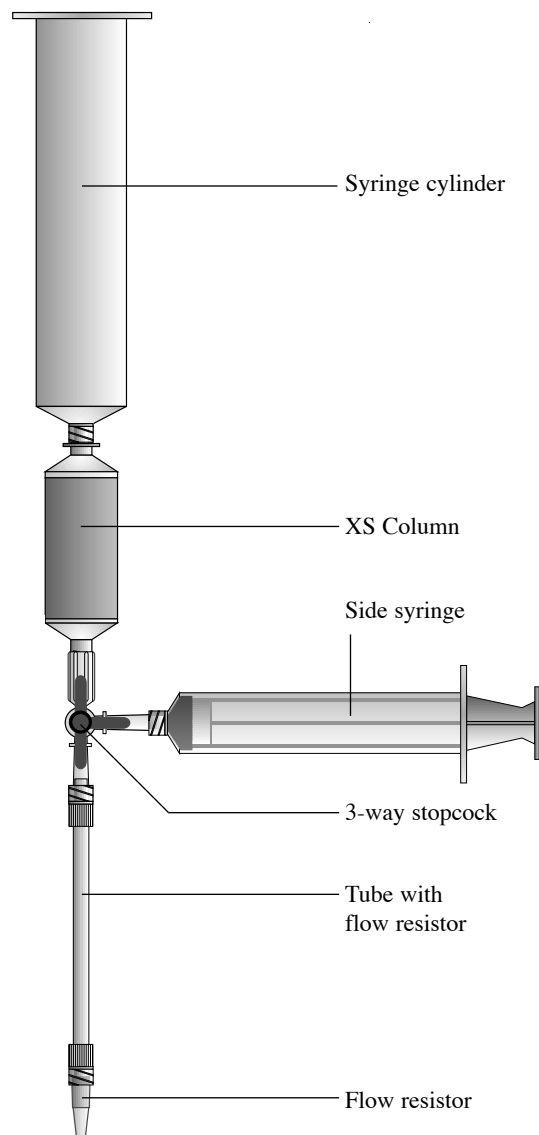
### Assembly of XS Separation Column for Use in SuperMACS

#### Set-up

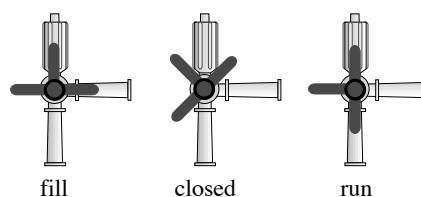


#### Preparation of the XS Separation Column

- Attach the cylinder of the 50 ml syringe to port A of the column.
- Attach the 3-way stopcock with port C to port B of the column.
- Attach the tube with flow resistor to port E of the 3-way stopcock and remove the plastic sheath.
- Fill the supplied 20 ml syringe (side syringe) with buffer and attach to port D of the 3-way stopcock.
- Move the adapter holder out of the magnetic field of the SuperMACS or SuperMACS II by turning the handle, mount the XS Column Adapter, and insert the assembled column (for details, see "Instructions for Use").
- Turn 3-way stopcock to position "fill".
- Carefully fill the column from the bottom with buffer from the syringe until the buffer reaches the syringe cylinder.
- Turn the 3-way stopcock to position "run" and rinse column by filling from the top with buffer. Allow buffer to run into the column. Then add more buffer. Rinse with 50 ml of buffer.
- Close 3-way stopcock; leave the side syringe attached to port D of the 3-way stopcock during separation, except when refilling with buffer.
- Move column in the magnetic field of the SuperMACS or SuperMACS II by turning the handle.



#### 3-way stopcock positions



### Cell Separation Using XS Separation Column

- Pass cells through 40  $\mu\text{m}$  nylon mesh or filter (e.g. Cell Strainer, Order No. 2340, from Becton Dickinson, San José, USA) to remove clumps.
- Apply up to  $10^9$  magnetically labeled cells in a maximum of  $2 \times 10^{10}$  total cells (up to  $10^9$  total cells per 5 ml) into the syringe cylinder that is set up on the XS Separation Column and turn 3-way stopcock to position "run". Allow the cells to pass through the column and rinse with 30 ml of buffer. Collect the flow through as negative fraction.
- Close 3-way stopcock and remove flow resistor from the tube by attaching the plastic sheath and turning counterclockwise.
- Wash column with 3 x 30 ml of buffer. Collect the flow through as wash fraction.
- Remove column out of the magnetic field of the SuperMACS by turning the handle (see "Instructions for Use").
- Detach syringe cylinder from port A of the XS Separation Column.
- Detach side syringe from port D of the 3-way stopcock, fill with buffer and attach side syringe to port A of the XS Separation Column.
- Elute retained cells with 20 ml buffer using the side syringe.
- (Optional) Repeat magnetic separation step: apply the eluted cells to a new prefilled XS Separation Column, wash, and elute retained cells in buffer.

- ▲ Do not use XS Separation 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 magnetic particles are used.

### 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.

### Important Notes

- ▲ For protocols using XS Separation Columns in a closed system, contact Miltenyi Biotec.
- ▲ 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 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. Use column immediately after filling to avoid formation of air bubbles in the column caused by warming up.
- ▲ Use a cell concentration of  $10^9$  cells per 5 ml of buffer when applying cells to the separation column.
- ▲ XS Separation Columns are not suitable for particles larger than 30  $\mu\text{m}$ . To remove clumps and prevent aggregate formation in sample, resuspend material carefully and pass through 40  $\mu\text{m}$  nylon mesh or filter (e.g. Cell Strainer, Order No. 2340, from Becton Dickinson, San José, USA) before separation.
- ▲ To increase purity, cells can be passed over a new freshly prepared column a second time.
- ▲ Do not use samples or buffers with too high a viscosity or with particles  $>30 \mu\text{m}$ .