µ Columns

Order no. 130-042-701

Contents

1. Description

Components	20 µ Columns for the isolation of magnetically labeled molecules. Sterile packed.
Storage	Store columns dry and protected from light. Do not use after expiration date.

▲ µ Columns are for molecule isolation only. Do not use µ Columns for cell separation.

▲ Do not use µ Columns in combination with magnetic particles other than µMACS™ MicroBeads. Magnetic forces in the column are very high and may damage biological material if other beads are used.

▲ µ Columns are not suitable for particles larger than 30 µm. To remove clumps and to prevent aggregates in the sample, resuspend material carefully and precipitate clumps by centrifugation before applying the sample on the column.

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

1.1 Background information

µ Columns are used for molecular biology and protein biochemistry applications such as isolation of mRNA, immunoprecipitated protein, or epitope tagged protein in combination with µMACS MicroBeads and a thermoMACS™ or µMACS Separator. As MACS MicroBeads are extremely small, superparamagnetic particles, a high-gradient magnetic field is required to retain the labeled molecules. µ Columns contain an optimized matrix to generate this strong magnetic field when placed in a permanent magnet, such as the thermoMACS or µMACS Separator. Washing and elution steps are performed by simply rinsing the column with an appropriate buffer as described in the individual µMACS MicroBeads data sheet or as tested experimentally.

1.2 Applications

- Isolation of up to 10 µg mRNA derived from a maximum of 10^7 cells, 30 mg human or animal tissue, 100 mg plant tissue, or 200 µg total RNA.
- Immunoprecipitation of proteins using 1–2 µg of monoclonal antibody, 10–100 µL of hybridoma supernatant, 0.1–1 µL of ascites, or 0.5–5 µL of serum containing 1–10% specific antibody in combination with Protein A or Protein G MicroBeads.
- Isolation of up to 20 pmol epitope tagged protein from a maximum of 10^7 cells.

1.3 Technical specifications

- For capacity of the µ Column, refer to the respective µMACS MicroBeads data sheet.
- Columns are flow stop and do not run dry.
- Void volume: 30 µL.
- Recommended filling volume: 1 mL.
- Typical flow rate for PBS: 300 µL/minute.
- The columns are for single use only.

1.4 Reagent and instrument requirements

- Equilibration buffer for column preparation: buffer supplemented with 1% detergent, e.g. SDS, Triton® X-100 or Igepal® CA-630 (formerly NP-40). Alternatively, 70% ethanol in H2O bidest can be used. Degas buffer before use, as air bubbles could block the column.
- Separation buffer: any buffer suitable for your magnetic separation. For details see the respective µMACS MicroBeads data sheet. If the separation buffer contains 1% detergent, it can also be used for column preparation. Degas buffer before use, as air bubbles could block the column.

  ▲**Note:** Use degassed buffer only! Degas buffer by applying vacuum or sonification for ten minutes, preferentially with buffer at room temperature. Excessive gas in running buffer will form bubbles in the matrix during isolation. This is particularly important, when the applied buffer has a different temperature as the µ Column, e.g. when using cold buffer on a column at room temperature. Air bubble formation in the µ Column may lead to clogging of the column and decrease the quality of isolation.
- µMACS MicroBeads for magnetic labeling of target molecules.
- µMACS Separator or thermoMACS Separator.

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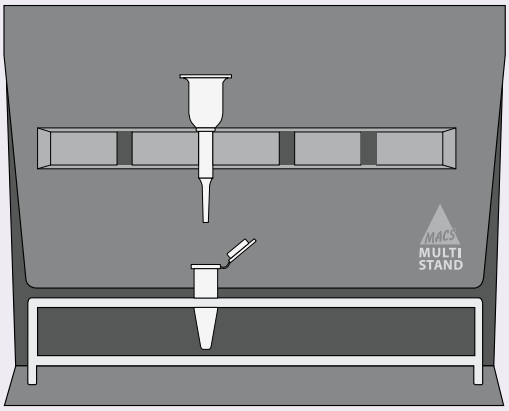
2. Preparation of the µ Column

1. Attach thermoMACS or µMACS Separator to the MultiStand.
2. Place the µ Column in the thermoMACS or µMACS Separator. Place a collection tube under the column (see figure below).



3. Apply 100 µL of degassed equilibration buffer on top of the column and let the solution run through.
4. If the separation buffer is different from the equilibration buffer, apply 100 µL of degassed separation buffer and let the solution run through. The µ Column is now ready for magnetic separation. Perform the magnetic separation as indicated in the individual µMACS MicroBeads data sheets.

▲ **Note:** Use column immediately after filling to avoid formation of air bubbles caused by warming up. An air bubble might be trapped on top of the column matrix and could lead to flow stop. To remove air bubbles, remove buffer still contained in the column reservoir. Pipette new buffer with maximum force exactly on top of the column matrix so that the column starts running again.

All protocols and data sheets are available at www.miltenyibiotec.com.

Warranty

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