Miltenyi Biotec

Anti-Cytokeratin MicroBeads
human

Order no. 130-123-094

Components	**2 mL Anti-Cytokeratin MicroBeads:** MicroBeads conjugated to monoclonal recombinant engineered antibodies (isotype: recombinant human IgG1).
Capacity	For up to 10^9 total cells from dissociated formalin-fixed, paraffin-embedded (FFPE) tissue, up to 50 separations.
Product format	All components are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2 – 8 °C. Do not freeze. The expiration date is indicated on the vial labels.

Safety information

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

Before use, please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Cell separation protocols

Fully automated cell labeling and separation using the autoMACS® Pro Separator

Alternatively:

Manual magnetic labeling

↓

Subsequent manual separation

or

↓

Subsequent automated cell separation using the autoMACS® Pro Separator

General notes

▲ For tips concerning sample preparation, magnetic labeling and separation, visit **www.miltenyibiotec.com/faq** and **www.miltenyibiotec.com/protocols**.

▲ For product-specific background information and applications of this product, refer to the respective product page at **www.miltenybiotec.com/130-123-094**.

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). Degas buffer before use, as air bubbles could block the column.
- (Optional) FFPE Tissue Dissociation Kit (# 130-118-052) for the generation of single-cell suspension from FFPE tumor tissue sections.
- (Optional) gentleMACS™ Octo Dissociator with Heaters (# 130-096-427)
- (Optional) gentleMACS C Tubes (# 130-093-237, # 130-096-334)
- (Optional) Pre-Separation Filters (20 µm) (# 130-101-812) to remove cell clumps.
- (Optional) DAPI (# 130-098-462) for nuclear staining.
- (Optional) Flurochrome-conjugated antibodies, e.g. Anti-Cytokeratin-FITC. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- Choose the appropriate MACS Separator and MACS Columns:

Column	Max. number of labeled cells	Max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS
autoMACS	2×10^8	4×10^9	autoMACS Pro

Fully automated cell labeling and separation using the autoMACS® Pro Separator

▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.

▲ All buffer temperatures should be ≥10 °C.

▲ Place tubes in the following Chill Rack positions:

position A = sample, **position B** = negative fraction, **position C** = positive fraction.

1. For appropriate resuspension volumes and cell concentrations, please visit **www.automacspro.com/autolabeling**.
2. Switch on the instrument for automatic initialization.
3. Go to the **Reagent** menu and select **Read Reagent**. Scan the 2D barcode of each reagent vial with the barcode scanner on the autoMACS Pro Separator. Place the reagent into the appropriate position on the reagent rack.
4. Place sample and collection tubes into the Chill Rack.
5. Go to the **Separation** menu and select the reagent name for each sample from the **Labeling** submenu (the correct labeling, separation, and wash protocols will be selected automatically).
6. Enter sample volume into the **Volume** submenu. Press **Enter**.

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7. Select **Run**.
8. Collect enriched cytokeratin-positive cell fraction at position C = positive fraction.

Manual magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions (2–8 °C).

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly.

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic labeling.

1. Prepare cells and determine cell number.
2. Centrifuge cell suspension at 1000×g for 5 minutes at 4 °C. Aspirate supernatant completely.
3. Resuspend cell pellet in 160 µL of buffer per 10^7 total cells.
4. Add 40 µL of Anti-Cytokeratin MicroBeads per 10^7 total cells.
5. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
6. Add buffer to a final volume of 1 mL.
7. Centrifuge cell suspension at 1000×g for 5 minutes at 4 °C. Aspirate supernatant completely.
8. Resuspend cell pellet in 500 µL of buffer.
9. Proceed to subsequent magnetic cell separation.

   ▲ **Note:** A minimum of 500 µL is required for magnetic separation. If necessary, add buffer to the cell suspension.

Subsequent manual cell separation

▲ Always wait until the column reservoir is empty before proceeding to the next step.

10. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to the MS Column data sheet.
11. Prepare column by rinsing with 500 µL of buffer.
12. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
13. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 12.

    ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
14. Remove column from the separator and place it on a suitable collection tube.
15. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
16. The eluted fraction has to be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 10 to 15 by using a new MS Column.

Subsequent automated cell separation using the autoMACS® Pro Separator

▲ Refer to the user manual for instructions on how to use the autoMACS® Pro Separator.

▲ All buffer temperatures should be ≥10 °C.

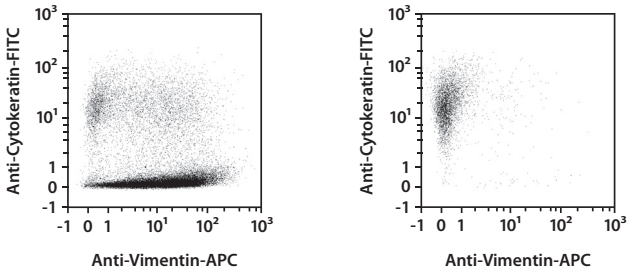
▲ Place tubes in the following Chill Rack positions:

position A = sample, **position B** = negative fraction, **position C** = positive fraction.

10. Prepare and prime the instrument.
11. Follow the instructions that are given in the user manual.
12. The program **"Posseld2"** is recommended. Collect enriched carcinoma cells at position C = positive fraction.

Example of a separation using Anti-Cytokeratin MicroBeads

Human carcinoma cells were purified from a mixture of dissociated FFPE tumor cells spiked in dissociated FFPE spleen cells using Anti-Cytokeratin MicroBeads, human. Isolation was performed on an autoMACS® Pro Separator. The cells were fluorescently stained with Anti-Cytokeratin-FITC, Anti-Vimentin-APC, and CD235a (Glycophorin A)-PE, and DAPI and analyzed by flow cytometry using the MACSQuant® Analyzer.
Aggregates were excluded from the analysis based on scatter signals and single-cells were gated on DAPI fluorescence.



For more information or assistance refer to our technical support.

Check out Miltenyi Biotec's flow cytometry solutions at **www.miltenyibiotec.com/MACSQuant** and explore the extensive antibody portfolio at **www.miltenyibiotec.com/antibodies.**

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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