Miltenyi Biotec

Basic MicroBeads

Order No. 130-048-001

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1. Description

Components	2 mL Basic MicroBeads
Size	For 4x10^{10} total cells, up to 4000 separations.
Product format	Basic MicroBeads (unconjugated) are supplied as a suspension in phosphate buffered saline (PBS) containing 5 mM EDTA and 0.05% sodium azide.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Principle of MACS® separation

First, cells are magnetically labeled with Basic MicroBeads. Then the cell suspension is loaded onto a column which is placed in the magnetic field of a MACS® Separator. The magnetically labeled cells are retained on the column. The unlabeled cells run through and this cell fraction is depleted of labeled cells. After removal of the column from the magnetic field, the magnetically retained material can be eluted as the positively selected fraction.

1.2 Background and product applications

Basic MicroBeads are unconjugated MicroBeads.

Examples of applications

- Depletion of material which non-specifically binds to MicroBeads, e.g. sticky or dead cells.
- Isolation of lysosomes after endocytosis of Basic MicroBeads by keratinocytes.[1]

1.3 Reagent and instrument requirements

- Buffer (degassed): PBS (phosphate buffered saline) pH 7.2, supplemented with 0.5% BSA and 2 mM EDTA. Keep buffer cold (4–8 °C).

  ▲ **Note:** EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as gelatin, human serum albumin, human serum or bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators:
  Basic MicroBeads can be used in combination with MS, LS or XS Columns. They can also be used in combination with LD, CS or D Columns and by using the autoMACS™ Separator. When depleting sticky or dead cells, we recommend using the same column type and the same autoMACS program as will be used in the subsequent experiment.

Column	max. number of labeled cells	max. number of total cells	Separator
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
XS	10^9	2×10^{10}	SuperMACS
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS

▲ **Note:** Column adapters are required to insert certain columns into VarioMACS or SuperMACS. For details, see MACS Separator data sheets.

- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filter (# 130-041-407).

2. Protocol

2.1 Sample preparation

Isolate single-cell suspension by standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume of all indicated reagent volumes and total volumes).

▲ For optimal performance it is important to obtain a single-cell suspension before magnetic separation. Pass cells through 30 µm nylon mesh (Pre-Separation Filter # 130-041-407) to remove cell clumps which may clog the column.

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1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Pipette off supernatant completely.
3. Resuspend cell pellet in 50 µL of buffer per 10^7 total cells.
4. Pre-dilute appropriate amount of Basic MicroBeads 1:10 with PBS.

   ▲ **Note:** To sterilize Basic MicroBeads, pass the pre-diluted Basic MicroBeads through a 0.2 µm GV4 filter (Millipore S.A., Molsheim, France)
5. Add 5 µL of pre-diluted Basic MicroBeads per 10^7 total cells.
6. Mix well and incubate for 15 minutes at 4–8 °C.

   ▲ **Note:** To increase the depletion efficiency, the incubation temperature or the time may be altered, e.g. to room temperature or to 30 minutes, respectiveley.
7. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
8. Resuspend up to 10^8 cells in 500 µL of buffer.

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

   ▲ **Note:** For depletion with LD Columns, resuspend cell pellet in 500 µL of buffer for up to 1.25×10^8 cells.
9. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of magnetically labeled cells (see table in section 1.3).

▲ When depleting sticky or dead cells, we recommend using the same column type and the same autoMACS program as will be used in the subsequent experiment.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator (see "Column data sheets").
2. Prepare column by rinsing with appropriate amount of buffer:

   MS: 500 µL LS: 3 mL.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times, each time once the column reservoir is empty.

   MS: 3×500 µL LS: 3×3 mL.

   Collect total effluent. This is the unlabeled cell fraction.
5. (Optional) Remove column from the separator and place it on a suitable collection tube.
6. (Optional) Pipette appropriate amount of buffer onto the column. Immediately flush out fraction with the magnetically labeled cells by firmly applying the plunger supplied with the column.

   MS: 1 mL LS: 5 mL.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation, refer to the "XS Column data sheet".

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator (see "LD Column data sheet").
2. Prepare column by rinsing with 2 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 2×1 mL of buffer. Collect total effluent. This is the unlabeled cell fraction.

Depletion with CS Columns

1. Assemble CS Column and place it in the magnetic field of a suitable MACS Separator (see "CS Column data sheet").
2. Prepare column by filling and rinsing with 60 mL of buffer. Attach a 22G flow resistor to the 3-way-stopcock of the assembled column (see "CS Column data sheet").
3. Apply cell suspension onto the column.
4. Collect unlabeled cells which pass through and wash column with 30 mL buffer from the top. Collect total effluent. This is the unlabeled cell fraction.

Depletion with D Columns

For instructions on column assembly and separation, refer to the "D Column data sheet".

Magnetic separation with the autoMACS™ Separator

▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS™ Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing the magnetically labeled cells in the autoMACS Separator. Choose a separation program according to the recommendations in the autoMACS User Manual.

   ▲ **Note:** Program choice depends on the isolation strategy, the strength of magnetic labeling and the frequency of magnetically labeled cells. For details see autoMACS User Manual: "autoMACS Cell Separation Programs".

3. Reference

1. Glombitza, GJ; Becker, E; Kaiser, HW; Sandhoff, K (1997) Biosynthesis, Processing, and Intracellular Transport of G_{M2} Activator Protein in Human Epidermal Keratinocytes. J. Biol. Chem. 272: 5199-5207. [478]

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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