Anti-FITC MicroBeads

Magnetic cell sorting

Order No. 130-048-701

Index

1. Description

Components	2 mL Anti-FITC MicroBeads: MicroBeads conjugated to monoclonal anti-FITC Isomer-1 antibody (isotype: mouse IgG1)
Size	For 2×10^9 total cells, up to 200 separations.
Product format	Anti-FITC MicroBeads are supplied in a solution containing stabilizer 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, the cells are stained with a FITC-conjugated primary antibody or ligand. Subsequently, the cells are magnetically labeled with Anti-FITC MicroBeads. Then the cell suspension is loaded on a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled cells are retained in the column while the unlabeled cells run through. After removal of the column from the magnetic field, the magnetically retained cells can be eluted as the positively selected cell fraction.

1.2 Background and product applications

Anti-FITC MicroBeads are developed for the separation of cells according to surface markers labeled with FITC-conjugated antibodies, peptides or ligands. After separation the FITC-labeled cells can be detected by flow cytometry or fluorescence microscopy.

▲ **Note:** Magnetic labeling with Anti-FITC MicroBeads may reduce the fluorescence intensity of the FITC staining.

Examples of applications

- Positive selection or depletion of cells labeled with FITC-conjugated antibodies.
- Positive selection or depletion of cells labeled with FITC-conjugated peptides or ligands.

1.3 Reagent and instrument requirements

- Buffer (degassed): Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA (bovine serum albumin) 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 (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 human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- FITC-conjugated primary antibody, peptide or ligand.

  ▲ **Note:** Use primary reagents conjugated with **FITC Isomer-1** only. Most commercially antibody suppliers use FITC Isomer-1.

- (Optional) PI (propidium iodide) or 7-AAD for exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- MACS Columns and MACS Separators: Cells labeled with Anti-FITC MicroBeads can be enriched by using MS, LS or XS Columns (positive selection). Anti-FITC MicroBeads can be used for depletion of cells on LD, CS or D Columns. Cells which strongly express the FITC-labeled antigen can also be depleted using MS, LS or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Separator.

Column	max. number of labeled cells	max. number of total cells	Separator
Positive selection			
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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS VarioMACS, SuperMACS
CS	2×10^8		VarioMACS, SuperMACS
D	10^9		SuperMACS
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS

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

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2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, PBMC should be isolated by density gradient centrifugation (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **Note:** Remove platelets after density gradient separation: resuspend cell pellet in buffer and centrifuge at 200xg for 10–15 minutes at 20 °C. Carefully remove supernatant. Repeat washing step and carefully remove supernatant.

When working with tissues, prepare a single-cell suspension by a standard preparation method (see "General Protocols" in the User Manuals or visit www.miltenyibiotec.com).

▲ **Note:** Dead cells may bind non-specifically to MACS MicroBeads. To remove dead cells, we recommend using density gradient centrifugation (e.g. Ficoll-Paque™) or using the Dead Cell Removal Kit (# 130-090-101).

2.3 Magnetic labeling

▲ Work fast, keep the cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and a non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for 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).

▲ Primary FITC-conjugated antibodies should be titrated to determine the optimal staining dilution. Staining should not increase fluorescence intensity of the negative population.

1. After preparation of single-cell suspension count cells and centrifuge cell sample.
2. Resuspend cell pellet and stain with the primary FITC-conjuated antibody according to the manufacturer's recommendations. For MACS FITC-conjugated antibodies, resuspend 10^7 total cells in 100 µL buffer and add 10 µL FITC-conjugate.
3. Mix well and incubate for 10 minutes in the dark at 4–8 °C or according to the manufacturer's recommendations.

   ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. Wash cells to remove unbound primary antibody by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes.

   ▲ **Note:** The optimal relative centrifugal force (RCF) and centrifugation time may be different depending on the cell sample.
5. (Optional) Repeat washing step.
6. Pipette off supernatant completely and resuspend cell pellet in 90 µL of buffer per 10^7 total cells.
7. Add 10 µL of Anti-FITC MicroBeads per 10^7 total cells.

   ▲ **Note:** The concentration of Anti-FITC MicroBeads used to achieve optimal magnetic separation is dependent on the intensity of FITC-conjugated antibody staining and on the frequency of target cells in suspension. Dimly FITC-stained target cells require a higher concentration of Anti-FITC MicroBeads to achieve optimal magnetic labeling and separation.
8. Mix well and incubate for 15 minutes at 4–8 °C.

   ▲ **Note:** Working on ice requires increased incubation time. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
9. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes.

   ▲ **Note:** The optimal relative centrifugal force (RCF) and centrifugation time may be different depending on the cell sample.
10. Pipette off supernatant completely.
11. 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 up to 1.25×10^8 cells in 500 µL of buffer.
12. 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 1.3).

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: 3×3mL.
   Collect total effluent. This is the unlabeled cell fraction.
5. Remove column from the separator and place it on a suitable collection tube.
6. 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.

   ▲ **Note:** To increase the purity of the magnetically labeled fraction, it can be passed over a new, freshly prepared column.

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 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 autoMACS™ Separator

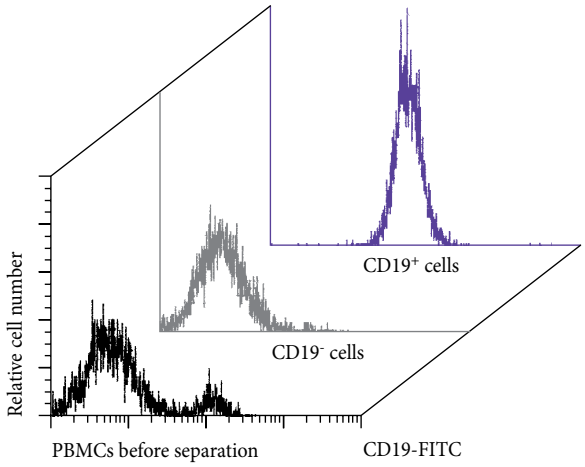
▲ Refer to the "autoMACS™ User Manual" for instructions on how to use the autoMACS Separator.

1. Prepare and prime autoMACS Separator.
2. Place tube containing magnetically labeled cells in autoMACS. 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. Example of a separation using Anti-FITC MicroBeads

Separation of human peripheral blood mononuclear cells (PBMCs) using CD19-FITC, Anti-FITC MicroBeads and a MiniMACS™ Separator with an MS Column.



Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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.

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