Miltenyi Biotec

Anti-Cy7 MicroBeads

Order no. 130-091-652

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1. Description

Components	2 mL Anti-Cy7 MicroBeads: MicroBeads conjugated to monoclonal anti-Cy7 antibodies (isotype: mouse IgG1).
Size	For 10^9 total cells, up to 100 separations.
Product format	Anti-Cy7 MicroBeads 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 label.

1.1 Principle of MACS® Separation

First, the cells are stained with a Cy7-conjugated primary antibody or ligand. Subsequently, the cells are magnetically labeled with Anti-Cy7 MicroBeads. Then, the cell suspension is loaded onto a MACS® Column which is placed in the magnetic field of a MACS Separator. The magnetically labeled cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of magnetically labeled cells. After removing 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-Cy7 MicroBeads are developed for the separation of cells labeled with primary antibodies conjugated to Cy7, Alexa Fluor® 750 or tandem conjugates thereof, like APC-Cy7 or PE-Cy7. After separation the labeled cells can be detected by flow cytometry or fluorescence microscopy without additional staining.

▲ **Note:** Anti-Cy7 MicroBeads may cross-react with Alexa Fluor 680, Cy5, Cy5.5, and tandem conjugates thereof.

Example applications

- Positive selection or depletion of cells labeled with APC-Cy7– or PE-Cy7–conjugated primary antibodies.
- Positive selection or depletion of cells labeled with Alexa Fluor 750 conjugated antibodies, peptides, or ligands.

1.3 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). Keep buffer cold (4–8 °C). Degas buffer before use, as air bubbles could block the column.

  ▲ **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.

- APC-Cy7–, PE-Cy7– or Alexa Fluor 750–conjugated primary antibody, peptide, or ligand.
- MACS Columns and MACS Separators: Cells labeled with Anti-Cy7 MicroBeads can be enriched by using MS, LS, or XS Columns (positive selection) or depleted with the use of LD, CS, or D Columns. Cells which strongly express the Cy7- or Alexa Fluor 750-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 the VarioMACS™ or SuperMACS™ Separators. For details see the respective MACS Separator data sheet.

- (Optional) Propidium iodide (PI) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

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140-001-126.05

2. Protocol

2.1 Sample preparation

When working with anticoagulated peripheral blood or buffy coat, peripheral blood mononuclear cells (PBMCs) should be isolated by density gradient centrifugation, e.g. using Ficoll-Paque™. For details see section General Protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

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

When working with tissues, prepare a single-cell suspension by a standard preparation method. For details see section General Protocols in the user manuals or visit www.miltenyibiotec.com/protocols.

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



2.2 Magnetic labeling

▲ Work fast, keep the cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and 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 Filters, # 130-041-407) to remove cell clumps which may clog the columns.

▲ Primary Cy7- or Alexa Fluor 750-conjugated antibodies should be titrated to determine the optimal staining dilution.

▲ The optimal relative centrifugal force (RCF) and centrifugation time may be different depending on the cell sample.

▲ Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet and stain with the primary Cy7- or Alexa Fluor 750-conjugated antibody according to the manufacturer's recommendations.
4. Mix well and refrigerate for 10 minutes in the dark (4–8 °C) or according to the manufacturer's recommendations.
5. 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. Aspirate supernatant completely.
6. (Optional) Repeat washing step.
7. Resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-Cy7 MicroBeads per 10^7 total cells.
9. Mix well and refrigerate for 15 minutes (4–8 °C).
10. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate 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. For details see table 1.3.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see respective MACS Column data sheet.
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 that pass through and wash column with appropriate amount of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
   MS: 3×500 µL LS: 3×3 mL
   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 an appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
   MS: 1 mL LS: 5 mL

   ▲ **Note:** To increase the purity of the magnetically labeled fraction pass the cells 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. For details 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 that 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. For details see CS Column data sheet.

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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. For details see CS Column data sheet.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that 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 autoMACS™ Separator

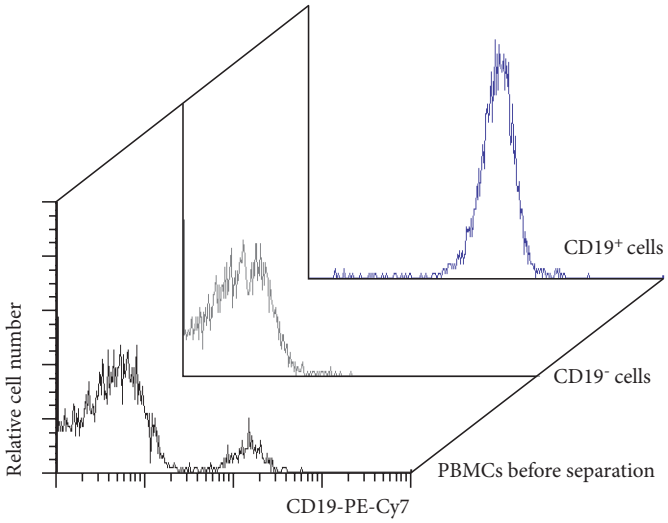
▲ Refer to the autoMACS™ User Manual for instructions on how to use the autoMACS Separator.

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, section autoMACS Cell Separation Programs.

3. Example of a separation using Anti-Cy7 MicroBeads

Separation of human peripheral blood mononuclear cells (PBMCs) using CD19-PE-Cy7, Anti-Cy7 MicroBeads, an MS Column, and a MiniMACS™ Separator. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.



Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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