Miltenyi Biotec

Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads

Order no. 130-091-395

Contents

1. Description
   - 1.1 Principle of the MACS® Separation
   - 1.2 Background information
   - 1.3 Applications
   - 1.4 Reagent and instrument requirements
2. Protocol
   - 2.1 Sample preparation
   - 2.2 Magnetic labeling
   - 2.3 Magnetic separation
3. Example of a separation using the Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads

1. Description

Components	2 mL Anti-Cy5/Anti-Alexa 647 MicroBeads: MicroBeads conjugated to monoclonal anti-Cy5 antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Anti-Cy5/Anti-Alexa 647 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 the MACS® Separation

First, the cells are stained with a Cy5™- or Alexa Fluor® 647-conjugated primary antibody or ligand. Subsequently, the cells are magnetically labeled with Anti-Cy5/Anti-Alexa Fluor 647 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 on 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 information

Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads have been developed for the separation of cells according to surface markers labeled with Cy5- or Alexa Fluor 647-conjugated primary antibodies. Also, cells labeled with primary antibodies conjugated to PE-Cy5 tandem conjugates such as PC5 (Beckman-Coulter), RPE-Cy5 (Serotec) or TRI-COLOR® (Invitrogen), can be separated using Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads. After separation the labeled cells can be detected by flow cytometry or fluorescence microscopy without additional staining.

▲ **Note:** Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads may cross-react with Alexa Fluor 680, Cy5.5, Cy7 and tandem conjugates thereof.

1.3 Applications

- Positive selection or depletion of cells labeled with Cy5- or Alexa Fluor 647-conjugated primary antibodies.
- Positive selection or depletion of cells labeled with PE-Cy5-conjugated antibodies, peptides or ligands.

1.4 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 (2–8 °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 bovine serum. Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- Cy5-, PE-Cy5- or Alexa Fluor 647-conjugated primary antibody, peptide or ligand.
- MACS Columns and MACS Separators: Cells labeled with Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads can be enriched by using MS, LS, or XS Columns or depleted with the use of LD, CS, or D Columns. Cells which strongly express the Cy5- or Alexa Fluor 647-labeled antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS or the autoMACS Pro 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, autoMACS Pro

▲ **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.

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- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.
- (Optional) Pre-Separation Filters (# 130-041-407) to remove cell clumps.

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, for example, using Ficoll-Paque™. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at 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 or lysed blood, prepare a single-cell suspension using standard methods. For details see the General Protocols section of the respective separator user manual. The General Protocols are also available at www.miltenyibiotec.com/protocols.

▲ 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 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 column. Wet filter with buffer before use.

▲ The recommended incubation temperature is 2–8 °C. Working on ice may require increased incubation times. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.

▲ Primary Cy5- or Alexa Fluor 647-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.

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 Alexa Fluor 647- or Cy5-conjugated antibody according to the manufacturer's recommendations.
4. Mix well and incubate for 10 minutes in the dark in the refrigerator (2–8 °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-Cy5/Anti-Alexa Fluor 647 MicroBeads per 10^7 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °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 in section 1.4.

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details see the respective MACS Column data sheet.
2. Prepare column by rinsing with the 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 the appropriate amount of buffer. Collect total effluent; this is the unlabeled cell fraction. 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
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the 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
7. (Optional) To increase the purity of the magnetically labeled cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

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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. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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.
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 the autoMACS™ Separator or the autoMACS™ Pro Separator

▲ Refer to the respective user manual for instructions on how to use the autoMACS™ Separator or the autoMACS Pro Separator.

▲ Buffers used for operating the autoMACS Separator or the autoMACS Pro Separator should have a temperature of ≥ 10 °C.

▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

Magnetic separation with the autoMACS™ Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and the appropriate port for the positive fraction.
3. Choose an appropriate program according to the recommendations in the autoMACS Separator user manual.

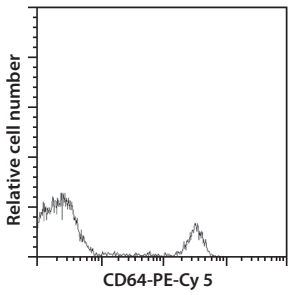
Magnetic separation with the autoMACS™ Pro Separator

1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. Choose an appropriate program according to the recommendations in the autoMACS Pro Separator user manual.

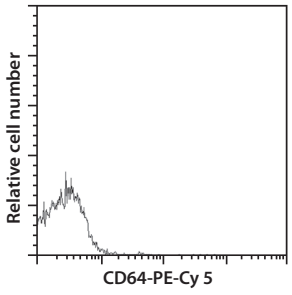
3. Example of a separation using the Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads

Separation of human peripheral blood mononuclear cells (PBMCs) using a PE-Cy5-conjugated CD64 antibody, Anti-Cy5/Anti-Alexa Fluor 647 MicroBeads and a MiniMACS™ Separator with an MS Column. Cell debris and dead cells are excluded from the analysis based on scatter signals and PI fluorescence.

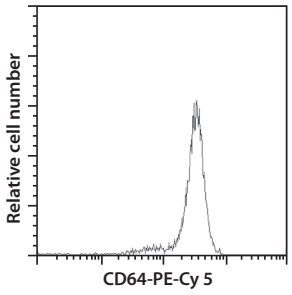
PBMCs before separation



$CD64^-$ cells



$CD64^+$ cells



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.

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.

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