Miltenyi Biotec

Anti-PE MicroBeads UltraPure

Order no. 130-105-639

Contents

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.

1. Description

This product is for research use only.

Components	2 mL Anti-PE MicroBeads UltraPure: UltraPure MicroBeads conjugated to monoclonal anti-PE antibodies (isotype: mouse IgG1)
Capacity	For 10^9 total cells, up to 100 separations.
Product format	Anti-PE MicroBeads UltraPure 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 R-Phycoerythrin (PE)-conjugated primary antibody or ligand. Subsequently, the cells are magnetically labeled with Anti-PE MicroBeads UltraPure. 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 information

Anti-PE MicroBeads UltraPure have been especially developed for highly efficient separation of cells from debris-rich samples or other biological materials according to surface markers labeled with PE-conjugated primary antibodies, peptides, or ligands. After separation the PE-labeled cells can be directly detected by flow cytometry or fluorescence microscopy.

▲ Fluorochrome tandem conjugates of R-Phycoerythrin and other fluorescent dyes that are often used in flow cytometry for third color analysis may also be recognized by Anti-PE MicroBeads UltraPure, e.g., PE-Cy™5, ECD, and PC5. For separation of cells labeled with primary antibodies conjugated to PE-Cy5, it is recommended to use Anti-Cy5/Anti-Alexa Fluor® 647 MicroBeads (# 130-091-395).

1.3 Applications

- For magnetic cell sorting from any biological starting material, from any species and any cell type.
- High-yield positive cell separation using any PE-labeled primary antibody, even from debris-rich samples and samples with increased amount of dead cells.
- Ultrapure cell isolation, especially when working with rare cells.
- Superior depletion of unwanted cells, even from low quality samples, using one or multiple PE-labeled antibodies, to obtain ultrapure and untouched target cells.

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 (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- MACS Columns and MACS Separators: Cells labeled with Anti-PE MicroBeads UltraPure 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 PE-labeled antigen can also be depleted using MS, LS, or XS Columns. Positive selection or depletion can also be performed by using the autoMACS Pro or the MultiMACS™ Cell24 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 II
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
XS	10^9	2×10^{10}	SuperMACS II
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
CS	2×10^8		VarioMACS, SuperMACS II

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D	10^9		SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro
Multi-24	10^8	10^9	MultiMACS Cell24

▲ **Note:** Column adapters are required to insert certain columns into the VarioMACS™ or SuperMACS™ Separators. For details refer to the respective MACS Separator data sheet.

- PE-conjugated primary antibody, peptide, or ligand.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Pre-Separation Filters, 30 µm (# 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™.

▲ **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 lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When working with tissues, prepare a single-cell suspension using the gentleMACS™ Dissociator.

For details refer to www.gentlemacs.com/protocols.

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 a 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, 30 µm, # 130-041-407) to remove cell clumps which may clog the column. Moisten filter with buffer before use.

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

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

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

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 PE-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.
6. (Optional) Repeat washing step.
7. Aspirate supernatant completely and resuspend cell pellet in 80 µL of buffer per 10^7 total cells.
8. Add 20 µL of Anti-PE MicroBeads UltraPure 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.
11. Aspirate supernatant completely.
12. 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.
13. 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 refer to the table in section 1.4.

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

Magnetic separation with MS or LS Columns

1. Place column in the magnetic field of a suitable MACS Separator. For details refer to 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. Collect flow-through containing unlabeled cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through and combine with the flow-through from step 3.

   MS: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

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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.

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 refer to the 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 refer to the 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 refer to the 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 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 MultiMACS™ Cell24 Separator Plus

Refer to the the MultiMACS™ Cell Separator Plus user manual for instructions on how to use the instrument.

Magnetic separation with the autoMACS® Pro Separator

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

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

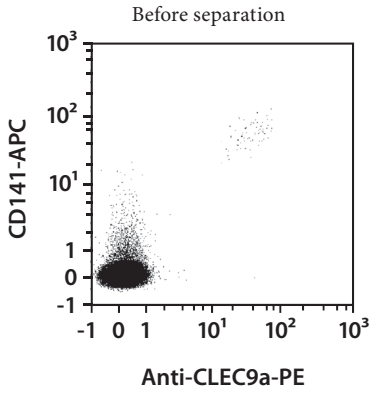
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose one of the following programs:

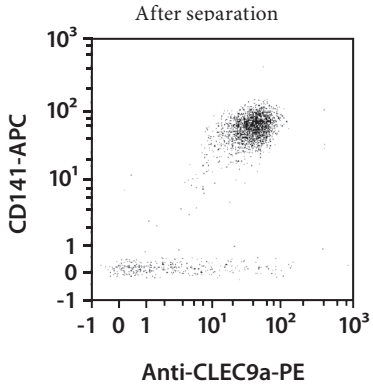
   Positive selection: Possel
   Collect positive fraction in row C of the tube rack.

   Depletion: Deplete
   Collect negative fraction in row B of the tube rack.

3. Example of a separation using Anti-PE MicroBeads UltraPure

Human CLEC9a$^+$ cells have been isolated from a debris-rich sample of peripheral blood mononuclear cells (PBMCs) using Anti-CLEC9a-PE, Anti-PE MicroBeads UltraPure, two MS Columns, and a MiniMACS™ Separator. Cells were stained with Anti-CLEC9a-PE, CD141 (BDCA-3)-APC, and Propidium Iodide Solution (# 130-093-233). Cells were analyzed after gating on viable lymphoid 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.

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