Miltenyi Biotec

Memory B Cell Isolation Kit
human

Order no. 130-093-546

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 B Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD14, CD16, CD36, CD43, and CD235a (Glycophorin A). **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1). **2 mL CD27 MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human CD27 antibodies (isotype: mouse IgG1).
Capacity	For 2×10^9 total cells.
Product format	All components 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

The isolation of memory B cells is performed in a two-step procedure. First, the non–B cells are indirectly magnetically labeled with a cocktail of biotin-conjugated antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. In between the two labeling steps no washing steps are required. The labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator.
In the second step, memory B cells are directly labeled with CD27 MicroBeads and isolated by positive selection from the pre-enriched memory B cell fraction.
After removing the column from the magnetic field, the magnetically retained memory B cells can be eluted as the positively selected cell fraction.

Human PBMCs: Depletion of non–B cells

1. Indirect magnetic labeling of non–B cells with Biotin-Antibody Cocktail and Anti-Biotin MicroBeads.
2. Magnetic separation using an LD Column or an autoMACS Column (program "Depl025").

Pre-enriched memory B cells (flow-through fraction): Positive selection of memory B cells

1. Direct magnetic labeling of $CD27^+$ memory B cells with CD27 MicroBeads.
2. Magnetic separation using an MS Column or an autoMACS Column (program "Possel").

Memory B cells

1.2 Background information

The Memory B Cell Isolation Kit has been developed for the isolation of memory B cells from human peripheral blood mononuclear cells (PBMCs). Memory B cells, defined as quiescent antigen-experienced B cells, are generated in response to T cell–dependent and T cell–independent antigens. They are able to react quickly to a recurrent antigenic challenge thereby providing serological immune protection.
CD27, a member of the TNF-receptor family, is expressed on most memory B cells. It allows their discrimination from naive B cells, which are $CD27^-$.
Memory B cells are isolated by depletion of unwanted non–B cells and subsequent positive selection with CD27 MicroBeads. Unwanted cells, for example, T cells, NK cells, monocytes, dendritic cells, granulocytes, platelets, and erythroid cells are depleted using a cocktail of biotinylated antibodies against CD2, CD14, CD16, CD36, CD43, and CD235a (Glycophorin A), and Anti-Biotin MicroBeads. For evaluation of MACS Separations, staining with CD19-APC (# 130-091-248) and CD27-PE (# 130-097-223) antibodies is recommended. Do not use clone M-T271 for CD27 staining.

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1.3 Application

- Isolation of memory B cells from human PBMCs.

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.

- MACS Columns and MACS Separators: Depletion of non–B cells can be performed on an LD Column. The subsequent positive selection of memory B cells can be performed on an MS Column. Depletion and positive selection 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
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS II
Positive selection or depletion			
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

  ▲ **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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19-APC (# 130-091-248). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD for flow cytometric exclusion of dead cells.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion 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 tissues or lysed blood, prepare a single-cell suspension using standard methods.

For details refer to the protocols section 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 of non–memory B cells

▲ 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 10^8 total cells. When working with fewer than 10^8 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 2×10^8 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.

1. Determine cell number.
2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
3. Resuspend cell pellet in 400 µL of cold buffer per 10^8 total cells.
4. Add 100 µL of the B Cell Biotin-Antibody Cocktail per 10^8 total cells.
5. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
6. Add 300 µL of cold buffer per 10^8 total cells and 200 µL of Anti-Biotin MicroBeads per 10^8 total cells.
7. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
8. Wash cells by adding 10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 total cells in 1 mL of cold buffer.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non–B cells

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of memory B cells. For details refer to table in section 1.4.

Depletion with LD Columns

1. Place LD Column in the magnetic field of a suitable MACS Separator. For details refer to 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 pre-enriched memory B cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column. This fraction represents the magnetically labeled non–B cells.
6. Proceed to 2.4 for the isolation of memory B cells.

Depletion with the autoMACS® Pro Separator or the autoMACS 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.

Magnetic separation with the autoMACS® Pro Separator

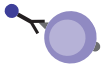
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Depletion: Depl025
   Collect negative fraction in row B of the tube rack.
4. Proceed to 2.4 for the isolation of memory B cells.

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 port pos1.
3. For a standard separation choose the following program:

   Depletion: Depl025
   Collect negative fraction from outlet port neg1.
4. Proceed to 2.4 for the isolation of memory B cells.



2.4 Magnetic labeling of memory B cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^8 total cells. For higher initial cell numbers, scale up all volumes accordingly.

1. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
2. Resuspend cell pellet in 100 µL of buffer.
3. Add 100 µL of CD27 MicroBeads.
4. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 10 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
6. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
7. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of memory B cells

Positive selection with MS Column

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 3×500 µL of buffer. Perform washing steps by adding buffer three times. Only add new buffer when the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column. This fraction represents the memory B cells.

Positive selection with the autoMACS® Pro Separator or the autoMACS Separator

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 fraction collection tubes in rows B and C.
3. For a standard separation choose the following program:

   Positive selection: Possel
   Collect positive fraction in row C of the tube rack. This is the enriched memory B cell fraction.

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 port pos1.
3. For a standard separation choose the following program:

   Positive selection: Possel
   Collect positive fraction from outlet port pos1. This is the enriched memory B cell fraction.

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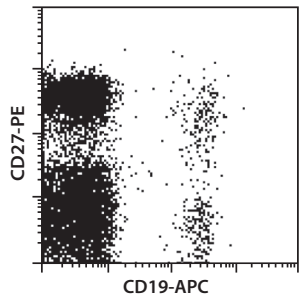
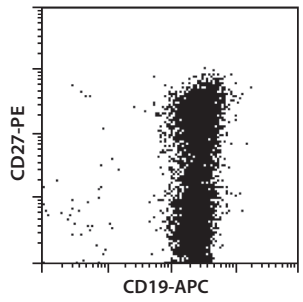
3. Example of a separation using the Memory B Cell Isolation Kit

Memory B cells were isolated from human PBMCs using the Memory B Cell Isolation Kit, an LD Column and a MidiMACS™ Separator, and an MS Column and a MiniMACS™ Separator. Cells are fluorescently stained with CD19-APC (# 130-091-248) and CD27-PE (# 130-097-223). Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.

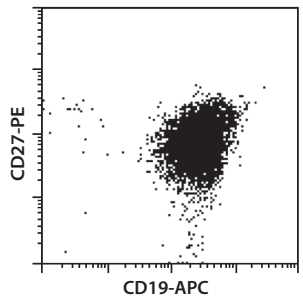
Before separation



Pre-enriched memory B cells



Isolated memory B cells



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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