Miltenyi Biotec

IgM+ Memory B Cell Isolation Kit
human

Order no. 130-093-619

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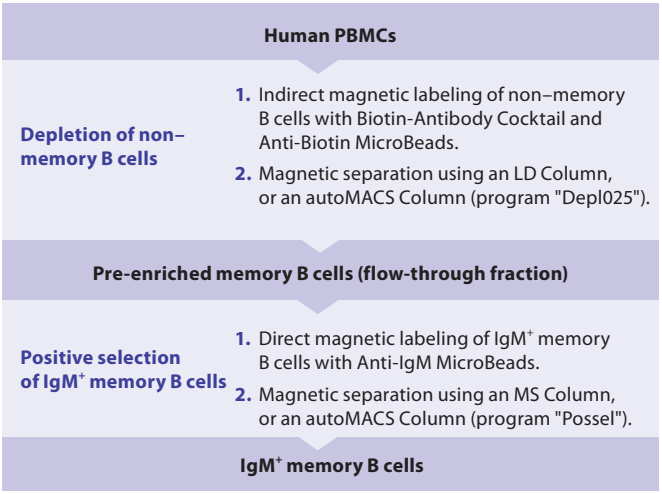
1. Description

Components	**2 mL IgM+ Memory B Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD14, CD16, CD23, CD36, CD43 and CD235a (Glycophorin A). **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1). **2 mL Anti-IgM MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human IgM 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 IgM+ memory B cells is performed in a two-step procedure. First, non–memory B cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies and Anti-Biotin MicroBeads. The labeled cells are subsequently depleted by separation over a MACS® Column.
In the second step, IgM+ memory B cells are directly labeled with Anti-IgM 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 IgM+ memory B cells can be eluted as the positively selected cell fraction.



1.2 Background information

The IgM+ Memory B Cell Isolation Kit has been developed for the isolation of IgM+ memory B cells from human peripheral blood mononuclear cells (PBMCs). IgM+ 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.
IgM+ Memory B cells are isolated by depletion of unwanted cells and subsequent positive selection with Anti-IgM 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, CD23, CD36, CD43, and CD235a (Glycophorin A), and Anti-Biotin MicroBeads. For evaluation of MACS Separations, staining with Anti-IgM-APC and CD27-PE is recommended.

1.3 Application

- Isolation of IgM+ 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.

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

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- MACS Columns and MACS Separators: Depletion of non–memory B cells is performed on an LD Column. The subsequent positive selection of IgM$^+$ memory B cells is 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
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, SuperMACS
Depletion and positive selection			
autoMACS	2×10^8	4×10^9	autoMACS, autoMACS Pro

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., Anti-IgM-APC (# 130-093-076) and CD27-PE (# 130-093-185).
- (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 (# 130-041-407) to remove cell clumps.

2. Protocol

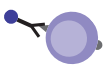
2.1 Sample preparation

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 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, # 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.

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 IgM$^+$ Memory 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 1000 µL of cold buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.

8. Proceed to magnetic separation (2.3).

2.3 Magnetic separation: Depletion of non–memory B cells

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

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 pre-enriched memory B cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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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–memory B cells.
6. Proceed to 2.4 for the isolation of IgM$^+$ memory B cells.

Depletion 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.

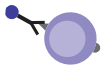
▲ Program choice depends on the isolation strategy, the strength of magnetic labeling, and the frequency of magnetically labeled cells. For details refer to the section describing the cell separation programs in the respective user manual.

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.
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 IgM$^+$ memory B cells.

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 IgM$^+$ memory B cells.



2.4 Magnetic labeling of IgM$^+$ 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 400 µL of buffer.
3. Add 100 µL of Anti-IgM 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.
7. Resuspend up to 10^8 cells in 500 µL of buffer.
   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
8. Proceed to magnetic separation (2.5).

2.5 Magnetic separation: Positive selection of IgM$^+$ memory B cells

Positive selection with MS Column

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details see 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 IgM$^+$ memory B cells.

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

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 IgM$^+$ memory B cell fraction.

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 IgM$^+$ memory B cell fraction.

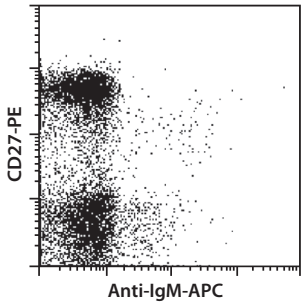
2.6 (Optional) Evaluation of IgM$^+$ memory B cell purity

The purity of the enriched IgM$^+$ memory B cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with fluorochrome-conjugated antibodies against IgM$^+$ memory B cell markers, for example, Anti-IgM-APC (# 130-093-076) and CD27-PE (# 130-093-185), as recommended in the respective data sheets. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non–memory B cells with the Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, for example, Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

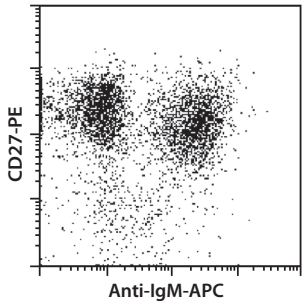
3. Example of a separation using the IgM$^+$ Memory B Cell Isolation Kit

IgM$^+$ memory B cells were isolated from human PBMCs using the IgM$^+$ 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 Anti-IgM-APC (# 130-093-076) and CD27-PE (# 130-093-185). Cell debris and dead cells are excluded from the analysis based on scatter signals and propidium iodide fluorescence.

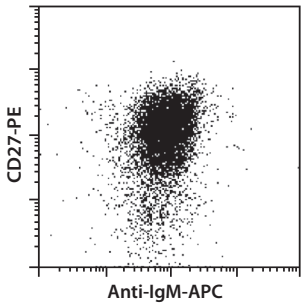
Before separation



Pre-enriched memory B cells



Isolated IgM$^+$ 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.

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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