Miltenyi Biotec

Memory B Cell Isolation Kit

mouse

Order no. 130-095-838

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 Memory B Cell Biotin-Antibody Cocktail, mouse: Cocktail of biotin-conjugated monoclonal anti-mouse antibodies.

2×2 mL Anti-Biotin MicroBeads: MicroBeads conjugated to monoclonal anti-biotin antibody.

0.2 mL Anti-IgG1-APC, mouse: Monoclonal anti-IgG1 antibody conjugated to APC (isotype: rat IgG1).

0.2 mL Anti-IgG2ab-APC, mouse: Monoclonal anti-IgG2ab antibody conjugated to APC (isotype: rat IgG1).

2 mL Anti-APC MicroBeads: MicroBeads conjugated to monoclonal anti-APC antibody.

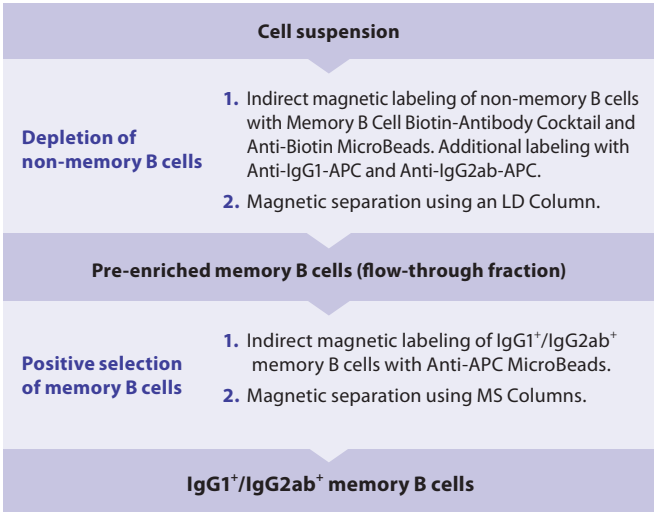
Capacity For 2×10^9 total cells, up to 20 separations.

Product format All components are supplied in buffer containing stabilizer and 0.05% sodium azide.

1.1 Principle of the MACS® Separation

The isolation of IgG1$^+$/IgG2ab$^+$ 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 antibodies, as primary labeling reagent and subsequently depleted using Anti-Biotin MicroBeads. During the first incubation step with the cocktail, cells are additionally labeled with Anti-IgG1-APC and Anti-IgG2ab-APC antibodies.
In the second step, pre-enriched memory B cells are labeled with Anti-APC MicroBeads and isolated by positive selection over a MACS Column, which is placed in the magnetic field of a MACS Separator.
After removing the column from the magnetic field, the magnetically retained IgG1$^+$/IgG2ab$^+$ memory B cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the IgG1$^+$/IgG2ab$^+$ memory B cells can be separated over a second column.

▲ **Note:** Do not use APC tandem conjugates for staining before performing magnetic separation, as they might be recognized by the Anti-APC MicroBeads.



1.2 Background information

The Memory B Cell Isolation Kit has been developed for the isolation of IgG1$^+$/IgG2ab$^+$ memory B cells from mouse spleen or lymph nodes. Memory B cells, defined as quiescent antigen-experienced B cells, are able to react quickly to a recurrent antigenic challenge thereby providing serological immune protection.

1.3 Applications

- Isolation of IgG1$^+$/IgG2ab$^+$ memory B cells from mouse spleen and lymph nodes for further characterization.

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140-003-075.04

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 mouse serum albumin, mouse serum, or fetal bovine serum (FBS). Buffers or media containing Ca^{2+} or Mg^{2+} are not recommended for use.

- (Optional) FcR Blocking Reagent, mouse (# 130-092-575) to avoid Fc receptor–mediated antibody labeling.

- MACS Columns and MACS Separators: Depletion of non-memory B cells can be performed on an LD Column. The subsequent positive selection of $IgG1^+$/$IgG2ab^+$ memory B cells can be performed on MS Columns.

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

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

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD45R (B220)-VioBlue®, mouse, CD38-PE, mouse, or Anti-IgM-FITC, mouse. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.

- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) 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 lymphoid organs, non-lymphoid tissues, or peripheral blood, prepare a single-cell suspension using manual methods or the gentleMACS™ Dissociator.

▲ 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 labeling. 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 380 µL of buffer per 10^8 total cells.
4. Add 100 µL of Memory B Cell Biotin-Antibody Cocktail, 10 µL Anti-IgG1-APC, and 10 µL Anti-IgG2ab-APC per 10^8 total cells.

   ▲ **Note:** Adding only one of the APC-conjugated antibodies while replacing the other with 50 µL of buffer, will result in the isolation of only one subpopulation of memory B cells (either $IgG1^+$ or $IgG2ab^+$).
5. Mix well and incubate for 5 minutes in the dark in the refrigerator (2–8 °C).
6. Add 300 µL of buffer and 200 µL of Anti-Biotin MicroBeads per 10^8 total cells.
7. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
8. Proceed to magnetic separation (2.3).

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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 IgG1$^+$/IgG2ab$^+$ memory B cells. For details refer to table in section 1.4.

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

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. Proceed to 2.4 for the labeling of IgG1$^+$/IgG2ab$^+$ memory B cells.

2.4 Magnetic labeling of IgG1$^+$/IgG2ab$^+$ 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-APC MicroBeads.
4. Mix well and incubate for 15 minutes in the dark in the refrigerator (2–8 °C).
5. Wash cells by adding 10× labeling volume 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 IgG1$^+$/IgG2ab$^+$ memory B cells

Positive selection with MS Columns

▲ To achieve highest purities, perform two consecutive column runs.

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. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.

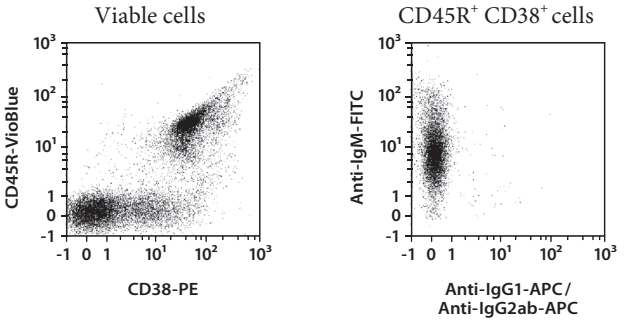
   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.

   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. (Optional) To increase purity of IgG1$^+$/IgG2ab$^+$ memory B cells, the eluted fraction must be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

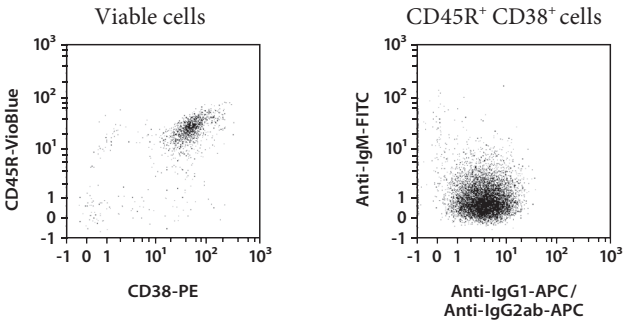
3. Example of a separation using the Memory B Cell Isolation Kit

Splenic IgG1$^+$/IgG2ab$^+$ memory B cells from an immunized BALB/c mouse were isolated using the Memory B Cell Isolation Kit. The cells were fluorescently stained with CD45R (B220)-VioBlue®, CD38-PE , and Anti-IgM-FITC and with Anti-IgG1-APC and Anti-IgG2ab-APC and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A) Before separation



B) Isolated IgG1$^+$/IgG2ab$^+$ memory B cells



4. References

1. Ridderstad, A. and Tarlinton, D. (1998) Kinetics of establishing the memory B cell population as revealed by CD38 expression. J. Immunol. 160: 4688–4695.
2. Anderson, S. *et al.* (2007) New markers for murine memory B cells that define mutated and unmutated subsets. J. Environ. Monit. 204 (9): 2103–2114.

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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