Miltenyi Biotec

Switched Memory B Cell Isolation Kit
human

Order no. 130-093-617

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1. Description

Components	**2 mL Switched Memory B Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD14, CD16, CD36, CD43, CD235a, IgM and IgD. **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin 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

Using the Switched Memory B Cell Isolation Kit, human switched memory B cells are isolated by depletion of non-target cells. Non-target cells are indirectly magnetically labeled with a cocktail of biotin-conjugated monoclonal antibodies, as primary labeling reagent, and anti-biotin monoclonal antibodies conjugated to MicroBeads, as secondary labeling reagent. The magnetically labeled non-target cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled switched memory B cells pass through the column.

1.2 Background information

The Switched Memory B Cell Isolation Kit has been developed for the isolation of untouched switched memory B cells from human peripheral blood mononuclear cells (PBMCs). Non-target cells are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies against CD2, CD14, CD16, CD36, CD43, CD235a (Glycophorin A), IgM, IgD, and Anti-Biotin MicroBeads. Isolation of untouched switched memory B cells is achieved by depletion of magnetically labeled cells.

Switched memory B cells are indicators of normal B cell activation and differentiation in germinal centers of lymph nodes or other secondary lymphoid tissues. Measurement of the amount of switched memory B cells in peripheral blood is a predictor of clinical complications in humoral immunodeficiencies, such as SAD (specific antibody deficiency) or CVID (common variable immunodeficiency).

1.3 Applications

- Studies on signal requirements for switched memory B cell activation, induction of proliferation, or differentiation.
- Studies on signal transduction in switched memory B cells.
- Analysis of immunoglobulin class switching and somatic hypermutation in switched memory B cells.
- Studies on antigen uptake and presentation by switched memory B cells.
- Studies on cognate interaction of switched memory B cells with T helper cells or dendritic 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: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

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

Column	Max. number of labeled cells	Max. number of total cells	Separator
LS	10^8	2×10^9	MidiMACS, QuadroMACS, VarioMACS, SuperMACS
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19-FITC (# 130-091-328), Anti-IgG-APC (# 130-093-194), Anti-IgA-APC (# 130-093-113), Anti-IgM-PE (# 130-093-075), or Anti-IgD-PE. For more information about fluorochrome-conjugated antibodies see www.miltenyibiotec.com.

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- (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™.

▲ **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 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

▲ 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, # 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. 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 buffer per 10^8 total cells.
4. Add 100 µL of Switched 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. Wash cells by adding 1–2 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
7. Add 800 µL of buffer per 10^8 total cells.
8. Add 200 µL of Anti-Biotin MicroBeads per 10^8 total cells.
9. Mix well and incubate for 15 minutes in the refrigerator (2–8 °C).
10. Wash cells by adding 10–20 mL of buffer per 10^8 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.
12. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

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

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

Magnetic separation with LS Columns

1. Place LS 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 3 mL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells, representing the enriched switched memory B cell fraction.
4. Wash column with 3×3 mL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3. This fraction represents the enriched switched memory B cells.

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. (Optional) Elute retained cells outside of the magnetic field. This fraction represents the magnetically labeled non-B cells and non-memory B cells.

Magnetic separation with the autoMACS™ Pro Separator or the autoMACS™ Separator

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

▲ Buffers used for operating the autoMACS Pro Separator or the autoMACS 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™ 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.

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3. For a standard separation choose the following program:
   Depletion: "Depletes"
   Collect negative fraction in row B of the tube rack. This fraction contains the enriched switched memory B cells.
4. (Optional) Collect positive fraction from row C. This fraction represents the magnetically labeled non-B cells and non-memory B cells.

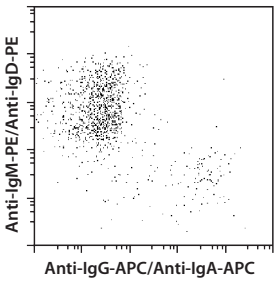
Magnetic separation with the autoMACS™ Separator

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: "Depletes"
   Collect negative fraction from outlet port neg1. This fraction contains the enriched switched memory B cells.
4. (Optional) Collect positive fraction from port pos1. This fraction represents the magnetically labeled non-B cells and non-memory B cells.

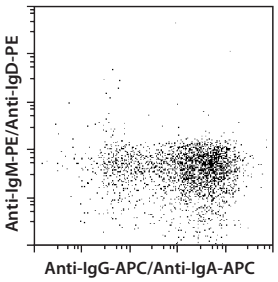
3. Example of a separation using the Switched Memory B Cell Isolation Kit

Untouched switched memory B cells were isolated from human PBMCs using the Switched Memory B Cell Isolation Kit, an LS Column, and a MidiMACS™ Separator. Cells are fluorescently stained with CD19-FITC, Anti-IgD-PE, Anti-IgM-PE, Anti-IgA-APC, and Anti-IgG-APC and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence. The cells are gated on $CD19^+$ cells.

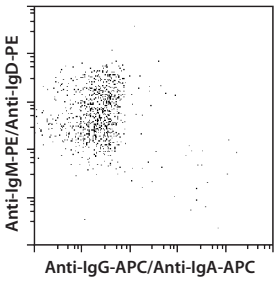
PBMCs before separation



Enriched switched memory B cells



Other 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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