B Cell Isolation Kit II
human

Order no. 130-091-151

Contents

1. Description

Components	**1 mL B Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD14, CD16, CD36, CD43, and CD235a (Glycophorin A). **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibodies (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
Product format	The Biotin-Antibody Cocktail and the Anti-Biotin MicroBeads are supplied in a solution 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 B Cell Isolation Kit II, human B cells are isolated by depletion of non-B cells (negative selection). Non-B 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. In between the two labeling steps no washing steps are required. The magnetically labeled non-B cells are depleted by retaining them on a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled B cells pass through the column.

1.2 Background information

The B Cell Isolation Kit II is an indirect magnetic labeling system for the isolation of untouched B cells from human peripheral blood mononuclear cells (PBMCs). Non-B cells, for example, T cells, NK cells, dendritic cells, monocytes, granulocytes, and erythroid cells, are indirectly magnetically labeled by using a cocktail of biotin-conjugated antibodies against CD2, CD14, CD16, CD36, CD43, CD235a (Glycophorin A), and Anti-Biotin MicroBeads. Isolation of highly pure B cells is achieved by depletion of magnetically labeled cells.

1.3 Applications

- Functional studies on B cells in which effects due to antibody-cross-linking of cell surface proteins should be avoided.
- Studies on signal requirements for B cell activation, induction of B cell proliferation, differentiation of B cells, induction of apoptosis in B cells, etc.
- Studies on signal transduction in B cells.
- Analysis of immunoglobulin class switching and somatic hypermutation in B cells.
- Studies on antigen presentation by B cells and cognate interaction of B cells with T helper cells or dendritic cells.

1.4 Reagent and instrument requirement

- 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: Choose the appropriate MACS Separator and MACS Columns according to the number of labeled cells and to the number of total cells.

Column	Max. number of labeled cells	Max. number of total cells	Separator
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
autoMACS	2×10^8	4×10^9	autoMACS Pro, autoMACS

▲ **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.

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- (Optional) Fluorochrome-conjugated antibodies for flow-cytometric analysis, for example, CD19-FITC (# 130-091-328), CD19-PE (#130-091-247), CD19-APC (# 130-091-248), CD20-FITC (# 130-091-108), CD20-PE (# 130-091-109), Anti-Biotin-PE (# 130-090-756), or Anti-Biotin-APC (# 130-090-856). 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) Pre-Separation Filters, 30 µm (# 130-041-407) to remove cell clumps.
- (Optional) Dead Cell Removal Kit (# 130-090-101) for the depletion of dead cells.

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 preparation 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

▲ Work fast, keep cells cold, 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 10^7 total cells. When working with fewer than 10^7 cells, use 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. Moistent 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 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of Biotin-Antibody Cocktail per 10^7 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Add 30 µL of buffer per 10^7 total cells.
7. Add 20 µL of Anti-Biotin MicroBeads per 10^7 total cells.
8. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
9. Proceed to magnetic separation (2.3).

   ▲ **Note:** A minimum of 500 µL is required for magnetic separation. If necessary, add buffer to the cell suspension.

2.3 Magnetic separation

▲ Choose an appropriate MACS Column and MACS Separator according to the number of labeled cells and to the number of total 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. Avoid formation of air bubbles. 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: 500 µL LS: 3 mL
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette an appropriate amount 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.

Magnetic separation with XS Columns

For instructions on the column assembly and the separation refer to the XS Column data sheet.

2.4 Autolabeling and separation using the autoMACS® Pro Separator

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

▲ If not using autolabeling or if using the autoMACS® Separator, choose the program **Depletes**.

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

▲ For appropriate resuspension volumes and cell concentrations, please visit www.automacspro.com/autolabeling.

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2.4.1 Autolabeling with the autoMACS® Pro Separator using the 2D code reader

1. Prepare and prime the instrument.
2. Go to the Reagent menu and highlight the position where the reagent vial will be placed in the MACS Reagent Rack. Four positions are available.
3. Select Read Reagent to activate the 2D code reader.
4. Hold the vial with the barcode facing the 2D code reader.
5. Select reagent name appearing on screen.
6. After scanning the vial, the next available MACS Reagent Rack position will be automatically highlighted.
7. Insert the vial into the appropriate rack position.
8. Proceed to magnetic separation (2.4.2).

2.4.2 Magnetic separation

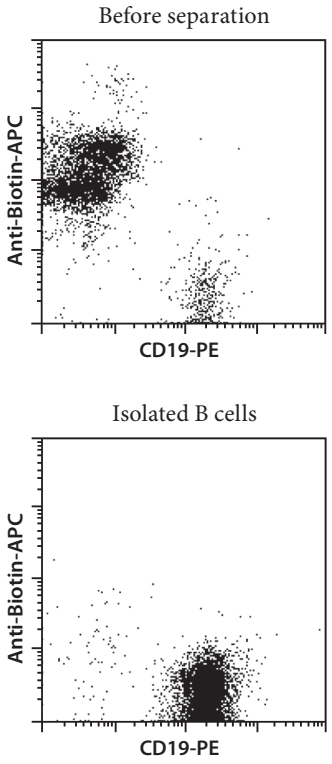
1. Highlight the desired position(s) in the sample separation template.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube in row A of the tube rack and the fraction collection tubes in rows B and C.
3. Choose reagent name from the labeling submenu.
4. (Optional) The recommended cell separation and wash program will be automatically displayed after choosing the program. It is possible to change the separation program or the wash program between samples or to assign the Sleep program after finishing the last sample. Highlight the desired cell separation and wash program in the Separation and Wash submenus, respectively.
5. Insert sample volume in the Volume submenu using the numeric keypad.
6. Select Run to start the cell separation.

2.5 (Optional) Evaluation of B cell purity

The purity of the enriched B cells can be evaluated by flow cytometry or fluorescence microscopy. Stain aliquots of the cell fractions with a fluorochrome-conjugated antibody against a B cell marker, for example, CD19-PE (# 130-091-247), as recommended in the respective data sheets. Analyze cells by flow cytometry or fluorescence microscopy. Labeling of non-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 B Cell Isolation Kit II

Isolation of untouched B cells from human PBMCs using the B Cell Isolation Kit II, an LS Column, and a MidiMACS™ Separator. Cells are fluorescently stained with CD19-PE (# 130-091-247) and Anti-Biotin-APC (# 130-090-856). Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



All protocols and data sheets are available at **www.miltenyibiotec.com**.

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.

Warranty

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