Miltenyi Biotec

Pan B Cell Isolation Kit
mouse

Order no. 130-095-813

Contents

1. Description

Components	**1 mL Pan B Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against CD4, CD11c, CD49b, CD90.2, Gr-1, and Ter119. **2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1).
Capacity	For 10^9 total cells, up to 100 separations.
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 Pan B Cell Isolation Kit, mouse B cells are isolated by depletion of non-target cells. 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 within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled B cells run through the column.

1.2 Background information

The mouse Pan B Cell Isolation Kit can be used for the untouched isolation of B-1 and B-2 B cell subsets from single cell suspensions of lymphoid tissues. The kit does not contain biotin-conjugated antibodies against CD43 or CD11b in the depletion cocktail which might be expressed on some malignant target cells. It is therefore perfectly suited to be used for isolation of B cells from different mouse models for human diseases, e.g., B cells from B-CLL mouse models.

1.3 Applications

- Isolation of untouched B cells from mouse lymphoid tissue for further phenotypical and functional analysis.
- Analysis of B cells from normal and tumor cell containing samples.
- Studies on signal requirements for B cell activation, induction of B cell proliferation, differentiation of B cells, induction of apoptosis in B cells.
- Studies on signal transduction in B cells.
- Analysis of immunoglobulin class switching and somatic hypermutation in B 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 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-B cells can be performed on LD Columns and by using the autoMACS Pro or the autoMACS 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
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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19-PE, mouse (# 130-092-041) or CD4-FITC, mouse (# 130-091-608). 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.

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- (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.
- (Optional) Red Blood Cell Lysis Solution (10×) (# 130-094-183) when working with mouse blood samples.

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.

For details refer to the protocols section at www.miltenyibiotec.com/protocols.

When using mouse blood samples a lysis of erythrocytes is necessary before magnetic labeling according to the protocol of the Red Blood Cell Lysis Solution (10×) (# 130-094-183).

▲ 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).

2.3 Magnetic labeling

▲ Cells can be labeled with MACS MicroBeads using the autolabeling function of the autoMACS Pro Separator. For more information refer to section 2.4.

▲ 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 10^7 total cells. When working with fewer than 10^7 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 2×10^7 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 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 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of Pan B Cell 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 cold buffer per 10^7 total cells.
7. Add 20 µL of Anti-Biotin MicroBeads per 10^7 cells.
8. Mix well and incubate for an additional 10 minutes in the refrigerator (2–8 °C).
9. Adjust the volume to a minimum of 500 µL of buffer.

   ▲ **Note:** Resuspend up to 10^8 cells in 500 µL of buffer. For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose the autoMACS Separator or an LD Column with an appropriate MACS Separator. 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 the 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 Pan 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 non-B cells by firmly pushing the plunger into the column.

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 **Depl05**.

▲ 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.

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.

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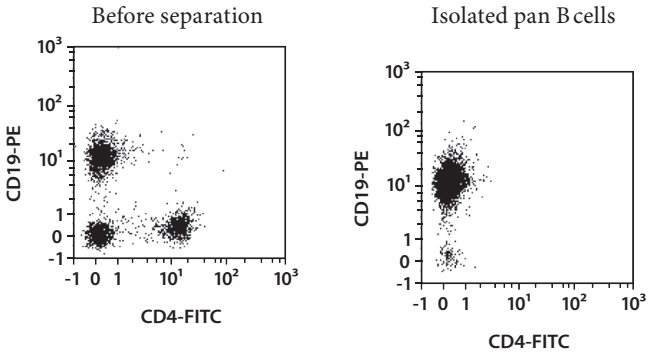
6. After scanning the vial, the next available MACS Reagent Rack position will be automatically highlighted.
7. Insert the vial into the appropriate rack position.
8. Proceed to magnetic separation (2.4.2).

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.

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

Pan B cells were isolated from spleen of a BALB/c mouse by using the Pan B Cell Isolation Kit, an LD Column, and a MidiMACS™ Separator. The cells were fluorescently stained with CD19-PE (# 130-092-041) and CD4-FITC (# 130-091-608) 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.



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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