Miltenyi Biotec

Naive B Cell Isolation Kit II
human

Order no. 130-091-150

Contents

1. Description

Components	**1 mL Naive B Cell Biotin-Antibody Cocktail, human:** Cocktail of biotin-conjugated monoclonal antibodies against CD2, CD14, CD16, CD27, CD36, CD43 and CD235a (Glycophorin A). **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 Naive B Cell Isolation Kit, human naive 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 MicroBeads, as secondary labeling reagent. In between the two labeling steps no washing steps are required. The magnetically labeled non-target cells are depleted by retaining them within a MACS® Column in the magnetic field of a MACS Separator, while the unlabeled naive B cells run through the column.

1.2 Background information

The Naive B Cell Isolation Kit II is an indirect magnetic labeling system for the isolation of untouched naive B cells from human peripheral blood mononuclear cells (PBMCs).

1.3 Applications

- Functional studies on naive B cells.
- Studies on signal requirements for naive B cell activation, induction of proliferation, differentiation of naive B cells, e.g. into memory B cells or plasma cells, induction of apoptosis in naive B cells, etc.
- Studies on signal transduction in naive B cells.
- Analysis of immunoglobulin class switching and somatic hypermutation in naive B cells.
- Studies on antigen uptake and presentation by naive B cells.
- Studies on cognate interaction of naive 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.

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.

- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD19-FITC (# 130-091-328), CD27-PE (# 130-093-185), and Anti-IgD-APC (# 130-099-221). 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) 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.

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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 10^7 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. 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 40 µL of buffer per 10^7 total cells.
4. Add 10 µL of Naive 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 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 additional 10 minutes in the refrigerator (2–8 °C).
9. Proceed to magnetic separation (2.3 or 2.4).

   ▲ **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 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 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. Collect flow-through containing unlabeled cells, representing the enriched naive B cells.
4. Wash column with the appropriate amount of buffer. Collect unlabeled cells that pass through, representing the enriched naive B cells, and combine with the flow-through from step 3.

   MS: 1×500 µL LS: 1×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots only when the column reservoir is empty.
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled non–B cells and non–naive B cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL

2.4 Cell separation with the autoMACS® Pro Separator and autoMACS Separator

▲ Refer to the 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.

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

2.4.1 Cell separation with the autoMACS® Pro Separator using autolabeling for a fully automated procedure

1. Turn on the instrument for automatic initialization (automated preparation and priming procedure).
2. Program autolabeling by selecting Read Reagent in the "reagent menu" tab and scan the 2D barcode on each reagent vial with the barcode scanner on the autoMACS Pro instrument. Place the reagent into the appropriate space on the reagent rack.
3. Place sample and collection tubes into the sample rack. Sample tube should be in row A, and the collection tubes in rows B and C.
4. Select the reagent name for each sample from the labeling submenu (the correct labeling, separation and wash protocols will be selected automatically).

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5. Enter sample volume into the Volume submenu.
6. Select run.

For more details on complete walk away automation on the autoMACS Pro Separator, please refer to the autoMACS Pro Separator user manual.

2.4.2 Magnetic separation with the autoMACS® Pro Separator using manual labeling

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. 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.
4. For a standard separation choose the following program:

   Depletion: "Depletes"

   Collect negative fraction in row B of the tube rack. This fraction represents the enriched naive B cells.
5. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non–B cells and non–naive B cells.

2.4.3 Magnetic separation with the autoMACS® Separator

1. Label the sample as described in section 2.2 Magnetic labeling.
2. Prepare and prime the instrument.
3. 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.
4. For a standard separation choose the following program:

   Depletion: "Depletes"

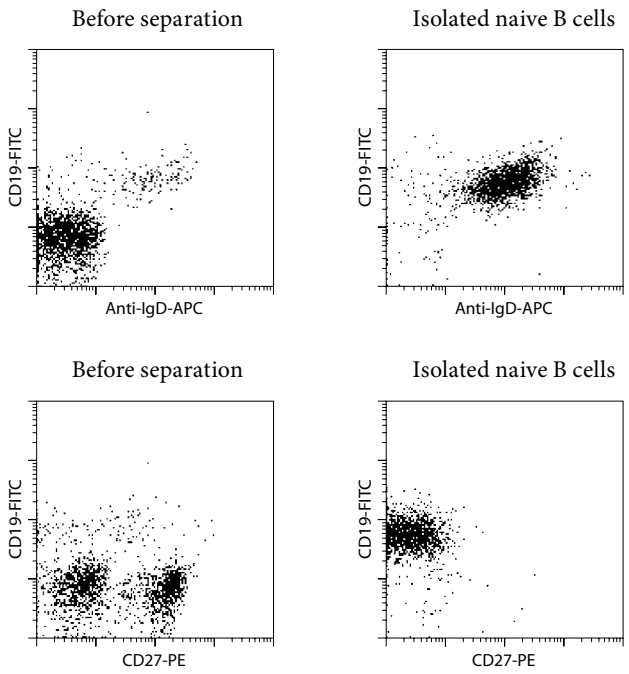
   Collect negative fraction from outlet port neg1. This fraction represents the enriched naive B cells.
5. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non–B cells and non–naive B cells.

2.5 (Optional) Evaluation of naive B cell purity

The purity of the enriched naive 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 lineage marker, e.g. CD19-FITC (# 130-091-328), or CD20-FITC (# 130-091-108), and a marker that allows the discrimination of naive B cells from other B cells, e.g. anti-IgD-APC for the identification of naive B cells or CD27-PE for the identification of memory B cells, activated B cells, and plasma cells as recommended by the manufacturer. Labeling of non–B cells and non–naive B cells with the Naive B Cell Biotin-Antibody Cocktail can be visualized by counterstaining with a fluorochrome-conjugated anti-biotin antibody, e.g., Anti-Biotin-PE (# 130-090-756) or Anti-Biotin-APC (# 130-090-856). Staining with fluorochrome-conjugated streptavidin is not recommended.

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

Isolation of untouched naive B cells from human PBMCs using the Naive B Cell Isolation Kit II and an LS Column. The cells are fluorescently stained with CD19-FITC (# 130-091-328), CD27-PE (# 130-093-185), and Anti-IgD-APC (# 130-099-221). 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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