Miltenyi Biotec

Marginal Zone and Follicular B Cell Isolation Kit
mouse

Order no. 130-100-366

Contents

1. Description

Components	**2 mL MZ & FO B Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against CD43, CD4, CD93, and Ter119. **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **1 mL CD23 MicroBeads, mouse:** MicroBeads conjugated to monoclonal anti-mouse CD23 antibody (isotype: rat IgG2a).
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.
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

The isolation of CD21^{hi}CD23$^{low/-}$ marginal zone (MZ) and CD21^{int}CD23^{hi} follicular (FO) B cells is performed in a two-step procedure. First, the non-B cells, the B-1 cells, and the transitional B cells are indirectly magnetically labeled with a cocktail of biotin-conjugated 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 labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator. The unlabeled fraction contains the pre-enriched MZ and FO B cells. In order to further separate these two subsets the FO B cells are directly labeled with CD23 MicroBeads in a second step and isolated by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator. The flow through contains the enriched MZ B cells. After removing the column from the magnetic field, the magnetically retained FO B cells can be eluted as the positively selected cell fraction.

Mouse splenocytes: Depletion of non-MZ and non-FO B cells

1. Indirect magnetic labeling of non-B cells, B1 cells, and transitional B cells with MZ & FO B Cell Biotin-Antibody Cocktail and Anti-Biotin MicroBeads.
2. Magnetic separation using an LS Column or an autoMACS Column (program "Depletes").

Pre-enriched MZ and FO B cells (flow-through fraction): Positive selection of FO B cells

1. Direct magnetic labeling of FO B cells with CD23 MicroBeads.
2. Magnetic separation using an LS Column or an autoMACS Column (program "Possels").

CD23^{hi} FO B cells (eluted fraction)
CD23$^{low/-}$ MZ B cells (flow-through fraction)

1.2 Background information

The Marginal Zone and Follicular B Cell Isolation Kit has been developed for the isolation of marginal zone (MZ) B cells and follicular (FO) B cells from spleens of untreated BALB/c and C57BL/6 mice.

1.3 Applications

- Isolation of MZ and FO B cells from mouse spleen for phenotyping and functional characterization.

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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.

- MACS Columns and MACS Separators: Depletion of non-MZ and non-FO B cells can be performed on an LS Column. The subsequent positive selection of $CD23^+$ FO B cells can be performed on an LS Column. Positive selection and depletion can also be performed by using the autoMACS Pro or the autoMACS Separator.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion or positive selection			
LS	10^8	2×10^9	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., CD45R (B220)-VioBlue, mouse (# 130-094-287), CD21/CD35-PE-Vio770, mouse (130-097-216), and CD23-PE, mouse (130-097-819). For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) FcR Blocking Reagent, mouse (# 130 092 575) to avoid Fc receptor–mediated antibody labeling.
- (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.

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 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 of non-MZ and non-FO 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. 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 MZ & FO B Cell Biotin-Antibody Cocktail per 10^8 total cells.
5. Mix well and incubate for 5 minutes in the refrigerator (2–8 °C).
6. Add 300 µL of cold 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. Wash cells by adding 10 mL of buffer per 10^8 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
9. Resuspend up to 10^8 cells in 500 µL of buffer.

   ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
10. Proceed to magnetic separation (2.3).

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2.3 Magnetic separation: Depletion of non-MZ and non-FO B cells

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

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

Depletion with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details see LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column.
4. Collect unlabeled cells that pass through and wash column with 1×3 mL of buffer. Collect total effluent; this is the unlabeled pre-enriched MZ and FO B cell fraction.
5. Proceed to 2.4 for the labeling of $CD21^{int}CD23^{hi}$ FO B cells.
6. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled non-MZ and non-FO B cells and all other non-B cells by firmly pushing the plunger into the column.

Depletion 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.
3. For a standard separation choose the following program:

   Depletion: Depletes
   Collect negative fraction in row B of the tube rack. This is the unlabeled pre-enriched MZ and FO B cell fraction.
4. Proceed to 2.4 for the labeling of $CD21^{int}CD23^{hi}$ FO B cells.
5. (Optional) Collect positive fraction from row C of the tube rack. This fraction represents the magnetically labeled non-MZ and non-FO B cells and all other non-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 is the unlabeled pre-enriched MZ and FO B cell fraction.
4. Proceed to 2.4 for the labeling of $CD21^{int}CD23^{hi}$ FO B cells.
5. (Optional) Collect positive fraction from outlet port pos1. This fraction represents the magnetically labeled non-MZ and non-FO B cells and all other non-B cells.

2.4 Magnetic labeling of $CD21^{int}CD23^{hi}$ FO 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 450 µL of buffer.
3. Add 50 µL of CD23 MicroBeads.
4. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
5. Wash cells by adding 10 mL 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 $CD21^{int}CD23^{hi}$ FO B cells

Positive selection with LS Columns

1. Place LS Column in the magnetic field of a suitable MACS Separator. For details refer to LS Column data sheet.
2. Prepare column by rinsing with 3 mL of buffer.
3. Apply cell suspension onto the column and collect flow-through.
4. Wash column with 3 mL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3. This fraction contains the unlabeled enriched MZ B cells.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette 5 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column. This is the enriched FO B cell fraction.

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Positive selection with the autoMACS® Pro Separator or the autoMACS® Separator

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.
3. For a standard separation choose the following program:

   Positive selection: Possels
   Collect negative fraction in row B of the tube rack. This fraction contains the unlabeled enriched MZ B cells.
   Collect positive fraction in row C of the tube rack. This is the enriched FO B cell fraction.

Magnetic separation with the autoMACS® Separator

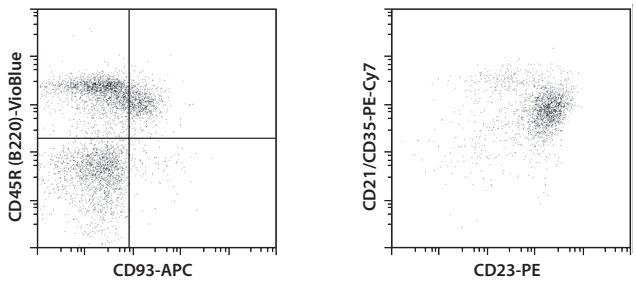
1. Prepare and prime the instrument.
2. Apply tube containing the sample and provide tubes for collecting the labeled and unlabeled cell fractions. Place sample tube at the uptake port and the fraction collection tubes at port neg1 and port pos1.
3. For a standard separation choose the following program:

   Positive selection: Possels
   Collect negative fraction in outlet port neg1 of the tube rack. This fraction contains the unlabeled enriched MZ B cells.
   Collect positive fraction from outlet port pos1. This is the enriched FO B cell fraction.

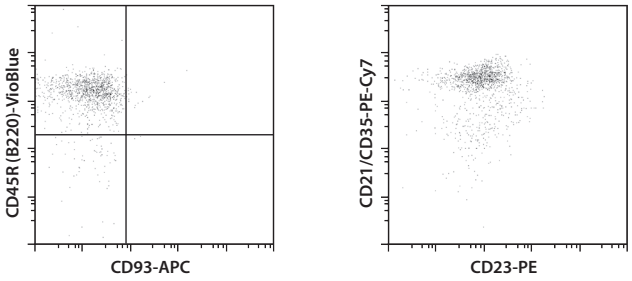
3. Example of a separation using the Marginal Zone and Follicular B Cell Isolation Kit

MZ and FO B cells were isolated from the spleens of 6-week-old BALB/c mice by using the Marginal Zone and Follicular B Cell Isolation Kit, mouse. The cells were fluorescently stained with CD45R (B220)-VioBlue (# 130-094-287), CD21/CD35-PE-Vio770 (# 130-097-216), CD23-PE (# 130-097-819), and CD93 (AA4.1)-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.

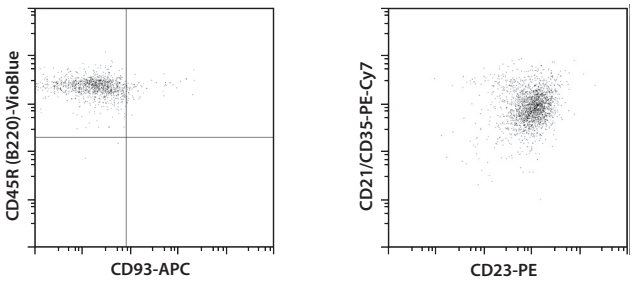
Before separation



Flow-through fraction: unlabeled enriched MZ B cells



Eluted fraction: enriched FO B cells



4. References

1. White, H. N. *et al.* (2012) Recruitment of a distinct but related set of VH sequences into the murine CD21^{hi}/CD23^{-} marginal zone B cell repertoire to that seen in the class-switched antibody response. J. Immunol. 188: 287–293.
2. Turchinovich, G. *et al.* (2011) Programming of marginal zone B-cell fate by basic Krüppel-like factor (BKLF/KLF3). Blood 117: 3780–3792.

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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