Miltenyi Biotec

Germinal Center B Cell (PNA) MicroBead Kit
mouse

Order no. 130-110-479

Contents

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.

1. Description

This product is for research use only.

Components	**2 mL PNA-Biotin:** Biotin-conjugated peanut agglutinin (PNA) **2×2 mL Anti-Biotin MicroBeads UltraPure:** UltraPure MicroBeads conjugated to monoclonal mouse anti-biotin antibodies (isotype: mouse IgG1).
Capacity	For 2×10^9 total cells, up to 200 separations.
Product format	All reagents 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

First, the germinal center B cells are indirectly magnetically labeled with PNA-Biotin and Anti-Biotin MicroBeads UltraPure. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled germinal center B cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of germinal center B cells. After removing the column from the magnetic field, the magnetically retained germinal center B cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the germinal center B cells must be separated over a second column.

1.2 Background information

Following primary immunization with T-dependent antigen, B cells in the lymphatic tissues can form germinal centers (GC). Here B cells proliferate, differentiate, and mutate their antibody genes through somatic hypermutation, and switch the class of their antibody.
B cells within GC express ligands for peanut agglutinin (PNA), allowing for their identification and isolation. PNA is a plant lectin protein derived from the fruits of *Arachis hypogae*, which specifically binds to terminal non-reducing galactose residues on the cellular membrane.

1.3 Applications

- Isolation of germinal center B cells from mouse spleen and lymph nodes of immunized mice for further characterization.

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: 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

  ▲ **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-Vio®770, mouse, CD38-APC, mouse, or CD95-PE, mouse. 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.

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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.

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™ Dissociators.

For details refer to www.gentleMACS.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. 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 PNA-Biotin 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 UltraPure per 10^7 total cells.
8. Mix well and incubate for 10 minutes in the refrigerator (2–8 °C).
9. Wash cells by adding 1–2 mL of buffer per 10^7 cells and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
10. Resuspend up to 10^8 cells in 500 µL of buffer.
    ▲ **Note:** For higher cell numbers, scale up buffer volume accordingly.
11. Proceed to magnetic separation (2.3).

2.3 Magnetic separation

▲ Choose an appropriate MACS® Column and MACS Separator according to the number of total cells and the number of germinal center B cells. For details refer to the table in section 1.4.

▲ When starting frequency of germinal center B cells is below 5%, the use of a second column is recommended.

▲ 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.
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: 3×500 µL LS: 3×3 mL

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
5. Remove column from the separator and place it on a suitable collection tube.
6. Pipette the appropriate amount of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.

   MS: 1 mL LS: 5 mL
7. (Optional) To increase the purity of germinal center B cells, the eluted fraction can be enriched over a second MS or LS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

Magnetic separation with XS Columns

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

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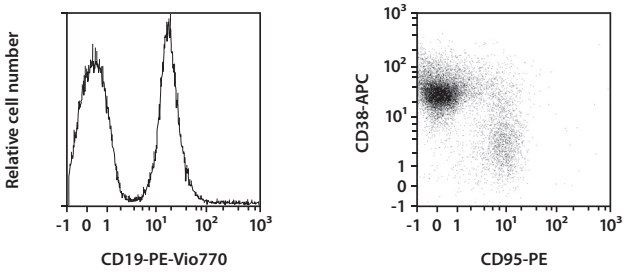
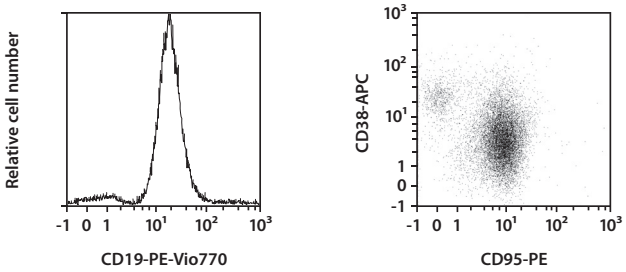
3. Example of a separation using the Germinal Center B Cell (PNA) MicroBead Kit

Isolation of germinal center B cells from mouse SRBC-immunized spleen using the Germinal Center B Cell (PNA) MicroBead Kit, an MS Column, and a MiniMACS™ Separator. Cells are fluorescently stained with CD19-PE-Vio®770, CD38-APC, and CD95-PE and analyzed by flow cytometer using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

A) Before separation



B) After separation



4. References

1. Shinall, S. et al. (2000) Identification of murine germinal center B cell subsets defined by the expression of surface isotypes and differentiation antigens. J. Immunol. 164: 5729–5738.

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.

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