Miltenyi Biotec

B-1a Cell Isolation Kit
mouse

Order no. 130-097-413

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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

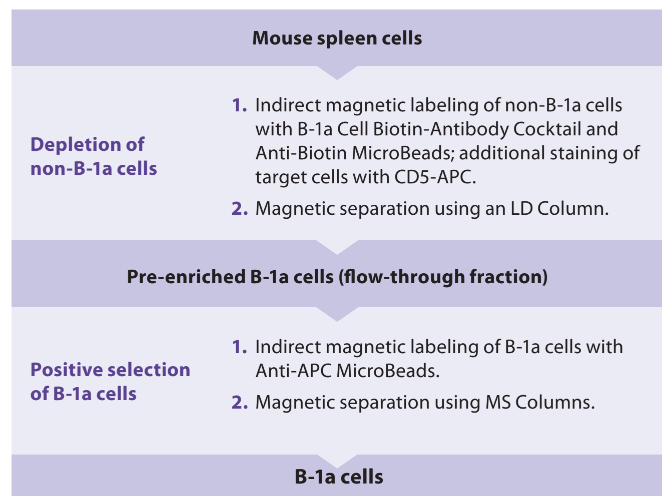
This product is for research use only.

Components	**2 mL B-1a Cell Biotin-Antibody Cocktail, mouse:** Cocktail of biotin-conjugated monoclonal anti-mouse antibodies against non-B-1a cells and additional APC-conjugated anti-mouse CD5 antibody. **2×2 mL Anti-Biotin MicroBeads:** MicroBeads conjugated to monoclonal anti-biotin antibody (isotype: mouse IgG1). **2 mL Anti-APC MicroBeads, mouse:** MicroBeads conjugated to monoclonal anti-APC antibody (isotype: mouse IgG1).
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 B-1a cells is performed in a two-step procedure. First, non-B-1a 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 parallel the target cells are stained with CD5-APC as primary labeling reagent. The magnetically labeled cells are subsequently depleted by separation over a MACS® Column, which is placed in the magnetic field of a MACS Separator.
In the second step, the B-1a cells are indirectly labeled with Anti-APC MicroBeads and isolated by positive selection from the pre-enriched B-1a cell fraction by separation over a MACS Column, which is placed in the magnetic field of a MACS Separator.
After removing the column from the magnetic field, the magnetically retained B-1a cells can be eluted as the positively selected cell fraction. To increase the purity, the positively selected cell fraction containing the B-1a cells can be separated over a second column.

▲ **Note:** Do not use APC tandem conjugates for staining before performing magnetic separation, as they might be recognized by the Anti-APC MicroBeads.



1.2 Background information

The B-1a Cell Isolation Kit has been developed for the isolation of $CD5^+$ B-1a cells from mouse spleen or body cavities. B-1 cells seem to be involved mainly in T cell-independent and innate-like immune responses and are the main producers of natural antibodies.
B-1 cells expressing CD5 are known as B-1a cells and those lacking expression of CD5, but having other hallmarks of B-1 cells, are known as B-1b cells.

1.3 Applications

- Isolation of B-1a cells from mouse spleen or body cavities for further phenotypical or functional characterization.

Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 877 591 1060
macs@miltenyibiotec.com

140-003-625.02

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 $Ca2^{+}$ or $Mg2^{+}$ are not recommended for use.

- MACS Columns and MACS Separators: Depletion of non-B-1a cells can be performed on an LD Column. The subsequent positive selection of B-1a cells can be performed on MS Columns.

Column	Max. number of labeled cells	Max. number of total cells	Separator
Depletion			
LD	10^8	5×10^8	MidiMACS, QuadroMACS, VarioMACS, SuperMACS II
Positive selection			
MS	10^7	2×10^8	MiniMACS, OctoMACS, VarioMACS, 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 (# 130-092-041). 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™ 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 of non-B-1a 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.

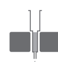
▲ Volumes for magnetic labeling given below are for up to 10^7 total cells. When working with fewer than 10^7 cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10^7 total cells, use twice the volume 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 B-1a 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 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. 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).



2.3 Magnetic separation: Depletion of non-B-1a cells

▲ Choose an appropriate MACS Column and MACS Separator according to the number of total cells and the number of B-1a cells. 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 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 pre-enriched B-1a cell fraction. Perform washing steps by adding buffer two times. Only add new buffer when the column reservoir is empty.

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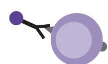
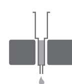
5. (Optional) Remove column from the separator and place it on a suitable collection tube. Pipette 3 mL of buffer onto the column. Immediately flush out the magnetically labeled non-B-1a cells by firmly pushing the plunger into the column.
6. Proceed to 2.4 for the labeling of B-1a cells.



2.4 Magnetic labeling of B-1a cells

▲ Volumes for magnetic labeling given below are for an initial starting cell number of up to 10^7 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 40 µL of buffer.
3. Add 10 µL of Anti-APC MicroBeads.
4. Mix well and incubate for 15 minutes in the refrigerator (2−8 °C).
5. Wash cells by adding 0.5–1 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 B-1a cells

Positive selection with MS Columns

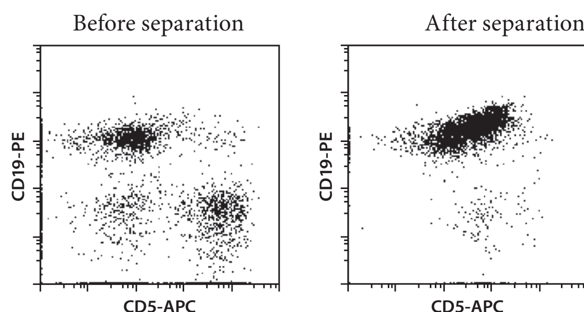
▲ To achieve highest purities when isolating splenic B-1a cells, perform two consecutive column runs.

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to MS Column data sheet.
2. Prepare column by rinsing with 500 µL of buffer.
3. Apply cell suspension onto the column. Collect flow-through containing unlabeled cells.
4. Wash column with 3×500 µL of buffer. Collect unlabeled cells that pass through and combine with the effluent from step 3.
   ▲ **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.
   ▲ **Note:** To perform a second column run, you may elute the cells directly from the first onto the second, equilibrated column instead of a collection tube.
6. Pipette 1 mL of buffer onto the column. Immediately flush out the magnetically labeled cells by firmly pushing the plunger into the column.
7. To increase purity of B-1a cells, the eluted fraction must be enriched over a second MS Column. Repeat the magnetic separation procedure as described in steps 1 to 6 by using a new column.

3. Example of a separation using the B-1a Cell Isolation Kit

B-1a cells were isolated from mouse spleen cell suspension (A) or peritoneal cavity (B) by using the B-1a Cell Isolation Kit, an LD and two MS Columns (A) or one MS Column (B), a MidiMACS™ and a MiniMACS™ Separator. The cells were fluorescently stained with CD19-PE (# 130-092-041) and CD3ε-FITC (# 130-092-962) 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.

A)



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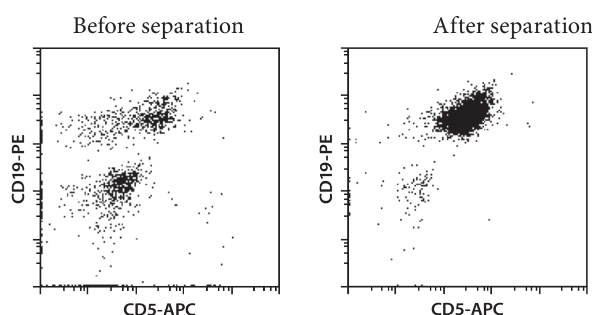

4. References

1. Hayakawa, K. *et al.* (1983) The "Ly-1 B" cell subpopulation in normal immunodefective, and autoimmune mice. J. Exp. Med. 157(1): 202–218.
2. Berland, R. and Wortis, H. H. (2002) Origins and functions of B-1 cells with notes on the role of CD5. Annu. Rev. Immunol. 20:253–300.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols.

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