Mouse B Cell Isolation Kit (Negative Isolation)

Cat. No.: M1522410 | Pack size: 10T; 50T; 100T | Storage: 2-8℃; Do not freeze

Overview

The Mouse B Cell Isolation Kit (Negative Isolation) is used to isolate B cells from mouse spleen, lymph nodes, or bone marrow by negative isolation. The principle is to use a cocktail of biotin-labeled monoclonal antibodies against non-target cells (non-B cells), followed by removal of these non-target cells using streptavidin-labeled magnetic beads, thereby achieving the isolation of mouse B cells. A magnetic separator is required for the isolation process.

Applications

Isolation of B cells from mouse spleen, lymph nodes, and bone marrow.

Kit Contents & Storage

Component Code	Component	Appearance	10T	50T	100T	Storage
M1522410A	Biotin-Antibody Mix	Liquid	20 µL	100 µL	200 µL	2-8℃
M1522410B	Streptavidin Magnetic Beads	Liquid	200 µL	1 mL	1 mL × 2	2-8℃

Protocol

Prepare single-cell suspension

Grind the spleen on a 70 µm cell strainer, rinse the strainer with pre-chilled PBS, and collect the cell suspension into a 50 mL centrifuge tube. For bone marrow, after preparing the cell suspension, filter it through a 70 µm cell strainer and collect into a 50 mL centrifuge tube. Centrifuge at 500 g for 5 min.

Lyse red blood cells

After centrifugation, discard the supernatant. Add 5 mL of ACK red blood cell lysis buffer per mouse, lyse at room temperature for 5 min, then add 20 mL PBS to stop lysis, and centrifuge at 500 g for 5 min.

Note: The red blood cell lysis step can be adjusted in volume and time depending on the tissue sample and the lysis buffer used. A small amount of red blood cell residue will not affect the purity of isolated cells.

Filter

After centrifugation, discard the supernatant and resuspend the splenocytes or bone marrow cells in PBS. Filter the cell suspension through a 70 µm cell strainer, then centrifuge at 500 g for 5 min.

Note: The cell suspension must be passed through a cell strainer to remove tissue and cell clumps; otherwise, subsequent cell isolation purity may be affected.

Adjust cell density

After centrifugation, resuspend the cells in Isolation Buffer to a density of 1×10^8 cells/mL.

Note: Isolation buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS), or PBS containing 2 mM EDTA and 0.5% BSA. The buffer must be pre-filtered through a 0.22 µm filter.

Add Biotin-Antibody Mix and wash

Add 100 µL of cell suspension (1×10^7 cells) to the bottom of a 1.5 mL centrifuge tube, then add 2 µL of Biotin-Antibody Mix. Mix well and incubate at 4℃ for 10 min. After incubation, add 1 mL of Isolation Buffer and centrifuge at 500 g for 5 min. Discard the supernatant, add 100 µL of Isolation Buffer to the cell pellet, resuspend the cells, and transfer to a sterile FACS tube.

Note: When adding the cell suspension, add it to the bottom of the centrifuge tube or FACS tube, avoiding the tube wall. Depending on the magnetic separator used, the entire process can be performed in a centrifuge tube. If isolating more cells, increase the volume of Biotin-Antibody Mix proportionally.

Add Streptavidin Magnetic Beads

Add 20 µL of washed Streptavidin Magnetic Beads to the FACS tube (the beads must be washed before use: vortex thoroughly to resuspend the beads, transfer the required volume of beads to a 1.5 mL centrifuge tube, add 1 mL of Isolation Buffer, centrifuge at 10,000 g for 1 min or use a magnetic separator for 3 min, then discard the supernatant. Add 1 mL of Isolation Buffer and wash the beads once more, then resuspend the beads in the same volume of Isolation Buffer as originally taken. For example, if 20 µL of beads are taken, resuspend them in 20 µL of Isolation Buffer after washing). Mix well and incubate at 4℃ for 10 min.

Note: If isolating more cells, increase the volume of Streptavidin Magnetic Beads proportionally. For example, to isolate 5×10^7 cells, add 10 µL of Biotin-Antibody Mix and 100 µL of Streptavidin Magnetic Beads to 500 µL of cell suspension. If isolating fewer than 1×10^7 cells, adjust the cell suspension volume to 100 µL, then add 2 µL of Biotin-Antibody Mix and 20 µL of Streptavidin Magnetic Beads.

Dilute and mix

After incubation, add 2.5 mL of Isolation Buffer to the FACS tube, and mix gently by pipetting up and down 5 times (avoid vigorous shaking or inverting).

Magnetic separation

Place the FACS tube containing the cell suspension on a magnetic separator and let stand for 5 min.

Collect B cells

Gently pour the cell suspension into a sterile centrifuge tube (do not remove the FACS tube from the magnetic separator while pouring). This cell suspension contains the purified mouse B cells. Centrifuge at 500 g for 5 min, then discard the supernatant and collect the cells.

Resuspend for further use

Wash the cells as required for your experiment, then resuspend them in the desired buffer or culture medium for subsequent molecular or cell biology experiments.

Isolation Performance

B cells were isolated from C57BL/6 mouse bone marrow and spleen cells. Cells before and after isolation were stained with FITC anti-mouse CD3 antibody (clone 145-2C11) and APC anti-mouse CD19 antibody (clone 6D5) and analyzed by flow cytometry.

Bone marrow cells: B cell purity before and after isolation was 24.6% and 95.8%, respectively.

Splenocytes: B cell purity before and after isolation was 62.9% and 97.8%, respectively.

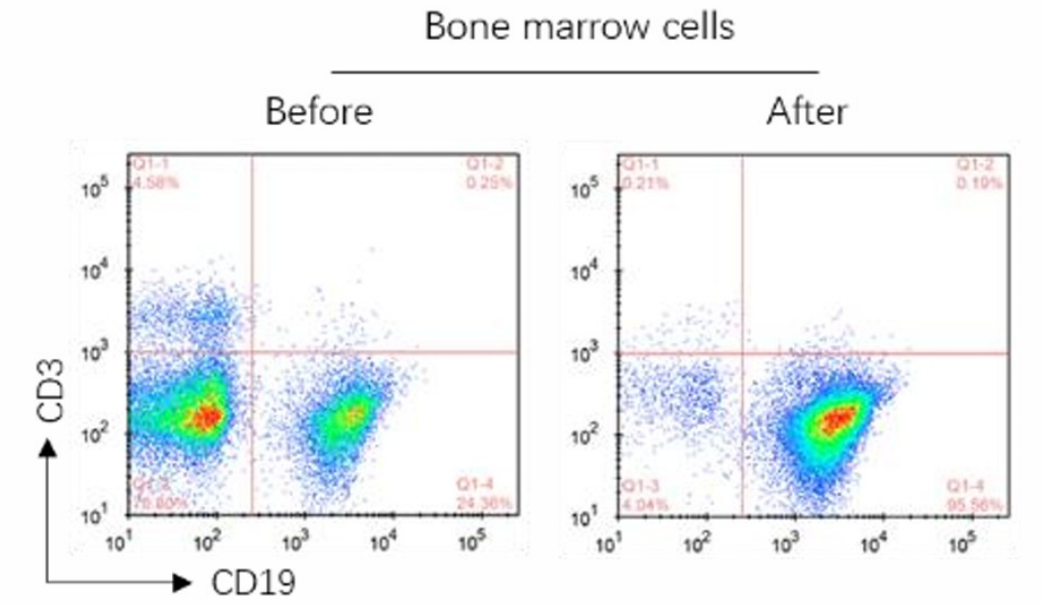

Figure 1. Mouse B cell isolation performance in bone marrow cells.

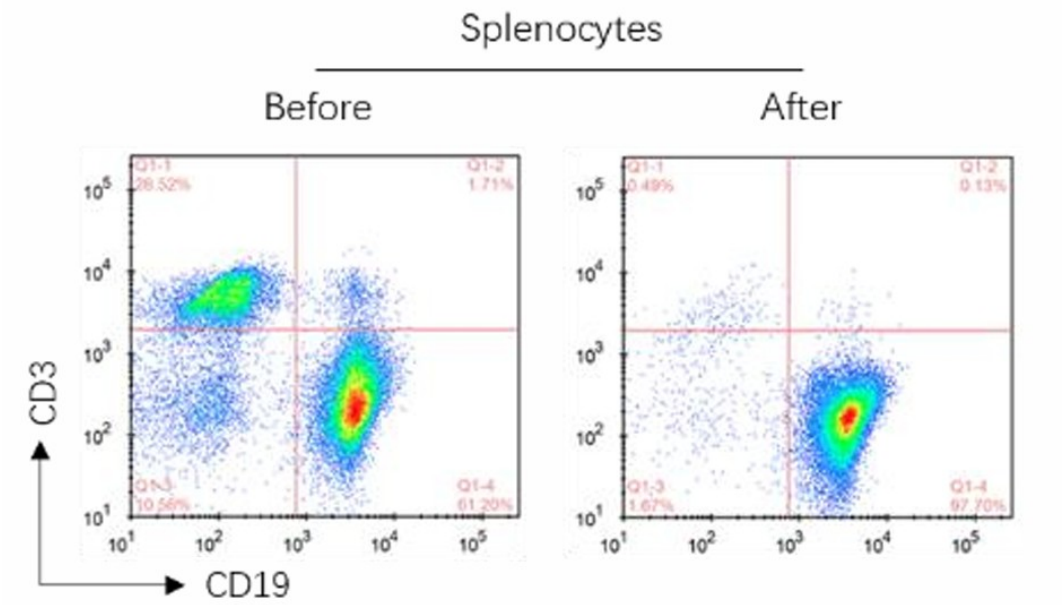

Figure 2. Mouse B cell isolation performance in splenocytes.

Precautions

Avoid freezing the magnetic beads during use and storage.

Low-retention pipette tips and centrifuge tubes are recommended to avoid loss of beads due to adsorption.

Before taking the magnetic beads, select a pipette with an appropriate volume range, thoroughly resuspend the beads by pipetting, and avoid generating bubbles during pipetting.

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Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

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