

Mouse CD8⁺ Cell Isolation Kit (Positive Isolation)

Cat. No.: M1522419 | Pack size: 10T; 100T | Storage: 2-8°C; Do not freeze

Overview

The Mouse CD8⁺ Cell Isolation Kit (Positive Isolation) is used to isolate CD8⁺ cells from mouse spleen or other tissue single-cell suspensions by positive isolation. The principle is to label CD8⁺ cells with a CD8 Capture Antibody, then capture the target cells using Releasable Magnetic Beads, and finally dissociate the beads from the cell surface using a Magnetic Beads Release Buffer, thereby obtaining magnetic bead-free mouse CD8⁺ cells. The isolated CD8⁺ cells can be used in downstream molecular and cell biology experiments.

Applications

Isolation of CD8⁺ cells from mouse lymphoid organs such as spleen and lymph nodes.

Kit Contents & Storage

Component Code	Component	Appearance	10T	100T	Storage
M1522419A	CD8 Capture Antibody	Liquid	20 µL	200 µL	2-8°C
M1522419B	Releasable Magnetic Beads	Liquid	200 µL	1 mL × 2	2-8°C
M1522419C	Magnetic Beads Release Buffer	Liquid	4 mL	40 mL	2-8°C

Protocol (Using Mouse Spleen CD8⁺ Cell Isolation as an Example)

1. Prepare single-cell suspension

Grind the spleen on a 70 µm cell strainer, rinse the strainer with pre-chilled PBS, collect the cell suspension into a 50 mL centrifuge tube, and centrifuge at 500 g for 5 min.

2. Lyse red blood cells

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

Note: A small amount of residual red blood cells will not affect subsequent isolation or cell purity.

3. Filter and count cells

After centrifugation, discard the supernatant, resuspend the splenocytes in PBS, filter the cell suspension through a 70 μm cell strainer, and count the cells. After counting, centrifuge at 500 g for 5 min.

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

4. Adjust cell density

After centrifugation, discard the supernatant, 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.

5. Add CD8 Capture Antibody

Add 500 μL of cell suspension (5×10^7 cells) to the bottom of a sterile FACS tube, then add 10 μL of CD8 Capture Antibody. Mix well and incubate at 4°C for 10 min.

Note: Add the cell suspension directly to the bottom of the tube, avoiding the tube wall. Depending on the magnetic separator used, a centrifuge tube can also be used for cell isolation. For other cell numbers, adjust the volume of CD8 Capture Antibody proportionally. If isolating fewer than 1×10^7 cells, adjust the cell suspension volume to 100 μL and add 2 μL of CD8 Capture Antibody.

6. Add Releasable Magnetic Beads

After incubation, add 100 μL of washed Releasable Magnetic Beads to the FACS tube, mix well, and incubate at 4°C for 10 min (the beads must be washed before use: vortex 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).

Note: For other cell numbers, adjust the volume of Releasable Magnetic Beads proportionally. If isolating fewer than 1×10^7 cells, use 20 μL of Releasable Magnetic Beads.

7. First magnetic separation and wash

After incubation, add Isolation Buffer to the FACS tube to a total volume of 2.5 mL, and mix by pipetting 5 times (avoid inverting the tube). Place the FACS tube on a magnetic separator and let stand for 5 min.

8. Discard supernatant and resuspend beads

Aspirate and discard the supernatant. Remove the tube from the magnetic separator, quickly add 2 mL of Isolation Buffer, and pipette repeatedly to disperse the beads. Place the tube back on the magnetic separator and let stand for 5 min.

9. Repeat washing

Repeat step 8 twice (thorough washing ensures high purity of target cells after subsequent release).

10. First elution

After magnetic separation, aspirate and discard the supernatant. Remove the tube from the magnetic separator, quickly add 1 mL of Magnetic Beads Release Buffer to resuspend the beads (avoid letting the beads dry), transfer the bead suspension to a 1.5 mL centrifuge tube, and rotate at room temperature for 10 min.

Note: For other cell numbers, adjust the volume of Magnetic Beads Release Buffer proportionally. If isolating fewer than 1×10^7 cells, use 200 μ L of Magnetic Beads Release Buffer for elution.

11. Collect first elution cells

After incubation, pipette repeatedly at least 10 times, transfer the bead suspension to a new FACS tube, add Isolation Buffer to 2.5 mL, mix by pipetting, place the tube on a magnetic separator, and let stand for 5 min.

12. Second elution

Transfer the supernatant to a 15 mL centrifuge tube and keep it (the supernatant contains the target cells; do not discard). Quickly resuspend the beads in 1 mL of Magnetic Beads Release Buffer (avoid drying), transfer to a 1.5 mL centrifuge tube, and rotate at room temperature for 10 min.

13. Collect second elution cells

After incubation, pipette repeatedly at least 10 times, transfer the bead suspension to a new FACS tube, add Isolation Buffer to 2.5 mL, mix by pipetting, place the tube on a magnetic separator, and let stand for 5 min.

14. Combine cells and centrifuge

Combine the supernatant with the cell supernatant from the first elution, centrifuge at 500 g for 5 min, discard the supernatant, and collect the magnetic bead-free CD8⁺ cells.

15. 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

CD8⁺ cells were isolated from C57BL/6 mouse splenocytes. Cells before and after isolation were stained with FITC-labeled anti-mouse CD8 antibody (clone 53-5.8) and analyzed by flow cytometry. The purity of CD8⁺ cells before and after isolation was 8.9% and 95.1%, respectively.

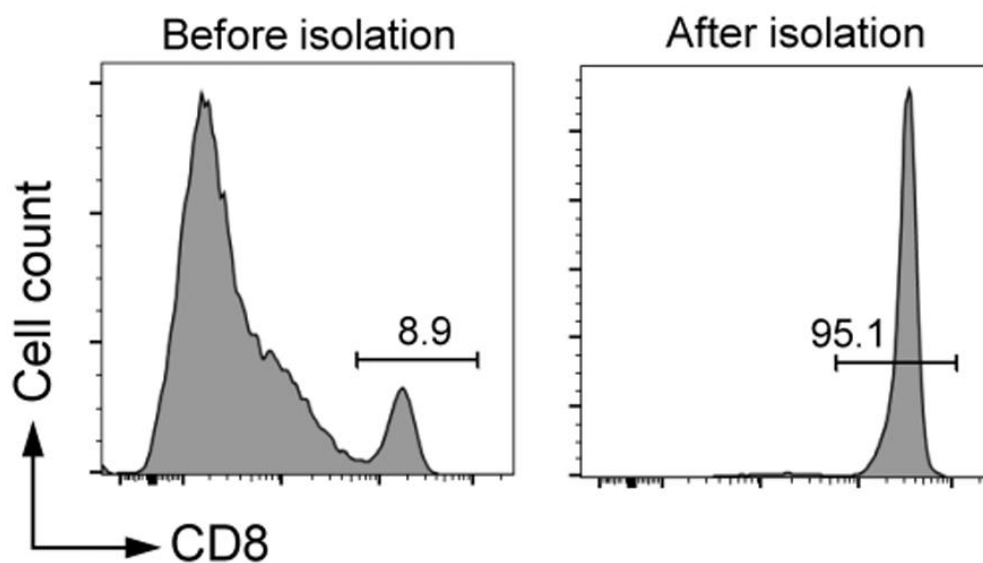


Figure 1. Mouse CD8⁺ cell positive-isolation performance.

Precautions

Avoid freezing any component of the kit during use and storage.

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

This product must be used with a magnetic separator.

For research use only.

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