

Mouse CD3⁺ T Cell Isolation Kit (Negative Isolation)

Cat. No.: M1522398 | Pack size: 10T; 50T; 100T | Storage: 2-8°C; Do not freeze

Overview

The Mouse CD3⁺ T Cell Isolation Kit (Negative Isolation) is used to isolate CD3⁺ T cells from mouse spleen or other tissue single-cell suspensions by negative isolation. The principle is to use a cocktail of biotin-labeled monoclonal antibodies against non-target cells (non-CD3⁺ T cells), followed by removal of these non-target cells using streptavidin-labeled magnetic beads, thereby achieving the isolation of mouse CD3⁺ T cells. A magnetic separator is required for the isolation process.

Applications

Isolation of CD3⁺ T cells from mouse spleen and lymph nodes.

Kit Contents & Storage

Component Code	Component	Appearance	10T	50T	100T	Storage
M1522398A	Biotin-Antibody Mix	Liquid	20 µL	100 µL	200 µL	2-8°C
M1522398B	Streptavidin Magnetic Beads	Liquid	200 µL	1 mL	1 mL × 2	2-8°C

Protocol (Using Mouse Spleen CD3⁺ T Cell Isolation as an Example)

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.

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: The red blood cell lysis step can be adjusted in volume and time depending on the lysis buffer used. A small amount of red blood cell residue will not affect subsequent isolation or cell purity.

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

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.

Add Biotin-Antibody Mix

Add 100 µL of cell suspension (1×10^7 cells) to the bottom of a sterile FACS tube, then add 2 µL of Biotin-Antibody Mix. Mix well and incubate at 4°C for 10 min.

Note:

When adding the cell suspension, add it 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.

If isolating more cells, increase the volume of Biotin-Antibody Mix proportionally.

Add Streptavidin Magnetic Beads

After incubation, add 20 µL of washed Streptavidin Magnetic Beads to the FACS tube (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). Mix well and incubate at 4°C 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 CD3⁺ T 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 CD3⁺ T 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

CD3⁺ T cells were isolated from C57BL/6 mouse splenocytes. Cells before and after isolation were stained with FITC anti- mouse CD3 antibody (clone 145-2C11) and analyzed by flow cytometry. The purity of CD3⁺ T cells before and after isolation was 35.3% and 97.6%, respectively.

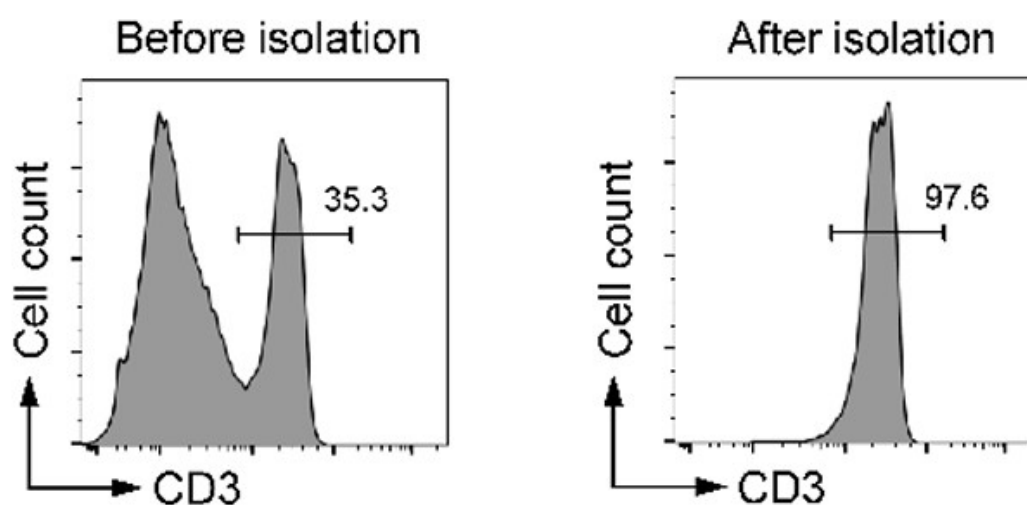


Figure 1. Mouse CD3⁺ T cell isolation performance.

Precautions

Avoid freezing the magnetic beads and antibody mix 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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