Mouse CD4$^+$ T Cell Isolation Kit (positive isolation)

Catalog No.: BRC0004

Product Description

The Mouse CD4$^+$ T Cell Isolation Kit (positive selection) is designed to isolate CD4$^+$ T cells from mouse splenocytes or single-cell suspensions of other tissues. The principle utilizes CD4 Capture Antibody to label CD4$^+$ cells, followed by capture with Releasable EnkiBeads™. The EnkiBeads™ Release Buffer is then used to dissociate the magnetic beads from the cell surface, yielding bead-free mouse CD4$^+$ cells. The isolated CD4$^+$ cells can be used for downstream molecular and cellular biology applications.

Kit Components

No.	Reagent Name	Size1 (For 10^9 cell)
Reagent1	CD4 Capture Antibody	200μl
Reagent2	Releasable EnkiBeads™	2 mL
Reagent3	EnkiBeads™ Release Buffer	40mL

Storage Conditions and Shelf Life

Storage Conditions: Store at 2–8°C. DO NOT FREEZE.
Shelf Life: 2 years

Applications

This kit is suitable for isolating CD4$^+$ T cells from mouse spleen and lymph nodes.

Protocol

Example: Isolation of CD4$^+$ T Cells from Mouse Spleen

1. Prepare single-cell suspension: Mince spleen through a 70 μm cell strainer. Rinse the strainer with ice-cold PBS and collect the cell suspension in a 50 mL centrifuge tube. Centrifuge at 500 g for 5 min.
2. Remove supernatant after centrifugation, add 5 mL erythrocyte lysis buffer (Cat. No. RC0011), and lyse at room temperature for 5 min. Add 20 mL PBS and centrifuge at 500 g for 5 min.

Note: Erythrocyte lysis conditions may vary depending on the specific lysis buffer used. Trace residual erythrocytes will not affect subsequent isolation or cell purity.

3. After centrifugation, remove supernatant, resuspend splenocytes in PBS, filter through a 70 μm cell strainer, and count cells. Centrifuge at 500 g for 5 min.

Note: The cell suspension must be filtered through a cell strainer to remove tissue debris and cell aggregates; otherwise, isolation purity will be compromised.

4. After centrifugation, remove supernatant and resuspend cells in isolation buffer at 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. Filter-sterilize through a 0.22 µm membrane before use.

5. Transfer 500 µL of cell suspension (5×10^7 cells) to the bottom of a sterile flow cytometry tube, add 10 µL CD4 Capture Antibody, mix well, and incubate at 4°C for 15 min.

Note: When adding cell suspension, pipette directly to the bottom of the tube; avoid dispensing along the tube wall. Depending on the magnetic separator used, centrifuge tubes may also be used for cell isolation. For different cell numbers, adjust CD4 Capture Antibody proportionally. For fewer than 1×10^7 cells, adjust cell suspension volume to 100 µL and add 2 µL CD4 Capture Antibody.

6. After incubation, add 100 µL washed Releasable EnkiBeads™ to the tube (magnetic beads must be washed before use: vortex to resuspend beads, pipette the required volume into a 1.5 mL centrifuge tube, add 1 mL isolation buffer, centrifuge at 10,000 g for 1 min, and remove supernatant. Repeat wash once with 1 mL isolation buffer, then resuspend beads in the same original volume of isolation buffer. For example, if 20 µL of beads were taken for washing, resuspend in 20 µL isolation buffer after washing), mix well, and incubate at 4°C for 10 min.

Note: For different cell numbers, adjust Releasable EnkiBeads™ proportionally. For fewer than 1×10^7 cells, use 20 µL Releasable EnkiBeads™.

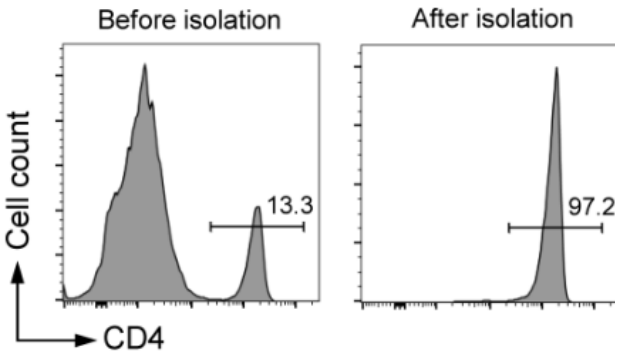
7. After incubation, add isolation buffer to the tube to a final volume of 2.5 mL, mix by pipetting up and down 5 times (avoid inversion mixing). Place the tube on the magnetic separator and let stand for 5 min.
8. Aspirate and discard the supernatant. Remove the tube from the magnetic separator, quickly add 2 mL isolation buffer, and resuspend beads by repeated pipetting. Place the tube on the magnetic separator and let stand for 5 min.
9. Repeat step 8 twice (thorough washing ensures subsequent elution of high-purity target cells).
10. After magnetic separation, aspirate and discard the supernatant. Remove the tube from the magnetic separator, quickly add 1 mL EnkiBeads™ Release Buffer to resuspend beads (avoid bead drying), transfer the bead suspension to a 1.5 mL centrifuge tube, and incubate with rotation at room temperature for 10 min.

Note: For different cell numbers, adjust EnkiBeads™ Release Buffer proportionally. For fewer than 1×10^7 cells, use 200 µL EnkiBeads™ Release Buffer to elute cells.

11. After incubation, pipette up and down at least 10 times, transfer the bead suspension to a new flow cytometry tube, add isolation buffer to 2.5 mL, mix by pipetting, and place the tube on the magnetic separator for 5 min.
12. Transfer the supernatant to a 15 mL centrifuge tube and set aside (the supernatant contains target cells, do not discard). Quickly resuspend beads in 1 mL EnkiBeads™ Release Buffer (avoid bead drying), transfer the bead suspension to a 1.5 mL centrifuge tube, and incubate with rotation at room temperature for 10 min.
13. After incubation, pipette up and down at least 10 times, transfer the bead suspension to a new flow cytometry tube, add isolation buffer to 2.5 mL, mix by pipetting, and place the tube on the magnetic separator for 5 min.
14. Pool the supernatant with the cell supernatant from the first elution, centrifuge at 500 g for 5 min, and remove supernatant to collect bead-free $CD4^+$ cells.
15. Wash cells as required for your experiment, then resuspend in appropriate buffer or culture medium for subsequent molecular or cellular biology applications.

Isolation Performance

CD4$^+$ cells were isolated from BALB/c mouse splenocytes and stained with PE-conjugated anti-mouse CD4 antibody (clone RM4-4) for flow cytometry analysis. CD4$^+$ cell purity increased from 13.3% to 97.2% pre- and post-isolation.



Important Notes

1. Avoid freezing magnetic beads and antibody mix during storage and use.
2. Use low-retention pipette tips and centrifuge tubes to minimize loss of beads and antibodies due to adsorption.
3. This product should be used with a magnetic separator.
4. For research use only.