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

Catalog No.: BRC0001

Product Description

The Mouse CD4$^+$ T Cell Isolation Kit is designed to isolate CD4$^+$ T cells from mouse splenocytes or single-cell suspensions of other tissues via negative selection. The principle involves labeling non-target cells (non- CD4$^+$ T cells) with biotin-conjugated monoclonal antibodies, followed by depletion using streptavidin-conjugated magnetic beads. This process enriches mouse CD4$^+$ T cells. A magnetic separator is required for the procedure.

Kit Components

No.	Reagent Name	Size1 (For 10^9 cell)	Size2 (For 5×10^8 cell)
Reagent1	Biotin-Antibody Mix	200μl	100μl
Reagent2	EnkiBeads™ Streptavidin	2 mL	1ml

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 100 μL of cell suspension (1×10^7 cells) to the bottom of a sterile flow cytometry tube,

add 2 µL Biotin-Antibody Mix, mix well, and incubate at 4°C for 10 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 larger cell numbers, increase Biotin-Antibody Mix proportionally.

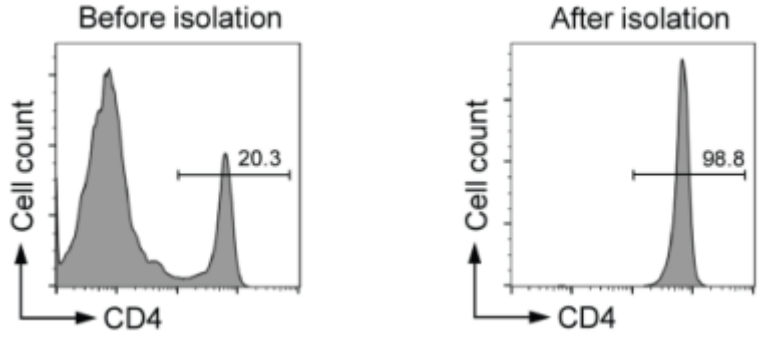
6. After incubation, add 20 µL washed EnkiBeads™ Streptavidin to the tube (magnetic beads must be washed before use: vortex to resuspend beads, pipette the required volume into a 1.5 mL centrifuge tube, add 1 mL isolation buffer, centrifuge at 10,000 g for 1 min, and remove supernatant. Repeat wash once 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 larger cell numbers, increase EnkiBeads™ Streptavidin proportionally. For example, to isolate 5×10^7 cells, add 10 µL Biotin-Antibody Mix and 100 µL EnkiBeads™ Streptavidin to 500 µL cell suspension. For fewer than 1×10^7 cells, adjust cell suspension volume to 100 µL and add 2 µL Biotin-Antibody Mix and 20 µL EnkiBeads™ Streptavidin.

7. After incubation, add 2.5 mL isolation buffer to the tube and mix gently by pipetting up and down 5 times (avoid vigorous shaking or inversion).
8. Place the tube containing cells on the magnetic separator and let stand for 5 min.
9. Gently pour the cell suspension into a sterile centrifuge tube (do not remove the tube from the magnetic separator during pouring). This suspension contains the purified mouse $CD4^+$ T cells. Centrifuge at 500 g for 5 min, then remove supernatant and collect cells.
10. Wash cells as required for your experiment, then resuspend in appropriate buffer or culture medium for subsequent molecular or cellular biology applications.

Isolation Performance

$CD4^+$ T cells were isolated from C57BL/6 mouse splenocytes. Pre- and post-isolation cells were stained with FITC anti-mouse CD4 antibody (clone GK1.5) and analyzed by flow cytometry. $CD4^+$ T cell purity increased from 20.3% to 98.8%.



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.