

Mouse CD3⁺ T Cell Isolation Kit (negative isolation)

Catalog No.: BRC0003

Product Description

The Mouse CD3⁺ T Cell Isolation Kit is designed to isolate CD3⁺ T cells from mouse splenocytes or single-cell suspensions of other tissues via negative selection. The principle involves labeling non-target cells (non- CD3⁺ T cells) with biotin-conjugated monoclonal antibodies, followed by depletion using streptavidin-conjugated magnetic beads. This process enriches mouse CD3⁺ 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 CD3⁺ T cells from mouse spleen and lymph nodes.

Protocol

Example: Isolation of CD3⁺ 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 1×10⁸ 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 (1×10⁷ 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.

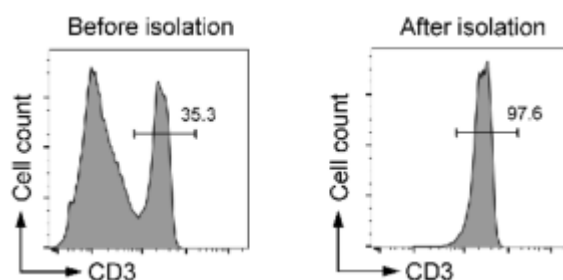
6. After incubation, add 20 μL washed EnkiBeads™ Streptavidin to the tube (magnetic beads must be washed before use: vortex to resuspend beads, pipette the required volume into a 1.5 mL centrifuge tube, add 1 mL isolation buffer, centrifuge at 10,000 g for 1 min, and remove supernatant. Repeat wash once 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 CD3⁺ 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

CD3⁺ T cells were isolated from C57BL/6 mouse splenocytes. Pre- and post-isolation cells were stained with FITC anti-mouse CD3 antibody (clone 145-2C11) and analyzed by flow cytometry. CD3⁺ T cell purity increased from 35.3% to 97.6%.



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.