Mouse CD4⁺ T Cell Isolation Kit

Description

The Mouse CD4⁺ T Cell Isolation Kit (B90001) isolates CD4⁺ T cells from single cell suspension of mouse spleen cells or other tissues by negative sorting. The principle is to use different biotin-labeled monoclonal antibodies to label non-target cells (non-CD4⁺ T cells), and then use streptavidin (streptavidin)-labeled magnetic beads to remove non-target cells, thereby to achieve the purpose of mouse CD4⁺ T cell sorting. The sorting process requires the use of a magnetic stand.

Components

Reagent	For 10^9 cell
Biotin-Antibody Mix	200 μl
Streptavidin	2 ml

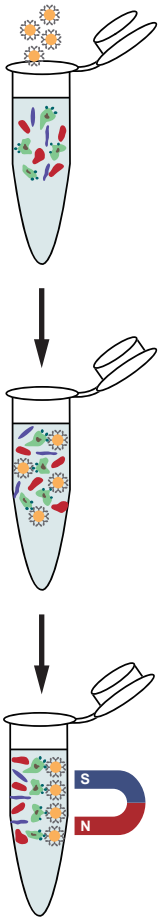
Storage

Store at 2-8°C, valid for two year, do not freeze.

Application

Isolation of mouse spleen or lymph CD4⁺ T cells.

Protocol



1. Add antibody-labeled magnetic beads to the cell suspension.

2. Magnetic beads bind to cells with corresponding antigens through specific antibodies.

3. Placed in a magnetic field, the cells attached to the magnetic beads are adsorbed by the magnetic field.

Taking the isolation of mouse spleen CD4⁺ T cells as an example:

1. Preparation of cell suspension: Grind the spleen on a 70 μm cell mesh, rinse the cell mesh with pre-cooled PBS, collect the cell suspension in a 50 ml centrifuge tube, centrifuge at 500 g for 5 min.

2. After centrifugation, discard the supernatant, add 5 ml of ACK erythrocyte lysate, lyse at room temperature for 5 min, then add 20 ml of PBS and centrifuge at 500 g for 5 min.
Tips: The amount and time of the red blood cell lysis step can be adjusted according to the different lysis solutions used. A small amount of residual red blood cells will not affect subsequent sorting and cell purity.

3. After centrifugation, the supernatant is discarded, the splenocytes are resuspended in PBS, and the cell suspension is filtered through a 70 μm cell mesh before counting. After counting, continue to centrifuge at 500 g for 5 min.
Tips: Cell suspension need to be passed through a cell strainer to remove tissue and cell clumps that would otherwise compromise the purity of subsequent cell isolation.

4. After centrifugation, discard the supernatant, resuspend the cells in sorting buffer, and adjust the cell density to 1×10^8 cells/ml.
Tips: The sorting buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS) or PBS containing 2 mM EDTA and 0.5% BSA, which needs to be sterilized by filtration through a 0.22 μm filter in advance.

5. Add 100 μl of cell suspension (1×10^7 cells) to the bottom of the sterile flow tube, then add 2 μl of Biotin-Ab Mix, and incubate at 4°C for 10 min after mixing.

※ **Please continue reading the protocol overleaf .**

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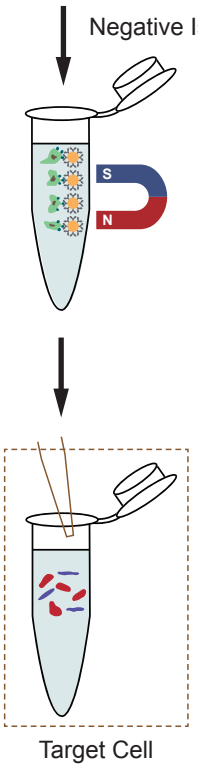


4. Aspirate the supernatant and leave the cells with the antigen in the tube and the other cells are in the aspirated supernatant.

For negative isolation, the supernatant should be analyzed. Target cells are in the supernatant.

Tips: When adding the cell suspension, add cells to the bottom of the flow tube and avoid adding them along the wall of the flow tube. Depending on the characteristics of the magnetic stand used, centrifuge tubes can also be used for cell sorting. If more cells were sorted, proportionally increase the amount of Biotin-Ab Mix.

6. After the incubation, add 20 µl Streptavidin beads to the flow tube (vortex to resuspend the magnetic beads before use), and incubate at 4°C for 10 min after mixing.
Tips: If more cells are isolated, proportionally increase the amount of Streptavidin beads. For example, if 5×10^7 cells are sorted, 10 µl Biotin-Ab Mix and 100 µl Streptavidin beads should be added to 500 µl cell suspension. If sorting less than 1×10^7 cells, make up the cell suspension volume to 100 µl, use 2 µl Biotin-Ab Mix and 20 µl Streptavidin beads.

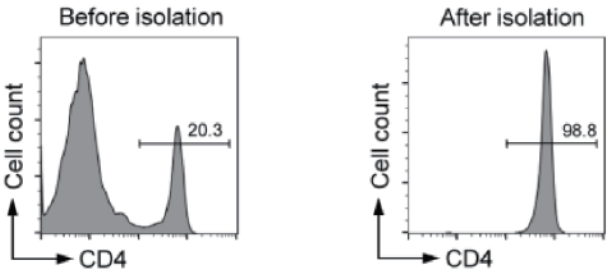
7. After incubation, add 2.5 ml of isolation buffer to the flow tube, and mix by pipetting up and down for 5 times (avoid vigorous shaking or inversion).

8. Place the sorting flow tube containing the cells on a magnetic stand and let stand for 5 min.

9. Gently pour the cell suspension into a sterile centrifuge tube (the flow tube should not fall off the magnetic stand during the pouring process), centrifuge at 500 g for 5 min. The cell suspension contains purified mouse $CD4^+$ T cells. After centrifugation, the supernatant is discarded and the cells are collected.

10. After washing the cells according to the needs of the experiment, resuspend the cells in the desired buffer or medium and use them for subsequent molecular biology or cell biology experiments.

Schematic Diagram of Isolation



$CD4^+$ T cells are isolated from spleen cells of C57BL/6 mice, and the cells before and after sorting are labeled with FITC anti-mouse CD4 antibody (clone number GK1.5) for flow cytometry analysis. $CD4^+$ T cells before and after sorting are analyzed by flow cytometry. Purities are 20.3% and 98.8%, respectively.

Notice

1. Freezing, high-speed centrifugation, etc. should be avoided during the use and storage of magnetic beads and antibody mixtures operate;
2. It is recommended to use low adsorption pipette tips and centrifuge tubes to avoid magnetic beads and loss of body;
3. This product needs to be used in conjunction with a magnetic separator;
4. This product is for research use only.

Product List

Cat.No.	Product Name	Size
B90001	Mouse $CD4^+$ T Cell Isolation Kit	For 10^9 cells
B90011	Mouse $CD8^+$ T Cell Isolation Kit	For 10^9 cells

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