Mouse CD8$^+$ T Cell Isolation Kit (Negative Selection)

Introduction

The Mouse CD8$^+$ T Cell Isolation Kit (Negative Selection) is used for the isolation of CD8$^+$ T cells from Single-cell suspension of mouse spleen or lymph nodes by negative selection. The isolation principle of this kit is to label unwanted cells with the biotin-labeled monoclonal antibody mix, and then add streptavidin-labeled magnetic beads to remove unwanted cells. Isolated cells are high-purity CD8$^+$ T cells.

This kit is easy to use and does not require a separation column, which only requires a magnetic separator to isolate cells in as little as 15 min. And the purity of the sorted cells can reach more than 95%. At the same time, the obtained cells are not labeled with antibodies and magnetic beads, so they will not be abnormally activated and will not affect downstream experiments.

Components and Storage

Components \ Size	50 Assays (for 5×10^8 cells)	100 Assays (for 10^9 cells)	Storage
Biotin-Antibody Mix	100 μL	200 μL	4°C
Streptavidin Beads	1 mL	2 mL	4°C
Shipping: Blue ice	Shelf life: 1 year		

Protocol

Take sorting mouse spleen CD8$^+$ T cells as an example

1. Preparation of sorting buffer: The sorting buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS), where 2% fetal bovine serum (FBS) can be replaced with 0.5% BSA. Sorting buffers need to be sterilized by 0.22 μm filter.

2. Preparation of single-cell suspension of mouse spleen:

    1) Grind the mouse spleen with a 70 μm cell sieve, rinse the sieve with pre-chilled PBS, collect the cell suspension in a 50 mL centrifuge tube, and then centrifuge at 500 g for 5 min.

    2) After centrifugation, discard the supernatant. Add 5 mL of Red Blood Cell Lysis Buffer and lyse at room temperature for 5 min. Subsequently, add 20 mL of PBS and centrifuge at 500 g for 5 min.

***Note:** The amount and duration of red blood cell lysate can be adjusted according to the specific experiment. A small amount of residual red blood cells does not affect subsequent sorting.

3) After centrifugation, discard the supernatant. Resuspend cells with PBS and filter with a 70 μm cell sieve. After counting, centrifuge at 500 g for 5 min.

***Note:** Cell suspension is sifted to remove tissue and cell clumps so as not to interfere with subsequent sorting.

4) Remove the supernatant, and resuspend cells in the sorting buffer. Adjust the cell density to 1×10^8 cells/mL.

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

***Note:** Please add the cell suspension directly to the bottom of the tube, not along the wall of the tube. Depending on the magnet used, centrifuge tubes can also be used for sorting. If more cells need to be sorted, scale up the volume of the Biotin-Antibody Mix.

4. Magnetic bead washing: Resuspend Streptavidin Beads by vortexing and shaking. Take an appropriate amount of Streptavidin Beads to a 1.5 mL centrifuge tube, and add the sorting buffer to a total volume of 1 mL. Centrifuge at 10,000 g for 1 min and discard the supernatant. Add 1 mL of sorting buffer to resuspend the beads, centrifuge at 10,000 g for 1 min, and discard the supernatant. Resuspend the beads with the same volume of sorting buffer as the original. If taking 20 μL of Streptavidin Beads, add 20 μL of sorting buffer.

5. After incubation, add 20 μL of washed Streptavidin Beads to the tube. Mix well and incubate at 4°C for 10 min.

***Note:** If more cells need to be sorted, scale up the volume of Streptavidin Beads. If sorting less than 1×10^7 cells, make up the cell suspension to 100 μL and add 2 μL of Biotin-Antibody Mix and 20 μL of washed Streptavidin Beads.

6. Add 2.5 mL of sorting buffer to the tube and mix gently. Do not shake vigorously or mix upside down.

7. Place the tube in a magnet for 5 min.

8. Gently pour the cell suspension into a sterile tube, and do not disengage the flow tube from the magnet during the pouring process. This cell suspension is the isolated Mouse $CD8^+$ T cells. Centrifuge at 500 g for 5 min and discard the supernatant.

9. Wash and resuspend cells with a suitable buffer or medium for subsequent experiments.

Note

1. Do not freeze both Biotin-Antibody Mix and Streptavidin Beads.

2. Streptavidin Beads should be avoided from drying during use and storage, and should not be placed in a magnetic field for a long time to avoid clumping of the beads and reduce the activity of the beads.

3. Try to use low-adsorption pipette tips and tubes to avoid loss due to adsorption.

4. This kit does not provide the magnet. You can purchase QuickSort Magnet (Cat. No. CS1010).

5. For your safety and health, please wear lab coats and gloves during the experiment.

6. For research use only. Not to be used in clinical diagnostic or clinical trials.

APExBIO Technology
www.apexbt.com
7505 Fannin street, Suite 410, Houston, TX 77054.
Tel: +1-832-696-8203 | Fax: +1-832-641-3177 | Email: info@apexbt.com