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Mouse CD8$^+$ Cell Positive Selection Kit

1 Packing list

Components	HY-K0310-2 mL (For 10^9 cells)
CD8 Capture Antibody	200 μL
Releasable Magnetic Beads	1 mL × 2
Magnetic Beads Release Buffer	40 mL

2 Introduction

MCE Mouse CD8$^+$ Cells Positive Selection Kit is designed for the isolation of CD8$^+$ cells from single cell suspensions of mouse spleen cells, lymph nodes or other tissues. Its principle is that CD8 Capture Antibody can label CD8$^+$ cells, these target cells are captured by Releasable Magnetic Beads, and finally, the Magnetic Beads Release Buffer dissociates the magnetic beads from the cell surface. This process effectively facilitates the sorting of mouse CD8$^+$ cells that are not labelled with magnetic beads.

MCE Mouse CD8$^+$ Cells Positive Selection Kit is user-friendly and portable, with components that are non-toxic to cells. It enables the isolation of high-purity cells, and the resulting cells can be utilized in a variety of downstream applications. These include flow cytometry, cell culture, Western blot analysis, immunoprecipitation (IP), reporter gene detection, and DNA/RNA extraction.

Features of MCE Mouse CD8$^+$ Cells Positive Selection Kit

- Separation Columns-free: Eliminates the need for separation columns by using a magnetic separator for swift cell separation;
- High Purity: Can yield sorted cells with a purity exceeding 95%;
- Broad Applicability: Can be used directly for the capture of target cells, and the final cells obtained are free of magnetic bead labeling.

3 General Protocol

Recommended Buffer	
Isolation Buffer	PBS, 0.5% BSA, 2 mM EDTA, pH 7.0 - 7.4

Note: a. BSA can be replaced with human serum albumin (HSA) or 2% fetal bovine serum (FBS).

b. It is recommended to prepare the Isolation Buffer using water-for-injection, followed by filtration through a 0.22 μm membrane for sterilization purposes. The prepared buffer should be stored at 4°C.

Taking the isolation of CD8$^+$ cells from mouse spleen as an example

1. To prepare a single-cell suspension from a mouse spleen: Disaggregate the spleen using a 70 μm cell sieve, and rinse the sieve with pre-cooled PBS. Transfer the resultant cell suspension to a 50 mL centrifuge tube, centrifuge at 500 × g for 5 minutes, and discard the supernatant.

2. Add 5 mL of ACK Lysis Buffer to the tube, lysed at room temperature for 5 min. Add 20 mL of PBS to resuspend the cells, centrifuged at 500 × g for 5 min, and discard the supernatant.

Note: a. The erythrocyte lysis step can be adjusted according to the different lysates used.

b. A small amount of residual red blood cells will not affect the subsequent sorting and cell purity.

3. Resuspend the cells in PBS; filter the cell suspension through a 70 μm cell sieve and count. Centrifuge the cells at 500 × g for 5 min and discard the supernatant.

Note: This step is to eliminate tissue and cell aggregates from the suspension, ensuring they do not affect the purity of the subsequently sorted cells.

4. Resuspend the cells in the Isolation Buffer, and adjust the cell density to 1×10^8 cells/mL.

5. Transfer 500 μL of the cell suspension (5×10^7 cells) to the bottom of a sterile tube. Add 10 μL of CD8 Capture Antibody, mix well and incubate at 4°C for 10 minutes.

Note: a. Transfer cell suspension directly to the bottom of the tube, avoiding adding along the wall of the tube.

b. If a larger quantity of cells requires sorting, the amount of CD8 Capture Antibody can be adjusted proportionally. If sorting less than 1×10^7 cells adjust the volume of the cell suspension to 100 μL and add 2 μL of CD8 Capture Antibody.

6. To prepare the Releasable Magnetic Beads: Resuspend the Releasable Magnetic Beads thoroughly and transfer 100 μL of these beads to a 1.5 mL EP tube. Add 1 mL of Isolation Buffer, mix and centrifuge at 10,000 × g for 1 min. Discard the supernatant and repeat wash the beads 1-2 times, resuspend the beads in 100 μL of Isolation Buffer.

Note: The volume of Isolation Buffer used for the beads resuspension should be equal to the initial volume of beads that was aspirated.

7. After cell-antibody incubation, add 100 μL of pre-washed beads to the tube, mix well and incubate at 4°C for 10 min.

Note: If a larger number of cells need to be sorted, the amount of magnetic beads can be increased proportionally. If sorting less than 1×10^7 cells adjust the volume of the cell suspension to 100 μL and add 2 μL of CD4 Capture Antibody along with 20 μL of beads.

8. After incubation, add 2 mL of Isolation Buffer to the tube and mix gently (avoid vigorous shaking or up-and-down mixing).

9. Place the tube in the magnetic separator for 5 min; discard the supernatant and repeat wash the beads-cells mixture 1-2 times.

10. Remove the tube from the magnetic rack and immediately add 1 mL of Magnetic Beads Release Buffer to resuspend the magnetic beads to prevent the beads drying out. Transfer the beads suspension to a 1.5 mL EP tube, rotate and incubate for 10 minutes at room temperature.

Note: If a larger number of cells need to be sorted, the amount of magnetic beads can be increased proportionally. If sorting less than 1×10^7 cells adjust the volume of the Magnetic Beads Release Buffer to 100 μL.

11. After incubation, mix gently with a pipette (avoiding vigorous shaking or excessive up-and-down mixing). Transfer the beads suspension to a new tube and place it on a magnetic separator for 5 minutes.

12. Carefully transfer the cell suspension into a sterile centrifuge tube (The supernatant contains the targeted cells.), immediately add 1 mL of Magnetic Beads Release Buffer to resuspend the magnetic beads to prevent the beads drying out. Transfer the beads suspension to a 1.5 mL EP tube, rotate and incubate for 10 minutes at room temperature.

13. After incubation, mix gently with a pipette (avoiding vigorous shaking or excessive up-and-down mixing). Transfer the beads suspension to a new tube and place it on a magnetic separator k for 5 minutes.

14. Combine the supernatant with the first elution (step 12). Place this mixture on a magnetic separator for 2 - 5 minutes to remove any residual beads.

15. Transfer the cell suspension into a sterile centrifuge tube, centrifuge at 500 × g for 5 minutes. Discard the supernatant and collect the $CD8^+$ cells that are not labelled with magnetic beads.

16. According to the requirements of experiment wash the cells, and resuspend the cells in the appropriate buffer or medium. The cells can be used for downstream molecular or cell biology experiments.

4 Storage

4°C， 2 years

Do not freeze the magnetic beads

5 Precautions

1. During the use and storage of the Biotin-Antibody Mix, avoid processes such as repeated freeze-thaw cycles and high-speed centrifugation.

2. It is recommended to use low-adsorption consumables, such as pipette tips and centrifuge tubes, in order to prevent the loss of beads and antibodies due to adsorption.

3. During the use and storage of Streptavidin Magnetic Beads, avoid high-speed centrifugation, desiccation, or freezing. Additionally, the beads should not be kept in a magnetic field for an extended period as this could lead to bead agglomeration, which may reduce their binding activity.

4. Before aspiration from the bead storage tubes, ensure that the beads are thoroughly mixed by gentle shaking. Handle gently to prevent the formation of air bubbles.

5. This kit is intended for use in conjunction with a magnetic separator.

6. The experimental procedures should be handled gently to prevent any mechanical damage to the cells.

7. This product is for R&D use only, not for drug, household, or other uses.

8. For your safety and health, please wear a lab coat and disposable gloves to operate.