MCE USA
Tel: 609-228-6898
Email: tech@MedChemExpress.com

# Mouse CD4$^+$ T Cells Negative Selection Kit

## 1 Packing list

| Components | HY-K0307-1 mL (For 5 × $10^8$ cells) | HY-K0307-2 mL (For $10^9$ cells) |
|---|---|---|
| Biotin-Antibody Mix | 100 μL | 200 μL |
| Streptavidin Magnetic Beads | 1 mL | 1 mL × 2 |

## 2 Introduction

MCE Mouse CD4$^+$ T Cells Negative Selection Kit is designed for the isolation of CD4$^+$ T cells from single cell suspensions of mouse spleen cells, lymph nodes or other tissues. Its principle hinges on utilizing a biotin-labeled monoclonal antibody to mark non-target cells (non-CD4$^+$ T-cells), which are subsequently removed using streptavidin-labeled magnetic beads. This process effectively facilitates the sorting of human CD4$^+$ T cells.

The 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 CD4$^+$ T Cells Negative Selection Kit:

- Efficiency: Enables sorting of target cells in as little as 15 minutes;
- 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 97%;
- High Activity: The sorted cells are free of antibody and magnetic bead labeling, and retain high activity and functionality for downstream experiments.

## 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 CD4$^+$ T 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 $1\times10^8$ cells/mL.

5. Transfer 100 μL of the cell suspension ($1 \times 10^7$ cells) to the bottom of a sterile tube. Add 2 μL of Biotin-Antibody Mix, 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, proportionally increase the volume of Biotin-Antibody Mix used.

6. To prepare the Streptavidin Magnetic Beads: Resuspend the Streptavidin Magnetic Beads thoroughly and transfer 20 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 20 μL of Isolation Buffer.

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

7. After cell-antibody incubation, add 20 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. For sorting $5 \times 10^7$ cells, add 10 μL of Biotin-Antibody Mix and 100 μL of beads to 500 μL of cell suspension. If sorting less than $1 \times 10^7$ cells adjust the volume of the cell suspension to 100 μL and add 2 μL of Biotin-Antibody Mix along with 20 μL of beads.

8. After incubation, add 2.5 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.

10. Carefully transfer the cell suspension into a sterile centrifuge tube (taking care to not detach the tube from the magnetic separator during this process). This tube contains the purified mouse $CD4^+$ T cells. Centrifuge at 300 × g for 5 minutes, discard the supernatant and collect the cells.

Note: During the transfer process, avoid contact between the pipette tip and the magnetic beads, and the cell suspension can also be collected by decantation.

11. 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.