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

## Introduction

The Human CD8$^+$ T Cell Isolation Kit (Negative Selection) is used for the isolation of CD8$^+$ T cells from human peripheral blood mononuclear cells (PBMCs) 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 30 min. And the purity of the sorted cells can reach 90%. 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

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 human PBMCs: Use Ficoll density gradient centrifugation to obtain PBMCs from human peripheral blood, then wash PBMCs with PBS and centrifuge. Resuspend PBMCs in the sorting buffer and 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 15 min. Add 10-fold volume sorting buffer to the tube and centrifuge at 500 g for 5 min. remove the supernatant and resuspend the cells in 100 μL of sorting buffer.

***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 10 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 $1\times10^7$ 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. Transfer the cells and beads to another sterile tube. 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 human $CD8^+$ T cells. Centrifuge at 500 g for 5 min and discard the supernatant.
9. (Optional) To further increase cell purity, resuspend cells with 100 μL of sorting buffer. Repeat steps 5-8 once to re-purify human $CD8^+$ T cells. The purity may increase 2-4% with a second separation.
10. 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.

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