Mouse CD8⁺ T Cell Isolation Kit

CS103

Version 24.1

Product Description

Mouse CD8⁺ T Cell Isolation Kit is designed to isolate CD8⁺ T cells from single-cell suspensions of mouse spleen, lymph nodes, and other tissues by the negative selection method. The principle is to use biotin-labeled monoclonal antibodies to label non-target cells, and then use streptavidin magnetic beads to remove non-target cells, thereby to achieve the purpose of mouse CD8⁺ T cell sorting. This product can obtain the target cells in only 16 min with the purity rate of up to 96%. There is no abnormal activation of cells after sorting, thus not affecting downstream experiments. The isolation process requires the use of magnetic poles or magnetic racks.

Components

Components	CS103-01 (for 1 × 10^9 cells)
CD8⁺ T cell Isolation Cocktail	1 ml
Streptavidin Beads	2 × 1 ml

Storage

Store at 2 ~ 8°C and ship on ice pack. Do not freeze.

Applications

It is applicable for isolating CD8⁺ T cells from mouse spleen, lymph nodes, and mixed samples.

Notes

1. Magnetic beads need to be mixed thoroughly on the vortex mixer before use. After mixing, high-speed centrifugation should not be performed.
2. Please avoid freezing during storage of this kit.
3. It is recommended to use low-adsorption tubes and tips to avoid the loss of magnetic beads and antibodies.
4. The separation process should be as gentle as possible to avoid mechanical damage to cells caused by magnetic beads.
5. If subsequent cell culture experiments are performed, the separation process needs to be finished in a sterile environment.
6. When sedimentation occurs during the addition of magnetic beads for incubation, pipette up and down every 1 - 2 min.

Experiment Process

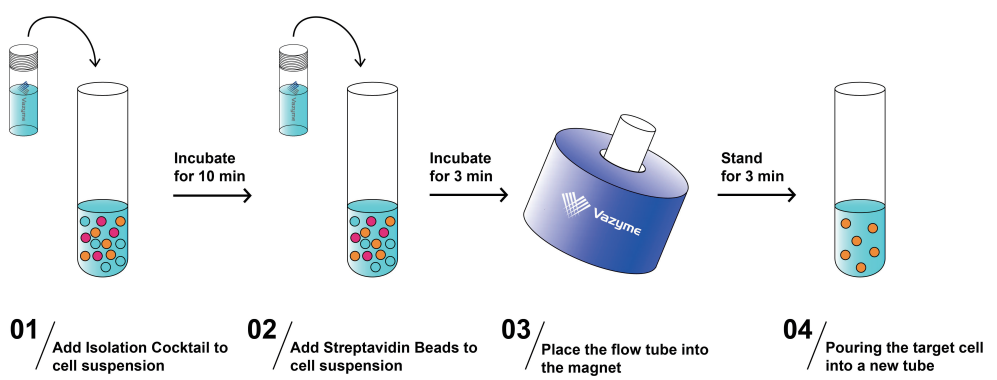
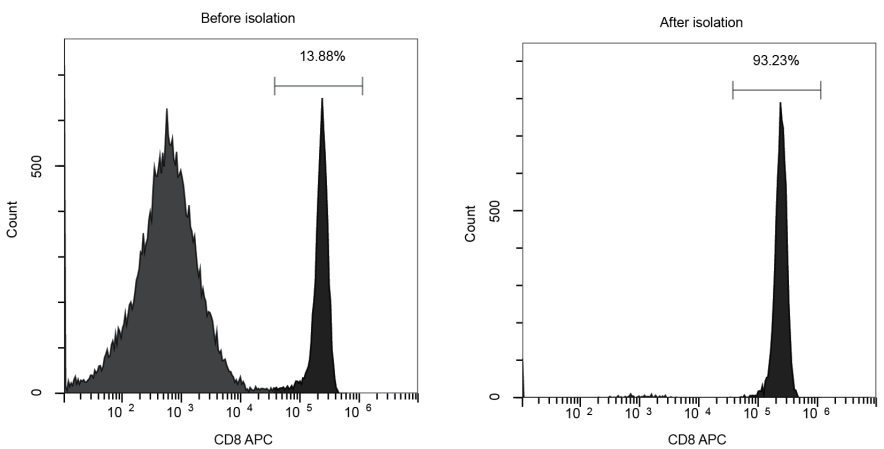


Fig 1. Workflow of vazyme negative sorting mouse CD8⁺ T cell

Taking the separation of CD8$^+$ T cells from mouse spleen as an example:

1. Preparation of single-cell suspension: Grind the cell on a 70 μm cell strainer, then rinse the cell strainer with pre-chilled sorting buffer and collect the suspension into a 50 ml centrifuge tube. Centrifuge at 2,000 rpm (500 × g) for 5 min, and discard the supernatant.
   ▲ The sorting buffer is PBS containing 1 mM EDTA and 2% fetal bovine serum (FBS), which needs to be sterilized by filtration through a 0.22 μm filter in advance.
2. (Optional) Add 3 ml of red blood cell (RBC) lysis buffer to the centrifuge tube, pipette up and down several times to mix thoroughly, lyse at room temperature for 10 min, then add 10 ml of PBS to stop the reaction. Centrifuge at 2,000 rpm (500 × g) for 5 min, and discard the supernatant.
   ▲ RBC lysis steps can be adjusted according to the amount of lysis buffer and lysis time. A small amount of residual RBC will not affect subsequent sorting and cell purity.
3. Resuspend spleen cells with 1 ml of sorting buffer, filter the cells using a 70 μm cell strainer, and then perform cell counting. Adjust the cell concentration to 1 × 10^8 cells/ml.
   ▲ This step of the cell suspension is recommended to pass through a cell strainer to avoid tissue and cell clumps affecting the purity of subsequent cell sorting.
4. Pipette 100 μl of cell suspension (1 × 10^7 cells) into the bottom of the flow tube, and add 10 μl of CD8$^+$ T cell Isolation Cocktail. Gently mix, then incubate on ice for 10 min.
   ▲ If a larger number of cells need to be sorted, the amount of CD8$^+$ T cell Isolation Cocktail should be increased according to the ratio of increased cells.
5. After incubation, add 20 μl of Streptavidin Beads, gently mix, and incubate at room temperature for 3 min.
   ▲ Please vortex the magnetic beads well before use. If a larger number of cells need to be sorted, the amount of Streptavidin Beads should be increased according to the ratio of increased cells.
6. After incubation, add 1 ml of sorting buffer and gently pipette up and down 3 - 4 times to mix thoroughly, then place the flow tube on the magnetic rack and let it stand for 3 min.
   ▲ The experimental system should not exceed 2.5 ml to avoid exceeding the sorting range of the magnetic pole.
7. Slowly pour the cell suspension into a sterile centrifuge tube, this cell suspension is the purified CD8$^+$ T cell suspension.
   ▲ During the pouring process, the flow tube must not detach from the magnetic rack.
8. Researchers can use PBS to wash the cells as the subsequent experiment requires. Centrifuge at 2,000 rpm (500 × g) for 5 min, and discard the supernatant. Resuspend the cells with a buffer or culture medium for subsequent molecular biology experiments or cell culture, etc.



A. Purity of CD8$^+$ T cells before sorting B. Purity of CD8$^+$ T cells after sorting

Fig 2. The purity of mouse spleen CD8$^+$ T cell before and after sorting

The purity of CD8$^+$ T cells isolated from C57BL/6 mouse splenocytes typically ranges from 91% - 96%. According to Fig 2, the purities of CD8$^+$ T cells before and after sorting are 13.88% and 93.23%, respectively.