Mouse CD8+ T Cell Isolation Kit for 5 × 10^8 cells, Negative

Description

Mouse CD8+ T cell sorting kit isolates CD8+ T cells from single cell suspension of mouse spleen cells or other tissues by negative sorting method. The principle is to select different biotin labeled monoclonal antibodies to label non-target cells (non-CD8+ T cells), and then clear non-target cells through streptavidin labeled magnetic beads, so as to achieve the purpose of mouse CD8+ T cells sorting. The sorting process requires the use of magnetic racks.

For custom sizes, formulations or bulk quantities please contact our customer service department.

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Characteristics

Constituent	Specification
Biotin-Antibody Mix	100 µL
Streptavidin	1 mL

Advantages

Simple and fast: the target cells can be sorted in 15 min;

No separation column required: The magnetic separator can be used to achieve rapid separation of the target cells;

High purity: the purity of the sorted cells can reach more than 95%;

High activity: more than 99% of active cells can be obtained after sorting, and the cells have good function.

Scope of application

This kit is suitable for sorting CD8+ T cells from mouse spleen and lymph nodes.

Operation process

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

1. Preparation of single-cell suspension: The spleen was ground on a 70 μm cell screen, the cell screen was rinsed with pre-cooled PBS, and the cell suspension was collected in a 50 mL centrifuge tube for 500 g and centrifuged for 5 min.

2. After centrifugation, discard the supernatant, add 5 mL of red blood cell lysate (ACK), pyrolysis at room temperature for 5 min, then add 20 mL of PBS, 500 g, centrifugation for 5 min.

Note: The amount and time of red blood cell lysis procedure can be adjusted according to the lysate used. A small amount of residual red blood cells did not affect subsequent sorting and cell purity.

3. After centrifugation, the supernatant was abandoned and the spleen cells were re-suspended on PBS. The cell suspension was filtered with a 70 μm cell screen and counted. After counting, centrifuge 500 g for 5 min.

Note: The cell suspension needs to pass through the cell screen to remove tissue and cell clumps, otherwise it will affect the purity of subsequent cell sorting.

4. After centrifugation, the supernatant was abandoned and the cells were re-suspended in the sorting buffer, and the cell density was adjusted to 1×10^8 cells/mL.

Note: The sorting buffer was PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS) or PBS containing 2 mM EDTA and 0.5% BSA, which was pre-filtered through 0.22 μm filter.

5. 100 μL cell suspension (1×10^7 cells) was added to the bottom of a sterile flow tube, followed by 2 μL Biotin-Antibody Mix, and incubated at 4°C for 10 min.

Note: When adding cell suspension, add cells to the bottom of the flow tube, avoid adding along the wall of the flow tube. Depending on the magnetic frame used, centrifuge tubes can also be used for cell sorting. If more cells are selected, the dosage of Biotin-Antibody Mix is increased proportionally.

6. After incubation, add 20 μL of cleaned Streptavidin into the flow tube (**Beads need to be cleaned with a sorting buffer before use:** The magnetic beads were re-suspended by vortex oscillation, and the magnetic beads needed for the experiment were absorbed into a 1.5 mL centrifuge tube, 1 mL of sorting buffer was added, centrifuge at 10000 g for 1 min, and the supernant was discarded. Add 1 mL sorting buffer and repeat washing magnetic beads once, then re-suspend magnetic beads with the same volume of sorting buffer as the original. If 20 μL magnetic beads are absorbed for cleaning, then re-suspension with 20 μL sorting buffer after cleaning), mixed and incubated at 4°C for 10 min.

Note: If more cells are sorted, the amount of Streptavidin is increased proportionally. For example, 5×10^7 cells were sorted and 500 µL cell suspension was supplemented with 10 µL Biotin-Antibody Mix and 100 µL Streptavidin. If fewer than 1×10^7 cells are sorted, cell suspension is supplemented to 100 µL using 2 µL Biotin-Antibody Mix and 20 µL Streptavidin.

7. After incubation, add 2.5 mL sorting buffer into the flow tube and mix it up and down with the pipette for 5 times (avoid violent oscillation or mixing upside down).

8. Place the sorting flow tube containing cells on the magnetic rack and stand for 5 min.

9. Gently pour the cell suspension into a sterile centrifuge tube (the flow tube should not be removed from the magnetic rack during the pouring process). The cell suspension contains purified mouse $CD8^+$ T cells, 500 g, and centrifuge for 5 min. After centrifugation, the supernatant was discarded and the cells were collected.

10. After washing the cells according to the needs of the experiment, the cells are re-suspended in the required buffer or medium, which can be used for subsequent molecular biology or cell biology experiments.

Isolating effect

$CD8^+$ T cells were sorted from spleen cells of C57BL/6 mice. The cells before and after sorting were labeled with FITC anti-mouse CD8 antibody (Clone No. 53-6.7) and analyzed by flow cytometry. The purity of $CD8^+$ T cells before and after sorting was 13.8% and 97.1%, respectively.

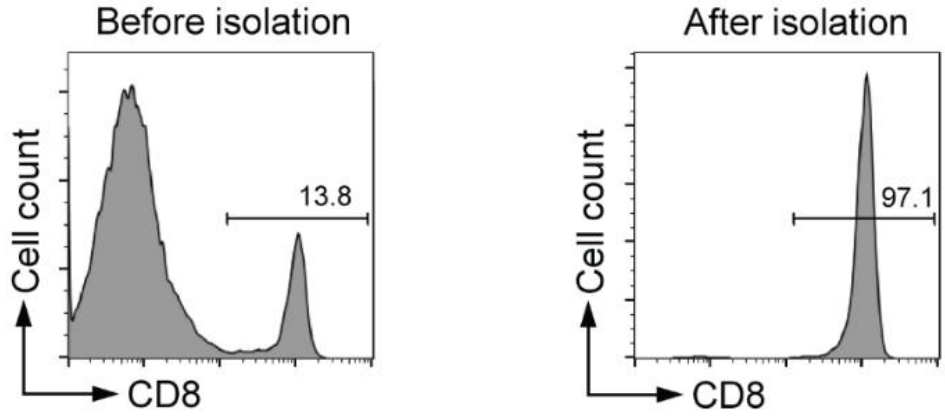

Figure 1. $CD8^+$ T cells were separated from spleen cells of C57BL/6 mice using Mouse $CD8^+$ T Cell Isolation Kit (negative selection).

Storage conditions and expiration date

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This product should be stored at 2 - 8°C. **DO NOT FREEZE**. The expiration date can be found on the test tube label.

Notes

1. Magnetic beads and antibody mixture should avoid freezing during use and storage;
2. It is recommended to choose a low adsorption pipette head and centrifugal tube to avoid the loss of magnetic beads and antibodies caused by adsorption;
3. This product should be used with a magnetic separator;
4. This product is for research use only.

Ordering Information

Website: www.abvigen.com

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