Mouse CD8⁺ Cell Isolation Kit for 1 × 10⁸ cells, Positive
PRODUCT DATA SHEET

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Description

Mouse CD8⁺ cell sorting kit (positive selection method) is suitable for sorting CD8⁺ cells from mouse spleen cells or other tissue single-cell suspension. The principle is to label CD8⁺ cells using CD8 Capture Antibody, capture target cells through Releasable Particles, and then use a Release Buffer to disassociate magnetic beads from the cell surface. Thus, mouse CD8⁺ cells without magnetic beads were obtained. The sorted CD8⁺ cells can be applied to the downstream molecular biology and cell biology experiments.

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

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Characteristics

Constituent	Specification
CD8 Capture Antibody	20 µL
Releasable Particles	0.2 mL
Release Buffer	4 mL

Advantages

The purity is more than 95%

The sample has wide applicability and can directly capture the target cells

Magnetic bead release technique cells are not labeled with magnetic beads

Scope of application

This kit is suitable for sorting CD8⁺ cells in mouse lymphatic organs such as spleen and lymph nodes.

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Operation process

Take the sorted mouse spleen $CD8^+$ 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: A small amount of red blood cell residue will 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: Cell suspensions need to be filtered with a 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. 500 μL cell suspension (5×10^7 cells) was added to the bottom of a sterile flow tube and 10 μL CD8 Capture Antibody was added, mixed and incubated at 4°C for 10 min.

Note: Add the cell suspension directly to the bottom of the flow tube and avoid adding along the wall of the flow tube. Depending on the magnetic frame used, centrifuge tubes can also be used for cell sorting. CD8 Capture Antibody levels can be proportionally adjusted for selection of other cells. If fewer than 1×10^7 cells are selected, cell suspension volume is supplemented to 100 μL and 2 μL CD8 Capture Antibody is added.

6. After incubation, add 100 μL of cleaned Releasable Particles into the flow tube, mix and incubate at 4°C for 10 min (**Clean with sorting buffer before using magnetic beads:** 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, and 1 mL of sorting buffer was added, centrifuged 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 the 20 μL

magnetic bead is absorbed for cleaning, the 20 μL sorting buffer is used for re-suspension after cleaning).

Note: The amount of Releasable Particles can be proportionally adjusted when sorting other quantities of cells. If fewer than 1×10^7 cells are sorted, use 20 μL Releasable Particles.

7. After incubation, add the sorting buffer to 2.5 mL in the flow tube and mix it with pipette for 5 times (avoid mixing upside down). Place the flow tube on the magnetic rack and let it stand for 5 min.

8. Suck out and discard the supernatant. Remove the flow tube from the magnetic rack, quickly add 2 mL sorting buffer, and blow the dispersing magnetic beads repeatedly with the pipette. Place the flow tube on the magnetic rack and let it stand for 5 min.

9. Repeat step 8 twice (**thorough cleaning ensures subsequent elution of high-purity target cells**).

10. After the magnet is finished, suck out and discard the supernatant. Remove the flow tube from the magnetic rack and quickly add 1 mL Release Buffer to re-suspend magnetic beads to avoid drying of magnetic beads. Transfer the suspension of magnetic beads to 1.5 mL centrifuging tubes and incubate for 10 min at room temperature.

Note: The amount of Release Buffer can be proportionally adjusted when sorting other quantities of cells. If fewer than 1×10^7 cells are sorted, cells are eluted using 200 μL Release Buffer.

11. After incubation, use a pipette to blow at least 10 times, transfer the magnetic bead suspension to a new flow tube, add the sorting buffer to 2.5 mL, blow and mix well, place the flow tube on the magnetic rack, and let it stand for 5 min.

12. Transfer the supernatant to a 15 mL centrifuge tube for use (**the supernatant contains the target cells, do not discard it**). Quickly resuspension the beads with 1 mL Release Buffer to avoid drying the beads, transfer the beads suspension to 1.5 mL centrifuge tube and incubate for 10 min at room temperature.

13. After incubation, blow the magnetic bead suspension at least 10 times with a pipette, transfer the magnetic bead suspension to a new flow tube, add the sorting buffer to 2.5 mL, blow and mix well, place the flow tube on the magnetic rack, and let it stand for 5 min.

14. Mix the supernatant with the cell supernatant after the first elution for 500 g, centrifuge for 5 min, discard the supernatant, and $CD8^+$ cells without magnetic bead labeling can be collected.

15. 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$^+$ cells were sorted from spleen cells of C57BL/6 mice, stained with FITC-labeled anti-mouse CD8 antibody (Clone No. 53-5.8), and flow cytometry was performed. The purity of CD8$^+$ cells before and after sorting was 8.9% and 95.1%, respectively.

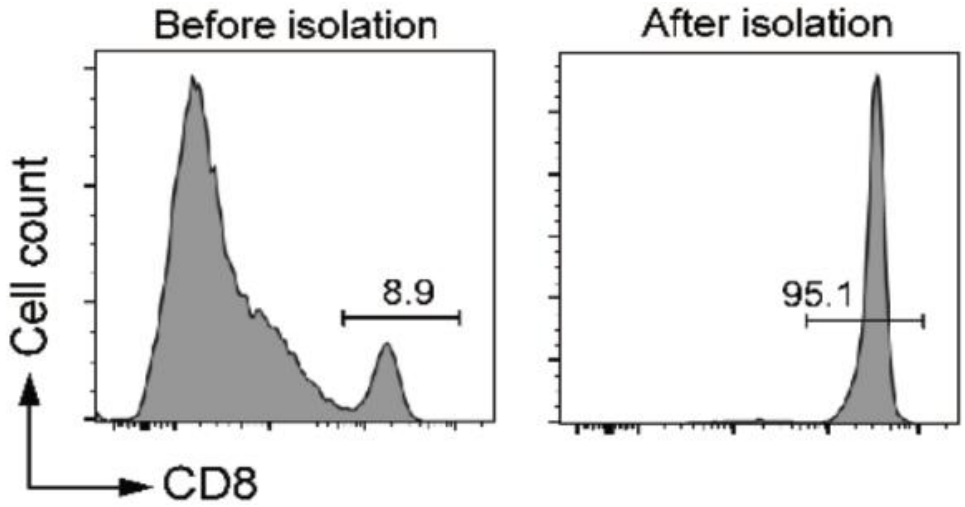

Figure 1. Mouse CD8$^+$ cell sorting kit (positive method) sorted mouse spleen cells, flow cytometry analysis.

Storage conditions and expiration date

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. Avoid freezing during the use and storage of each component of the kit;
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 needs to be used with the magnetic rack;
4. This product is for research use only.

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Ordering Information

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