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

Catalog No.: BRC0009

Product Description

This product isolates CD8$^+$ T cells from human peripheral blood mononuclear cells (PBMC) via negative selection. The principle is to label non-target cells (non-CD8$^+$ T cells) with biotin-conjugated monoclonal antibodies, followed by depletion with streptavidin-conjugated magnetic beads, thereby achieving purification of human CD8$^+$ T cells. A magnetic stand is required for the separation process.

Kit Components

No.	Reagent Name	Size1 (For 10^9 cell)	Size2 (For $5*10^8$ cell)
Reagent1	Biotin-Antibody Mix	200μl	100μl
Reagent2	EnkiBeads™ Streptavidin	2 mL	1ml

Storage Conditions and Shelf Life

Storage Conditions: Store at 2–8°C. DO NOT FREEZE.
Shelf Life: 2 years

Applications

This kit is suitable for isolating CD8$^+$ T cells from freshly isolated or frozen human PBMC.

Protocol

1. Prepare human PBMC: Isolate PBMC from human peripheral blood by Ficoll density gradient centrifugation. Collect PBMC, wash with PBS, centrifuge, and resuspend in separation buffer at a density of 1×10^8 cells/mL.

Note: Separation buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS) or PBS containing 2 mM EDTA and 0.5% BSA. Filter-sterilize through a 0.22 μm membrane before use.

2. Add 100 μL cell suspension (1×10^7 cells) to the bottom of a sterile 1.5 mL centrifuge tube. Add 2 μL Biotin-Antibody Mix, mix well, and incubate at 4°C for 15 min. Add 10 volumes of separation buffer, centrifuge at 500 g for 5 min, and discard the supernatant. Resuspend cells in 100 μL separation buffer.

Note: Scale up the amount of Biotin-Antibody Mix proportionally if processing more cells. 15 mL or 50 mL centrifuge tubes can be used. For example, for 5×10^7 cells, add 10 μL Biotin-Antibody Mix to 500 μL cell suspension, wash with 5 mL separation buffer, and resuspend in 500 μL separation buffer after centrifugation.

3. Wash magnetic beads: Vortex thoroughly to resuspend beads. Transfer 20 μL of beads to a sterile 1.5 mL centrifuge tube, add separation buffer to a total volume of 1 mL, centrifuge at 10,000 g for 1 min, and discard the supernatant. Add 1 mL separation buffer to resuspend beads, centrifuge at 10,000 g for 1 min, discard the supernatant, and resuspend beads in 20 μL separation buffer.

4. Add 10 µL of washed EnkiBeads™ Streptavidin to the cell suspension, mix well, and incubate at 4°C for 10 min.

Note: Scale up the amount of EnkiBeads™ Streptavidin proportionally if processing more cells. For example, for 5×10^7 cells, add 50 µL EnkiBeads™ Streptavidin to 500 µL cell suspension. If processing fewer than 1×10^7 cells, adjust the cell suspension volume to 100 µL and add 2 µL Biotin-Antibody Mix and 10 µL EnkiBeads™ Streptavidin.

5. After incubation, transfer the cell-bead mixture to a sterile flow tube, add separation buffer to 2.5 mL, and mix by pipetting up and down 5 times (avoid vigorous vortexing or inversion mixing).
6. Place the flow tube containing cells on the magnetic stand and let stand for 5 min.
7. Gently pour the cell suspension into a sterile centrifuge tube (do not remove the flow tube from the magnetic stand during pouring). The cell suspension now contains purified human CD8$^+$ T cells, ready for downstream biological experiments or flow cytometry analysis. For higher purity, centrifuge the cell suspension at 500 g for 5 min, discard the supernatant, resuspend cells in 100 µL separation buffer, then proceed with the second round purification (Steps 8–11).

Note: Scale up the resuspension volume proportionally if processing more cells. For example, for 5×10^7 cells, resuspend in 500 µL separation buffer after centrifugation.

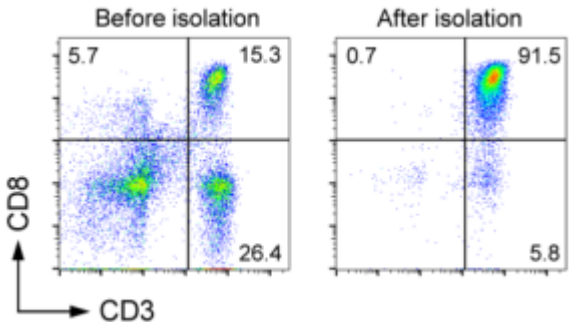
8. Add 10 µL of washed EnkiBeads™ Streptavidin, mix well, and incubate at 4°C for 10 min.

Note: Scale up the amount of EnkiBeads™ Streptavidin proportionally if processing more cells. For example, for 5×10^7 cells, add 50 µL EnkiBeads™ Streptavidin to 500 µL cell suspension.

9. After incubation, add separation buffer to 2.5 mL, mix by pipetting 5 times, and transfer the cell-bead mixture to a sterile flow tube.
10. Place the flow tube on the magnetic stand and let stand for 5 min.
11. Transfer the cell suspension to a sterile centrifuge tube (do not remove the flow tube from the magnetic stand during transfer). The cell suspension now contains second-round purified human CD8$^+$ T cells. The second round can improve CD8$^+$ T cell purity by 2–4%.
12. Wash cells as required, then resuspend in appropriate buffer or culture medium for downstream molecular or cellular biology applications.

Isolation Performance

CD8$^+$ T cells were isolated from human PBMC. Cells before and after isolation were stained with PE anti-human CD8a antibody (clone RPA-T8) and APC anti-human CD3 antibody (clone OKT3) and analyzed by flow cytometry. Purity of CD3$^+$CD8$^+$ T cells was 15.3% pre-isolation and 91.5% post-isolation.



Important Notes

1. Avoid freezing magnetic beads and antibody mix during storage and use.
2. Use low-retention pipette tips and centrifuge tubes to minimize loss of beads and antibodies due

to adsorption.
3. This product should be used with a magnetic separator.
4. For research use only.