Human CD3$^+$ T Cell Isolation Kit (Negative Selection)

Catalog No.: BRC0015

Product Description

The Human CD3$^+$ T Cell Isolation Kit isolates CD3$^+$ T cells from human peripheral blood mononuclear cells (PBMC) via negative selection. The principle is to label non-target cells (non-CD3$^+$ T cells) with biotin-conjugated monoclonal antibodies, followed by depletion with streptavidin-conjugated magnetic beads, thereby achieving purification of human CD3$^+$ 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)
Reagent 1	Biotin-Antibody Mix	200μL	100μL
Reagent 2	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 CD3$^+$ T cells from human PBMC.

Protocol

1. Sample 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 flow tube, then add 2 μL Biotin-Antibody Mix. Mix well and incubate at 4°C for 10 min.

Note: When adding cell suspension, pipette directly to the bottom of the tube; avoid pipetting along the tube wall. Centrifuge tubes may also be used depending on the magnetic stand. Scale up the amount of Biotin-Antibody Mix proportionally if processing more cells.

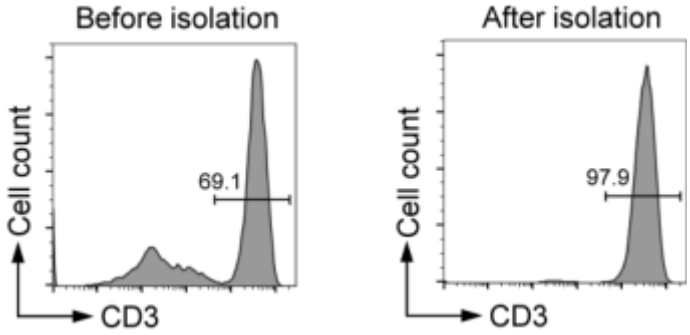
3. After incubation, add 20 μL of washed EnkiBeads™ Streptavidin to the flow tube (Wash magnetic beads before use: Resuspend beads by vortexing, transfer the required volume to a 1.5 mL centrifuge tube, add 1 mL separation buffer, centrifuge at 10,000 g for 1 min, and discard the supernatant. Repeat wash once with 1 mL separation buffer, then resuspend beads in the original volume of separation buffer. For example, if 20 μL of beads were taken for washing, resuspend in 20 μL separation buffer after washing). 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 10 µL Biotin-Antibody Mix and 100 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 20 µL EnkiBeads™ Streptavidin.

4. After incubation, add 2.5 mL separation buffer to the flow tube. Mix by pipetting up and down 5 times (avoid vigorous vortexing or inversion mixing).
5. Place the flow tube containing cells on the magnetic stand and let stand for 5 min.
6. Gently pour the cell suspension into a sterile centrifuge tube (do not remove the flow tube from the magnetic stand during pouring). This cell suspension now contains purified human $CD3^+$ T cells. Centrifuge at 500 g for 5 min, discard the supernatant, and collect the cells.
7. Wash cells as required, then resuspend in appropriate buffer or culture medium for downstream molecular or cellular biology applications.

Isolation Performance

$CD3^+$T cells were isolated from human PBMC. Cells before and after isolation were stained with PE anti-human CD3 antibody (clone OKT3) and analyzed by flow cytometry. Purity of $CD3^+$ T cells was 69.1% pre-isolation and 97.9% 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.