

Human CD4⁺ T Cell Isolation Kit (Negative Isolation)

Cat. No.: H1522401 | Pack size: 10T; 50T; 100T | Storage: 2-8°C; Do not freeze

Overview

The Human CD4⁺ T Cell Isolation Kit (Negative Isolation) is used to isolate CD4⁺ T cells from human peripheral blood mononuclear cells (PBMCs) by negative isolation. The principle is to use a cocktail of biotin-labeled monoclonal antibodies against non-target cells (non-CD4⁺ T cells), followed by removal of these non-target cells using streptavidin-labeled magnetic beads, thereby achieving the isolation of human CD4⁺ T cells. A magnetic separator is required for the isolation process.

Applications

Isolation of CD4⁺ T cells from freshly isolated or cryopreserved human PBMCs.

Kit Contents & Storage

Component Code	Component	Appearance	10T	50T	100T	Storage
H1522401A	Biotin-Antibody Mix	Liquid	20 µL	100 µL	200 µL	2-8°C
H1522401B	Streptavidin Magnetic Beads	Liquid	200 µL	1 mL	1 mL × 2	2-8°C

Protocol Example

Prepare human PBMCs

Isolate PBMCs from human peripheral blood using Ficoll density gradient centrifugation. Collect the PBMCs, wash the cells with PBS, and after centrifugation, resuspend the PBMCs in Isolation Buffer to a density of 1×10⁸ cells/mL.

Note: Isolation buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS), or PBS containing 2 mM EDTA and 0.5% BSA. The buffer must be pre-filtered through a 0.22 µm filter.

Add Biotin-Antibody Mix and wash

Add 100 µL of cell suspension (1×10⁷ cells) to the bottom of a sterile 1.5 mL centrifuge tube, then add 2 µL of Biotin-Antibody Mix. Mix well and incubate at 4°C for 15 min. Add 10 volumes of Isolation

Buffer, centrifuge at 500 g for 5 min, and discard the supernatant. Resuspend the cells in 100 μ L of Isolation Buffer.

Note: If isolating more cells, increase the volume of Biotin-Antibody Mix proportionally. A 15 mL or 50 mL centrifuge tube can be used. For example, to isolate 5×10^7 cells, add 10 μ L of Biotin-Antibody Mix to 500 μ L of cell suspension, wash with 5 mL of Isolation Buffer, and after centrifugation, resuspend the cells in 500 μ L of Isolation Buffer.

Wash the magnetic beads

Vortex thoroughly to resuspend the beads. Transfer 20 μ L of beads to a sterile 1.5 mL centrifuge tube, add Isolation Buffer to a total volume of 1 mL, centrifuge at 10,000 g for 1 min (or use a magnetic separator for 3 min), and discard the supernatant. Add 1 mL of Isolation Buffer, wash the beads once more, and then resuspend the beads in 20 μ L of Isolation Buffer.

First addition of Streptavidin Magnetic Beads

Add 10 μ L of washed Streptavidin Magnetic Beads to the cell suspension, mix well, and incubate at 4°C for 10 min.

Note: If isolating more cells, increase the volume of Streptavidin Magnetic Beads proportionally. For example, to isolate 5×10^7 cells, add 50 μ L of Streptavidin Magnetic Beads to 500 μ L of cell suspension. If isolating fewer than 1×10^7 cells, adjust the cell suspension volume to 100 μ L and use 2 μ L of Biotin-Antibody Mix and 10 μ L of Streptavidin Magnetic Beads for the isolation.

Transfer and dilute

After incubation, transfer the cell and bead mixture to a sterile FACS tube, add Isolation Buffer to a total volume of 2.5 mL, and mix by pipetting 5 times.

First magnetic separation

Place the FACS tube containing the cell suspension on a magnetic separator and let stand for 5 min.

Collect once-purified cells (optional second purification)

Transfer the cell suspension to a sterile centrifuge tube (do not remove the FACS tube from the magnetic separator while transferring). This cell suspension contains the purified human CD4⁺ T cells and can be used for downstream biological experiments or flow cytometry analysis.

To further increase the purity of CD4⁺ T cells, centrifuge the cell suspension at 500 g for 5 min, discard the supernatant, resuspend the cells in 100 μ L of Isolation Buffer, and proceed with the second purification as described below.

Note: If isolating more cells, increase the resuspension volume accordingly. For example, to isolate 5×10^7 cells, after centrifugation, resuspend the cells in 500 μ L of Isolation Buffer.

Second addition of Streptavidin Magnetic Beads

Add 10 μL of washed Streptavidin Magnetic Beads, mix well, and incubate at 4°C for 10 min.

Note: If isolating more cells, increase the volume of Streptavidin Magnetic Beads proportionally. For example, to isolate 5×10^7 cells, add 50 μL of Streptavidin Magnetic Beads to 500 μL of cell suspension.

Second dilution and transfer

After incubation, add Isolation Buffer to a total volume of 2.5 mL, mix by pipetting 5 times, and transfer the cell and bead mixture to a sterile FACS tube.

Second magnetic separation

Place the FACS tube containing the cell suspension on a magnetic separator and let stand for 5 min.

Collect twice-purified cells

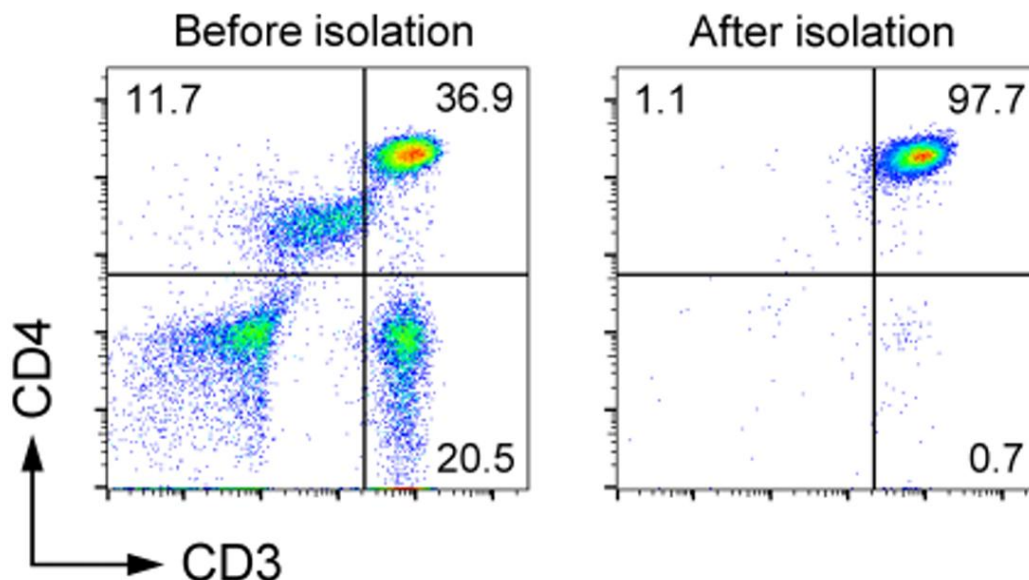
Transfer the cell suspension to a sterile centrifuge tube (do not remove the FACS tube from the magnetic separator while transferring). This cell suspension contains the twice-purified human CD4⁺ T cells. The second purification can increase the purity of CD4⁺ T cells by 2-4%.

Resuspend for further use

Wash the cells as required for your experiment, then resuspend them in the desired buffer or culture medium for subsequent molecular or cell biology experiments.

Isolation Performance

CD4⁺ T cells were isolated from human PBMCs. Cells before and after isolation were stained with PE-labeled anti-human CD4 antibody (clone RPA-T4) and APC-labeled anti-human CD3 antibody (clone OKT3) and analyzed by flow cytometry. The purity of CD3⁺CD4⁺ T cells before and after isolation was 36.9% and 97.7%, respectively.



Precautions

Avoid freezing the magnetic beads and antibody mix during use and storage.

Low-retention pipette tips and centrifuge tubes are recommended to avoid loss of beads and antibodies due to adsorption.

This product must be used with a magnetic separator.

For research use only.

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