

Human CD3⁺T Cell Sorting Kit (Negative Selection)

Cat. No.: H1522404 | Pack size: 10T / 50T / 100T | Storage: Store at 2-8°C, Do not freeze

Overview

The Human CD3⁺ T Cell Isolation Kit (Negative Isolation) is used to isolate CD3⁺ 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-CD3⁺ T cells), followed by removal of these non-target cells using streptavidin-labeled magnetic beads, thereby achieving the isolation of human peripheral blood CD3⁺ T cells. A magnetic separator is required for the isolation process.

Key Features

- ☐ Designed for cell separation or fractionation workflows described in the product file.
- ☐ Storage condition: Store at 2-8°C, Do not freeze.

Kit Contents & Storage

Cat. No.	Component	Appearance	10T	50T	100T	Storage
H1522404A	Biotin-Antibody Mix	Liquid	20 µL	100 µL	200 µL	2-8°C
H1522404B	Streptavidin Magnetic Beads	Liquid	200 µL	1 mL	1 mL×2	2-8°C

Storage: Store at 2-8°C, Do not freeze

Materials Required But Not Supplied

Item	Recommended Specification	Purpose
Sterile pipettes, tubes, and centrifuge	Cell culture grade	Sample processing
PBS or isotonic buffer	Sterile, calcium/magnesium-free if specified	Cell washing and dilution
Appropriate separator or instrument	As required by protocol	Cell separation or downstream processing

Specifications

Attribute	Value
Storage	Store at 2-8°C, Do not freeze

Protocol

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 1×10^8 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 μ m filter.

Add Biotin-Antibody Mix

Add 100 μ L of cell suspension (1×10^7 cells) to the bottom of a sterile FACS tube, then add 2 μ L of Biotin-Antibody Mix. Mix well and incubate at 4°C for 10 min.

Note: Add the cell suspension directly to the bottom of the FACS tube, avoiding the tube wall. Depending on the magnetic separator used, a centrifuge tube can also be used for cell isolation. If isolating more cells, increase the volume of Biotin-Antibody Mix proportionally.

Add Streptavidin Magnetic Beads

After incubation, add 20 μ L of washed Streptavidin Magnetic Beads to the FACS tube, mix well, and incubate at 4°C for 10 min (the beads must be washed before use: vortex thoroughly to resuspend the beads, transfer the required volume of beads to a 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, then discard the supernatant. Add 1 mL of Isolation Buffer and wash the beads once more, then resuspend the beads in the same volume of Isolation Buffer as originally taken. For example, if 20 μ L of beads are taken, resuspend them in 20 μ L of Isolation Buffer after washing).

Note: If isolating more cells, increase the volume of Streptavidin Magnetic Beads proportionally. For example, to isolate 5×10^7 cells, add 10 μ L of Biotin-Antibody Mix and 100 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, then add 2 μ L of Biotin-Antibody Mix and 20 μ L of Streptavidin Magnetic Beads.

Dilute and mix

After incubation, add 2.5 mL of Isolation Buffer to the FACS tube, and mix by pipetting 5 times (avoid vigorous shaking or inverting).

Magnetic separation

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

Collect CD3⁺ T cells

Gently pour the cell suspension into a sterile centrifuge tube (do not remove the FACS tube from the magnetic separator while pouring). This cell suspension contains the purified human CD3⁺ T cells. Centrifuge at 300 g for 5 min, then discard the supernatant and collect the cells.

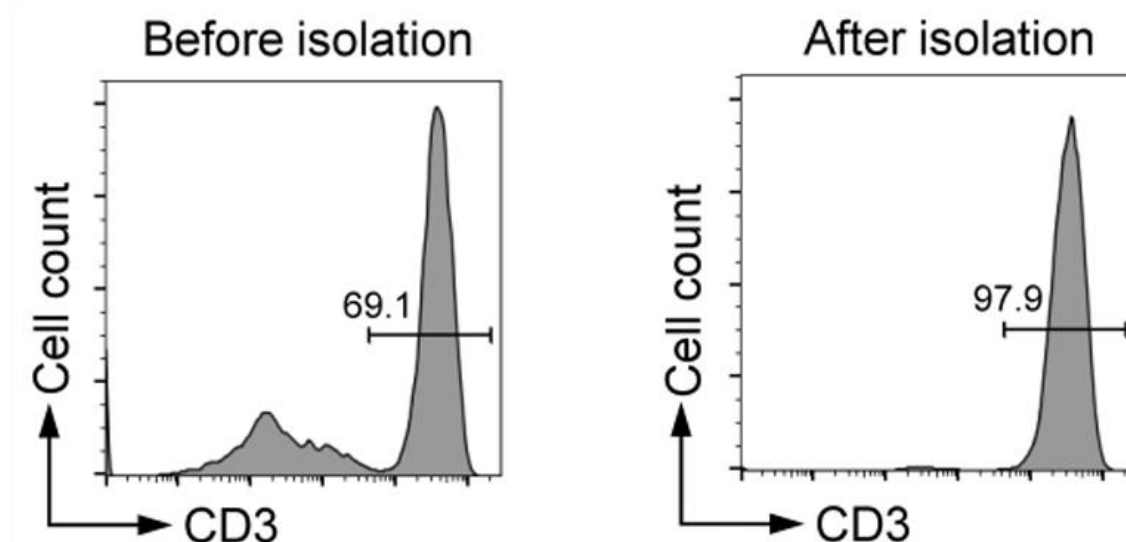
Resuspend for further use

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.

Performance Characteristics

Isolation Performance

CD3⁺ T cells were isolated from human PBMCs. Cells before and after isolation were stained with PE-labeled anti-human CD3 antibody (clone OKT3) and analyzed by flow cytometry. The purity of CD3⁺ T cells before and after isolation was 69.1% and 97.9%, respectively.



Sample Handling

Process blood, tissue, or cell suspensions as soon as possible after collection. Keep samples at the temperature specified in the protocol and avoid harsh mixing that may reduce cell recovery or viability.

Use clean, sterile buffers and tubes. Prepare a single-cell suspension or clarified sample as required before loading onto the separation medium or magnetic separation workflow.

Safety & Precautions

1. Read the current SDS before use. Wear lab coat, gloves, and eye protection. Handle reagents according to local laboratory safety and EHS procedures.
2. Use clean or sterile consumables as required by the application and avoid contamination during handling.
3. Follow the storage condition on the product label. Protect from light or freezing where specified.
4. Avoid freezing the magnetic beads and antibody mix during use and storage.
5. Low-retention pipette tips and centrifuge tubes are recommended to avoid loss of beads and antibodies due to adsorption.
6. This product must be used with a magnetic separator.
7. For research use only.

Quality Control

QC Item	Method	Acceptable Range
Appearance and sterility-related condition	Visual inspection / lot COA	No leakage, turbidity outside specification, or contamination
Separation performance	Representative sample or control workflow	Cell fraction, purity, or recovery meets expected application requirements
Storage condition verification	Receipt and storage review	Stored as specified; do not freeze where indicated

Troubleshooting

Issue	Possible Causes	Corrective Action
Low cell recovery	• Sample age or condition unsuitable • Centrifugation or magnetic separation parameters not optimized • Loss of target fraction during transfer	• Use fresh samples and recommended buffers • Optimize time/speed according to sample volume • Aspirate/transfer layers carefully

Issue	Possible Causes	Corrective Action
Low purity	• Incorrect buffer or density condition • Disturbed interface or bead carryover • Insufficient washing	• Use specified buffer and temperature • Avoid disturbing non-target fractions • Repeat wash/separation steps as appropriate
Reduced cell viability	• Delayed processing • Improper temperature • Harsh mixing	• Process samples promptly • Maintain recommended temperature • Mix gently and avoid vigorous shaking

Recommended Applications

Isolation of CD3⁺ T cells from human PBMCs.

Contact & Global Offices

Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to assist. Please contact the office for your region; for general inquiries, the North American office is the corporate primary.

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Limitations & Disclaimer

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- ☐ This product is not a CE-marked in vitro diagnostic device under IVDR (EU) 2017/746 and is not an FDA-cleared device under 21 CFR. Use is restricted to verified businesses, institutions, and qualified professionals for research and development purposes.
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