

Mouse CD4⁺ T Cell Isolation Kit (Negative Isolation)

Cat. No.: M1522407 | Pack size: 10T; 50T; 100T | Storage: 2-8°C; Do not freeze

Overview

The Mouse CD4⁺ T Cell Isolation Kit (Negative Isolation) is used to isolate CD4⁺ T cells from mouse spleen or other tissue single-cell suspensions 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 mouse CD4⁺ T cells. A magnetic separator is required for the isolation process.

Applications

Isolation of CD4⁺ T cells from mouse spleen and lymph nodes.

Kit Contents & Storage

Component Code	Component	Appearance	10T	50T	100T	Storage
M1522407A	Biotin-Antibody Mix	Liquid	20 µL	100 µL	200 µL	2-8°C
M1522407B	Streptavidin Magnetic Beads	Liquid	200 µL	1 mL	1 mL × 2	2-8°C

Protocol (Using Mouse Spleen CD4⁺ T Cell Isolation as an Example)

Prepare single-cell suspension

Grind the spleen on a 70 µm cell strainer, rinse the strainer with pre-chilled PBS, collect the cell suspension into a 50 mL centrifuge tube, and centrifuge at 500 g for 5 min.

Lyse red blood cells

After centrifugation, discard the supernatant, add 5 mL of ACK red blood cell lysis buffer, lyse at room temperature for 5 min, then add 20 mL PBS and centrifuge at 500 g for 5 min.

Note: The red blood cell lysis step can be adjusted in volume and time depending on the lysis buffer used. A small amount of red blood cell residue will not affect subsequent isolation or cell purity.

Filter and count cells

After centrifugation, discard the supernatant, resuspend the splenocytes in PBS, filter the cell suspension through a 70 µm cell strainer, and count the cells. After counting, centrifuge at 500 g for 5 min.

Note: The cell suspension must be passed through a cell strainer to remove tissue and cell clumps; otherwise, subsequent cell isolation purity may be affected.

Adjust cell density

After centrifugation, discard the supernatant, resuspend the cells 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 µm filter.

Add Biotin-Antibody Mix

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

Note:

When adding the cell suspension, add it to the bottom of the 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 µL of washed Streptavidin Magnetic Beads to the FACS tube (the beads must be washed before use: vortex to resuspend the beads, transfer the required volume of beads to a 1.5 mL centrifuge tube, add 1 mL of Isolation Buffer, 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 µL of beads are taken, resuspend them in 20 µL of Isolation Buffer after washing). 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 10 µL of Biotin-Antibody Mix and 100 µL of Streptavidin Magnetic Beads to 500 µL of cell suspension.

If isolating fewer than 1×10^7 cells, adjust the cell suspension volume to 100 μL , then add 2 μL of Biotin- Antibody Mix and 20 μL of Streptavidin Magnetic Beads.

Dilute and mix

After incubation, add 2.5 mL of Isolation Buffer to the FACS tube, and mix gently by pipetting up and down 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 CD4⁺ 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 mouse CD4⁺ T cells. Centrifuge at 500 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.

Isolation Performance

CD4⁺ T cells were isolated from C57BL/6 mouse splenocytes. Cells before and after isolation were stained with FITC anti- mouse CD4 antibody (clone GK1.5) and analyzed by flow cytometry. The purity of CD4⁺ T cells before and after isolation was 20.3% and 98.8%, respectively.

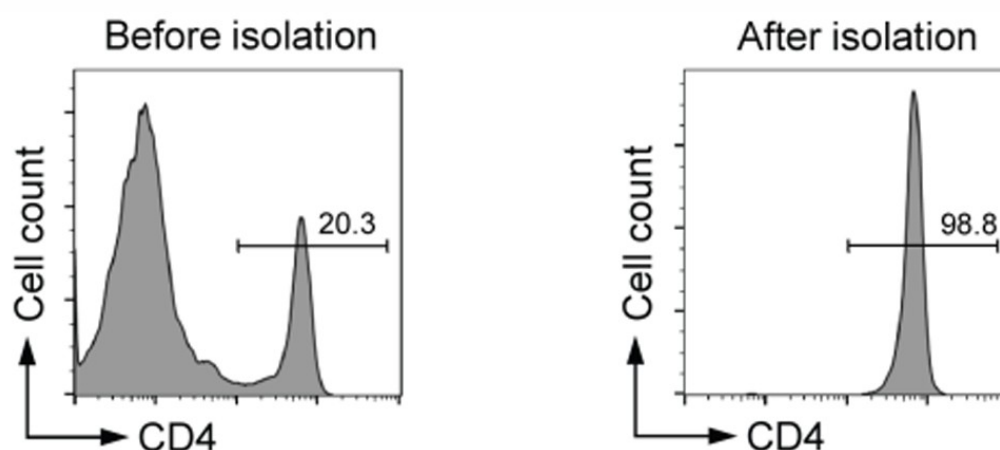


Figure 1. Mouse CD4⁺ T cell isolation performance.

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.

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 help.

NORTH AMERICAN SALES, SUPPORT & GENERAL INQUIRIES

Aladdin Scientific Corporation

14078 Meridian Parkway, Riverside, CA 92518, USA

Phone: 1-833-552-7181

Sales: sales@aladdinsci.com

Customer Service: custserv@aladdinsci.com

EU SALES, LOGISTICS & LOCALIZED SUPPORT

Aladdin Biochem Deutschland GmbH

Westring 2, 33142 Büren, Germany

Phone: +02951 9383958

Support: support.eu@aladdinsci.com

Limitations & Disclaimer

- For Research Use Only (RUO). Not for use in human or animal diagnostics, therapeutics, or in vivo applications. Not for food, cosmetic, or household use.
- 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.
- Where any kit component is classified as hazardous under CLP (EC) 1272/2008 or OSHA HCS (29 CFR 1910.1200), the product Safety Data Sheet (SDS) takes precedence over this document for handling, storage, and disposal information.
- Performance depends on sample type, sample condition, handling, and operator technique. Users are responsible for validating the kit for their specific application.
- Aladdin product labels, SDS, COA, and approved specifications take precedence over this document. If product formulation, label, SDS, storage conditions, pack size, or quality specifications change, this document must be reviewed and reissued.