Instruction Manual

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Mouse CD4+ T Cell Isolation Kit (negative selection)

Description

TargetMol's Mouse CD4+ T Cell Isolation Kit (negative selection) provides superparamagnetic beads for the isolation of CD4+ T cells from single-cell suspensions prepared from mouse spleen or other tissues using negative selection. The kit uses different biotin-labeled monoclonal antibodies to label non-target cells (non-CD4+ T cells), which are subsequently depleted using streptavidin-labeled magnetic beads, thereby isolating mouse CD4+ T cells.

Recommended Products

1. Mouse Cells

Cell Type	Spleen	Lymph Node	Peripheral Blood	Bone Marrow	Tumor Tissue
CD3+ T Cell	C0061		/	/	/
CD4+ Cell	C0062 (Preferred), C0067 (Optional)		C0067	/	C0067
CD8+ Cell	C0063 (Preferred), C0068 (Optional)		C0068	/	C0068
Neutrophil	C0064	/	C0064	C0064	/
CD3+ Cell Removal	C0152	C0152	/	/	/
CD3/CD28 T Cell Activation	C0180	/	/	/	/
B Cell	C0218	C0218	/	C0218	/

2. Human Cells

Cell Type	Peripheral Blood	Umbilical Cord Blood
CD3+ T Cell	C0065	/
CD34+ Cell Enrichment	C0066	C0066
CD4+ T Cell	C0148	/
CD8+ T Cell	C0149	/
CD3/CD28 T Cell Activation	C0150	/
CD66b+ Cell	C0151	/
Neutrophil	C0216	/
CD3+ Cell Removal	C0217	/

Product Features

1. High Purity: High-purity target cell population can be acquired after isolation with efficient enrichment of target cells.
2. High Viability: Cell function remains intact after isolation, with no abnormal activation and no antibody or magnetic bead labeling.
3. Easy to Operate: No separation column is required; target cells can be isolated using a magnetic rack.
4. Fast: Target cells can be obtained in as little as 15 min.

Product Applications

Suitable for the isolation of CD4+ T cells from mouse spleen, lymph nodes, and mixed samples thereof.

Packing List

Catalog No.	Product Name	Packing (for 5 × 10^8 cells)	Packing (for 1 × 10^9 cells)
C0062-1	Biotin-Antibody Mix	100 µL	200 µL
C0062-2	Streptavidin Magnetic Beads	1 mL	2 mL

Instructions

Example: Isolation of CD4+ T Cells from Mouse Spleen

1. Preparation of Single-Cell Suspension: Place the spleen on a 70 µm cell strainer, gently grind the tissue, and rinse the strainer with pre-cooled PBS. Collect the cell suspension into a 50 mL centrifuge tube and centrifuge at 500 × g for 5 min.
2. After centrifugation, discard the supernatant. Add 5 mL RBC Lysis Buffer (ACK) and lyse at room temperature for 5 min. Then add 20 mL PBS and centrifuge at 500 × g for 5 min.
Note: The lysis time and volume may be adjusted based on the buffer used. A small number of residual red blood cells will not significantly affect downstream separation or purity.
3. After centrifugation, discard the supernatant. Resuspend the splenocytes in PBS and filter the suspension through a 70 µm cell strainer. After cell counting, centrifuge again at 500 × g for 5 min.
Note: To prevent tissue debris and cell clumps from affecting the purity of subsequent cell isolation, the cell suspension must be filtered through a cell strainer.
4. After centrifugation, discard the supernatant. Resuspend the cells in Isolation Buffer and adjust the cell concentration to 1 × 10^8 cells/mL.
Note: Recommended Isolation Buffer formulations: PBS containing 2 mM EDTA and 2% FBS; or PBS containing 2 mM EDTA and 0.5% BSA. The buffer should be sterilized in advance by filtration through a 0.22 µm membrane filter.
5. Add 100 µL of cell suspension (containing 1 × 10^7 cells) to the bottom of a sterile flow cytometry tube, followed by 2 µL Biotin-Antibody Mix. Mix thoroughly and incubate at 4°C for 10 min.
Note: When adding the cells to the flow cytometry tube, avoid dispensing the suspension along the tube wall. If more cells are to be isolated, increase the amount of Biotin-Antibody Mix proportionally. Depending on the magnetic rack used, a centrifuge tube may also be used for cell isolation.
6. Magnetic Bead Pretreatment: Vortex to fully resuspend the magnetic beads. Transfer the required amount of magnetic beads into a 1.5 mL centrifuge tube, add 1 mL Isolation Buffer, and centrifuge at 10,000 × g for 1 min. Discard the supernatant. Repeat the above washing step once. Resuspend the magnetic beads in the same volume of Isolation Buffer as the original bead volume. If 20 µL magnetic beads are used for washing, resuspend the washed beads in 20 µL Isolation Buffer.
7. After cell incubation, add 20 µL pretreated Streptavidin Magnetic Beads to the flow cytometry tube. Mix thoroughly and incubate at 4°C for 10 min.
Note: If a larger number of cells is being isolated, increase the amount of Streptavidin Magnetic Beads proportionally. For example, when isolating 5 × 10^7 cells, add 10 µL Biotin-Antibody Mix and 100 µL Streptavidin Magnetic Beads to 500 µL cell suspension. If fewer than 1 × 10^7 cells are being isolated, adjust the cell suspension volume to 100 µL, and add 2 µL Biotin-Antibody Mix and 20 µL Streptavidin Magnetic Beads.
8. After incubation, add 2.5 mL Isolation Buffer to the flow cytometry tube. Gently pipette up and down 5 times to mix. Avoid vigorous shaking or repeatedly inverting the tube to mix.
9. Place the flow cytometry tube containing the cells on a magnetic rack and allow it to stand for 5 min.
10. Gently pour the cell suspension containing the purified CD4+ T cells into a sterile centrifuge tube while keeping the flow cytometry tube on the magnetic rack during transfer. Centrifuge at 500 × g for 5 min, discard the supernatant, and collect the cells.
11. Wash the cells as required for the experiment, then resuspend them in the desired buffer or culture medium for subsequent molecular biology or cell biology experiments.

Storage Conditions

Store at 4°C for 2 years.

Precautions

1. Avoid freezing. Store beads in the solution to prevent drying.
2. Before removing beads from the tube, ensure they are evenly suspended by gentle shaking. Handle gently to avoid introducing bubbles.
3. It is recommended to use high-quality pipette tips and centrifuge tubes to avoid loss of beads due to adhesion.
4. This product is not suitable for the isolation of naive CD4+ T cells.
5. This product is for scientific research use by professionals only and must not be used for clinical diagnosis or treatment, food or drug applications, and must not be stored in residential areas.
6. For your safety and health, wear lab coats and disposable gloves during operation.

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