Human CD8⁺ T Cell Isolation Kit (negative selection)

Introduction

The TargetMol's Human CD8⁺ T Cell Isolation Kit (Negative Selection) provides superparamagnetic microbeads and employs a negative selection method to isolate CD8⁺ T cells from human peripheral blood mononuclear cells (PBMCs). The principle involves using biotin-labeled monoclonal antibodies to mark non-target cells (non-CD8⁺ T cells), which are then removed using streptavidin-labeled magnetic beads. This process enables the isolation of human CD8⁺ T cells from human peripheral blood mononuclear cells (PBMCs).

Recommended Products

Mouse Cells

	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 Depletion	C0152	C0152	/	/	/
CD3/CD28 T Cell Activation	C0180	/	/	/	/

Human Cells

	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	/

Product Features

1. High Purity: Isolated cells exhibit high purity, reaching over 90%.
2. High Activity: After isolation, cell function remains intact with no abnormal activation, and no antibody or magnetic bead labeling.
3. Easy Operation: No separation column is required, target cell isolation can be achieved using a magnetic separator.

Application

Suitable for isolating CD8⁺ T cells from human peripheral blood mononuclear cells (PBMCs).

Packing Information

Catalog No.	Product Name	Packing (for 5×10^8 cells)	Packing (for 1×10^9 cells)
C0149-1	Biotin-Antibody Mix	100 μL	200 μL
C0149-2	Streptavidin Magnetic Beads	1 mL	2 mL

Instructions

1. To prepare human PBMCs: Isolate peripheral blood mononuclear cells (PBMCs) from human peripheral blood using Ficoll density gradient centrifugation. Wash the collected cells with PBS and centrifuge. After centrifugation, discard the supernatant and resuspend the PBMCs in isolation buffer. Adjust the cell concentration to 1×10^8 cells/mL.

Note: Recommended isolation buffer: a. PBS（2 mM EDTA and 2% FBS）; b. PBS（2 mM EDTA and 0.5% BSA）. The buffer should be pre-filtered using a 0.22 μm membrane for sterilization.

1. Transfer 100 μL of the cell suspension (1×10^7 cells) to the bottom of a sterile flow tube. Add 2 μL of Biotin-Antibody Mix, mix thoroughly, and incubate at 4°C for 15 minutes. Then add 10 volumes of isolation buffer, centrifuge at 500 g for 5 minutes, and discard the supernatant. Finally, resuspend the cells in 100 μL of isolation buffer.

Note: a. Transfer cell suspension directly to the bottom of the tube, avoiding adding along the wall of the tube.

b. If a larger quantity of cells requires sorting, proportionally increase the volume of Biotin-Antibody Mix used. Depending on the magnetic separator used, centrifuge tubes may also be suitable.

3. To prepare the magnetic beads: Vortex to resuspend the beads. Transfer the required amount of beads to a 1.5 mL centrifuge tube. Add 1 mL of isolation buffer, and centrifuge at 10,000 g for 1 minute. Discard the supernatant and repeat the washing step once. The volume of isolation buffer used for beads resuspension should be equal to the initial volume of beads that was aspirated. E.g., if 20 μL of beads are used for washing, resuspend in 20 μL of isolation buffer.

4. Add 10 μL of pre-washed Streptavidin Magnetic Beads to the cell suspension from Step 2, mix thoroughly, and incubate at 4°C for 10 minutes.

Note: If a larger number of cells need to be sorted, the amount of Streptavidin Magnetic Beads can be increased proportionally. For sorting 5×10^7 cells, add 10 μL of Biotin-Antibody Mix and 50 μL of Streptavidin Magnetic Beads to 500 μL of cell suspension. If sorting less than 1×10^7 cells, adjust the volume of the cell suspension to 100 μL and add 2 μL of Biotin-Antibody Mix along with 10 μL of Streptavidin Magnetic Beads.

5. After incubation, add 2.5 mL of isolation buffer to the tube and mix 5 times gently (avoid vigorous shaking or up-and-down mixing).

6. Place the tube in the magnetic separator for 5 minute.

7. Gently pour the cell suspension containing purified $CD8^+$ T cells into a sterile centrifuge tube, keeping the flow tube on the magnetic separator during the transfer. The cells can be used directly for downstream biological experiments or flow cytometry analysis. To further improve purity, centrifuge the suspension at 500g for 5 minutes, discard the supernatant, and resuspend the cells in 100 μL of isolation buffer for a second round of purification following the steps below.

Note: If processing a larger number of cells, increase the resuspension volume accordingly. For example, when sorting 5×10^7 cells, resuspend the centrifuged pellet in 500 μL of isolation buffer.

8. Add 10 μL of pre-washed Streptavidin Magnetic Beads, gently mix, and incubate at 4°C for 10 minutes.

Note: If a larger number of cells are being sorted, increase the volume of Streptavidin Magnetic Beads proportionally. For example, when sorting 5×10^7 cells, add 50 μL of Streptavidin Magnetic Beads to 500 μL of cell suspension.

9. After incubation, add isolation buffer to bring the volume to 2.5 mL. Gently pipette up and down 5 times to mix, then transfer the mixture to a sterile flow tube.

10. Place the flow tube containing the cells on the magnetic separator and let it stand for 5 minutes.

11. Gently pour the cell suspension containing purified $CD8^+$ T cells into a sterile centrifuge tube, keeping the flow tube on the magnetic separator during the transfer. At this stage, the $CD8^+$ T cells are secondarily purified, with a potential increase in purity of 2–4%.

12. Wash cells according to the requirements of experiment, and resuspend the cells in the appropriate buffer or medium for downstream molecular or cell biology experiments.

Storage

Store at 4 ℃ 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 prevent the bubbles.
3. It is recommended to use high-quality pipette tips and centrifuge tubes to avoid loss of beads due to adhesion.
4. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
5. Please wear a lab coat and disposable gloves.

Negative Selection VS Positive Selection

MACS	Negative Selection	Positive Selection
Samples	Diverse	Diverse
Capture	Magnetic beads binding to non-target cells	Magnetic beads binding to target cells
Isolation Required or Not	No	Yes
Antibody Labeling	No	Yes
Purity	>97%	>95%
Activity	High	High
Features	High purity of target cells; No antibody or magnetic bead residue; Better cell viability, suitable for downstream functional experiments.	Broader sample range.

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