

Human CD8+ T Cell Isolation Kit (negative selection)

Description

TargetMol's Human CD8+ T Cell Isolation Kit (negative selection) utilizes superparamagnetic beads to isolate CD8+ T cells from human peripheral blood mononuclear cells (PBMCs) via negative selection. Non-target cells (non-CD8+ T cells) are indirectly immunolabeled with a cocktail of biotin-conjugated monoclonal antibodies, followed by magnetic depletion using streptavidin-coated magnetic beads. Unlabeled human CD8+ T cells are thus efficiently isolated.

Cell Isolation Product Recommendations

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: Efficient depletion of non-target cells enables the isolation of highly enriched target cells.
2. High Viability: Isolated cells maintain intact functional activity without abnormal activation, free of antibodies and magnetic beads
3. Easy Operation: Column-free separation using a simple magnetic rack.

Applications

Suitable for isolating CD8+ T cells from human PBMCs.

Kit Components

Catalog No.	Component Name	Packaging Size (for 5×10^8 cells)	Packaging Size (for 1×10^9 cells)
C0149-1	Biotin-Antibody Mix	100 μ L	200 μ L
C0149-2	Streptavidin Magnetic Beads	1 mL	2 mL

Instructions

- Human PBMC Preparation: Isolate PBMCs from human peripheral blood using Ficoll density gradient centrifugation. Wash the collected cells with PBS and centrifuge. Discard the supernatant, resuspend PBMCs in Isolation Buffer, and adjust cell concentration to 1×10^8 cells/mL.
Note: Recommended Isolation Buffer: PBS containing 2 mM EDTA and 2% FBS; or PBS containing 2 mM EDTA and 0.5% BSA. The buffer must be sterile-filtered through a 0.22 μ m membrane filter before use.
- Add 100 μ L cell suspension (1×10^7 cells) directly to the bottom of a sterile flow cytometry tube. Add 2 μ L Biotin-Antibody Mix, mix thoroughly, and incubate at 4°C for 15 min. Add 10 volumes of Isolation Buffer, centrifuge at 500 x g for 5 min, and discard the supernatant. Resuspend cells in 100 μ L Isolation Buffer.
Note: Avoid running cell suspensions down the tube wall when adding cells to the bottom. Scale up the Biotin-Antibody Mix volume proportionally when isolating more cells. Centrifuge tubes may also be used depending on the compatibility of the magnetic rack.
- Bead Pretreatment: Resuspend magnetic beads by vortexing. Transfer the required amount of beads into a 1.5 mL tube, add 1 mL Isolation Buffer, centrifuge at 10,000 x g for 1 min, and discard the supernatant. Repeat the washing step once. Resuspend beads in Isolation Buffer back to their original volume. (e.g., if 20 μ L beads were washed, resuspend in 20 μ L Isolation Buffer after washing).
- Add 10 μ L pretreated Streptavidin Magnetic Beads to the cell suspension from Step 2, mix well, and incubate at 4°C for 10 min.
Note: Scale up Streptavidin Magnetic Beads proportionally for larger cell numbers. For example, when isolating 5×10^7 cells, add 10 μ L Biotin-Antibody Mix and 50 μ L Streptavidin Magnetic Beads to 500 μ L cell suspension. For $< 1 \times 10^7$ cells, adjust cell suspension volume to 100 μ L, then add 2 μ L Biotin-Antibody Mix and 10 μ L Streptavidin Magnetic Beads.
- After incubation, add 2.5 mL Isolation Buffer to the flow tube and gently pipette up and down 5 times to mix. Avoid vigorous vortexing or tube inversion.
- Place the flow tube on a magnetic rack and let it stand for 5 min.
- Gently pour the cell suspension containing purified CD4⁺ T cells into a sterile centrifuge tube while keeping the flow tube on the magnetic rack. The isolated cells are ready for downstream biological assays or flow cytometry analysis. To further increase purity, perform a second-round purification by centrifuging the cell suspension at 500 x g for 5 min, discarding the supernatant, resuspending in 100 μ L Isolation Buffer, and executing the following steps.
Note: Scale up the resuspension volume accordingly for larger cell numbers. For example, when isolating 5×10^7 cells, resuspend the cell pellet in 500 μ L Isolation Buffer after centrifugation.
- Add 10 μ L pre-washed Streptavidin Magnetic Beads, mix gently, and incubate at 4°C for 10 min.
Note: Scale up Streptavidin Magnetic Beads proportionally for larger cell numbers. For example, when isolating 5×10^7 cells, add 50 μ L Streptavidin Magnetic Beads to 500 μ L cell suspension.

9. After incubation, top up Isolation Buffer to 2.5 mL, pipette gently 5 times to mix, and transfer the mixture into a sterile flow tube.
10. Place the flow tube on the magnetic rack and let it stand for 5 min.
11. Gently pour the cell suspension into a sterile tube while keeping the flow tube on the magnetic rack. The twice-purified human CD4⁺ T cells are collected.
12. Wash cells according to experimental requirements and resuspend them in desired buffer or culture medium for downstream molecular or cell biology experiments.

Storage

4°C, 2 years.

Precautions

1. Do not freeze any kit components. Magnetic beads should be stored in their storage solution to prevent drying.
2. Resuspend the beads thoroughly by vortexing before pipetting from the stock tube, taking care to avoid air bubbles.
3. High-quality low-binding pipette tips and reaction tubes are recommended to prevent bead and solution loss due to wall adhesion.
4. This product is intended for scientific research purposes by professionals only and must not be used for clinical diagnosis or treatment, food, or medicine, nor stored in residential areas.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.

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