

EasySort™ Human CD4⁺ T Cell Isolation Kit

Cat. No: MIH002N

Size: 10 Assays/100 Assays/200 Assays

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIH002NA	EasySort™ Human CD4 ⁺ T Beads Streptavidin 1.0-N	220 µL	1.1 mL×2	1.1 mL×4	2-8°C
MIH002NB	EasySort™ Human CD4 ⁺ T Cell Isolation Cocktail	290 µL	1.45 mL×2	1.45 mL×4	2-8°C
	Manual			1 copy	

Storage

Store at 2-8°C with shading light for 1 year. Avoid freezing and thawing.

Description

Human CD4⁺ T cell isolation kit utilizes a negative selection strategy to isolate CD4⁺ T cells from fresh or frozen human PBMC sample. The principle of this kit is to use different biotinylated monoclonal antibodies labeling non-CD4⁺ T cells, followed by streptavidin conjugated magnetic beads incubation. Unwanted labeled cells are efficiently removed by magnetic isolation, and high purity CD4⁺ T cells are isolated.

EasySort™ Human CD4⁺ T Cell Isolation Kit can help researcher isolate high purity human CD4⁺ T cells with simple experimental procedure. The kit is suitable for isolation of CD4⁺ T cells from fresh human PBMC or frozen PBMC, and the isolated CD4⁺ T cells can be directly used for downstream applications. The CD4⁺ T cells isolated from normal PBMC using this kit is typically 96.6±1.1%.

Reagents and Materials Not Supplied

1. Reagents:

PBS, fetal bovine serum (FBS), EDTA, Human peripheral blood mononuclear cells separation solution、DNase I

2. Materials:

70 µm mesh nylon strainer, 1.5 mL/2 mL EP tube, 15 mL/50 mL centrifuge tube, flow tube

3. Instrument:

Optical microscope, centrifuge, 5 mL magnetic rack

Experimental Operation

NOTE: The following operations must be performed under sterile conditions

➤ Isolation buffer preparation

Add fetal bovine serum (final concentration of 2%) and EDTA (final concentration of 2 mM) to PBS

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buffer and filter the prepared buffer with 0.22 μ m filter.

NOTE: Sealed store the prepared buffer at 4°C and use within 1 week. In addition, 2% fetal bovine serum can be replaced by 0.5% BSA.

➤ Sample Preparation and Processing

1. Fresh human PBMC: PBMC sample is obtained from fresh human whole blood by density gradient centrifugation. Wash PBMC twice with isolation buffer, centrifuge at 300 g for 5 minutes, filter the PBMC through a 70 μ m mesh nylon strainer and adjust the cell density to 1×10^8 cells/mL for cell isolation.

Note: The best separation effect can be achieved when the freshly collected human blood is separated within 1 hour. Approximately 1×10^7 PBMC can be obtained from 10 mL of human blood.

2. Frozen PBMC: incubate the frozen PBMC should be incubated with DNase I solution (PBS) at a concentration of 100 μ g/mL for 15 minutes at room temperature before cell isolation. Wash sample twice with isolation buffer, centrifuged at 300 g for 5 minutes. Filter aggregated suspensions through a 70 μ m mesh nylon strainer and adjust cell density at 1×10^8 cells/mL.

➤ Cell Isolation

- a) Prepare 100 μ L of cell suspension (about 1×10^7 cells), add 29 μ L Human CD4 $^+$ T Cell Isolation Cocktail, mix fully and incubate for 5 minutes at room temperature.

Note: Please make sure the cells are single-cell suspension.

- b) Add isolation buffer to a final volume of 2 mL, centrifuge at 300 g for 5 minutes. Discard the supernatant, and then resuspend the cells with 100 μ L isolation buffer.

- c) Wash Beads Streptavidin 1.0-N: Vortex beads for 20 seconds, add 22 μ L Beads in 1.5 mL EP tube. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 30 seconds. Remove the supernatant, then resuspend beads with 1 mL isolation buffer, and stand for 5 minutes at room temperature. Remove the supernatant, then resuspend beads with 22 μ L isolation buffer.

- d) Transfer the cells to the bottom of the flow tube (**Note:** Avoid adding along tube walls), add 22 μ L washed Human CD4 $^+$ T Beads Streptavidin 1.0-N, mix gently and incubate at room temperature for 5 minutes.

Note:

❖ If more than 1×10^7 cells are to be isolated, increase the amount of Human CD4 $^+$ T Cell Isolation Cocktail and Human CD4 $^+$ T Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains 1×10^8 cells/mL. If fewer than 1×10^7 cells are to be isolated, resuspend the cells with 100 μ L isolation buffer, add 29 μ L Human CD4 $^+$ T Cell Isolation Cocktail and 22 μ L washed Human CD4 $^+$ T Beads Streptavidin 1.0-N.

❖ The 5 mL flow tube is suitable for less than 1×10^8 cells.

- e) Add isolation buffer to a final volume of 2.5 mL, mix gently with a pipette by blowing up and down for 7-8 times until no particles of magnetic beads are visible. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 5 minutes.

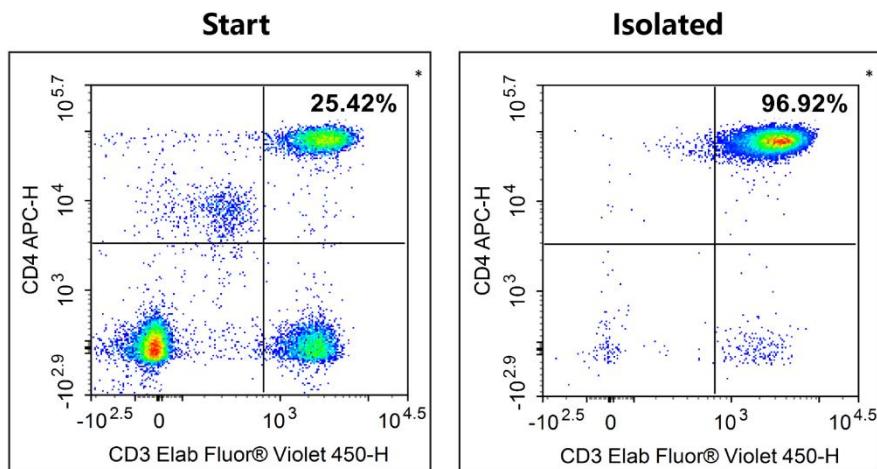
Note: Please mix the liquid thoroughly to avoid the magnetic beads clumping and affecting the

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isolation efficiency.

f) Transfer the cell suspension to a clean centrifuge tube, centrifuge at 300 g for 5 minutes. Discard the supernatant, resuspend the cells with buffer required for the subsequent experiments.

Typical data



The CD4⁺ T cells isolated from normal PBMC were stained with Elab Fluor® Violet 450 Anti-Human CD3 Antibody[OKT-3] (E-AB-F1001Q) and APC Anti-Human CD4 Antibody[SK3] (E-AB-F1352E) . The purities of the start and final isolated fractions were 25.42% and 96.92%, respectively.

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Avoid freezing and thawing during the use and storage of the beads.
4. Sample differences, sample preparation and experimental operation have an important impact on the final isolated cell purity. The statistical results for cell purity in this product are based on normal human PBMC samples.
5. The quality of pre-isolated PBMC sample is critically impacts the separation efficiency of this product. It is recommended to test whether the percentage of CD3⁺CD4⁺ T cells is in the normal physiological range (25%-60%) after the preparation of PBMC sample. It is recommended to re-prepare the PBMC sample when percentage of target cell population is lower than it's normal distribution.
6. The cell clusters in the cell suspension will affect the purity of cell isolation. Therefore, cell suspension should be filtered with a 70 µm mesh nylon sieve before formal isolation.
7. Cell suspension should be isolated immediately after preparation, the longer the storage time, the greater the impact on cell activity.
8. The cell suspension and reagents should be added directly to the bottom of flow tube to avoid sticking to the wall, resulting in insufficient reaction and affecting the isolation efficiency.
9. In order to ensure the activity of the cells, the whole process of the experiment should be

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completed on ice as much as possible, except for the incubation at room temperature.

10. It is recommended to use low adsorption pipette tips and centrifuge tubes to avoid the loss of magnetic beads and antibodies due to adsorption.
11. The kit should be used in combination with a magnetic rack.

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