EasySort™ Memory CD4⁺ T Cell Isolation Kit

Cat. No: MIH009N **Size: 10 Assays/100 Assays/200 Assays**

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIH009NA	EasySortTM Human Memory CD4+T Beads Streptavidin 1.0-N	250 μL	1.25 mL×2	1.25 mL×4	2-8°C
MIH009NB	EasySortTM Human Memory CD4+T Cell Isolation Cocktail	400 μL	1.4 mL×3	1.4 mL×6	2-8°C
	Manual	1 copy			

Storage

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

Description

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

EasySort™ Human Memory CD4⁺T Cell Isolation Kit can help researcher isolate high purity Human Memory CD4⁺T cells with simple experimental procedure. The kit is suitable for isolation of Memory CD4⁺T cells from fresh human PBMC or frozen PBMC, and the isolated CD3⁺T cells can be directly used for downstream applications. The Memory CD4⁺T cells isolated from normal PBMC using this kit is typically 86%-96%.

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

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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 buffer and filter the prepared buffer with 0.22 μm 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 min, filter the PBMC through a 70 μm mesh nylon strainerand 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 μg/mL for 15 min at room temperature before cell isolation. Wash sample twice with isolation buffer, centrifuged at 300 g for 5 min. Filter aggregated suspensions through a 70 μm mesh nylon strainer and adjust cell density at 1×10^8 cells/mL.

- **Cell Isolation**

a) Prepare 100 μL of cell suspension (about 1×10^7 cells), add 39.3 μL Human Memory $CD4^+$T Cell Isolation Cocktail, mix fully and incubate for 5 min at room temperature.

   Note: Please ensure that the cells are in a single-cell suspension. Before sample dilution, filter the samples through a 70 μm cell sieve. Before freezing and storing the PBMC samples, they need to be treated with DNase Iand then filtered through a 70 μm cell sieve again.

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

c) Wash Beads Streptavidin 1.0-N: Vortex beads for 20 seconds, add 25 μL 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 min at room temperature. Remove the supernatant, then resuspend beads with 25 μL isolation buffer.

d) Transfer the cells to the bottom of the flow tube (Note: Avoid adding along tube walls), add 25 μL washed Human Memory $CD4^+$T Beads Streptavidin 1.0-N, mix gently and incubate at room temperature for 5 min.

   Note:

   ✧ If more than 1×10^7 cells are to be isolated, increase the amount of Human Memory $CD4^+$T

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Cell Isolation Cocktail and Human Memory 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 µL isolation buffer, add 39.3 µL Human Memory CD4$^+$T Cell Isolation Cocktail and 25 µL washed Human Memory CD4$^+$T Beads Streptavidin 1.0-N.

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

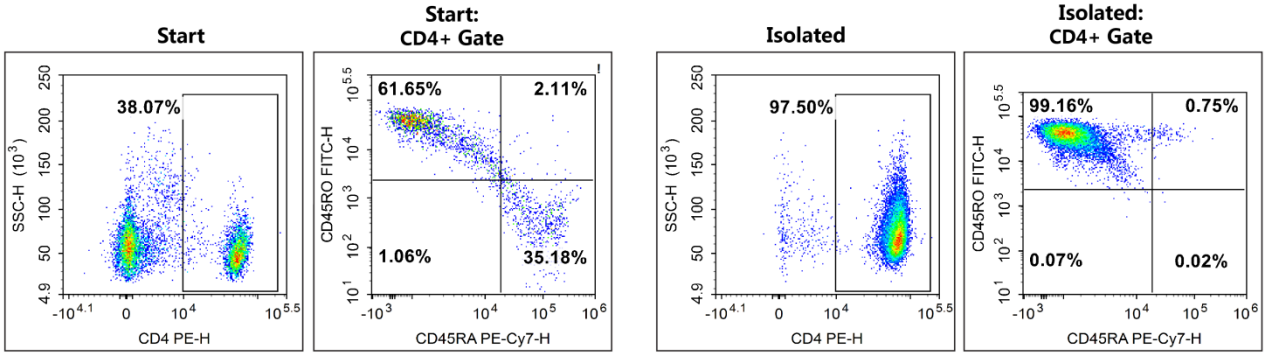
e) Add isolation buffer to a final volume of 2.5 mL, mix gently with a pipette by blowing up and down for 7-8 times until no particles of magnetic beads are visible. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 5 min.Collect the supernatant after magnetic attraction into a 15 mL centrifuge tube. This is the target cell fractionated and obtained.

f) Resuspend the cells adsorbed on the wall of the flow tube with 2.5 mL of isolation buffer, perform magnetic separation for 5 min, collect the supernatant after magnetic separation, and transfer it to the previously used 15 mL centrifuge tube, thereby obtaining the target cells from the second round of recovery.

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

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

Typical data



As shown in the above figure, the purity of Memory CD4+ T cells before and after sorting was analyzed by flow cytometry using the flow antibodies listed in the table below. The purities of the start and final isolated fractions were 23.47%和96.68%, respectively.

Products	Cat.	manufacturer
PE Anti-Human CD4 Antibody[RPA-T4]	E-AB-F1109D	Elabscience
PE/Cyanine7 Anti-Human CD45RA Antibody[HI100]	E-AB-F1052H	Elabscience
FITC Anti-Human CD45RO Antibody[UCHL1]	E-AB-F1139C	Elabscience

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.

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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.
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 Memory $CD4^+$T cells is in the normal physiological range (8%-30%) 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, thegreaterthe 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 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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