EasySort™ Human Naïve Pan T Cell Isolation Kit

Cat. No: MIH006N **Size: 10/100/200 Assays**

Component	Component Name				Storage
MIH006NA	EasySort™ Human Naïve Pan T Beads Streptavidin 1.0-N	300 μL	1.0 mL×3	1.0 mL×6	2-8°C
MIH006NB	EasySort™ Human Naïve Pan T Cell Isolation Cocktail	375 μL	1.25 mL×3	1.25 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 Naïve Pan T cell isolation kit utilizes a negative selection strategy to isolate naïve pan T cells from fresh or frozen human PBMC sample. The principle of this kit is to use different biotinylated monoclonal antibodies labeling non-Naïve Pan T cells, followed by streptavidin conjugated magnetic beads incubation. Unwanted labeled cells are efficiently removed by magnetic isolation, and high purity Naïve Pan T cells are isolated.

EasySort™ Human Naïve Pan T Cell Isolation Kit can help researcher isolate high purity human Naïve Pan T cells with simple experimental procedure. The kit is suitable for isolation of Naïve Pan T cells from fresh human PBMC or frozen PBMC, and the isolated Naïve Pan T cells can be directly used for downstream applications. The Naïve Pan T cells isolated from normal PBMC using this kit is typically 94.2 ± 1.6%.

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 37.5 μL Human Naïve Pan T Cell Isolation Cocktail, mix fully and incubate for 5 min 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 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 30 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 minutes at room temperature. Remove the supernatant, then resuspend beads with 30 μL isolation buffer.

d) Transfer the cells to the bottom of the flow tube (Note: Avoid adding along tube walls), add 30 μL washed Human Naïve Pan 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 Naïve Pan T Cell Isolation Cocktail and Human Naïve Pan 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 37.5 μL Human Naïve Pan T Cell Isolation Cocktail and 30 μL washed Human Naïve Pan T Beads Streptavidin 1.0-N.

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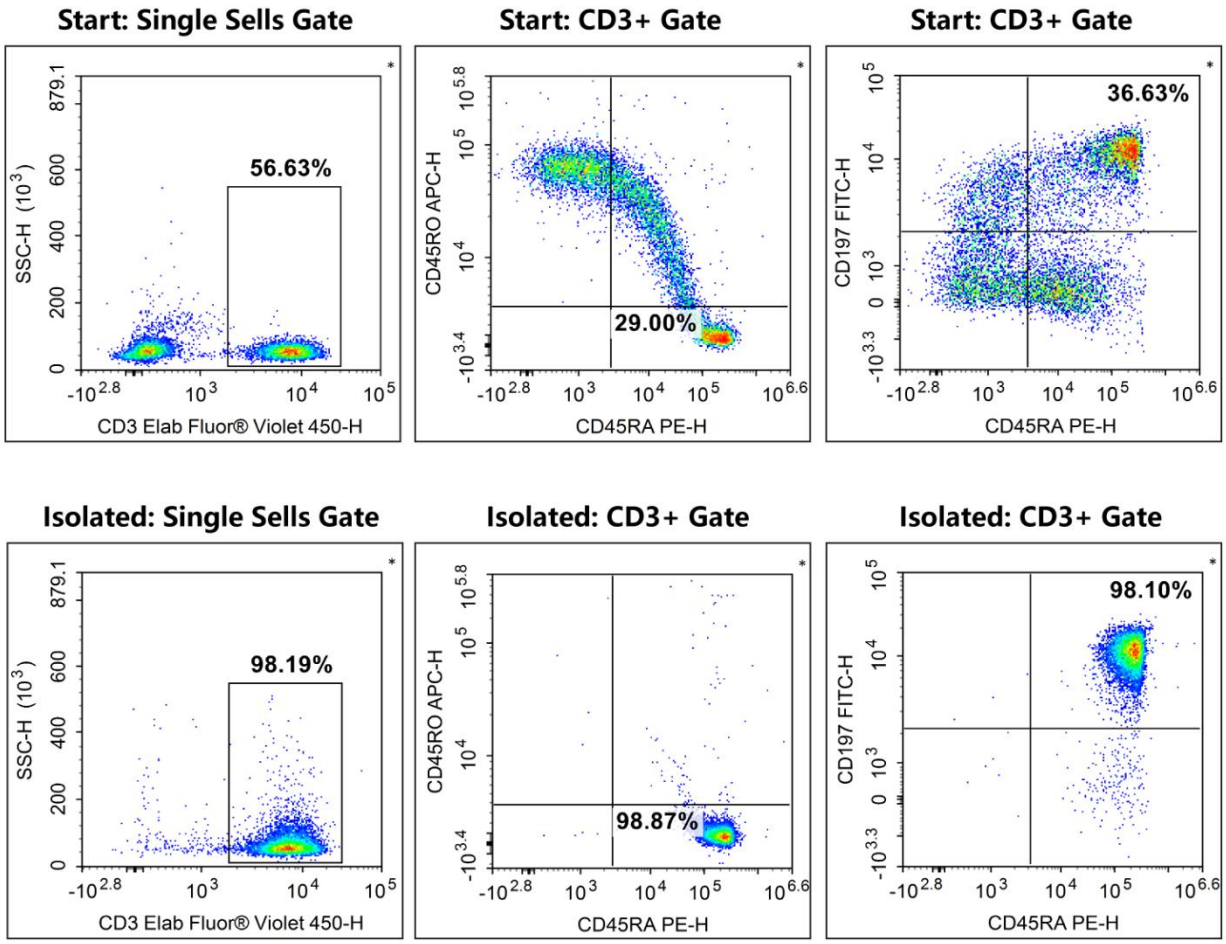
✧ The 5 mL flow tube is suitable for less than 1×10^8 cells.

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.

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

f) 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



In the above example, the purities of human naïve pan T cells (CD3+CD45RA+CD45RO-CD197+) in normal human peripheral blood mononuclear cell samples was 6.0% before sorting and 95.2% after sorting.

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Fluorochrome-conjugated antibody	Cat.	Company
Elab Fluor® Violet 450 Anti-Human CD3 Antibody[OKT-3]	E-AB-F1001Q	Elabscience
PE Anti-Human CD45RA Antibody[HI100]	E-AB-F1052D	Elabscience
APC Anti-Human CD45RO Antibody[UCHL1]	E-AB-F1139E	Elabscience
FITC Anti-Human CD197/CCR7 Antibody[G043H7]	E-AB-F1159C	Elabscience

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. The data presented for this product are based on testing of peripheral blood mononuclear cells (PBMCs) from healthy individuals. When processing peripheral blood from non-healthy individuals, to ensure optimal experimental outcomes, first assess the naïve pan T-cell content in the sample. If the naïve pan T-cell content is below 6%, it is recommended to perform a pre-sorting test, appropriately increase the amount of antibodies and beads per assay, or conduct a secondary sorting step.
5. 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.
6. Cell suspension should be isolated immediately after preparation, the longer the storage time, thegreaterthe impact on cell activity.
7. 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.
8. 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.
9. It is recommended to use low adsorption pipette tips and centrifuge tubes to avoid the loss of magnetic beads and antibodies due to adsorption.
10. The kit should be used in combination with a magnetic rack.

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