EasySort™ Human Naïve Pan T Cell Isolation Kit

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

Component	Component Name	10 Assays	100 Assays	200 Assays	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	400 μL	1.35 mL×3	1.35 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

The EasySort™ Human Naïve Pan T cell isolation kit is a product that enables rapid and simple isolation of high-purity human Naïve Pan T cells. This kit uses a negative selection method and is suitable for isolating Naïve Pan T from human PBMC sample. Different biotinylated monoclonal antibodies are used to label non-target cells (non-human naïve pan T cells). Subsequently, streptavidin-conjugated magnetic beads are employed to deplete these non-target cells, thereby obtaining highly purified human Naïve Pan T cells. The isolated human Naïve Pan T cells are free of any antibodies and magnetic bead labels, remain in an unstimulated, naïve state, and are ready for direct use in downstream applications.

The EasySort™ Human Naïve Pan T Cell Isolation Kit has been tested by magnetic cell separation followed by flow cytometric analysis of cells from fresh human PBMC sample. An assay is defined as 40 μL antibody and 30 μL beads to be used to isolate $1x10^7$ cells.

Reagents and Materials Not Supplied

1. **Reagents:**

   Phosphate buffered saline (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, horizontal centrifuge, 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.
2. Frozen PBMC: 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.

   Note: Generally,approximately 1×10^7 PBMC cells can be obtained from 10 mL of human blood. After preparing a single-cell suspension from fresh human blood , perform the cell isolation experiment within 1-2 hours, as a longer interval will affect the final isolated cell purity and cell viability.

- **Cell Isolation**

a) Prepare 100 μL of cell suspension (about 1×10^7 cells), add 40 μL Human Naïve Pan T Cell Isolation Cocktail, gently pipette up and down 6-8 times with a pipette to mix, then 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. The frozen PBMC sample need to be treated with DNase I and then filtered through a 70 μm cell sieve again before isolating.

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.

Note:

- If the total volume of the cell suspension exceeds 1 mL, the volume of the added isolation buffer shall be no less than the total volume of the cell suspension.For example, if the total volume of the cell suspension is 1.5 mL, the volume of the isolation buffer added shall be ≥ 1.5 mL.
- To maintain consistent cell density, the volume of cell isolation buffer for cell resuspension shall be identical to that of the input cell suspension. In the protocol example, if 100 μL of cell suspension is used as the starting input, cells should be resuspended with an equal volume of 100 μL cell isolation buffer.

c) Wash Human Naïve Pan T Beads Streptavidin 1.0-N:Place a clean flow cytometry tube or a centrifuge tube compatible with the magnetic rack into a tube rack. Pipette 1 mL of isolation buffer into the tube, then add 30 µL of magnetic beads directly into the aforementioned 1 mL of isolation buffer. Mix by pipetting up and down 6-8 times. Place the flow cytometry tube or centrifuge tube on a magnetic rack (provided by the user) and magnetically separate at room temperature for 5 min. At this point, the magnetic beads are attracted to the tube wall. Keep the tube on the magnetic rack, discard the supernatant, and then remove the tube from the magnetic rack.

Note： If the total volume of magnetic beads to be washed is greater than 1 mL, use a 1:1 volume ratio of isolation buffer to beads during the washing step.

d) Resuspend the magnetic beads using the cell suspension from step b): Aspirate the cell suspension and pipette the beads off the tube wall to the bottom of the tube (Note: avoid generating bubbles). Mix by pipetting up and down 6-8 times, then 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 40 µL Human Naïve Pan T Cell Isolation Cocktail and 30 µL washed Human Naïve Pan T Beads Streptavidin 1.0-N.
- ➢ The 5 mL flow tube is suitable for isolation of cell suspension ≤ 1 mL (1×10^8 cells). 10 mL or 15 mL centrifuge tube is suitable for isolation of cell suspension ≤ 4 mL (4×10^8 cells).

e) Add isolation buffer to a final volume of 2.5 mL (If the volume of the cell suspension for isolation is >1 mL, resuspend in an equal volume of isolation buffer), mix gently with a pipette by blowing up and down for 6-8 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

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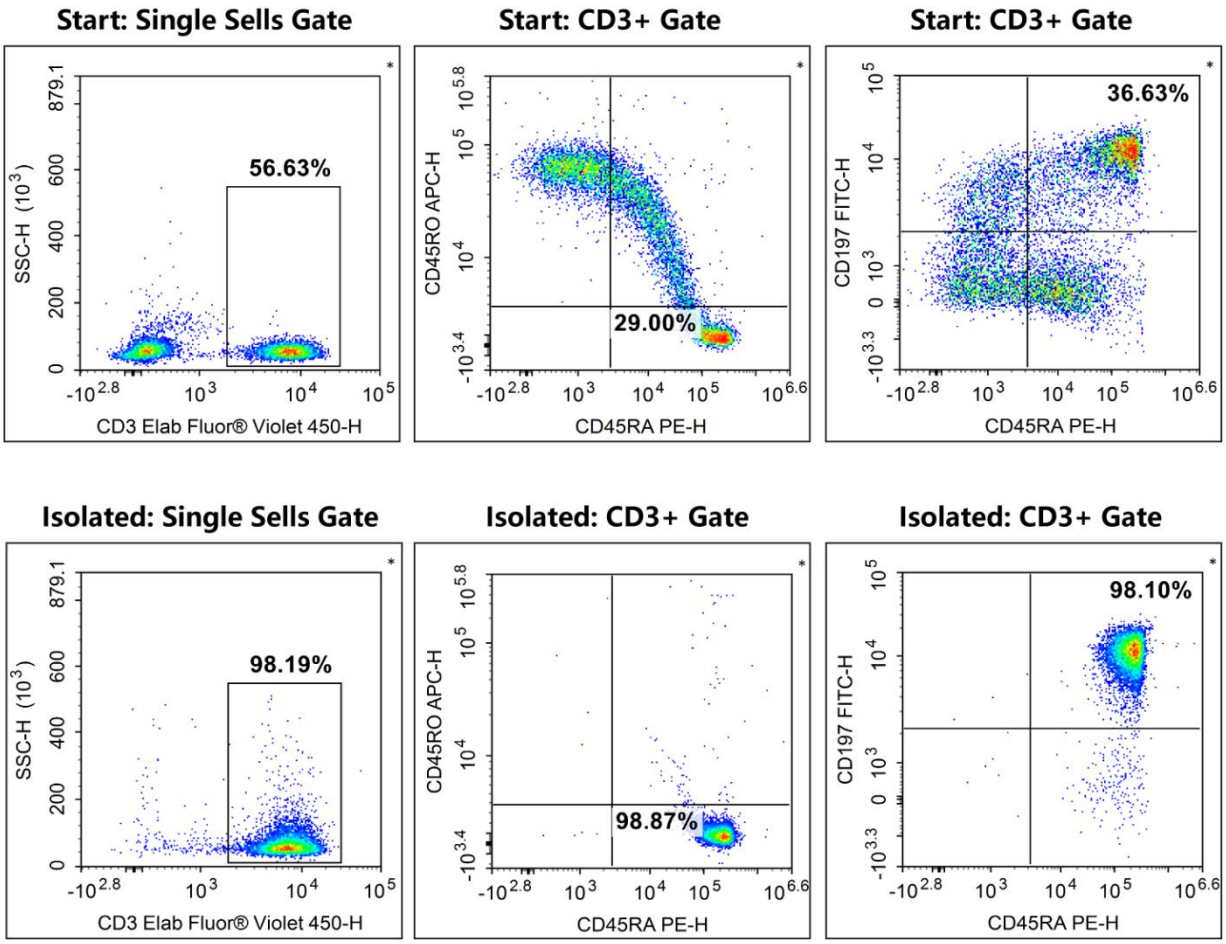

In the above example, the purities of human naïve pan T cells ($CD3^+CD45RA^+CD45RO^-CD197^+$) in normal human peripheral blood mononuclear cell samples was 6.0% before sorting and 95.2% after sorting.

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. All components of the kit should be stored at 2-8℃ and protected from freezing and thawing.
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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