EasySort™ Human Naïve CD4+ T Cell Isolation Kit

Cat. No: MIH007N **Size: 10Assays/100Assays/200Assays**

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIH007NA	EasySort™ Human Naïve CD4+ Beads	350 μL	1.2 mL×3	1.2 mL×6	2-8℃
MIH007NB	EasySort™ Human Naïve CD4+ Cell Isolation Cocktail	490 μL	1.65 mL×3	1.65 mL×6	2-8℃
	Manual	1 copy			

Storage

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

Description

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

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

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using this kit is typically 90% -97%.

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 buffer and filter the prepared buffer with 0.22 μm filter.

  NOTE: Sealed store the prepared buffer at 4℃ 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**

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a) Prepare 100 µL of cell suspension (about 1×10^7 cells), add 49 µL Human Naïve CD4+ 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 35 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 35 µL isolation buffer.

d) Transfer the cells to the bottom of the flow tube (Note: Avoid adding along tube walls), add 35 µL washed Human Naïve CD4+ 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 CD4+ Cell Isolation Cocktail and Human Naïve CD4+ Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains 1×108 cells/mL. If fewer than 1×107 cells are to be isolated, resuspend the cells with 100 µL isolation buffer, add 12 µL Human Naïve CD4+ Cell Isolation Cocktail and 16 µL washed Human Naïve CD4+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

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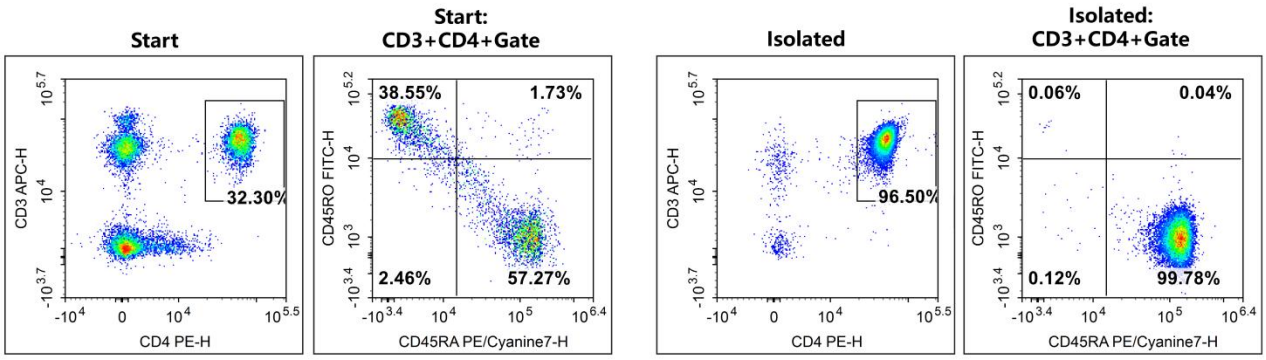
down for 7-8 times until no particles of magnetic beads are visible. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 5 min.

f) Transfer the cell suspension to a clean centrifuge tube, which is the sorted human Naïve CD4+ cells, precipitate and add the sorting buffer to a total volume of 2.5 mL, and mix evenly with a pipette up and down for 7-8 times until there are no visible magnetic beads, and it on a 5 mL sorting magnetic stand (self-provided) for static magnetic adsorption for 5 min.

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

g) Transfer the cell suspension to the centrifuge tube of the supernatant of step f, 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 CD4+ T cells (CD3+CD4+CD45RA+CD45RO-) in normal human peripheral blood mononuclear cell samples was 18.49% before sorting and 96.28% after sorting.

Fluorochrome-conjugated antibody	Cat.	Company
APC Anti-Human CD3 Antibody[OKT-3]	E-AB-F1001E	Elabscience
PE Anti-Human CD4 Antibody[RPA-T4]	E-AB-F1109D	Elabscience

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FITC Anti-Human CD45RO Antibody[UCHL1]	E-AB-F1139C	Elabscience
PE/Cyanine7 Anti-Human CD45RA Antibody[HI100]	E-AB-F1052H	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. 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 Naïve CD4+ cells is in the normal physiological range (7%-25%) 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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