

EasySort™ Human Naïve CD4+ T Cell Isolation Kit

Cat. No: MIH007N

Size: 10 Assays/100 Assays/200 Assays

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIH007NA	EasySort™ Human Naïve CD4+T Beads Streptavidin 1.0-N	350µL	1.2 mL×3	1.2 mL×6	2-8°C
MIH007NB	EasySort™ Human Naïve CD4+T Isolation Cocktail Manual	500µL	1.7 mL×3	1.7 mL×6 1 copy	2-8°C

Storage

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

Detection Principle

The EasySort™ Human Naïve CD4+ T Cell Isolation Kit is a product that enables rapid and simple isolation of high-purity human naïve CD4+ T cells. This kit employs a negative selection method and is suitable for isolating naïve CD4+ T cells from either fresh or cryopreserved human PBMC samples. Different biotinylated monoclonal antibodies are used to label non-target cells (non-human naïve CD4+T cells). Subsequently, streptavidin-conjugated magnetic beads are employed to deplete these non-target cells, thereby obtaining highly purified human naïve CD4+ T cells. The isolated human naïve CD4+ T cells carry no antibody or magnetic bead labels, remain in an undisturbed, naïve state, and are ready for downstream applications.

The EasySort™ Human Naïve CD4+ T Cell Isolation Kit has been validated for magnetic separation using fresh or cryopreserved human PBMC samples, and the isolated cells were analyzed and characterized by flow cytometry. An assay is defined as 35 µL of magnetic beads and 50 µL of antibody cocktail to be used to isolate 1×10^7 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% bovine serum albumin (BSA) .

➤ Sample Preparation and Processing

- a) 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 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.

- b) 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.

➤ Cell Isolation

- a) Prepare 100 µL of cell suspension (about 1×10^7 cells), add 50 µL Human Naive CD4+T 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 Naive CD4+ T Cell Streptavidin 1.0-N: Place a clean flow cytometry tube or a

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centrifuge tube compatible with the magnetic rack into a tube rack. Pipette 1 mL of isolation buffer into the tube, then add 35 μ L 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:

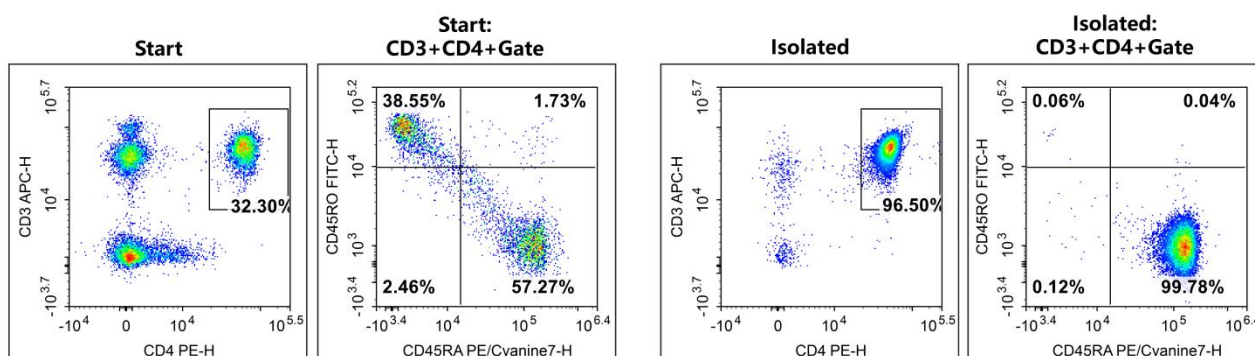
- If more than 1×10^7 cells are to be isolated, increase the amount of Human Naïve CD4+ T Cell Isolation Cocktail and Human Naïve CD4+ T Cell Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains 1×10^8 cells/mL. If fewer than 1×10^7 cells are to be isolated, resuspend the cells with 100 μ L isolation buffer, add 50 μ L Mouse Monocyte Isolation Cocktail and 50 μ L washed Human Naïve CD4+ T Cell 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 fully 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) Keep the sample on the magnetic rack, transfer the cell suspension to another clean centrifuge tube. This yields the Human Naïve CD4+ T Cell from the first time isolation.
- g) Remove the flow cytometry tube or centrifuge tube from the magnetic rack, add isolation buffer again to a total volume of 2.5 mL, mix by pipetting up and down 6-8 times until no visible magnetic bead particles remain, then place the tube on the magnetic rack for 5 min.
- h) Keep the sample on the magnetic rack, transfer the cell suspension to the centrifuge tube from step f), mix the Human Naïve CD4+ T Cell suspensions obtained from two times isolation. centrifuge at 300 g for 5 min. Discard the supernatant, resuspend the cells with buffer required for the downstream 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-F1013E	Elabscience
PE Anti-Human CD4 Antibody[RPA-T4]	AN00428E	Elabscience
FITC Anti-Human CD45RO Antibody[UCHL1]	E-AB-F1092E	Elabscience
PE/Cyanine7 Anti-Human CD45RA Antibody[HI100]	AN00629E	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°C and protected from freezing and thawing.
4. Sample type, 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. Single-cell suspension for cell isolation shall be filtered through a cell strainer to remove cell clumps and tissue debris, preventing cell aggregation from compromising isolation purity.
7. Perform isolation immediately after preparing the cell suspension, as cell viability will decrease with longer storage time.
8. When adding the antibody cocktail and aspirating the magnetic beads for washing, pipette them directly to the bottom of the tube to avoid adhesion to the wall, which would result in loss of components.
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.

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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.