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EasySort™ Human Naïve CD8+T Cell Isolation Kit

Cat. No: MIH008N Size: 10/100/200 Assays

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
	EasySort™ Human Naïve				
MIH008NA	CD8+T Beads	300 μL	1.5 mL×2	1.5 mL×4	2-8°C
	Streptavidin 1.0-N				
	EasySort™ Human Naïve				
MIH008NB	CD8 ⁺ T Cell Isolation	500 μL	1.7 mL×3	1.7 mL×6	2-8°C
	Cocktail				
Manual		1 сору			

Storage

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

Description

Human Naïve CD8+T cell isolation kit utilizes a negative selection strategy to isolate Naïve CD8+T cells from fresh or frozen human PBMC sample. The principle of this kit is to use different biotinylated monoclonal antibodies labeling non-Naïve CD8+T cells, followed by streptavidin conjugated magnetic beads incubation. Unwanted labeled cells are efficiently removed by magnetic isolation, and high purity Naïve CD8+T cells are isolated.

EasySort[™] Human Naïve CD8⁺T Cell Isolation Kit can help researcher isolate high purity human Naïve CD8⁺T cells with simple experimental procedure. The kit is suitable for isolation of Naïve CD8⁺T cells from fresh human PBMC or frozen PBMC, and the isolated Naïve CD8⁺T cells can be directly used for downstream applications. The Naïve CD8⁺T cells isolated from normal PBMC using this kit is typically 94.3 ± 2.4%.

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

For Research Use Only

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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 1×10⁸ 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 1×10⁷ 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 1×10⁸ cells/mL.

Cell Isolation

a) Prepare 100 μL of cell suspension (about 1×10⁷ cells), add 48.4 μL Human Naïve CD8⁺T Cell Isolation Cocktail, mix fully and incubate for 10 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 CD8⁺T Beads Streptavidin 1.0-N, mix gently and incubate at room temperature for 5 min.

Note:

- ♦ If more than 1×10⁷ cells are to be isolated, increase the amount of Human Naïve CD8⁺T Cell Isolation Cocktail and Human Naïve CD8⁺T 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 48.4 μL Human Naïve CD8⁺T Cell Isolation Cocktail and 30 μL washed Human Naïve CD8⁺T Beads Streptavidin 1.0-N.
- ♦ The 5 mL flow tube is suitable for less than 1×108 cells.

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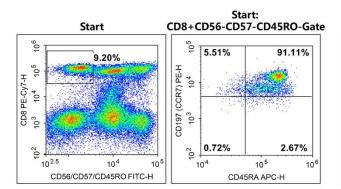
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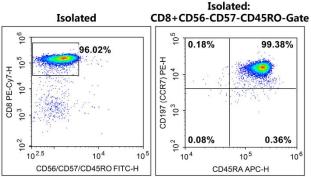
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- e) Add isolation buffer to a final volume of 2.5 mL, mix gently with a pipette by blowing up and down for 7-8 times until no particles of magnetic beads are visible. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 5 min.
 - Note: 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





As shown in the above figure, the purity of Naive CD8⁺T cells before and after sorting was analyzed by flow cytometry using the flow cytometry antibodies in the table below. The proportions of Naive CD8⁺T cells before and after sorting were 8.4% and 95.4%, respectively.

Products	Cat.	manufacturer	
PE/Cyanine7 Anti-Human CD8a Antibody[OKT-8]	E-AB-F1110H	Elabscience	
FITC Anti-Human CD56/NCAM Antibody[5.1H11]	E-AB-F1239C	Elabscience	
FITC Anti-Human CD57 Antibody[HNK-1]	E-AB-F1067C	Elabscience	
FITC Anti-Human CD45RO Antibody[UCHL1]	E-AB-F1139C	Elabscience	
APC Anti-Human CD45RA Antibody[HI100]	E-AB-F1052E	Elabscience	
PE Anti-Human CD197/CCR7 Antibody[G043H7]	E-AB-F1159D	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.

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- 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 CD8⁺T cells is in the normal physiological range (5%-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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