EasySort™ Mouse Naïve CD8⁺T Cell Isolation Kit

Cat. No: MIM008N **Size: 10Assays/100Assays/200Assays**

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIM008NA	EasySort™ Mouse Naïve CD8⁺T Beads Streptavidin 1.0-N	135 μL	1.35 mL	1.35 mL×2	2-8°C
MIM008NB	EasySort™ Mouse Naïve CD8⁺T Cell Isolation Cocktail	54 μL	540 μL	540 μL×2	2-8°C
	Manual	1 copy			

Storage

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

Detection Principle

Mouse Naïve CD8⁺T cell isolation is a negative selection method to isolate Naïve CD8⁺T cells from single cell suspensions of mouse spleen cells or other tissues. The principle is to use different biotinylated monoclonal antibodies to label non target cells (non-Naïve CD8⁺T cells), and then remove them by streptavidin-labelled magnetic beads, so the unstimulated primitive state Naïve CD8⁺T cells were isolated.

EasySort™ mouse Naïve CD8⁺T cell isolation kit is a product that can isolate high purity mouse Naïve CD8⁺T cells quickly and easily. The kit is suitable for isolation of Naïve CD8⁺T cells from mouse spleen and lymph nodes, and the isolated cells can be directly used for downstream applications.

Reagents and Materials Not Supplied

1. **Reagents:**

   PBS, fetal bovine serum (FBS), EDTA

2. **Materials:**

   Disposable sterile syringe, 70 μm mesh nylon strainer, ophthalmic scissors, ophthalmic forceps, 1.5 mL/2 mL EP tube, 15 mL centrifuge tube, flow tube

3. **Instrument:**

   Optical microscope, centrifuge, 5 mL magnetic rack

Experimental Operation

Toll-free: 1-888-852-8623 Tel: 1-832-243-6086 Fax: 1-832-243-6017
Web: www.elabscience.com Email: techsupport@elabscience.com

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.

- **Mouse spleen single cell suspension preparation**

a) Take the fresh mouse spleen to avoid excessive connective tissue attached.

b) Grind the spleen through a 70 μm mesh nylon strainer, rinse the cell sieve with pre-cooled PBS, and collect the cell suspension in a 15 mL centrifuge tube and centrifuge at 300 g for 5 min.

c) Discard the supernatant, resuspend the splenocytes with isolation buffer, and filter the cells through a 70 μm mesh nylon strainer, then count the cells. Adjust the cell density to 2×10^8 cells/mL.

Note: Generally, about $2\text{-}4\times10^8$ splenocytes can be obtained from each mouse.

- **Cell Sorting**

a) Prepare 50 μL of cell suspension (about 1×10^7 cells), add 5.4 μL Mouse Naïve $CD8^+$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 50 μL isolation buffer.

c) Wash Beads Streptavidin 1.0-N: Vortex beads for 20 seconds, add 13.5 μL Beads in 1.5 mL EP tube. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 30 s. Remove the supernatant by magnetic separation, then resuspend beads with 1 mL isolation buffer, and stand for 5 minutes at room temperature. Remove the supernatant by magnetic separation, then resuspend Beads with 13.5 μL isolation buffer.

d) Transfer the cells to the bottom of the flow tube (Note: Avoid adding along tube walls), add 13.5 μL washed Mouse Naïve $CD8^+$T Beads Streptavidin 1.0-N, mix fully and incubate at room temperature for 5 min.

Note:

- ✧ If more than 1×10^7 cells are to be sorted, increase the amount of Mouse Naïve $CD8^+$T Cell Isolation Cocktail and Mouse Naïve $CD8^+$T Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains 2×10^8 cells/mL. If fewer than 1×10^7 cells are to be sorted, resuspend the cells with 50 μL sorting buffer, add 5.4 μL Mouse Naïve $CD8^+$T Cell Isolation Cocktail and 13.5 μL washed Mouse Naïve $CD8^+$T Beads Streptavidin 1.0-N.
- ✧ The 5 mL flow tube is suitable for $\leq2\times10^8$ cells.

e) Add isolation buffer to a final volume of 2.5 mL, mix fully 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, this is the Naïve $CD8^+$T cells obtained from the first isolation. Add isolation buffer to a final volume of 2.5 mL into the flow tube, mix 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.

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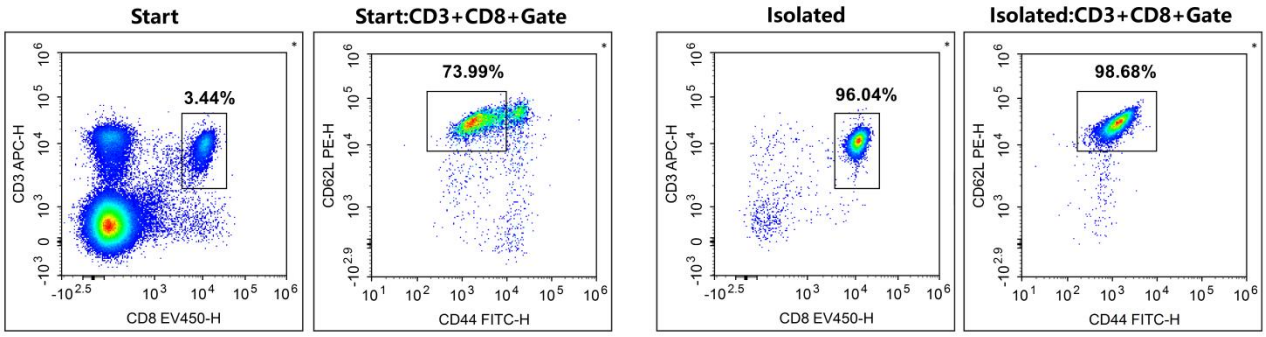
g) Transfer the cell suspension to the centrifuge tube in step f, mix the cell suspension obtained from step e and f, centrifuge at 300 g for 5 min. Discard the supernatant, resuspend the cells with isolation buffer required for the subsequent experiments.

Typical data



Naïve CD8$^+$T(CD3$^+$CD8$^+$CD44$^{-/low}$CD62L^{high}) cells were isolated from the spleen cells of C57BL/6 mice, and were stained with APC Anti-Mouse CD3ε Antibody[145-2C11](E-AB-F1103E)、Elab Fluor® Violet 450 Anti-Mouse CD8a Antibody[53-6.7](E-AB-F1104Q)、FITC Anti-Mouse CD44 Antibody[NIM-R8](AN00917C) and PE Anti-Mouse CD62L Antibody[MEL-14](E-AB-F1011D). The purities of start and final isolated fractions were 2.55% and 94.77%, respectively.

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 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.
5. Cell suspension should be isolation immediately after preparation, the longer the storage time, the greater the impact on cell activity.
6. The cell suspension and magnetic beads 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.
7. 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.
8. It is recommended to use low adsorption pipette tips and centrifuge tubes to avoid the loss of magnetic beads and antibodies due to adsorption.
9. The kit should be used in conjunction with a magnetic rack.
10. Sample type, sample preparation and experimental operation have an important impact on the final isolation cell purity.

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