EasySort™ Mouse Naïve CD8⁺T Cell Isolation Kit

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

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIM008NA	EasySort™ Mouse Naïve CD8⁺T Cell 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	60 μL	600 μL	600 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

The EasySort™ Mouse Naïve CD8⁺T Cell Isolation Kit is a product that enables rapid and simple isolation of high-purity mouse Naïve CD8⁺T cells. This kit uses a negative selection method and is suitable for isolating Naïve CD8⁺T cells from mouse spleen or lymph node samples. Different biotinylated monoclonal antibodies are used to label non-target cells (non-mouse naïve CD8⁺ T cells). Subsequently, streptavidin-conjugated magnetic beads are employed to deplete these non-target cells, thereby obtaining highly purified mouse Naïve CD8⁺T cells. The isolated mouse Naïve CD8⁺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™ Mouse Naïve CD8⁺T cell Isolation Kit has been tested by magnetic cell separation followed by flow cytometric analysis of cells from mouse spleen or lymph node tissues. An assay is defined as 6 μL antibody and 13.5 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

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, 5cm Culture Dish

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) .

- **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 70μm mesh nylon strainer, then count the cells. Adjust the cell density to 2×10^8 cells/mL.

Note: Generally, approximately $2\text{-}4\times10^8$ splenocytes can be obtained from each mouse.After preparing a single-cell suspension from mouse spleen 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 50 μL of cell suspension (about 1×10^7 cells), add 6 μL Mouse Naïve $CD8^+$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 make sure the cells are single-cell suspension; cell suspension volume should more than 50 μL.

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.

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 50 μL of cell suspension is used as the starting input, cells should be resuspended with an equal volume of 50 μL cell isolation buffer.

c) Wash Mouse Naïve $CD8^+$T Cell 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 13.5 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.

d) 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.

e) 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 Mouse Naïve $CD8^+$T Cell Isolation Cocktail and Mouse Naïve $CD8^+$T Cell 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 isolated, resuspend the cells with 50 µL isolation buffer, add 6 µL Mouse Naïve $CD8^+$T Cell Isolation Cocktail and 13.5 µL washed Mouse Naïve $CD8^+$T Cell Beads Streptavidin 1.0-N.
- The 5 mL flow tube is suitable for isolation of cell suspension ≤ 1 mL (2×10^8 cells). 10 mL or 15 mL centrifuge tube is suitable for isolation of cell suspension ≤ 4 mL (8×10^8 cells).

f) 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.

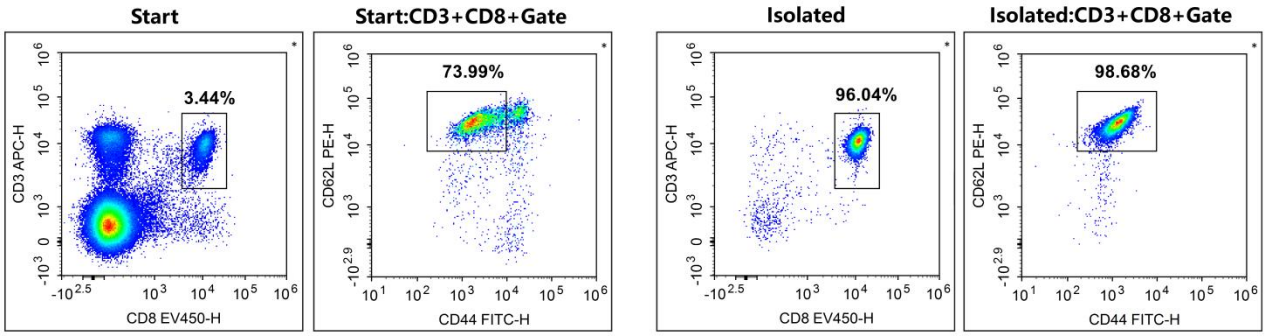
g) Keep the sample on the magnetic rack, transfer the cell suspension to another clean centrifuge tube. This yields the mouse Naïve $CD8^+$T cells from the first time isolation.

h) 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.

i) Keep the sample on the magnetic rack, transfer the cell suspension to the centrifuge tube from step f), mix the mouse Naïve $CD8^+$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.

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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. 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.
5. Perform isolation immediately after preparing the cell suspension, as cell viability will decrease with longer storage time.
6. 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.
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 isolated cell purity.

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