EasySort™ Mouse Memory CD4⁺T Cell Isolation Kit

Cat. No: MIM009N **Size: 10 Assays/100 Assays/200 Assays**

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIM009NA	EasySort™ Mouse Memory CD4⁺T Beads Streptavidin 1.0-N	500 μL	1.25 mL×4	1.25 mL×8	2-8°C
MIM009NB	EasySort™ Mouse Memory CD4⁺T Cell Isolation Cocktail	180 μL	0.9 mL×2	0.9 mL×4	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 memory CD4⁺T cell Isolation Kit is a product that enables rapid and simple isolation of high-purity mouse memory CD4⁺T cell. This kit uses a negative selection method and is suitable for isolating memory CD4⁺T from mouse spleen and lymph node samples. The principle involves labeling non-target cells (non-memory CD4⁺T) with a combination of biotin-conjugated monoclonal antibodies, followed by depletion of these cells using streptavidin-labeled magnetic beads, thereby obtaining highly purified mouse memory CD4⁺T cells. The isolated mouse memory CD4⁺T cell 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 memory CD4⁺T cell Isolation Kit has been tested by magnetic cell separation followed by flow cytometric analysis of cells from mouse spleen or lymph nodes. A assay is defined as 18 μL antibody and 50 μL beads to be used to isolate $4x10^7$ 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

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% 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, approximately $2\text{-}4\times10^8$ spleen cells can be obtained per 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 Sorting**

a) Prepare 200 μL of cell suspension (about 4×10^7 cells), add 18 μL Mouse Memory $CD4^+$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.

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 200 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 200 μL of cell suspension is used as the starting input, cells should be resuspended with an equal volume of 200 μL cell isolation buffer.

c) Wash Mouse memory $CD4^+$T 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 50 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

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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 4×10^7 cells are to be sorted, increase the amount of Mouse Memory $CD4^+$T Cell Isolation Cocktail and Mouse Memory $CD4^+$T Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains 2×10^8 cells/mL. If fewer than 4×10^7 cells are to be sorted, resuspend the cells with 200 µL sorting buffer, add 18 µL Mouse Memory $CD4^+$T Cell Isolation Cocktail and 50 µL washed Mouse Memory $CD4^+$T 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).

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) Keep the sample on the magnetic rack, transfer the cell suspension to another clean centrifuge tube. This yields the mouse memory $CD4^+$T cells 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 mouse memory $CD4^+$T cells 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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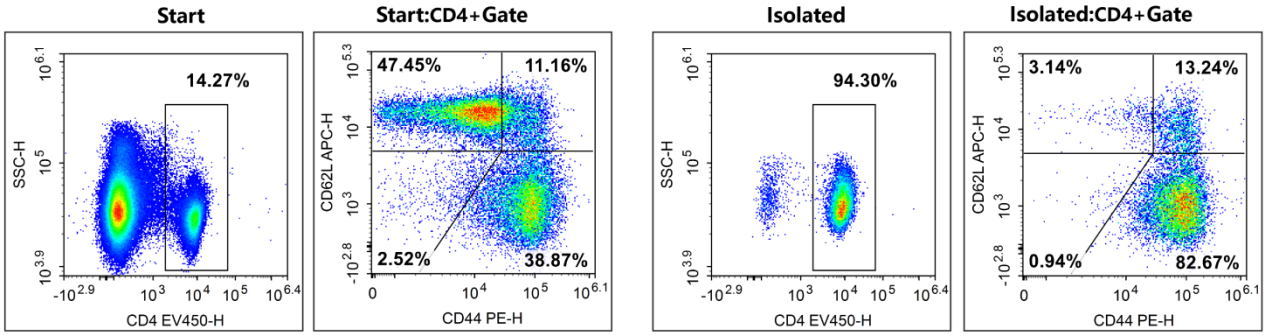
Typical data



In the above example,The purity of memory CD4⁺T cells (CD4⁺CD62L^{high}CD44$^{high/int}$ central memory and CD4⁺CD62L-CD44$^{high/int}$ effector memory) from normal C57BL/6 spleen samples was 7.14% before isolation and 90.44% after isolation, as analyzed by flow cytometry using the antibodies listed in the table below.

Fluorochrome-conjugated antibody	Cat	Company
EV450 Anti-Mouse CD4 Antibody [RM4-5]	E-AB-F1353Q	Elabscience
PE Anti-Human/Mouse CD44 Antibody[IM7]	E-AB-F1100D	Elabscience
APC Anti-Mouse CD62L Antibody[MEL-14]	E-AB-F1011E	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. 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 isolation cell purity. The purity of the cells in this product is obtained after sorting normal mouse spleen samples.

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