EasySort™ Mouse Pan-Naïve T Cell Isolation Kit

Cat. No: MIM006N **Size: 10Assays/100Assays/200Assays**

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIM006NA	EasySort™ Mouse Pan-Naïve T Cell Beads Streptavidin 1.0-N	100 μL	1 mL	1 mL×2	2-8°C
MIM006NB	EasySort™ Mouse Pan-Naïve T Cell Isolation Cocktail	30 μL	300 μL	600 μL	2-8°C
	Manual	1 copy			

Storage

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

Description

The EasySort™ Mouse Pan-Naïve T Cell Isolation Kit is a product that enables rapid and simple isolation of high-purity mouse mouse pan-naïve T cells. This kit uses a negative selection method and is suitable for isolating mouse pan-naïve T cells from mouse spleen or lymph node samples. Different biotinylated monoclonal antibodies are used to label non-target cells (non-mouse pan-naïve T cells). Subsequently, streptavidin-conjugated magnetic beads are employed to deplete these non-target cells, thereby obtaining highly purified mouse naïve T cells . The mouse naïve T cells isolated using this kit are free of any antibody and magnetic bead labeling, maintaining an unstimulated, original state suitable for direct downstream applications.

EasySort™ Mouse Pan-Naïve T Cell Isolation Kit has been validated by magnetic separation using mouse spleen or lymph node samples, with post-sorting cells analyzed and identified by flow cytometry. The single-experiment system of this kit uses 10 μL of magnetic beads and 3 μL of antibody cocktail, sufficient to meet the isolation and sorting needs of 1×10^7 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:**

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

Optical microscope, centrifuge, magnetic rack

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

- **Cell Isolation**

a) Prepare 50 μL of cell suspension (about 1×10^7 cells), add 3 μL Mouse Pan-Naïve 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.

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 Pan-Naïve 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 10 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

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

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 Mouse Pan-Naïve T cell Isolation Cocktail and Mouse Pan-Naïve 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 3 µL Mouse Pan-Naïve T cell Isolation Cocktail and 10 µL washed Mouse Pan-Naïve 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).

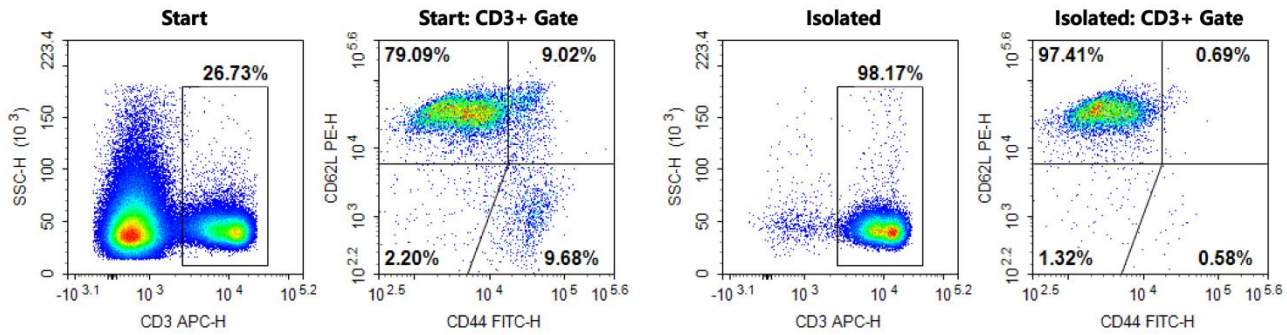
e) Add isolation buffer to a final volume of 2.5 mL(If the volume of the cell suspension for isolation is >1 mL, resuspend in an equal volume of isolation buffer), mix fully with a pipette by blowing up and down for 6-8 times until no particles of magnetic beads are visible. Put the tube on a 5 mL magnetic 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) Transfer the cell suspension to a clean centrifuge tube, centrifuge at 300 g for 5 min. Discard the supernatant, resuspend the cells with isolation buffer required for the subsequent experiments.

Note: Please repeat step e) -Step f) and mix the cell suspension obtained by twice if the experiment requires a high recovery rate.

Typical data



In the above example,the purity of C57BL/6 mouse splenocytes was analyzed by flow cytometry using the antibodies listed in the table below. The purity of naïve T cells ($CD3^+CD44^{-/low}CD62L^{high}$) in normal mouse spleen samples before and after sorting was 21.15% and 95.63%, respectively.

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

检测抗体	货号	厂家
APC Anti-Mouse CD3 Antibody[17A2]	E-AB-F1013E	Elabscience
PE Anti-Mouse CD62L[MEL-14]	E-AB-F1011D	Elabscience
FITC Anti-Mouse CD44[NIM-R8]	AN00917C	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. 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.

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