EasySort™ Mouse CD4 Nanobeads

Cat. No: MIM011P **Size: 10 Assays/200 Assays**

Component	Component Name	10 Assays	200 Assays	Storage
MIM011P	EasySort™ Mouse CD4 Nanobeads	100 μL	1 mL×2	2-8°C
	Manual	1 copy		

Storage

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

Description

EasySort™ Mouse CD4 Nanobeads are designed for the isolation of high-purity $CD4^+$ cells from single-cell suspensions derived from mouse spleen, lymph nodes, and other tissues via positive selection. This product consists of superparamagnetic nanobeads covalently conjugated to an anti-mouse CD4 monoclonal antibody, which specifically recognizes and labels the CD4 antigen on the surface of target cells. Add the labeled cell suspension to a sorting column placed within a magnetic field. Under the influence of the magnetic field, the $CD4^+$ cells labeled with the magnetic beads are retained within the sorting column, while non-target cells ($CD4^-$ cells) flow out with the flow through. After removing the magnetic field, elute the sorting column with the isolation buffer to collect the $CD4^+$ cells retained on the column, thereby achieving the isolation of the target cell population.

EasySort™ Mouse CD4 Nanobeads have been validated for magnetic sorting of mouse spleen and lymph node samples, with the sorted cells analyzed and identified using flow cytometry. For a single experiment with this kit, 10 μL of magnetic beads is sufficient to isolate and sort 1×10^7 cells.

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, 5cm Culture Dish

3. **Instrument:**

   Optical microscope, horizontal centrifuge, magnetic Separator, MS columns, LS columns

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

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 and filter the prepared buffer with 0.22 μm filter.

  NOTE: Sealed store the prepared buffer at 4°C and use within 1 week.

- **Mouse spleen single cell suspension preparation**

1. Take the fresh mouse spleen, taking care to remove excessive connective tissue attached.
2. 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, then centrifuge at 300 g for 5 min.
3. 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 1×10^8 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 100 μL of cell suspension (about 1×10^7 cells), add 10 μL EasySort™ Mouse CD4 Nanobeads, gently pipette up and down 6-8 times with a pipette to mix, then incubate for 10 min at room temperature.

   Note: Please make sure the cells are single-cell suspension.

b) After incubation, add 500 μL isolation buffer and mix well (MS column: 500 μL; LS column: 1 mL) to prepare for magnetic sorting.

c) Place the cell sorting column in the appropriate sorting magnetic separator. Before passing the cell suspension through the column, first rinse it once with 500 μL isolation buffer (MS column: 500 μL; LS column: 2 mL) along the sidewall of the sorting column. Wait until the liquid in the column has completely drained, then add the cell suspension prepared in step (b). Wait until the liquid in the column has completely drained, then rinse three times with 500 μL isolation buffer (MS column: 500 μL; LS column: 2 mL).

   Note:

- To prevent cell aggregates from forming during incubation and clogging the sorting column, it is recommended to filter the cell suspension through a 70 μm cell strainer before loading it onto the column.
- When loading the sample, ensure that the liquid added to the sorting column is free of bubbles.
- During sample loading and each wash cycle, be sure to wait until the liquid in the column has completely drained (leaving only the last drop suspended beneath the bottom of the column membrane) before proceeding to the next step.

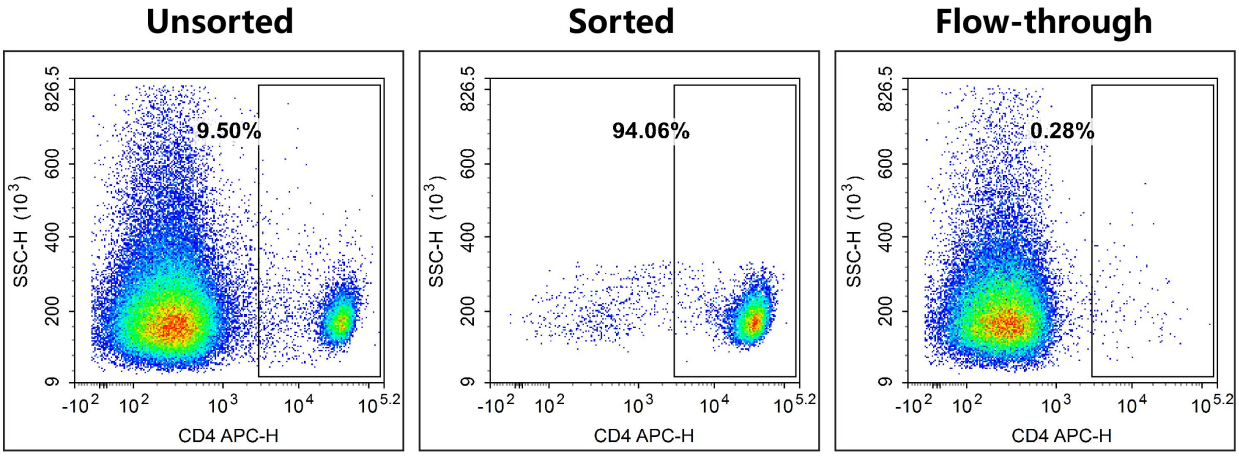
d) After the rinsing is completed, quickly remove the column from the magnetic field, add 1 mL

isolation buffer (1 mL for MS column; 2 mL for LS column), and immediately use the piston that comes with the column to rapidly push the cell suspension in the column into a 15 mL centrifuge tube. These are the mouse CD4⁺ cells.

e) (Optional) If you need to increase the cell yield, slowly and completely withdraw the piston supplied with the column, then add another 1 mL isolation buffer (MS column: 1 mL; LS column: 2 mL), and immediately use the piston provided with the column to quickly push the cell suspension from the column into the 15 mL centrifuge tube prepared in step d). Mix the cell suspensions obtained from the two sorting runs; These are the mouse CD4⁺ cells.

f) Centrifuge the suspension of sorted mouse CD4⁺ cells at 300 g for 5 minutes, discard the supernatant, resuspend the cells in the buffer required for subsequent experiments, and use them directly for cell culture or other downstream experiments.

Typical data



As shown in the above figure, CD4⁺ cells were sorted from spleen cells of C57BL/6 mice. Flow cytometric analysis was performed on the mouse spleen cells before and after sorting using the APC Anti-Mouse CD4 Antibody [RM4-4] (AN00417E). Left figure: The proportion of CD4⁺ cells before sorting was 9.50%; Middle figure: The proportion of CD4⁺ cells obtained after sorting was 94.06%; Right figure: The proportion of residual CD4⁺ cells in the flow-through fraction after sorting was 0.28%.

Note: When assessing the purity of CD4 cells after sorting, we recommend using the CD4 antibody with clone number RM4-4; clones GK1.5 and RM4-5 have been verified as unsuitable for analyzing the purity of cells sorted using this product.

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

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. Cell suspension should be isolated immediately after preparation. The longer the storage time, the greater the impact on cell activity.
6. The cell suspension and the nanobeads should be added directly to the bottom of the EP tube to avoid sticking to the wall, resulting in insufficient reaction and affecting the isolation efficiency.
7. All steps, except the room-temperature incubation, should be performed on ice whenever possible to maintain cell viability.
8. This kit should be used in conjunction with the magnetic Separator and columns.
9. Sample differences, sample preparation and experimental operation have an important impact on the final isolated cell purity. The cell purity of this product is determined based on the results obtained after sorting spleen samples from normal mice.
10. Follow the instructions to control the total number of cells (no more than 2×10^8 for MS columns and no more than 2×10^9 for LS columns); do not overload the column. If the total number of cells is too large, please pass them through the column in multiple batches.

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