

Mouse CD3+ Cell Separation Kit (Column Based,Positive) Instructions

Product Information

Product Name	Model	Product Components	Specification
Mouse CD3+ Cell Separation Kit-100T (Column Based,Positive)	K1301-10	Anti-Mouse CD3 Biotin Antibody	1 mL
		Streptavidin NanoBeads	1 mL
Mouse CD3+ Cell Separation Kit-20T (Column Based,Positive)	K1301-10T	Anti-Mouse CD3 Biotin Antibody	200 µL
		Streptavidin NanoBeads	200 µL

Product Description

The Mouse CD3+ Cell Separation Kit (Column Based,Positive) (the “Kit”) separates CD3+ cells from spleen, thymus and lymph node tissues of mice via column-based magnetic separation. CD3+ cells are labeled with biotin antibody and streptavidin nanobeads, then are separated via a magnetic separation column.

The Kit enables rapid and easy separation of CD3+ cells from single cell suspensions. Featuring small particle size and good biocompatibility, it ensures minimal interference with downstream applications. The purified CD3+ cells can be directly used for applications including flow cytometry, next-generation sequencing (NGS) and cell culture.

Test Capacity

- ✧ K1301-10 can process up to 1*10⁹ of total cells.
- ✧ K1301-10T can process up to 2*10⁸ of total cells.

Storage & Transportation

- ✧ The Kit should be transported at 2 ~ 8°C.
- ✧ The Kit should be stored at 2 ~ 8°C. Protect from light. Do not freeze.
- ✧ The Kit is valid for 12 months from the date of manufacture.

Consumables & Instruments

Reagents	Consumables	Instruments
Buffer	LarSep Columns (RWD: #HCSC-25)	Refrigerated Microcentrifuge (RWD)
Red Blood Cell Lysis Buffer	Magnetic Pole (RWD: #LSC-S1)	Fluorescent Automated Cell Counter (RWD)
	Separation Support (RWD: #SS01)	Single Cell Suspension Dissociator (RWD)

Operation

Preparation before Experiment

- Preparation of Buffer: Filter PBS (pH 7.2 ~ 7.4, containing 0.5% BSA and 2 mM EDTA) through a 0.22 µm membrane under 2 ~ 8°C for sterilization and store at 2 ~ 8°C.
⚠ Note: PBS containing Ca²⁺or Mg²⁺ is not recommended.

Sample Preparation

- For peripheral blood samples, isolate peripheral blood mononuclear cells (PBMCs) by density gradient centrifugation;

For solid tissue samples (e.g., spleen and lymph node), before cell separation (cell enrichment), dissociate the tissue into a single cell suspension using the single cell suspension dissociator.

- Wash the 40 µm cell strainer with pre-cooled buffer, filter the sample through the cell strainer and collect the cell suspension to a 50 mL centrifuge tube. Centrifuge the suspension at 500×g, 4°C for 5 min. After centrifugation, discard the supernatant and place the suspension at 2 ~ 8°C for short-term preservation.
⚠ Note: Excessive red blood cells, dead cells and cell clumps may affect the separation efficiency. It is recommended to perform red blood cell lysis and removal of dead cells and cell clumps.

Cell Labeling

⚠ Note: Quickly process cells using pre-cooled buffer and incubate at 2 ~ 8°C, keeping cells cold throughout. The recommended reagent volumes below can process 1*10⁷ of total cells. If the cell count is less than 1*10⁷, use the same volume as for 1*10⁷ cells. If the cell count exceeds 1*10⁷, increase the reagent volumes proportionally. During incubation of antibody and nanobeads, gently mix the cell suspension every 5 min.

- Cell Concentration Adjustment:** Adjust the cell concentration to 1*10⁸ cells/mL.
- Antibody Incubation:** Add 100 µL of cell suspension (1*10⁷ cells) to the centrifuge tube (or flow cytometry tube). Add 10 µL of anti-mouse CD3 biotin antibody to the tube, mix the suspension evenly and incubate at 2 ~ 8°C for 10 min.
- Washing:** After incubation, wash the cells with 1 ~ 2 mL of buffer, and centrifuge the suspension at 500×g, 4°C for 5 min. After centrifugation, discard the supernatant and add 100 µL of buffer to resuspend the cells.
- NanoBeads Incubation:** Add 10 µL of streptavidin nanobeads to the cell suspension, mix evenly and incubate at 2 ~ 8°C for 15 min.
- Washing:** After incubation, wash the cells with 1 ~ 2 mL of buffer, and centrifuge the suspension at 500×g, 4°C for 5 min. After centrifugation, discard the supernatant and add appropriate buffer to resuspend the cells for cell separation.

⚠ Note: If the cell count is less than 1.25*10⁸, resuspend the cells with 0.5 ~ 1 mL of buffer. If the cell count exceeds 1.25*10⁸, increase the buffer volume proportionally. If cell clumps are still present after resuspension, filter the cells through the 40 µm cell strainer before magnetic cell separation.

Cell Separation

- Preparation:** Install the separation support and magnetic pole, and place the separation column in the magnetic pole.
- Wetting:** Place a 15 mL centrifuge tube under the separation column, add 2 ~ 3 mL of buffer to the column, and collect the effluent.
⚠ Note: The pre-wetted column should be used immediately after preparation.
- Column Loading:** After the buffer has completely drained, discard the effluent and replace with a new 15 mL centrifuge tube. Load the nanobeads-labeled cell suspension onto the separation column and allow the suspension to flow through passively. Then, collect the effluent containing unlabeled cells to the tube.
⚠ Note: Before loading the buffer or cell suspension at each step, ensure that the solution previously loaded to the separation column has completely drained (no continuous drops falling from the bottom of the column).
- Washing:** Wash the separation column with 2 ~ 3 mL of buffer, repeat 2 ~ 3 times and collect the effluent to the centrifuge tube mentioned in step (3). After collection, remove the tube and label it as “Negative Fraction”.
- Collection:** Remove the separation column from the magnetic field and place a new 15 mL centrifuge tube underneath the column. Add 1 ~ 2 mL of buffer to the column and press the matching piston down. Label the

tube as “Positive Fraction”, in which CD3+ cells are contained in the collected suspension.

(Optional) To improve purity of magnetically labeled cells, reload the collected CD3+ cells (Positive Fraction) through a new separation column, repeating step (2) to (5).

Results

Label the CD3+ cells of mice with Mouse CD3+ Cell Separation Kit (Column Based,Positive) in combination with LarSep columns (RWD: #HCSC-25) and magnetic pole (RWD: #LSC-S1), and separate the CD3+ cells from spleen of C57 mice, as shown in Figure (a). Fluorescently stain the cells with PE anti-mouse CD3 Antibody (Clone: #17A2) and analyze by flow cytometry using Beckman Coulter (BECKMAN: #CytoFLE X).

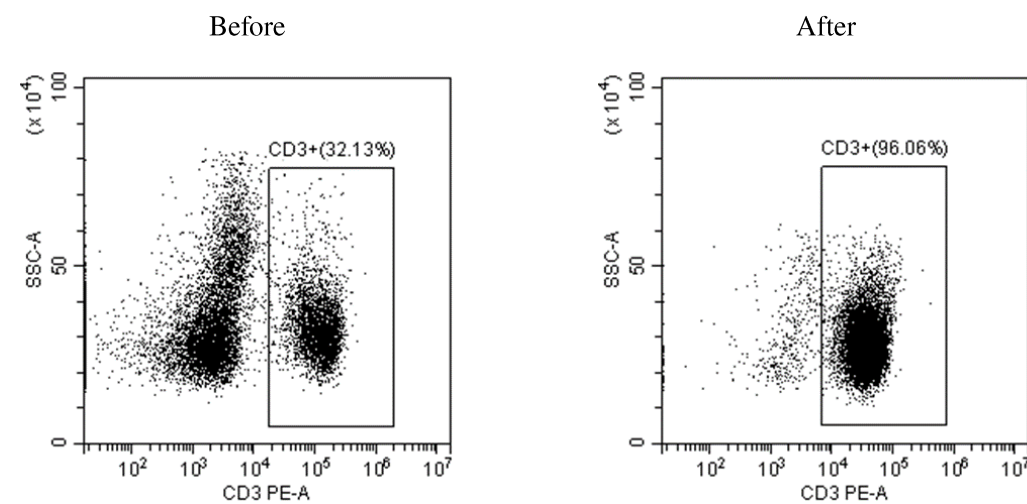


Figure (a) Before vs. After Separation

Precautions

- (1) The Kit is for scientific research only, not intended for any diagnostic or therapeutic use.
- (2) The Kit is valid for 12 months and RWD shall not guarantee the validity of expired products.
- (3) Ensure that all steps are performed under sterile conditions.
- (4) Do not mix kits of different models/lots.

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RWD Life Science Co., Ltd.

Add: 10410 Corporate Drive, Sugar Land, TX 77478, USA

Add: (Floor 9, 19&20 Building 7A, Floor 9 Building 7D) Room 1901, Building 7A, International Innovation Valley, Dashi 1st Road, Xili Community, Nanshan District, Shenzhen 518000, Guangdong, P. R. China

Web: www.rwdstco.com

E-Mail: service@rwdls.com

Tel: 0086-755-86111281

001-858-900-6602(USA)