Streptavidin NanoBeads (Column Based,Universal) Instructions

Product Information

Product Name	Model	Product Components	Specification
Streptavidin NanoBeads-100T (Column Based,Universal)	K1200-10	Streptavidin NanoBeads	1 mL
Streptavidin NanoBeads-20T (Column Based,Universal)	K1200-10T	Streptavidin NanoBeads	200 μL

Product Description

The Streptavidin NanoBeads (Column Based,Universal) (the "Kit") can be paired with biotin antibodies targeting any epitope, enabling rapid and easy separation of cells expressing the specific epitope from single cell suspensions via column-based magnetic separation. Featuring small particle size and good biocompatibility, it ensures minimal interference with downstream applications. The purified target cells can be directly used for applications including flow cytometry, next-generation sequencing (NGS) and cell culture.

Test Capacity

- ✧ K1200-10 can process up to $1*10^9$ of total cells.
- ✧ K1200-10T can process up to $2*10^8$ 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

(1) 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 Ca^{2+}or Mg^{2+} is not recommended.

Sample Preparation

(1) 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 (enrichment), dissociate the tissue into a single cell suspension using the single cell suspension dissociator.

(2) 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 $1*10^7$ of total cells. If the cell count is less than $1*10^7$, use the same volume as for $1*10^7$ cells. If the cell count exceeds $1*10^7$, increase the reagent volumes proportionally. During incubation of antibody and nanobeads, gently mix the cell suspension every 5 min.

(1) **Cell Concentration Adjustment:** Resuspend the cells with appropriate volume of buffer (the volume is related to the working concentration of antibody incubation), and it is recommended that the cell concentration be adjusted to $1*10^8$ cells/mL.

(2) **Antibody Incubation:** Add 100 μL of cell suspension ($1*10^7$ cells) to the centrifuge tube (or flow cytometry tube), and add the amount of biotin antibody recommended by manufacturer, or perform antibody titration test in advance to determine the most applicable amount. Mix the suspension evenly and incubate at 2 ~ 8°C for 10 min.

(3) **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 90 μL of buffer to resuspend the cells.

(4) **NanoBeads Incubation:** Add 10 μL of streptavidin nanobeads to the cell suspension, mix evenly and incubate at 2 ~ 8°C for 15 min.

(5) **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 $1.25*10^8$, resuspend the cells with 0.5 ~ 1 mL of buffer. If the cell count exceeds $1.25*10^8$, increase the buffer volume proportionally. If cell clumps are still present after resuspension, filter the cells through the 40 μm cell strainer before magnetic separation.

Cell Separation

(1) **Preparation:** Install the separation support and magnetic pole, and place the separation column in the magnetic pole.

(2) **Wetting:** Place a 15 mL centrifuge tube under the separation column, add 4 mL of buffer to the column, and collect the effluent.

⚠ Note: The pre-wetted column should be used immediately after preparation.

(3) **Column Loading:** After the buffer has completely drained, discard the effluent and replace with a new 15 mL centrifuge tube. Load the nanobead-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).

(4) **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".

(5) **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

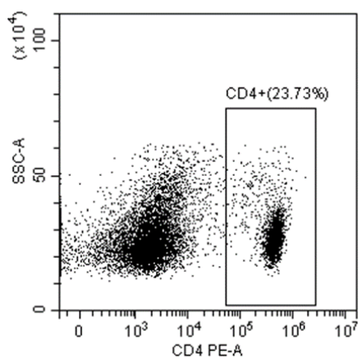
tube as “Positive Fraction”, in which cells expressing the specific epitope are contained in the collected suspension.

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

Results

Label the CD4+ cells of mice with Streptavidin NanoBeads (Column Based,Universal) in combination with LarSep columns (RWD: #HCSC-25) and magnetic pole (RWD: #LSC-S1), and separate the CD4+ cells from spleen of C57 mice, as shown in Figure (a). Fluorescently stain the cells with PE anti-mouse CD4 Antibody (Clone: #GK1.5) and analyze by flow cytometry using Beckman Coulter (BECKMAN: #CytoFLE X).

Before



After

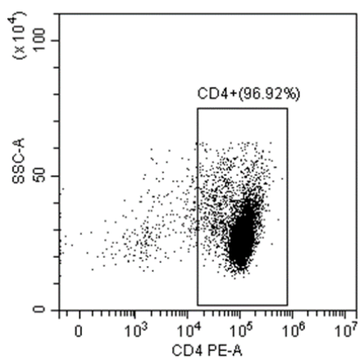


Figure (a) Before vs. After Separation

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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