Human NK Cell Isolation Kit (Column Based,Negative) Instructions

Product Information

Product Name	Model	Product Components	Specification
Human NK Cell Isolation Kit-100T (Column Based,Negative)	K1207-10	Human NK Cell Isolation Cocktail	2 mL
		Streptavidin NanoBeads	2 mL
Human NK Cell Isolation Kit-10T (Column Based,Negative)	K1207-10T	Human NK Cell Isolation Cocktail	200 µL
		Streptavidin NanoBeads	200 µL

Product Description

The Human NK Cell Isolation Kit (Column Based,Negative) (the "Kit") isolates NK cells from human PBMCs via column-based cell isolation. Non-NK cells are labeled with isolation cocktail and streptavidin nanobeads, then are isolated via a magnetic separation column to obtain the target NK cells.

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

Test Capacity

✧ K1207-10 can process up to 1×10^9 of total cells.

✧ K1207-10T can process up to 1×10^8 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 Red Blood Cell Lysis Buffer	LS Columns (RWD: #HCSC-25) Magnetic Pole (RWD: #LSC-S1) Separation Support (RWD: #SS01)	Refrigerated Microcentrifuge (RWD) Fluorescent Automated Cell Counter (RWD) 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 at 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 isolation (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 into a 50 mL centrifuge tube. Centrifuge the suspension at 4°C, 500×g 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 isolation 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 the cells 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 the antibody and nanobeads, gently mix the cell suspension every 5 min.

(1) **Cell Concentration Adjustment:** Adjust the cell concentration 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). Add 20 µL of human NK cell isolation cocktail to the tube, mix the suspension evenly and incubate the suspension at 2 ~ 8°C for 5 min.

(3) **NanoBeads Incubation:** Add 20 µL of streptavidin nanobeads to the cell suspension, mix evenly and incubate at 2 ~ 8°C for 10 min.

(4) **Washing:** After incubation, resuspend the cells with 1 ~ 2 mL of buffer, mix evenly for ***cell isolation***.

⚠ 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 isolation.

Cell Isolation

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

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

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

(3) **Column Loading:** After the buffer has completely drained, discard the effluent and replace the tube with a new 15 mL centrifuge tube. Load the nanobead-labeled cell suspension onto the LS column and allow the suspension to flow through passively. Then, collect the effluent containing unlabeled cells into the tube.

⚠ Note: Before loading the buffer or cell suspension at each step, ensure that the solution previously loaded onto the LS column has completely drained (no continuous drops falling from the bottom of the column).

(4) **Washing:** Wash the LS column with 2 ~ 3 mL of buffer, repeat 2 ~ 3 times, and collect the effluent into the centrifuge tube mentioned in step (3). Label the tube as "Negative Fraction", which contains the NK cells.

(Optional) To improve the purity of target cells, reload the collected NK cells (Negative Fraction) onto a new LS column, repeating steps (2) to (4).

Results

Isolate the NK cells from the human PBMCs using the Human NK Cell Isolation Kit (Column Based,Negative) in combination with the LS columns (RWD: #HCSC-25) and magnetic pole (RWD: #LSC-S1), as shown in Figure (a).Fluorescently stain the cells with APC anti-human CD3 Antibody (Clone: #HIT3a) and PE anti-human CD56

(NCAM) Antibody (Clone: #HCD56), and analyze by flow cytometry using Beckman Coulter (BECKMAN: #CytoFLE X).

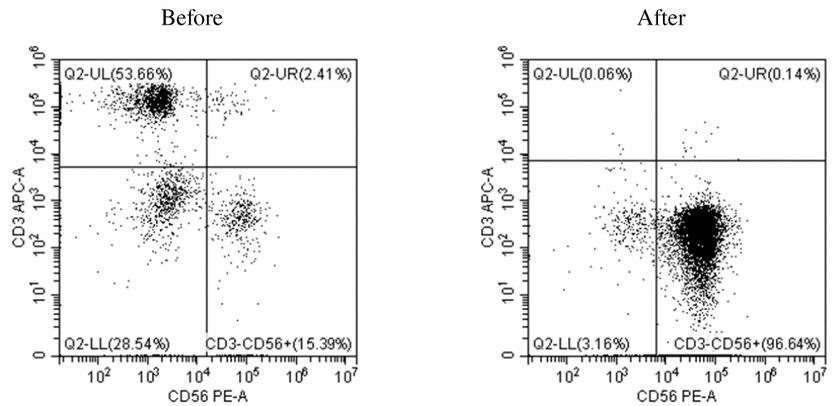

Figure (a) Before vs. After Isolation

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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