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

Product Information

Product Name	Model	Product Components	Specification
Human NK Cell Isolation Kit-100T (Column Free,Negative)	F1401-10	Human NK Cell Isolation Cocktail	2 mL
		Streptavidin MicroBeads	2 mL
Human NK Cell Isolation Kit-10T (Column Free,Negative)	F1401-10T	Human NK Cell Isolation Cocktail	200 µL
		Streptavidin MicroBeads	200 µL

Product Description

The Human NK Cell Isolation Kit (Column Free,Negative) (the "Kit") is designed for labeling the non-NK cells using isolation cocktail and streptavidin microbeads, and removing the labeled cells via the RWD magnetic pole, achieving column-free cell isolation.

The Kit enables rapid, easy, and high-yield isolation of NK cells from peripheral blood mononuclear cells, spleen or lymph node tissue of humans. The isolated cells are free of antibody and microbeads labeling, can be directly used for downstream applications including flow cytometry and cell culture.

Test Capacity

- ✧ F1401-10 can process up to $1*10^9$ of total cells.
- ✧ F1401-10T 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	Magnetic Pole (RWD: #CFMS-S1)	Refrigerated Microcentrifuge (RWD)
High Efficiency Debris Removal Kit (RWD)	5 mL Flow Cytometry Tube	Fluorescent Automated Cell Counter (RWD)
	40 µm Cell Strainer	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+} or biotin 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 (cell enrichment), dissociate the tissue into a single cell suspension using the single cell suspension dissociator.

(2) Filter the cell suspension through the 40 µm cell strainer to remove debris and cell clumps.

⚠ Note: It is not recommended to perform red blood cell lysis, which may compromise the cell viability and surface antigen integrity. Since the dead cells may have non-specific binding with antibody or microbeads, it is recommended to perform density gradient centrifugation or use High Efficiency Debris Removal Kit (RWD: #DHDR-5006) to remove dead cells.

Cell Labeling

⚠ Note: 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.

(1) **Cell Concentration Adjustment:** Adjust the cell concentration to $1*10^8$ cells/mL.

(2) **Antibody Labeling:** Add 100 µL of sample and 20 µL of human NK cell isolation cocktail to the 5 mL flow cytometry tube. Mix them evenly and incubate at room temperature for 5 min.

⚠ Note: Add no more than 1 mL of sample per tube (cell count not exceeding $1*10^8$).

(3) **MicroBeads Labeling:** Add 20 µL of streptavidin microbeads to the sample.

⚠ Note: Vortex or pipette to mix the sample before adding the microbeads.

(4) **Volume Standardization:** Add the buffer to adjust the total sample volume to 2.5 mL. Mix gently and incubate at room temperature for 2 min.

⚠ Note: For optimal labeling efficiency, gently mix the suspension 2 ~ 3 times during incubation.

Cell Isolation

(1) Insert the flow cytometry tube containing sample to the magnetic pole for magnetic capture at room temperature for 3 min.

⚠ Note: Ensure that the liquid in the tube is completely within the magnetic field of the magnetic pole.

(2) Take out the magnetic pole and quickly pour the sample to a new 5 mL flow cytometry tube. After pouring, pull out the tube from the magnetic pole.

⚠ Note: Do not pour out the last drop of liquid. It may impact the cell purity.

⚠ Note: Do not pull out the tube from the pole until the pouring is complete. After pouring, directly discard the remaining liquid in the tube.

Results

Isolate the NK cells of humans from the PBMCs of humans using the Human NK Cell Isolation Kit (Column Free,Negative) in combination with the RWD magnetic pole (RWD: #CFMS-S1). The isolated cells are labeled with APC anti-human CD3 Antibody (Clone: #HIT3a) and PE anti-human CD56 (NCAM) Antibody (Clone: #HCD56) followed by flow cytometric analysis. The percentages of NK cells (CD3-CD56+) before and after isolation are 15.75% and 94.62% respectively.

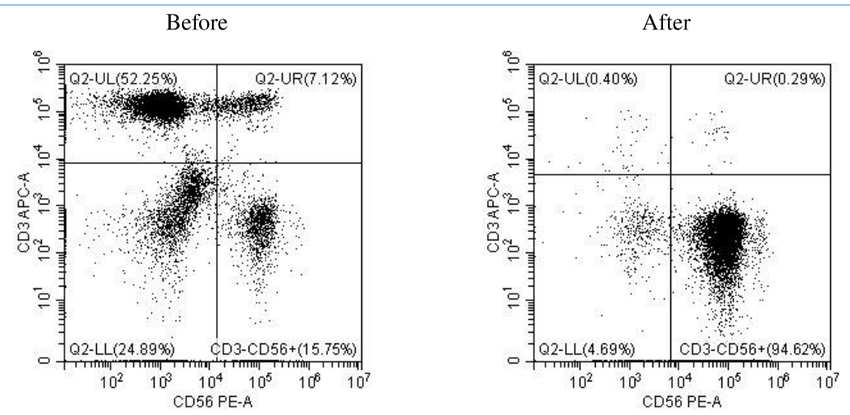

Figure (a) Before vs. After Isolation

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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