EasySort™ Human NK Cell Isolation Kit

Cat. No: MIH005N **Size:10 Assays/100 Assays/200 Assays**

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIH005NA	EasySort™ Human NK Beads Streptavidin 1.0-N	400 μL	1.35 mL×3	1.35 mL×6	2-8°C
MIH005NB	EasySort™ Human NK Cell Isolation Cocktail	510 μL	1.7 mL×3	1.7 mL×6	2-8°C
	Manual	1 copy			

Storage

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

Description

EasySort™ Human NK Cell Isolation Kit is a product designed for rapid and convenient isolation of highly purified human NK cells. This kit employs a negative selection method and is suitable for isolating NK cells from either fresh human PBMC samples or cryopreserved PBMC samples. The principle involves using different biotinylated monoclonal antibodies to label non-target cells (non-NK cells), followed by depletion of these non-target cells using streptavidin-conjugated magnetic beads, thereby yielding highly purified human NK cells. The human NK cells isolated using this kit carry no antibody or bead labeling, remaining in an unstimulated, native state and ready for direct downstream applications.

EasySort™ Human NK Cell Isolation Kit has been validated for magnetic separation using fresh human PBMC samples and cryopreserved PBMC samples, with post-sort cells analyzed and identified by flow cytometry. The single-experiment scale of this kit uses 40 μL of magnetic beads and 51 μL of antibody cocktail, sufficient to meet the isolation needs of 1×10^7 cells.

Reagents and Materials Not Supplied

1. **Reagents:**

   Phosphate buffered saline (PBS), fetal bovine serum (FBS), EDTA, Human peripheral blood mononuclear cells separation solution, DNase I

2. **Materials:**

   70 μm mesh nylon strainer, 1.5 mL/2 mL EP tube, 15 mL/50 mL centrifuge tube, flow tube

3. **Instrument:**

   Optical microscope, horizontal centrifuge, magnetic rack

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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 buffer and filter the prepared buffer with 0.22 μm filter.

  Note: Sealed store the prepared buffer at 4°C and use within 1 week. In addition, 2% fetal bovine serum can be replaced by 0.5% BSA.

- **Sample Preparation and Processing**

a） Fresh human PBMC: PBMC sample is obtained from fresh human whole blood by density gradient centrifugation. Wash PBMC twice with isolation buffer, centrifuge at 300 g for 5 min, filter the PBMC through a 70 μm mesh nylon strainerand and adjust the cell density to 1×10^8 cells/mL for cell isolation.

Note: The best separation effect can be achieved when the freshly collected human blood is separated within 1 hour. Approximately 1×10^7 PBMC can be obtained from 10 mL of human blood.

b） Frozen PBMC: incubate the frozen PBMC should be incubated with DNase I solution (PBS) at a concentration of 100 μg/mL for 15 min at room temperature before cell isolation. Wash sample twice with isolation buffer, centrifuged at 300 g for 5 min. Filter aggregated suspensions through a 70 μm mesh nylon strainer and adjust cell density at 1×10^8 cells/mL.

- **Cell Isolation**

a) Prepare 100 μL of cell suspension (about 1×10^7 cells), add 51 μL Human NK Cell Isolation Cocktail, gently pipette up and down 6-8 times with a pipette to mix, then incubate for 5 min at room temperature.

Note: Please ensure that the cells are in a single-cell suspension. Before sample dilution, filter the samples through a 70 μm cell sieve. The frozen PBMC sample need to be treated with DNase I and then filtered through a 70 μm cell sieve again before isolating.

b) Add isolation buffer to a final volume of 2 mL, centrifuge at 300 g for 5 min. Discard the supernatant, and then resuspend the cells with 100 μL isolation buffer.

Note:

- If the total volume of the cell suspension exceeds 1 mL, the volume of the added isolation buffer shall be no less than the total volume of the cell suspension.For example, if the total volume of the cell suspension is 1.5 mL, the volume of the isolation buffer added shall be ≥ 1.5 mL.
- To maintain consistent cell density, the volume of cell isolation buffer for cell resuspension shall be identical to that of the input cell suspension. In the protocol example, if 100 μL of cell suspension is used as the starting input, cells should be resuspended with an equal volume of 100 μL cell isolation buffer.

c) Wash Human NK Beads Streptavidin 1.0-N: Place a clean flow cytometry tube or a centrifuge

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tube compatible with the magnetic rack into a tube rack. Pipette 1 mL of isolation buffer into the tube, then add 40 µL of magnetic beads directly into the aforementioned 1 mL of sorting buffer. Mix by pipetting 6–8 times, then divide evenly into two tubes (20 µL of magnetic beads per tube). Place the two flow cytometry tubes or centrifuge tubes on a separation magnetic rack (self-provided) and let stand at room temperature for 5 minutes.At this point, the magnetic beads are attracted to the tube wall. Keep the tube on the magnetic rack, discard the supernatant, and then remove the tube from the magnetic rack.

Note： If the total volume of magnetic beads to be washed is greater than 1 mL, use a 1:1 volume ratio of isolation buffer to beads during the washing step.

d) Resuspend the magnetic beads using the cell suspension from step b): Aspirate the cell suspension and pipette the beads off the tube wall to the bottom of the tube (Note: avoid generating bubbles). Mix by pipetting up and down 6-8 times, no incubation is required.

Note:

- ✧ If more than 1×10^7 cells are to be isolated, increase the amount of Human NK Cell Isolation Cocktail and Human NK Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains 1×10^8 cells/mL. If fewer than 1×10^7 cells are to be isolated, resuspend the cells with 100 µL isolation buffer, add 51 µL Human NK Cell Isolation Cocktail and 40 µL washed Human NK Beads Streptavidin 1.0-N.
- ✧ The 5 mL flow tube is suitable for isolation of cell suspension ≤ 1 mL (2×10^8 cells). 10 mL or 15 mL centrifuge tube is suitable for isolation of cell suspension ≤ 4 mL (4×10^8 cells).

e) Add isolation buffer to a final volume of 2.5 mL, (If the volume of the cell suspension for isolation is >1 mL, resuspend in an equal volume of isolation buffer),mix gently with a pipette by blowing up and down for 6-8 times until no particles of magnetic beads are visible. Put the tube on a 5 mL magnetic rack (self-provided) and stand for 5 min.

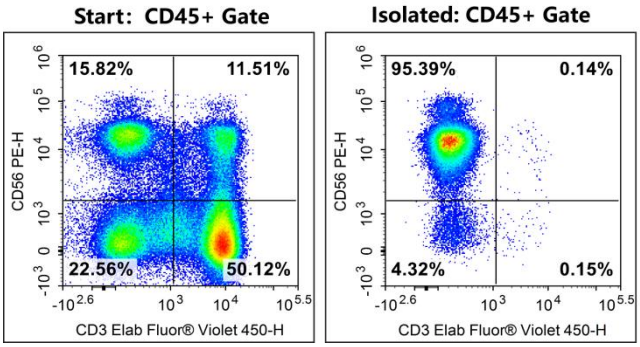
Note: Please mix the liquid thoroughly to avoid the magnetic beads clumping and affecting the isolation efficiency.

f) Transfer the 2.5 mL cell suspension from step e) into the flow cytometry tube containing the remaining 20 µL of washed Human NK Beads Streptavidin 1.0-N.

g) Mix by pipetting up and down 6–8 times until no visible magnetic bead particles remain and no incubation is required, then place on the separation magnetic rack and let stand for magnetic separation for 5 minutes.

h) Transfer the cell suspension to a clean centrifuge tube, centrifuge at 300 g for 5 min. Discard the supernatant, resuspend the cells with buffer required for the subsequent experiments.

Typical data



In the above example,The purity of NK cells （CD45+CD3-CD56+） from normal human PBMC samples was 15.82% before isolation and 95.39% , as analyzed by flow cytometry using the antibodies listed in the table below.

Fluorochrome-conjugated antibody	Cat.	Company
APC Anti-Human CD45 Antibody[HI30]	E-AB-F1137E	Elabscience
Elab Fluor® Violet 450 Anti-Human CD3 Antibody[OKT-3]	E-AB-F1001Q	Elabscience
PE Anti-Human CD56/NCAM Antibody[5.1H11]	E-AB-F1239D	Elabscience

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. All components of the kit should be stored at 2-8℃ and protected from freezing and thawing.
4. Sample type, sample preparation and experimental operation have an important impact on the final isolated cell purity.
5. The quality of pre-isolated PBMC sample is critically impacts the separation efficiency of this product. It is recommended to test whether the percentage of NK cells is in the normal physiological range (5%-15%) after the preparation of PBMC sample. It is recommended to re-prepare the PBMC sample when percentage of target cell population is lower than it's normal distribution.
6. 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.
7. Perform isolation immediately after preparing the cell suspension, as cell viability will decrease with longer storage time.
8. When adding the antibody cocktail and aspirating the magnetic beads for washing, pipette them directly to the bottom of the tube to avoid adhesion to the wall, which would result in loss of components.
9. In order to ensure the activity of the cells, the whole process of the experiment should be completed on ice as much as possible, except for the incubation at room temperature.

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10. It is recommended to use low adsorption pipette tips and centrifuge tubes to avoid the loss of magnetic beads and antibodies due to adsorption.
11. The kit should be used in combination with a magnetic rack.

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