EasySort™ Human Neutrophil Isolation Kit

Cat. No: MIH013N **Size: 10 Assays/100 Assays/200 Assays**

Component	Component Name	10 Assays	100 Assays	200 Assays	Storage
MIH013NA	EasySort™ Human Neutrophil Beads Streptavidin 1.0-N	350 μL	1.17 mL×3	1.17 mL×6	2-8°C
MIH013NB	EasySort™ Human Neutrophil Isolation Cocktail	480 μL	1.20 mL×4	1.20 mL×8	2-8°C
Manual			1 copy		

Storage

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

Description

The EasySort™ Human Neutrophil Isolation Kit is a product that enables rapid and simple isolation of high-purity human neutrophils. This kit uses a negative selection method and is suitable for isolating human neutrophils from fresh human peripheral blood leukocyte samples. The principle involves labeling non-target cells (non-Human Neutrophil) with a combination of biotin-conjugated monoclonal antibodies, followed by depletion of these cells using streptavidin-labeled magnetic beads, thereby obtaining highly purified human neutrophils. The isolated human neutrophils are free of any antibodies and magnetic bead labels, remain in an unstimulated, naïve state, and are ready for direct use in downstream applications.

The EasySort™ Human Neutrophil Isolation Kit has been tested by magnetic cell separation followed by flow cytometric analysis of cells from human fresh human peripheral blood leukocyte samples. A assay is defined as 48 μL antibody and 35 μL beads to be used to isolate 1×10^7 cells.

Reagents and Materials Not Supplied

1. **Reagents:**

   Red Blood Cell Lysis Buffer (without fixative), Phosphate Buffered Saline (PBS), Fetal Bovine Serum (FBS), EDTA

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, 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.

- **Fresh Human Peripheral Blood Leukocytes samples Preparation and Processing**

a) Collect fresh human whole blood in a heparin sodium anticoagulation tube.

b) Add 9 parts 1×ACK erythrocyte lysis solution to 1 part whole blood and mix well.

c) Incubate for 15 minutes. Centrifuge at 300 g for 5 minutes with the brake on low.

d) Discard supernatant and wash pellet with 1×PBS, centrifuging at 300 g for 5 minutes with the brake off.

e) Repeat step d) to wash once

f) Discard supernatant and resuspend cells at 1×10^8 cells/mL in recommended isolation buffer.

Note: Approximately $2\sim3\times10^7$ fresh human peripheral blood leukocytes can be obtained from 5 mL of human whole blood.

- **Cell Isolation**

a) Prepare 100 μL of cell suspension (about 1×10^7 cells), add 48 μL Human Neutrophil Isolation Cocktail, mix fully and incubate for 5 min at room temperature.

Note: Please make sure the cells are single-cell suspension.

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 Neutrophil Beads Streptavidin 1.0-N: Place a clean flow cytometry tube or a centrifuge tube compatible with the magnetic rack into a tube rack. Pipette 1 mL of isolation buffer into the tube, then add 35 μL of magnetic beads directly into the aforementioned 1 mL of sorting buffer. Mix by pipetting 6–8 times. Place the tube or centrifuge tube on a separation

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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:

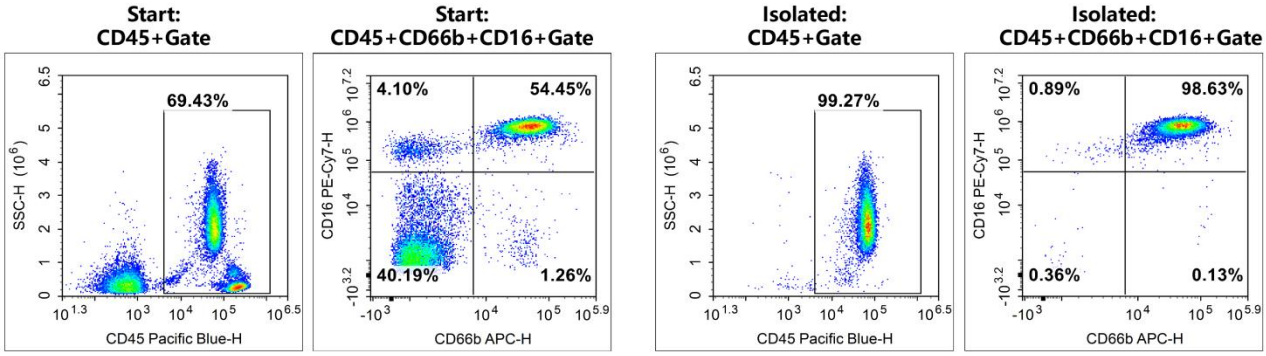
- ✧ If more than 1×10^7 cells are to be isolated, increase the amount of Human Neutrophil Isolation Cocktail and Human Neutrophil Beads Streptavidin 1.0-N proportionally while ensuring the cell density remains 1×10^8 cells/mL. If fewer than 1×10^7 cells are to be isolated, resuspend the cells with 100 µL isolation buffer, add 48 µL Human Neutrophil Isolation Cocktail and 35 µL washed Human Neutrophil 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 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 cell suspension to a clean centrifuge tube, centrifuge at 300 g for 5 min. Discard the supernatant, resuspend the cells with isolation buffer required for the subsequent experiments.

Typical data



In the above example,the purity of human neutrophils ($CD45^+CD66b^+CD16^+$) from human peripheral blood leukocyte samples was 37.80% before isolation and 97.91% after isolation, as analyzed by

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flow cytometry using the antibodies listed in the table below.

Fluorochrome-conjugated antibody	Cat.	Company
Elab Fluor® Violet 450 Anti-Human CD45 Antibody[HI30]	E-AB-F1137Q	Elabscience
APC Anti-Human CD66b Antibody[G10F5]	E-AB-F1267E	Elabscience
PE/Cyanine7 Anti-Human/Monkey CD16 Antibody[3G8]	E-AB-F1236H	Elabscience

Cautions

1. This kit is for research use only.
2. Please take safety precautions and follow the procedures of laboratory reagent operation.
3. Avoid freezing and thawing during the use and storage of the beads.
4. The data presented for this product are based on testing of fresh human peripheral blood leukocyte samples from healthy individuals. When processing peripheral blood from non-healthy individuals, to ensure optimal experimental outcomes, first assess the neutrophil content in the sample. If the neutrophil content is below 35%, it is recommended to perform a pre-sorting test, appropriately increase the amount of antibodies and beads per assay, or conduct a secondary sorting step.
5. To ensure the lysis effect of red blood cells, it is recommended that 1× red blood cell lysis buffer be preheated in a 37°C oven for 5 minutes before use.
6. The cell clusters in the cell suspension will affect the purity of cell isolation. Therefore, cell suspension should be filtered with a 70 μm mesh nylon sieve before formal isolation.
7. Cell suspension should be isolated immediately after preparation, the longer the storage time, the greater the impact on cell activity.
8. The cell suspension and reagents should be added directly to the bottom of flow tube to avoid sticking to the wall, resulting in insufficient reaction and affecting the isolation efficiency.
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.
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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