Mouse Neutrophil Isolation Kit for 1 × 10^9 cells
PRODUCT DATA SHEET

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Description

The mouse neutrophil sorting kit isolates neutrophils from single-cell suspensions of mouse bone marrow or other tissue samples by negative sorting. The principle is to select biotin labeled monoclonal antibody to label non-target cells (non-$CD11b^+$ Ly-$6G^+$ cells), and then clear non-target cells through streptavidin labeled magnetic beads, so as to achieve the purpose of mouse neutrophils sorting. The sorting process requires the use of magnetic racks.

For custom sizes, formulations or bulk quantities please contact our customer service department.

Website: www.abvigen.com Phone: +1 929-202-3014 Email: info@abvigenus.com

Characteristics

Constituent	Specification
Biotin-Antibody Mix	200 µL
Streptavidin	2 mL

Advantages

Simple and fast operation: no separation column is required, with a magnetic separator, the fastest 30min to complete the operation;

High activity, high purity: The target cells are free of antibodies and magnetic beads, and the purity is up to 95%.

Scope of application

This kit is suitable for sorting mouse neutrophils from bone marrow, peripheral blood or spleen.

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Cell sample preparation

1. Mouse bone marrow, spleen or peripheral blood cells were obtained in a centrifuge tube for red blood cell lysis.

Note: The procedure of red blood cell lysis can be adjusted according to the amount and time of different tissue samples and lysates used. Usually, it takes longer for mouse peripheral blood cell lysis. A small amount of residual red blood cells did not affect the purity of the sorted cells, and the bone marrow cells could also be directly sorted without lysing red blood cells, but the purity of the sorted cells may decrease slightly with the increase of the proportion of red blood cells within 5%.

2. After lysis, the cells were re-suspended by PBS, and the cell suspension was filtered with a 70 μm cell screen, 500 g, and centrifuged for 5 min.

Note: The cell suspension needs to pass through the cell screen to remove tissue and cell clumps, otherwise it will affect the purity of subsequent cell sorting.

3. After centrifugation, the cells were resuspended in the sorting Buffer, washed, centrifuged at 500 g for 5 min, and the supernatant was discarded.

Note: The sorting buffer was PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS) or PBS containing 2 mM EDTA and 0.5% BSA, which was pre-filtered through 0.22 μm filter.

4. Resuspend the cells in the sorting buffer and adjust the cell density to 1×10^8 cells/mL.

Isolating process

1. Add 100 μL cell suspension (1×10^7 cells) to the bottom of a 1.5 mL centrifuge tube and add 2 μL Biotin-Antibody Mix, and incubate at 4°C for 10 min.

Note: When adding cell suspension, add cells to the bottom of the centrifuge tube, avoid adding along the tube wall. If more cells are selected, the dosage of BiotinAntibody Mix is increased proportionally.

2. After incubation, wash the cells once: Buffer to 1.5 mL, centrifuge at 500 g for 5 min.

Note: If too many cells are sorted, use a 15 mL centrifuge tube, fill the sorting Buffer to 5-10 mL, and then centrifuge.

3. After centrifugation, the supernatant was abandoned and the cells were re-suspended with a 100 μL sorting Buffer.

Note: If more cells are sorted, the amount of sorted buffer is increased proportionally.

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4. Add 20 μL of cleaned Streptavidin to the centrifuging tube (**Beads need to be cleaned with a sorting buffer before use:** The magnetic beads were re-suspended by vortex oscillation, and the magnetic beads needed for the experiment were absorbed into a 1.5 mL centrifuge tube, and 1 mL of sorting buffer was added, centrifuged at 10000 g for 1 min, and the supernant was discarded. Add 1 mL sorting buffer and repeat washing magnetic beads once, then re-suspend magnetic beads with the same volume of sorting buffer as the original. If 20 μL magnetic beads are absorbed for cleaning, then re-suspension with 20 μL sorting buffer after cleaning), fully mixed and incubated at 4°C for 10 min.

Note: If more cells are sorted, the amount of Streptavidin is increased proportionally. For example, 5×10^7 cells were sorted and 500 μL cell suspension was supplemented with 10 μL Biotin-Antibody Mix and 100 μL Streptavidin. If fewer than 1×10^7 cells are sorted, cell suspension is supplemented to 100 μL with 2 μL Biotin-Antibody Mix and 20 μL Streptavidin.

5. After incubation, transfer to the flow tube, fill the sorting buffer to 2.5 mL, and mix it up and down with the pipette (avoid violent shaking or mixing upside down).

6. Place the sorting flow tube containing cells on the magnetic rack and stand for 5 min.

7. The cell suspension was gently poured into a sterile centrifuge tube (the flow tube should not be removed from the magnetic rack during the pouring process). The cell suspension contained purified mouse neutrophils, 500 g, and centrifuged for 5 min. After centrifugation, the supernatant was discarded and the cells were collected.

8. After washing the cells according to the needs of the experiment, the cells are re-suspended in the required buffer or medium, which can be used for subsequent molecular biology or cell biology experiments.

Isolating effect

Neutrophils were sorted from C57BL/6 mouse bone marrow cells. The cells before and after sorting were labeled with FITC anti-mouse Ly-6G antibody (clonal No. 1A8) and PE anti-mouse CD11b antibody (clonal No. M1/70) and then analyzed by flow cytometry. The purity of neutrophils before and after sorting were 43.5% and 95.1%, respectively.

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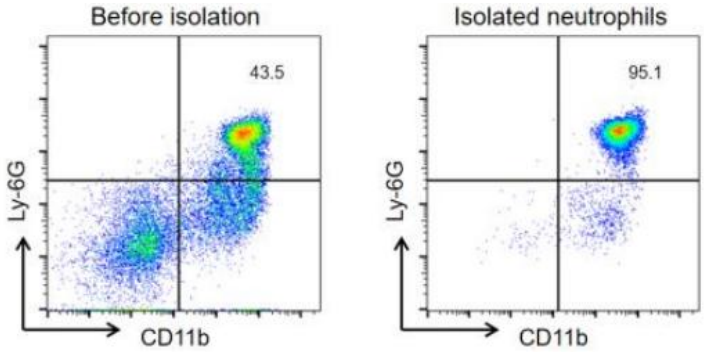

Storage conditions and expiration date

This product should be stored at 2 - 8°C. **DO NOT FREEZE**. The expiration date can be found on the test tube label.

Notes

1. Magnetic beads and antibody mixture should avoid freezing during use and storage;
2. It is recommended to choose a low adsorption pipette head and centrifugal tube to avoid the loss of magnetic beads and antibodies caused by adsorption;
3. This product should be used with a magnetic separator;
4. This product is for research use only.

Ordering Information

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