

Mouse Neutrophil Isolation Kit (Negative Isolation)

Cat. No.: M1522422 | Pack size: 10T; 50T; 100T | Storage: 2-8°C; Do not freeze

Overview

The Mouse Neutrophil Isolation Kit (Negative Isolation) is used to isolate neutrophils from mouse bone marrow or other tissue single-cell suspensions by negative isolation. The principle is to use a cocktail of biotin-labeled monoclonal antibodies against non-target cells (non-CD11b⁺ Ly-6G⁺ cells), followed by removal of these non-target cells using streptavidin-labeled magnetic beads, thereby achieving the isolation of mouse neutrophils. A magnetic separator is required for the isolation process.

Applications

Isolation of neutrophils from mouse bone marrow, peripheral blood, or spleen.

Kit Contents & Storage

Component Code	Component	Appearance	10T	50T	100T	Storage
M1522422A	Biotin-Antibody Mix	Liquid	20 µL	100 µL	200 µL	2-8°C
M1522422B	Streptavidin Magnetic Beads	Liquid	200 µL	1 mL	1 mL × 2	2-8°C

Cell Sample Preparation

Obtain cells and lyse red blood cells

Collect mouse bone marrow, spleen, or peripheral blood cells in a centrifuge tube and perform red blood cell lysis.

Note: The red blood cell lysis step can be adjusted in volume and time depending on the tissue sample and the lysis buffer used. Typically, mouse peripheral blood requires a longer lysis time. A small amount of red blood cell residue will not affect the purity of isolated cells. Bone marrow cells

can be processed directly without red blood cell lysis, but the purity of isolated neutrophils may decrease slightly (within 5%) as the proportion of red blood cells increases.

Filter

After lysis, resuspend the cells in PBS, filter the cell suspension through a 70 μ m cell strainer, and centrifuge at 500 g for 5 min.

Note: The cell suspension must be passed through a cell strainer to remove tissue and cell clumps; otherwise, subsequent cell isolation purity may be affected.

Wash cells

After centrifugation, resuspend the cells in Isolation Buffer, wash the cells, centrifuge at 500 g for 5 min, and discard the supernatant.

Note: Isolation buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS), or PBS containing 2 mM EDTA and 0.5% BSA. The buffer must be pre-filtered through a 0.22 μ m filter.

Adjust cell density

Resuspend the cells in Isolation Buffer to a density of 1×10^8 cells/mL.

Isolation Protocol

Add Biotin-Antibody Mix

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

Note: When adding the cell suspension, add it to the bottom of the tube, avoiding the tube wall. If isolating more cells, increase the volume of Biotin-Antibody Mix proportionally.

Wash cells once

After incubation, add Isolation Buffer to bring the volume to 1.5 mL, and centrifuge at 500 g for 5 min.

Note: If isolating a very large number of cells, use a 15 mL centrifuge tube, add Isolation Buffer to 5-10 mL, then centrifuge.

Resuspend cells

After centrifugation, discard the supernatant and resuspend the cells in 100 μ L of Isolation Buffer.

Note: If isolating more cells, increase the volume of Isolation Buffer proportionally.

Add Streptavidin Magnetic Beads

Add 20 μ L of washed Streptavidin Magnetic Beads to the centrifuge tube (the beads must be washed before use: vortex to resuspend the beads, transfer the required volume of beads to a 1.5 mL centrifuge tube, add 1 mL of Isolation Buffer, centrifuge at 10,000 g for 1 min or use a magnetic separator for 3 min, then discard the supernatant. Add 1 mL of Isolation Buffer and wash the beads once more, then resuspend the beads in the same volume of Isolation Buffer as originally taken. For example, if 20 μ L of beads are taken, resuspend them in 20 μ L of Isolation Buffer after washing). Mix thoroughly and incubate at 4°C for 10 min.

Note: If isolating more cells, increase the volume of Streptavidin Magnetic Beads proportionally. For example, to isolate 5×10^7 cells, add 10 μ L of Biotin-Antibody Mix and 100 μ L of Streptavidin Magnetic Beads to 500 μ L of cell suspension. If isolating fewer than 1×10^7 cells, adjust the cell suspension volume to 100 μ L, then add 2 μ L of Biotin-Antibody Mix and 20 μ L of Streptavidin Magnetic Beads.

Transfer and dilute

After incubation, transfer the mixture to a FACS tube, add Isolation Buffer to a total volume of 2.5 mL, and mix thoroughly by pipetting up and down (avoid vigorous shaking or inverting).

Magnetic separation

Place the FACS tube containing the cell suspension on a magnetic separator and let stand for 5 min.

Collect neutrophils

Gently pour the cell suspension into a sterile centrifuge tube (do not remove the FACS tube from the magnetic separator while pouring). This cell suspension contains the purified mouse neutrophils. Centrifuge at 500 g for 5 min, then discard the supernatant and collect the cells.

Resuspend for further use

Wash the cells as required for your experiment, then resuspend them in the desired buffer or culture medium for subsequent molecular or cell biology experiments.

Isolation Performance

Neutrophils were isolated from C57BL/6 mouse bone marrow cells. Cells before and after isolation were stained with FITC anti-mouse Ly-6G antibody (clone 1A8) and PE anti-mouse CD11b antibody (clone M1/70) and analyzed by flow cytometry. The purity of neutrophils before and after isolation was 43.5% and 95.1%, respectively.

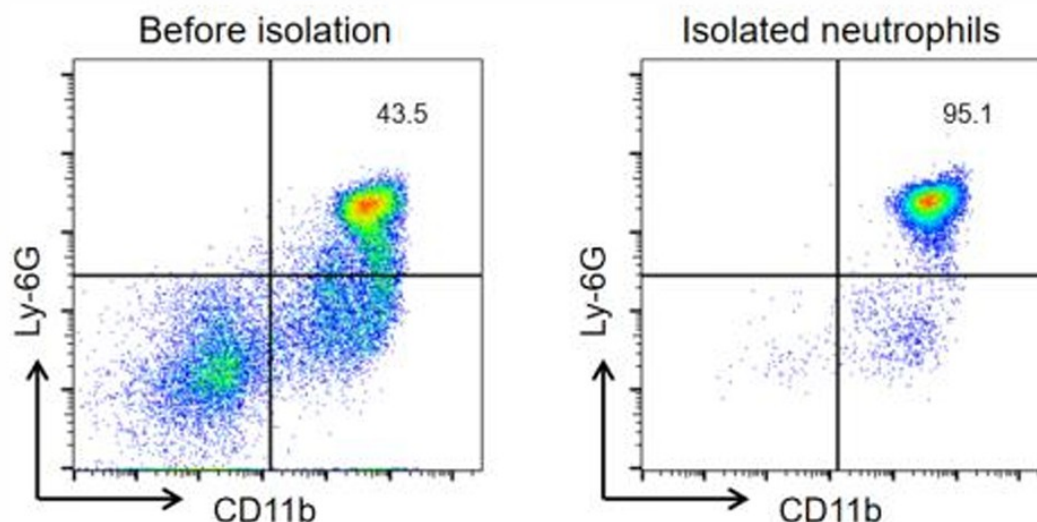


Figure 1. Mouse neutrophil isolation performance.

Precautions

Avoid freezing the magnetic beads and antibody mix during use and storage.

Low-retention pipette tips and centrifuge tubes are recommended to avoid loss of beads and antibodies due to adsorption.

This product must be used with a magnetic separator.

For research use only.

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