Mouse Neutrophil Isolation Kit (negative isolation)

Catalog No.: BRC0007

Product Description

The Mouse Neutrophil Isolation Kit isolates neutrophils from single-cell suspensions of mouse bone marrow or other tissue samples via negative selection. The principle is to label non-target cells (non-$CD11b^+Ly\text{-}6G^+$ cells) with biotin-conjugated monoclonal antibodies, followed by depletion with streptavidin-conjugated magnetic beads, thereby achieving purification of mouse neutrophils. A magnetic stand is required for the separation process.

Kit Components

No.	Reagent Name	Size1 (For 10^9 cell)	Size2 (For $5*10^8$ cell)
Reagent1	Biotin-Antibody Mix	200μl	100μl
Reagent2	EnkiBeads™ Streptavidin	2 mL	1ml

Storage Conditions and Shelf Life

Storage Conditions: Store at 2–8°C. DO NOT FREEZE.
Shelf Life: 2 years

Applications

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

Protocol

1. Obtain mouse bone marrow, spleen, or peripheral blood cells in a centrifuge tube and perform red blood cell (RBC) lysis.

Note: RBC lysis conditions (volume and time) should be optimized based on the tissue sample and lysis buffer used. Mouse peripheral blood typically requires longer lysis time. Residual RBCs will not affect cell purity; bone marrow can be processed without RBC lysis, though purity may decrease by ≤5% as the RBC proportion increases.

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

Note: Filtering through a cell strainer is required to remove tissue debris and cell aggregates, which will otherwise compromise cell purity.

3. Centrifuge cells, resuspend in separation buffer, wash, centrifuge at 500 g for 5 min, and discard the supernatant.

Note: Separation buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS) or PBS containing 2 mM EDTA and 0.5% BSA. Filter-sterilize through a 0.22 μm membrane before use.

4. Resuspend cells in separation buffer at a density of 1×10^8 cells/mL.
5. Add 100 μL cell suspension (1×10^7 cells) to the bottom of a sterile flow tube, then add 2 μL Biotin-Antibody Mix. Mix well and incubate at 4°C for 10 min.

Note: When adding cell suspension, pipette directly to the bottom of the tube; avoid pipetting along the tube wall. Centrifuge tubes may be used depending on the magnetic stand. Scale up the amount of Biotin-Antibody Mix proportionally if processing more cells.

6. After incubation, wash cells once: add separation buffer to 1.5 mL total volume and centrifuge at 500 g for 5 min.

Note: For larger cell numbers, use a 15 mL centrifuge tube, add separation buffer to 5–10 mL, then centrifuge.

7. After centrifugation, discard the supernatant and resuspend cells in 100 µL separation buffer.

Note: Scale up the separation buffer volume proportionally if processing more cells.

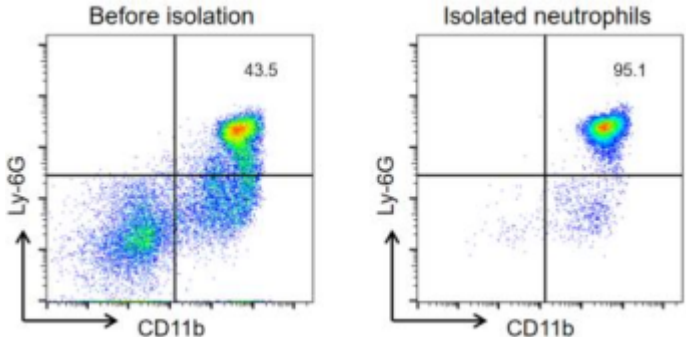
8. Add 20 µL of washed EnkiBeads™ Streptavidin (Wash magnetic beads before use: Resuspend beads by vortexing, transfer the required volume to a 1.5 mL centrifuge tube, add 1 mL separation buffer, centrifuge at 10,000 g for 1 min, and discard the supernatant. Repeat wash once with 1 mL separation buffer, then resuspend beads in the original volume of separation buffer. For example, if 20 µL of beads were taken, resuspend in 20 µL separation buffer after washing). Mix well and incubate at 4°C for 10 min.

Note: Scale up the amount of EnkiBeads™ Streptavidin proportionally if processing more cells. For example, for 5×10^7 cells, add 10 µL Biotin-Antibody Mix and 100 µL EnkiBeads™ Streptavidin to 500 µL cell suspension. If processing fewer than 1×10^7 cells, adjust the cell suspension volume to 100 µL and add 2 µL Biotin-Antibody Mix and 20 µL EnkiBeads™ Streptavidin.

9. After incubation, transfer to a flow tube, add separation buffer to 2.5 mL total volume, and mix thoroughly by pipetting up and down (avoid vigorous vortexing or inversion mixing).
10. Place the flow tube on the magnetic stand and let stand for 5 min.
11. Gently pour the cell suspension into a sterile centrifuge tube (do not remove the flow tube from the magnetic stand during pouring). The cell suspension now contains purified mouse neutrophils. Centrifuge at 500 g for 5 min, discard the supernatant, and collect the cells.
12. Wash cells as required, then resuspend in appropriate buffer or culture medium for downstream molecular or cellular biology applications.

Isolation Performance

Neutrophils were isolated from bone marrow cells of C57BL/6 mice. 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. Purity: 43.5% pre-isolation and 95.1% post-isolation.



Important Notes

1. Avoid freezing magnetic beads and antibody mix during storage and use.
2. Use low-retention pipette tips and centrifuge tubes to minimize loss of beads and antibodies due to adsorption.
3. This product should be used with a magnetic separator.
4. For research use only.