

Human Neutrophil Cell Isolation Kit (negative selection)

Description

TargetMol Human Neutrophil Cell Isolation Kit (Negative Selection) provides superparamagnetic beads and uses a negative selection method to isolate neutrophils from fresh peripheral blood. The principle is based on labeling non-target cells with biotin-conjugated monoclonal antibodies, followed by depletion of the labeled non-target cells using streptavidin-coated magnetic beads, thereby enabling the isolation of human neutrophils.

Cell Isolation Product Recommendations

1. Mouse Cells

Cell Type	Spleen	Lymph Node	Peripheral Blood	Bone Marrow	Tumor Tissue
CD3+ T Cell	C0061		/	/	/
CD4+ Cell	C0062 (Preferred), C0067 (Optional)		C0067	/	C0067
CD8+ Cell	C0063 (Preferred), C0068 (Optional)		C0068	/	C0068
Neutrophil	C0064	/	C0064	C0064	/
CD3+ Cell Removal	C0152	C0152	/	/	/
CD3/CD28 T Cell Activation	C0180	/	/	/	/
B Cell	C0218	C0218	/	C0218	/

2. Human Cells

Cell Type	Peripheral Blood	Umbilical Cord Blood
CD3+ T Cell	C0065	/
CD34+ Cell Enrichment	C0066	C0066
CD4+ T Cell	C0148	/
CD8+ T Cell	C0149	/
CD3/CD28 T Cell Activation	C0150	/
CD66b+ Cell	C0151	/
Neutrophil	C0216	/
CD3+ Cell Removal	C0217	/

Product Features

1. High Purity: High-purity target cell populations can be obtained after isolation, with efficient target cell enrichment.
2. High Cell Viability: Non-CD3+ cells maintain good viability and normal functionality.
3. Easy to Use: CD3+ cell enrichment or depletion can be completed in a single step.
4. Fast Processing: CD3+ cell enrichment or depletion can be completed in just 35 minutes.

Applications

Suitable for the isolation of neutrophils from fresh human peripheral blood. Red blood cells should be removed prior to isolation.

Product Information

Catalog No.	Product Name	Package Size (for 5×10^8 cells)	Package Size (for 1×10^9 cells)
C0216-1	Biotin-Antibody Mix	100 μ L	200 μ L
C0216-2	Streptavidin Magnetic Beads	1mL	2mL

Instructions

The following protocol uses the removal of CD3+ cells from mouse spleen tissue as an example.

1. Preparation of Single-Cell Suspension: Collect fresh anticoagulated peripheral blood into a centrifuge tube and perform red blood cell lysis.

Note: The incubation time and volume for red blood cell lysis can be adjusted according to the lysis buffer used. A small amount of residual red blood cells generally has minimal impact on subsequent cell isolation and purity. Typically, it is recommended to add lysis buffer at 3–5 times the volume of peripheral blood and incubate at 4 °C or on ice. If the lysis efficiency is unsatisfactory, the procedure may be repeated once. Minor residual red blood cells usually do not affect the purity of subsequent cell isolation.

2. After lysis is complete, resuspend the cells in PBS and filter through a cell strainer. After cell counting, centrifuge at 500 $\times$ g for 5 min.

3. After centrifugation, discard the supernatant and repeat the washing step once with PBS to thoroughly remove the lysis buffer and cell debris. Centrifuge again, discard the supernatant, resuspend the cells in sorting buffer, and adjust the cell concentration to 1×10^8 cells/mL.

Note: Recommended sorting buffer composition: PBS containing 2 mM EDTA and 2% FBS. The buffer should be sterilized in advance by filtration through a 0.22 μ m membrane filter.

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

Note: When adding cells to the flow cytometry tube, avoid dispensing along the tube wall. If sorting a larger number of cells, the amount of Biotin-Antibody Mix should be increased proportionally. Depending on the magnetic separator used, centrifuge tubes may also be used for cell isolation.

5. Magnetic Bead Preparation: Resuspend the magnetic beads thoroughly by vortexing. Transfer the required volume of beads into a 1.5 mL centrifuge tube, add 1 mL of sorting buffer, and centrifuge at 10,000 g for 1 min. Discard the supernatant. Repeat the washing step once more. Resuspend the beads in sorting buffer to the original volume. For example, if 20 μ L of beads are used, resuspend the washed beads in 20 μ L of sorting buffer.

6. Add 20 μ L of the pretreated Streptavidin Magnetic Beads to the cells, mix well, and incubate at room temperature for 10 min.

Note: If a larger number of cells is being isolated, the amount of Streptavidin Magnetic Beads should be increased proportionally. For example, when isolating 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 fewer than 1×10^7 cells are used, adjust the cell suspension volume to 100 μ L and add 2 μ L of Biotin-Antibody Mix and 20 μ L of Streptavidin Magnetic Beads.

7. After incubation, add 2.5 mL of sorting buffer to the flow cytometry tube. Gently pipette up and down 5 times to mix thoroughly. Avoid vigorous shaking or repeated inversion.
8. Place the flow cytometry tube containing the cells onto a magnetic separator and allow it to stand for 5 min.
9. Carefully pour the cell suspension into a sterile centrifuge tube while keeping the flow cytometry tube on the magnetic separator. Centrifuge at 500 g for 5 min, discard the supernatant, and collect the cells.
10. Wash the cells according to experimental requirements, then resuspend them in the desired buffer or culture medium for subsequent molecular biology or cell biology experiments.

Storage

4°C, 2 years.

Precautions

1. Do not freeze any kit components. Magnetic beads should be stored in their storage solution to prevent drying.
2. Resuspend the beads thoroughly by vortexing before pipetting from the stock tube, taking care to avoid air bubbles.
3. High-quality low-binding pipette tips and reaction tubes are recommended to prevent bead and solution loss due to wall adhesion.
4. A magnetic separator with a magnetic field strength greater than 7000 Gs is recommended, as insufficient magnetic strength may affect the isolation efficiency.
5. This product is intended for scientific research purposes by professionals only and must not be used for clinical diagnosis or treatment, food, or medicine, nor stored in residential areas.
6. For your safety and health, please wear a lab coat and disposable gloves during operation.

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