# 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.

## Recommended Products

### 1. Mouse Cells

| | Spleen | Lymph Nodes | Peripheral Blood | Bone Marrow | Tumor Tissue |
|---|---|---|---|---|---|
| $CD3^+$ T Cells | C0061 | | / | / | / |
| $CD4^+$ Cells | C0062 (Preferred), C0067 (Optional) | | C0067 | / | C0067 |
| $CD8^+$ Cells | C0063 (Preferred), C0068 (Optional) | | C0068 | / | C0068 |
| Neutrophils | C0064 | / | C0064 | C0064 | / |
| CD3+ Cell Depletion | C0152 | | / | / | / |
| CD3/CD28 T Cell Activation | C0180 | / | / | / | / |
| B Cells | C0218 | | / | C0218 | / |

### 2. Human Cells

| | Peripheral Blood | Cord Blood |
|---|---|---|
| $CD3^+$ T Cells | C0065 | / |
| $CD34^+$ Cell Enrichment | C0066 | C0066 |
| $CD4^+$ T Cells | C0148 | / |
| $CD8^+$ T Cells | C0149 | / |
| CD3/CD28 T Cell Activation | C0150 | / |
| $CD66b^+$ Cells | C0151 | / |
| Neutrophils | C0216 | / |
| CD3+ Cell Depletion | C0217 | / |

## Product Features

1. High Purity: The purity of the isolated cells can reach up to 98%.
2. High Viability: The isolated cells retain intact functionality without abnormal activation, and are free of antibody and magnetic bead labeling.
3. Easy Operation: No separation columns are required; target cell isolation can be achieved simply using a magnetic separator.
4. Fast Procedure: Target cells can be obtained in as little as 25 minutes.

## Application

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

## Components

| Cat. No. | Product Name | Packing (for 5×10^8 cells) | Packing (for 1×10^9 cells) |
|---|---|---|---|
| C0216-1 | Biotin-Antibody Mix | 100 μL | 200 μL |
| C0216-2 | Streptavidin Magnetic Beads | 1 mL | 2 mL |

## Instructions

**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 × 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 μm membrane filter.

**4.** Add 100 μL of the cell suspension (containing 1 × 10^7 cells) to the bottom of a sterile 1.5 mL flow cytometry tube, then add 2 μL 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 μL of beads are used, resuspend the washed beads in 20 μL of sorting buffer.

**6.** Add 20 μL 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 μL of Biotin-Antibody Mix and 100 μL of Streptavidin Magnetic Beads to 500 μL of cell suspension. If fewer than 1 × 10^7 cells are used, adjust the cell suspension volume to 100 μL and add 2 μL of Biotin-Antibody Mix and 20 μL 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

Store at 4 °C for 2 years.

## Precautions

1. Avoid freezing any components of the kit. Magnetic beads should be stored in the storage solution to prevent drying.
2. Before removing the magnetic beads from the storage vial, thoroughly resuspend them by vortexing or gentle mixing to ensure a uniform suspension. Avoid generating bubbles during handling.

3. It is recommended to use high-quality pipette tips and reaction tubes to minimize sample loss caused by the adhesion of magnetic beads and solutions.
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. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
6. Please wear a lab coat and disposable gloves.

## Negative Selection VS Positive Selection

| Magnetic Cell Isolation Technology | Negative Selection | Positive Selection |
|---|---|---|
| Magnetic Cell Separation Technology | Diverse | Diverse |
| Capture Method | Magnetic beads binding to non-target cells | Magnetic beads binding to non-target cells |
| Dissociation Required | No | Yes |
| Target Cells Antibody-Labeled | No | Yes |
| Cell Purity | >97% | >95% |
| Cell Viability | High | High |
| Features | High purity of target cells;<br>No residual antibodies or magnetic beads on the cells;<br>Better cell viability, suitable for downstream functional assays. | Broader Sample Compatibility |