Human CD34+ Cell Enrichment Kit for 1 × 10^9 cells, Negative

Description

This product can enrich CD34+ cells from human cell samples by negative sorting. The principle is to use biotin labeled monoclonal antibody to label non-target cells (non-CD34+ cells), and then clear non-target cells through streptavidin labeled magnetic beads, so as to achieve the purpose of enriching CD34+ cells. The sorting process requires the use of magnetic racks.

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

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Characteristics

Constituent	Specification
Biotin-Antibody Mix	200 µL
Streptavidin	2 mL

Advantages

One-step enrichment reaches 10 times enrichment, and two-step enrichment reaches 30 times enrichment;

CD34+ cell enrichment can be completed in 30 min;

No separation column is required and can be used with a magnetic separator.

Scope of application

This kit is suitable for enrichment of CD34+ cells from human umbilical cord blood mononuclear cells (CBMC) or peripheral blood mononuclear cells (PBMC).

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Operation process

1. Preparation of human CBMC or PBMC: Mononuclear cells were isolated from human umbilical cord blood or peripheral blood by Ficoll density gradient centrifugation, washed with PBS, and then resuspended in the sorting buffer after centrifugation to adjust the cell density to 1×10^8 cells/mL.

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.

2. 100 μL cell suspension (1×10^7 cells) was added to the bottom of a sterile flow tube, followed by 2 μL Biotin-Antibody Mix, and incubated at 4°C for 10 min.

Note: Add the cell suspension directly to the bottom of the flow tube and avoid adding along the wall of the flow tube. Depending on the magnetic frame used, centrifuge tubes can also be used for cell sorting. If more cells are selected, the dosage of Biotin-Antibody Mix is increased proportionally.

3. After incubation, 20 μL of cleaned Streptavidin is added into the flow tube, mixed and incubated at 4°C for 10 min. (**The magnetic beads need to be cleaned with a sorting buffer before use:** After vortex oscillation, the magnetic beads were completely re-suspended, and the magnetic beads required for the experiment were added to the 1.5 mL centrifuge tube, the sorting buffer was added until the total volume was 1 mL, 10000 g, centrifuge for 1 min, and the supernant was discarded. Add 1 mL of sorted buffer resuspended magnetic beads, 10000 g, centrifuge for 1 min, discard the supernatant. The beads are re-suspended with the same volume of sorting buffer as at first. If the 20 μL magnetic bead is cleaned, it is re-suspended with a 20 μL sorting buffer after cleaning).

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.

4. After incubation, add 2.5 mL sorting buffer into the flow tube and mix with pipette for 5 times.

5. Place the flow tube containing cells on the magnetic rack and let it stand for 5 min.

6. Transfer the cell suspension into a sterile centrifuge tube (the flow tube does not detangle from the magnetic rack), which contains enriched human $CD34^+$ cells. Centrifuge 300 g for 5 min. Discard supernatant and collect cells.

Note: Steps 2-6 can be repeated once according to experimental needs to further improve the enrichment effect of $CD34^+$ cells.

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7. 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

$CD34^+$ cells were enriched from human CBMC, and the cells before and after sorting were labeled with FITC anti-human CD45 (clonal HI30) and PE anti-human CD34 antibody (clonal 581) and analyzed by flow cytometry. The purity of $CD34^+$ cells before sorting was 1.2%, the purity of $CD34^+$ cells after the first sorting was 13.9% and that of $CD34^+$ cells after the second sorting was 33.8%.

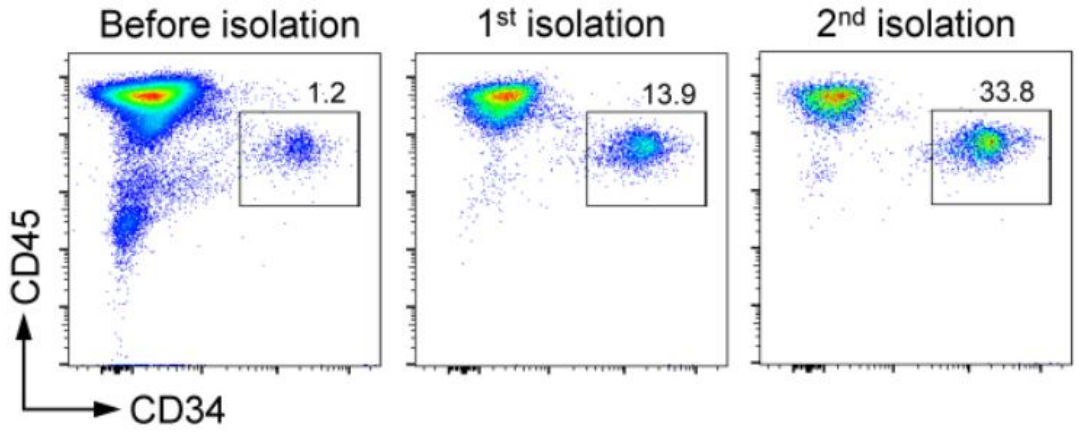

Figure 1. Purity analysis of CD34+ cells before and after enrichment.

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 needs to be used with the magnetic rack;
4. This product is for research use only.

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Ordering Information

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