Human CD34$^+$ Cell Enrichment Kit

Catalog No.: BRC0006

Product Description

This product enriches CD34$^+$ cells from human cell samples through negative selection. The principle is to label non-target cells (non-CD34$^+$ cells) with biotin-conjugated monoclonal antibodies, followed by depletion using streptavidin-conjugated magnetic beads, thereby achieving enrichment of CD34$^+$ cells. 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 enriching CD34$^+$ cells from human cord blood mononuclear cells (CBMC) or peripheral blood mononuclear cells (PBMC).

Protocol

1. Prepare human CBMC or PBMC: Isolate mononuclear cells from human cord blood or peripheral blood by Ficoll density gradient centrifugation. Wash cells with PBS, centrifuge, and resuspend CBMC or PBMC in separation buffer at a density of 1×10^8 cells/mL.

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.

2. Add 100 μL of cell suspension (1×10^7 cells) to the bottom of a sterile flow tube, then add 2 μL of 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 flow tube; avoid pipetting along the tube wall. Centrifuge tubes may also be used for cell separation depending on the magnetic stand. Scale up the amount of Biotin-Antibody Mix proportionally if processing more cells.

3. After incubation, add 20 μL of washed EnkiBeads™ Streptavidin to the flow tube (Magnetic beads must be washed 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 the wash once with 1 mL separation buffer, then resuspend the beads in the original volume of separation buffer. For example, if 20 μL of beads were taken for washing, 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, when processing 5×10^7 cells, add 10 µL of Biotin-Antibody Mix and 100 µL of EnkiBeads™ Streptavidin to 500 µL of cell suspension. If processing fewer than 1×10^7 cells, adjust the cell suspension volume to 100 µL and add 2 µL of Biotin-Antibody Mix and 20 µL of EnkiBeads™ Streptavidin.

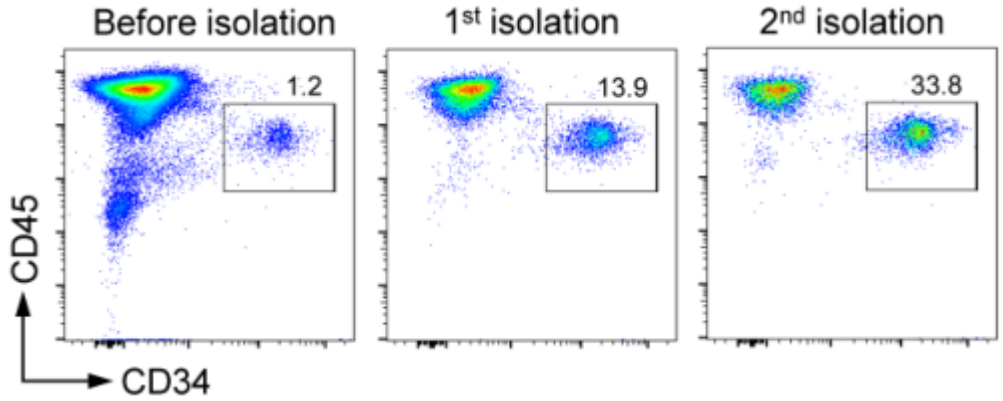
4. After incubation, add 2.5 mL of separation buffer to the flow tube. Mix by pipetting up and down 5 times (avoid vigorous vortexing or inversion mixing).
5. Place the flow tube containing cells on the magnetic stand and let stand for 5 min.
6. Gently pour the cell suspension into a sterile centrifuge tube (do not remove the flow tube from the magnetic stand during pouring). The cell suspension now contains purified human $CD34^+$ cells. Centrifuge at 500 g for 5 min, discard the supernatant, and collect the cells.

Note: Repeat Steps 2–6 once if needed to further improve $CD34^+$ cell purity.

7. Wash cells as required for your experiment, then resuspend in appropriate buffer or culture medium for downstream molecular or cellular biology applications.

Isolation Performance

Enrichment of $CD34^+$ cells from human CBMC. Cells before and after separation were stained with FITC anti-human CD45 (clone HI30) and PE anti-human CD34 (clone 581) antibodies and analyzed by flow cytometry. Purities: 1.2% pre-selection, 13.9% after first selection, and 33.8% after second selection.



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.