Human CD66$^+$ Cell Isolation Kit (positive isolation)

Catalog No.: BRC0013

Product Description

The Human CD66b$^+$ Cell Isolation Kit (Positive Selection) is designed to isolate CD66b$^+$ cells from human peripheral blood single-cell suspensions. The principle involves labeling CD66b$^+$ cells with CD66b Capture Antibody, followed by capture using **Releasable EnkiBeads™**. The beads are then released from the cell surface using **EnkiBeads™ Release Buffer**, yielding bead-free human CD66b$^+$ cells. CD66b is a 95–100 kDa glycosylphosphatidylinositol (GPI)-linked protein expressed on neutrophils and eosinophils, but not on basophils or lymphocytes. The isolated CD66b$^+$ cells are suitable for downstream molecular and cellular biology applications.

Kit Components

No.	Reagent Name	Size (For 10^9 cell)
Reagent 1	CD66b Capture Antibody	200μL
Reagent 2	Releasable EnkiBeads™	2 mL
Reagent 3	EnkiBeads™ Release Buffer	40mL

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 human peripheral blood CD66b$^+$ cells.

Protocol

Sample Preparation

1. **RBC Lysis:** Place fresh anticoagulated whole blood in a centrifuge tube and perform red blood cell lysis.
2. **Wash:** After lysis, resuspend cells in PBS, wash, and centrifuge at 500 g for 5 min.
3. **Resuspend:** Resuspend cells 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.

Cell Labeling

1. Add 500 μL cell suspension (5×10^7 cells) to the bottom of a flow tube, then add 10 μL CD66b Capture Antibody. Mix well and incubate at 4°C for 15 min.

Note: Pipette cell suspension directly to the bottom of the tube; avoid pipetting along the tube

wall. Centrifuge tubes may be used depending on the magnetic stand. Scale the volume of CD66b Capture Antibody proportionally for other cell numbers. If processing fewer than 1×10^7 cells, adjust the cell suspension volume to 100 µL and add 2 µL CD66b Capture Antibody.

2. After incubation, add 100 µL of washed Releasable EnkiBeads™ to the cell suspension (Wash beads before use: Vortex thoroughly to resuspend, transfer the required volume to a sterile 1.5 mL centrifuge tube, add separation buffer to a total volume of 1 mL, centrifuge at 10,000 g for 1 min, and discard the supernatant. Resuspend beads in 1 mL separation buffer, centrifuge again at 10,000 g for 1 min, discard the supernatant, and resuspend beads in the original required volume of separation buffer). Mix well and incubate at 4°C for 10 min.

Note: Scale the volume of Releasable EnkiBeads™ proportionally for other cell numbers. If processing fewer than 1×10^7 cells, use 20 µL Releasable EnkiBeads™.

Magnetic Separation and Washing

1. After incubation, add separation buffer to 2.5 mL and mix thoroughly by pipetting up and down (avoid vigorous vortexing or inversion mixing). Place the flow tube on the magnetic stand for 5 min.
2. Aspirate and discard the supernatant. Remove the tube from the magnetic stand, quickly add 2 mL separation buffer, and resuspend beads by repeated pipetting. Place the tube back on the magnetic stand for 5 min.
3. Repeat Step 2 two additional times (thorough washing ensures high purity of target cells in subsequent elution).

Bead Release

1. After the final wash, aspirate and discard the supernatant. Remove the tube from the magnetic stand, quickly add 1 mL EnkiBeads™ Release Buffer to fully resuspend beads (avoid bead drying), transfer the bead suspension to a 1.5 mL centrifuge tube, and incubate with rotation at room temperature for 10 min.

Note: Scale the volume of EnkiBeads™ Release Buffer proportionally for other cell numbers. If processing fewer than 1×10^7 cells, use 200 µL EnkiBeads™ Release Buffer for elution.

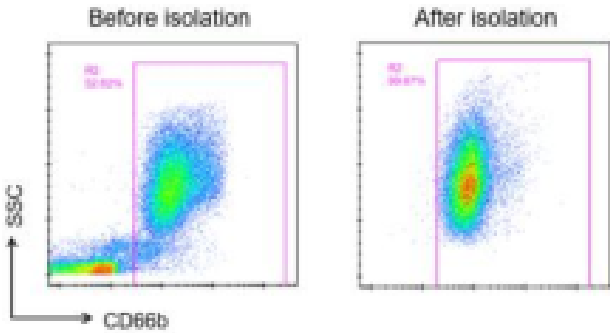
2. After incubation, pipette up and down at least 10 times, transfer the bead suspension to a new flow tube, and place on the magnetic stand for 5 min.

Note: For smaller cell numbers, add separation buffer to 1.5 mL before magnetic separation. For example, when processing 2×10^7 cells (in 400 µL EnkiBeads™ Release Buffer), after transferring to the new flow tube, add separation buffer to 1.5 mL, mix by pipetting, then place on the magnetic stand.

3. Transfer the supernatant to a new flow tube for storage (the supernatant contains target cells—do not discard). Quickly resuspend beads in 1 mL EnkiBeads™ Release Buffer (avoid bead drying), transfer to a 1.5 mL centrifuge tube, and incubate with rotation at room temperature for 10 min.
4. After incubation, pipette up and down at least 10 times, transfer the bead suspension to a new flow tube, and place on the magnetic stand for 5 min.
5. Pool the supernatant with the first eluted cell fraction, place on the magnetic stand for 5 min to remove any residual beads.
6. Transfer the supernatant to a centrifuge tube, centrifuge at 500 g for 5 min, and discard the supernatant to collect bead-free $CD66b^+$ cells.

Isolation Performance

CD66b$^+$ cells were isolated from human peripheral blood cells. Cells before and after isolation were stained with Alexa Fluor 647 anti-CD66b antibody (clone 6/40c) and analyzed by flow cytometry. Purity of CD66b$^+$ cells was 52.62% pre-isolation and 99.67% 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.