

Human CD66b+ Cell Isolation Kit (positive selection)

Description

TargetMol's Human CD66b+ Cell Isolation Kit (positive selection) utilizes superparamagnetic beads covalently coated with monoclonal antibodies specific for the human CD66b antigen. CD66b is a glycosylphosphatidylinositol (GPI)-anchored glycoprotein predominantly expressed on the surface of neutrophils and eosinophils and is commonly used as a marker for these granulocyte populations. It plays a critical role in cell adhesion, cell migration, and pathogen recognition.

The principle of the Human CD66b Cell Isolation Kit (positive selection) involves immunolabeling CD66b+ cells with CD66b Capture Antibody, capturing target cells using Releasable Beads, and subsequently detaching the magnetic beads from the cell surface with Beads Release Buffer. This yields bead-free human CD66b+ cells. This rapid, gentle column-free isolation method helps ensure high purity, recovery, and viability of the isolated CD66b+ cells. The resulting CD66b+ cells can be readily used for downstream molecular and cell biology experiments.

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: Efficiently captures CD66b+ cells to obtain highly enriched target cells.
2. High Viability: The gentle isolation process helps maintain high cell viability and preserves cells for downstream applications.
3. Easy Operation: Column-free separation using a simple magnetic rack.

Applications

Suitable for isolating CD66b+ cells from human peripheral blood.

Kit Components

Catalog No.	Component Name	Packaging Size (for 1×10^9 cells)
C0151-1	CD66b Capture Antibody	200 μ L
C0151-2	Releasable Magnetic Beads	2 mL
C0151-3	Magnetic Beads Release Buffer	40 mL

Instructions

1. Cell Sample Preparation: Collect fresh anticoagulated whole blood into a centrifuge tube and perform red blood cell (RBC) lysis.
Note: EDTA-anticoagulated whole blood is recommended. The volume of lysis buffer and lysis time can be adjusted as needed. If initial lysis is incomplete, a second round of lysis may be performed. A minor residual amount of RBCs will not significantly affect isolation purity.
2. Following RBC lysis, resuspend the cell pellet in PBS and wash by centrifuging at $500 \times g$ for 5 min.
3. Resuspension: Discard the supernatant, resuspend cells in Isolation Buffer, and adjust cell concentration to 1×10^8 cells/mL.
Note: Recommended Isolation Buffer: PBS containing 2 mM EDTA and 2% FBS. The buffer must be sterile-filtered through a $0.22 \mu\text{m}$ membrane filter prior to use.
4. Add 500 μ L cell suspension (5×10^7 cells) directly to the bottom of a sterile flow cytometry tube, add 10 μ L CD66b Capture Antibody, mix thoroughly, and incubate at 4°C for 15 min.
Note: Avoid running cell suspensions down the tube wall when adding cells. Scale up the CD66b Capture Antibody volume proportionally when isolating larger cell numbers. If isolating $< 1 \times 10^7$ cells, top up the cell suspension volume to 100 μ L and add 2 μ L CD66b Capture Antibody. Centrifuge tubes may also be used depending on the compatibility of the magnetic rack.
5. Bead Pretreatment: Resuspend magnetic beads by vortexing. Transfer the required amount of beads into a 1.5 mL tube, add 1 mL Isolation Buffer, centrifuge at $10,000 \times g$ for 1 min, and discard the supernatant. Repeat the wash step once. Resuspend beads in Isolation Buffer back to their original volume. (e.g., if 20 μ L beads were washed, resuspend in 20 μ L Isolation Buffer after washing).
6. After antibody incubation, add 100 μ L pretreated Releasable Magnetic Beads to the flow tube, mix thoroughly, and incubate at 4°C for 15 min.
Note: Scale up Releasable Magnetic Beads volume proportionally for larger cell numbers. If isolating $< 1 \times 10^7$ cells, add 20 μ L Releasable Magnetic Beads.

7. After incubation, add 2.5 mL Isolation Buffer to the flow tube and gently pipette up and down to mix. Avoid vigorous vortexing or tube inversion. Place the flow tube on a magnetic rack and let it stand for 5 min.
8. Discard the supernatant. Remove the flow tube from the magnetic rack, immediately add 2 mL Isolation Buffer, and gently pipette up and down several times to disperse the beads. Place the tube back onto the magnetic rack and let it stand for 5 min.
9. Repeat Step 8 two additional times.
Note: Thorough washing is critical to achieving high purity of target cells during downstream elution.
10. After magnetic separation, discard the supernatant. Remove the tube, immediately add 1 mL Magnetic Beads Release Buffer to resuspend the beads and prevent them from drying out. Transfer the bead slurry to a 1.5 mL microcentrifuge tube and incubate on a rotary mixer at room temperature for 10 min.
Note: The volume of Magnetic Beads Release Buffer can be scaled according to cell input. If isolating $< 1 \times 10^7$ cells, use 200 μ L Magnetic Beads Release Buffer for elution.
11. After incubation, pipette up and down at least 10 times, transfer the slurry to a new flow tube, top up Isolation Buffer to 1.5 mL, and mix gently by pipetting. Place the flow tube on the magnetic rack and let it stand for 5 min.
12. The supernatant contains the target cells; transfer this supernatant to a 15 mL centrifuge tube for collection. Immediately add 1 mL Magnetic Beads Release Buffer to the remaining magnetic beads to keep them moist, transfer the slurry to a 1.5 mL tube, and incubate on a rotary mixer at room temperature for 10 min.
13. Repeat the cell elution steps (Step 11) once more and retain the supernatant.
14. Combine the second eluate supernatant with the first eluate, centrifuge at 500 x g for 5 min, and discard the supernatant to yield bead-free CD66b+ cells.
15. Wash cells as required by downstream protocols, then resuspend in the desired buffer or culture medium for subsequent molecular or cellular 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. 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.
5. For your safety and health, please wear a lab coat and disposable gloves during operation.

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