Mouse B Cell Isolation Kit (negative selection)

Description

TargetMol's Mouse B Cell Isolation Kit (Negative Selection) utilizes superparamagnetic beads to isolate B cells from mouse spleen, lymph nodes, and bone marrow. The kit employs a negative selection strategy in which non-target cells are labeled with biotin-conjugated monoclonal antibodies. These labeled cells are subsequently removed using streptavidin-coated magnetic beads, enabling the efficient enrichment and isolation of untouched mouse B cells.

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	C0152	/	/	/
CD3/CD28 T Cell Activation	C0180	/	/	/	/
B Cells	C0218	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 97%.
2. High Viability: The isolated cells retain normal cellular functions 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 B cells from mouse spleen, lymph nodes, and bone marrow.

Components

Cat No.	Product Name	for 1×10^8 cells	for 5×10^8 cells	for 1×10^9 cells
C0218-1	Biotin-Antibody Mix	20 μL	100 μL	200 μL
C0218-2	Streptavidin Magnetic Beads	200 μL	1 mL	2 mL

Instructions

1. Preparation of Single-Cell Suspension

Place the spleen on a 70 μm cell strainer and gently dissociate the tissue. Rinse the strainer with pre-chilled PBS and collect the cell suspension into a 50 mL centrifuge tube. Centrifuge at 500 × g for 5 min.

2. After centrifugation, discard the supernatant. Add 5 mL of ACK red blood cell lysis buffer per mouse and incubate at room temperature for 5 min. Subsequently, add 20 mL PBS and centrifuge at 500 × g for 5 min.

Note: The incubation time and volume of RBC lysis buffer may be adjusted according to the specific lysis reagent used. A small amount of residual red blood cells generally has minimal impact on subsequent cell separation and purity.

3. Discard the supernatant and resuspend the splenocytes in PBS. Filter the cell suspension through a 70 μm cell strainer. After counting the cells, centrifuge again at 500 × g for 5 min.

Note: To avoid interference from tissue debris and cell aggregates during cell separation, the cell suspension should be filtered through a cell strainer before use.

4. Discard the supernatant and resuspend the cells in separation buffer. Adjust the cell concentration to 1 × 10^8 cells/mL.

Note: Recommended separation buffer composition: PBS containing 2 mM EDTA and 2% FBS, or PBS containing 2 mM EDTA and 0.5% BSA. The buffer should be sterilized by filtration through a 0.22 μm membrane before use.

5. Transfer 100 μL of cell suspension (containing 1 × 10^7 cells) to the bottom of a sterile 1.5 mL microcentrifuge tube. Add 2 μL of Biotin-Antibody Mix, mix gently, and incubate at 4°C for 10 min. After incubation, add 1 mL separation buffer and centrifuge at 500 × g for 5 min. Discard the supernatant and resuspend the cell pellet in 100 μL separation buffer. Transfer the suspension to a sterile FACS tube.

Note: Add the cell suspension directly to the bottom of the tube rather than along the tube wall. For larger cell numbers, increase the amount of Biotin-Antibody Mix proportionally. Depending on the magnetic separator used, centrifuge tubes may also be utilized for cell separation.

6. Magnetic Bead Preparation

Vortex the magnetic beads thoroughly to obtain a homogeneous suspension. Transfer the required volume of beads into a 1.5 mL centrifuge tube. Add 1 mL separation buffer and centrifuge at 10,000 × g for 1 min. Discard the supernatant. Repeat the washing step once more. Finally, resuspend the beads in the same volume of separation buffer as the original bead volume. For example, if 20 μL of beads are washed, resuspend them in 20 μL separation buffer.

7. Add 20 μL of the prepared Streptavidin Magnetic Beads to the cell suspension. Mix gently and incubate at 4°C for 10 min.

Note: For larger numbers of cells, increase the amount of Streptavidin Magnetic Beads proportionally. For example, when separating 5 × 10^7 cells, add 10 μL Biotin-Antibody Mix and 100 μL 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 Biotin-Antibody Mix plus 20 μL Streptavidin Magnetic Beads.

8. Following incubation, add 2.5 mL separation buffer to the FACS tube. Gently pipette up and down five times to mix. Avoid vigorous vortexing or repeated inversion.

9. Place the FACS tube containing the cells on a magnetic separator and allow it to stand for 5 min.

10. While keeping the tube on the magnetic separator, gently pour the cell suspension into a sterile centrifuge tube. Centrifuge at 500 × g for 5 min, discard the supernatant, and collect the cells.

11. Wash the cells as required for downstream applications. Resuspend the cells in the desired buffer or culture medium for subsequent molecular biology or cell biology experiments.

Storage

Store at 4 ℃ for 2 years.

Precautions

1. Avoid freezing any components of the kit. Magnetic beads should be stored in the storage solution to prevent drying.

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.

2. 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.
3. A magnetic separator with a magnetic field strength greater than 7000 Gs is recommended, as insufficient magnetic strength may affect the isolation efficiency.
4. The product is for R&D use only, not for diagnostic procedures, food, drug, household or other uses.
5. Please wear a lab coat and disposable gloves.

Negative Selection VS Positive Selection

Magnetic Cell Isolation Technology	Negative Selection	Positive Selection
Sample Type	Diverse	Diverse
Capture Method	Magnetic beads bind to non-target cells	Magnetic beads bind to 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; No residual antibodies or magnetic beads on the cells; Better cell viability, suitable for downstream functional assays.	Broader Sample Compatibility