Mouse Pan B Cell Isolation Kit (Negative Selection)

Introduction

The Mouse Pan B Cell Isolation Kit (Negative Selection) is used for the isolation of total B cells from Single-cell suspension of mouse spleen, lymph nodes, bone marrow cells, or peritoneal lavage fluid by negative selection. The isolation principle of this kit is to label unwanted cells with the biotin-labeled monoclonal antibody mix, and then add streptavidin-labeled magnetic beads to remove unwanted cells. Isolated cells are high-purity total B cells.

This kit is easy to use and does not require a separation column, which only requires a magnetic separator. At the same time, the obtained cells are not labeled with antibodies and magnetic beads, so they will not be abnormally activated and will not affect downstream experiments.

Components and Storage

Components \ Size	50 Assays (for 5×10^8 cells)	100 Assays (for 10^9 cells)	Storage
Biotin-Antibody Mix	100 µL	200 µL	4°C
Streptavidin Beads	1 mL	2 mL	4°C
Shipping: Blue ice	Shelf life: 2 years		

Protocol

1. Preparation of sorting buffer: The sorting buffer consists of PBS supplemented with 2 mM EDTA and 2% fetal bovine serum (FBS). Alternatively, 0.5% BSA may be used in place of FBS. Filter the prepared buffer through a 0.22 µm membrane to sterilize.
2. Preparation of single-cell suspension:
    1) For mouse spleen, grind the spleen with a 70 µm cell strainer, rinse the strainer with pre-chilled PBS, and collect the cell suspension in a 50 mL centrifuge tube. Bone marrow cell suspensions should also be filtered through a 70 µm strainer.
    2) Then centrifuge the cell suspension at 500 × *g* for 5 min.
    3) Discard the supernatant. Add 5 mL of Red Blood Cell Lysis Buffer per mouse and incubate at room temperature for 5 min. Subsequently, add 20 mL of PBS and centrifuge at 500 × *g* for 5 min.

***Note:** The volume and incubation time can be adjusted based on the experiment. Minimal residual red blood cells will not affect sorting. Omit this step for peritoneal lavage fluid, as it lacks red blood cells.

4) Discard the supernatant. Resuspend cells in PBS, filter through a 70 μm cell strainer, and count. Centrifuge at 500 × *g* for 5 min.

***Note:** Filtering removes tissue debris and cell clumps that may interfere with sorting.

5) Remove the supernatant, and resuspend cells in the sorting buffer. Adjust the cell density to 1×10^8 cells/mL.

3. Antibody incubation: Transfer 100 μL of cell suspension (1×10^7 cells) to a sterile tube. Add 2 μL of Biotin-Antibody Mix to the tube. Mix well and incubate at 4°C for 10 min.

***Note:** Please add the cell suspension directly to the bottom of the tube, not along the wall of the tube. Depending on the magnet used, centrifuge tubes can also be used for sorting. If more cells need to be sorted, scale up the volume of the Biotin-Antibody Mix.

4. Magnetic bead washing

1) Resuspend Streptavidin Beads by vortexing. Transfer the required amount of Streptavidin Beads to a 1.5 mL centrifuge tube, and add 1 mL of sorting buffer.

2) Centrifuge at 10,000 × *g* for 1 min and discard the supernatant.

3) Add 1 mL of sorting buffer to resuspend the beads, centrifuge at 10,000 × *g* for 1 min, and discard the supernatant.

4) Resuspend the beads in a volume of sorting buffer equal to the original volume of beads taken. For example, if taking 20 μL of Streptavidin Beads, add 20 μL of sorting buffer.

5. After incubation, add 20 μL of washed Streptavidin Beads to the tube. Mix well and incubate at 4°C for 10 min.

***Note:** If more cells need to be sorted, scale up the volume of Streptavidin Beads. If sorting less than 1×10^7 cells, make up the cell suspension to 100 μL and add 2 μL of Biotin-Antibody Mix and 20 μL of washed Streptavidin Beads.

6. Add 2.5 mL of sorting buffer to the tube and mix gently. Do not shake vigorously or invert the tube.

7. Place the tube in a magnet for 5 min.

8. Gently pour the cell suspension into a sterile tube, and do not disengage the flow tube from the magnet during the pouring process. This cell suspension is the isolated Mouse B cells. Centrifuge at 500 × *g* for 5 min and discard the supernatant.

9. Wash and resuspend cells with a suitable buffer or medium for subsequent experiments.

Note

1. Do not freeze both Biotin-Antibody Mix and Streptavidin Beads.

2. Streptavidin Beads should be avoided from drying during use and storage, and should not be placed in a

magnetic field for a long time to avoid clumping of the beads and reduce the activity of the beads.

3. Try to use low-adsorption pipette tips and tubes to avoid loss due to adsorption.
4. This product can be removed by a magnet, for example QuickSort Magnet (Cat. No. CS1010) or QuickFour Magnet (Cat. No. CS1011).
5. For your safety and health, please wear lab coats and gloves during the experiment.
6. For research use only. Not to be used in clinical diagnostic or clinical trials.

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