Mouse CD3$^+$ Cell Removal Beads

Cat. No.: R1522425 | Pack size: 250μL; 1mL | Storage: 2-8°C; Do not freeze

Overview

Mouse CD3$^+$ Cell Removal Beads are generated by covalently coupling anti-mouse CD3 antibody to magnetic beads. They can be used for rapid and efficient depletion of CD3$^+$ cells from single-cell suspensions such as mouse splenocytes.

Applications

Depletion of CD3$^+$ cells from mouse spleen and lymph nodes.

Beads Capacity

Beads Volume (suspension)	Total Cell Number
250 μL	5×10^8 cells
1 mL	2×10^9 cells

Protocol (Using Depletion of CD3$^+$ Cells from Mouse Spleen as an Example)

Prepare single-cell suspension

Grind the spleen on a 70 μm cell strainer, rinse the strainer with pre-chilled PBS, collect the cell suspension into a 50 mL centrifuge tube, and centrifuge at 500 g for 5 min.

Lyse red blood cells

After centrifugation, discard the supernatant. Add 5 mL of ACK red blood cell lysis buffer and lyse at room temperature for 5 min. Then add 20 mL PBS and centrifuge at 500 g for 5 min. If spleens from multiple mice are pooled for the same batch, increase the volume of red blood cell lysis buffer accordingly, following the manufacturer's instructions.

Note: The red blood cell lysis step can be adjusted in volume and time depending on the lysis buffer used. A small amount of residual red blood cells will not affect subsequent depletion or cell purity.

Resuspend splenocytes

After centrifugation, discard the supernatant and resuspend the splenocytes in PBS. Filter the cell suspension through a 70 µm cell strainer and count the cells. After counting, centrifuge at 500 g for 5 min.

Note: The cell suspension must be filtered through a cell strainer to remove tissue and cell clumps; otherwise, subsequent cell depletion purity may be affected.

Adjust cell concentration

After centrifugation, discard the supernatant and resuspend the cells in Isolation Buffer to a density of 2×10^8 cells/mL.

Note: Isolation buffer is PBS containing 2 mM EDTA and 2% fetal bovine serum (FBS), or PBS containing 2 mM EDTA and 0.5% BSA. The buffer must be pre-filtered through a 0.22 µm filter.

Incubate cells with beads

Add 500 µL of cell suspension (1×10^8 cells) to the bottom of a 1.5 mL centrifuge tube, then add 50 µL of washed and resuspended beads (Mouse $CD3^+$ Cell Removal Beads must be washed before use: select a pipette with an appropriate volume range, thoroughly resuspend the beads by pipetting, avoiding bubbles. Transfer the required volume of beads to a 1.5 mL centrifuge tube, add 1 mL of Isolation Buffer, centrifuge at 10,000 g for 1 min or use a magnetic separator for 3 min, then discard the supernatant. Add 1 mL of Isolation Buffer, wash the beads once more, and then resuspend the beads in the same volume of Isolation Buffer as originally taken. For example, if 50 µL of beads are taken, resuspend them in 50 µL of Isolation Buffer after washing). Mix the liquid in the tube by pipetting, and incubate at room temperature for 30 min using a rotator.

Note: For more cells, increase the volume of Mouse $CD3^+$ Cell Removal Beads proportionally. For example, to process 1.5×10^8 cells, add 750 µL of cell suspension and 75 µL of Mouse $CD3^+$ Cell Removal Beads in a 1.5 mL centrifuge tube. For $\leq5\times10^7$ cells, adjust the cell suspension volume to 250 µL and add 25 µL of Mouse $CD3^+$ Cell Removal Beads.

Magnetic separation

After incubation, add Isolation Buffer to bring the total volume to 3 mL (the liquid can be transferred from the 1.5 mL centrifuge tube to a FACS tube in several steps). Mix thoroughly by pipetting up and down 10 times (avoid vigorous shaking or inverting). Then place the FACS tube on a magnetic separator and let stand for 5 min.

Collect target cells

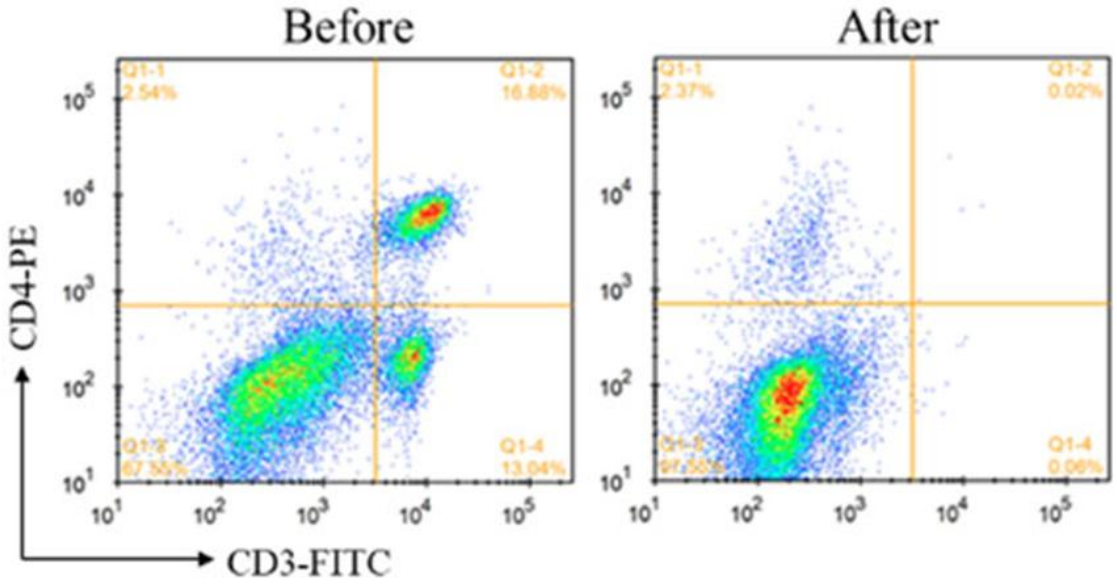
After magnetic separation, the target cells are in the supernatant. Carefully collect the supernatant (avoid taking up any beads), centrifuge at 500 g for 5 min, discard the supernatant, and collect the cells.

Resuspend for further use

Wash the cells as required for your experiment, then resuspend them in the desired buffer or culture medium for subsequent molecular or cell biology experiments.

Depletion Performance

$CD3^+$ cells were depleted from C57BL/6 mouse splenocytes. Cells before and after depletion were stained with FITC anti-mouse CD3 antibody (clone 145-2C11) and PE anti-mouse CD4 antibody (clone RM4-4) and analyzed by flow cytometry. The purity of $CD3^+$ T cells before and after depletion was 29.92% and 0.08%, respectively.



Precautions

Avoid freezing the beads during use and storage.

Low-retention pipette tips and centrifuge tubes are recommended to avoid loss of beads due to adsorption.

Before taking the beads, select a pipette with an appropriate volume range, thoroughly resuspend the beads by pipetting, and avoid generating bubbles during pipetting.

For research use only.

Specifications

Attribute	Value
Specifications & Purity	BioReagent
Stability and Storage	Store at 2-8°C long term (12 months). Do not freeze.
Storage Conditions	Store at 2-8°C; Do not freeze
Shipped In	Wet ice; Do not freeze. This product requires cold chain shipping. Ground and other economy services are not available.
Grade	BioReagent

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Whether you have a technical question, need help with a quotation, or want to inquire about an order, our regional teams are ready to help.

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