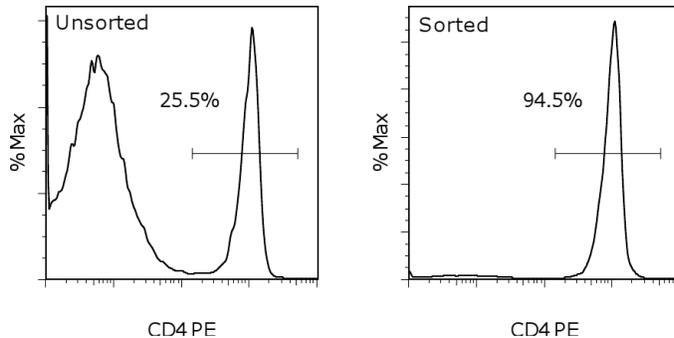


MagniSort™ Mouse CD4 T cell Enrichment Kit

Catalog Number: 8804-6821

RUO: For Research Use Only. Not for use in diagnostic procedures.



Mouse splenocytes were unsorted (left) or sorted with the MagniSort® Mouse CD4 T cell Enrichment Kit (right) then stained with Anti-Mouse CD4 PE (cat. 12-0041). Total viable cells were used for analysis.

Product Information

Contents: MagniSort™ Mouse CD4 T cell Enrichment Kit

Catalog Number: 8804-6821

Handling Conditions: For sorting sterile cells, perform all steps in the hood.

Formulation: aqueous buffer, 0.09% sodium azide, may contain carrier protein/stabilizer

Temperature Limitation: Store at 2-8°C. Do not freeze.

Batch Code: Refer to vial

Use By: Refer to vial

Contains sodium azide

REF



LOT



Description

The MagniSort® Mouse CD4 T cell Enrichment Kit is designed for the magnetic separation of mouse CD4 T cells by negative selection. It has been optimized for the isolation of mouse CD4 T cells from spleens or lymph nodes utilizing a biotinylated antibody cocktail and streptavidin-coated magnetic beads. Undesired cells are bound by antibody and then magnetic beads that, when placed in a magnetic field, leave CD4 T cells untouched and free in solution.

The MagniSort® Mouse CD4 T cell Enrichment Antibody Cocktail contains the following antibodies:

- Anti-Mouse CD8 Biotin
- Anti-Mouse CD11b Biotin
- Anti-Mouse CD19 Biotin
- Anti-Mouse CD24 Biotin
- Anti-Mouse CD45R (B220) Biotin
- Anti-Mouse CD49b (Integrin alpha 2) Biotin
- Anti-Mouse Ly-6G (Gr-1) Biotin
- Anti-Mouse $\gamma\delta$ TCR Biotin
- Anti-Mouse TER-119 Biotin

Components

MagniSort® Mouse CD4 T cell Enrichment Antibody Cocktail (cat. MS22-7762): 200 tests, 20 μ L/test; store at 2-8°C.

MagniSort® Negative Selection Beads B (cat. NB-6001): 4 mL; store at 2-8°C.

Applications Reported

The MagniSort® Mouse CD4 T cell Enrichment Kit has been reported for use in magnetic cell separation.

Applications Tested

The MagniSort® Mouse CD4 T cell Enrichment Kit has been tested by magnetic cell separation followed by flow cytometric analysis of cells from mouse secondary lymphoid tissues. A test is defined as the amount of antibody or beads to be used to stain 1×10^7 cells in 100 μ L.

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MagniSort™ Mouse CD4 T cell Enrichment Kit

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RUO: For Research Use Only. Not for use in diagnostic procedures.

This MagniSort® kit can sort 2×10^9 total cells.

Special Notes

For isolation of mouse CD4 T cells from splenocytes, follow the protocol provided. For isolation of mouse CD4 T cells from lymph node cells, reduce the amount of MagniSort® Negative Selection Beads to be used to 5 μ L/test to maximize recovery. The amount of beads to use to maximize recovery of cells from mixtures of splenocytes and lymph node cells should be determined empirically.

Related Products

01-1234 123count™ eBeads Counting Beads

12-0042 eBioscience™ Anti-Mouse CD4 PE (RM4-5)

MAG-4902 MagniSort™ Magnet

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MagniSort™ Negative Selection Protocol III

Introduction

The following protocol is a general guideline for the MagniSort™ Enrichment Kits, which are designed for the isolation of desired cells through negative selection. In negative selection, undesired cells are labeled with a cocktail of biotinylated antibodies followed by streptavidin-coated magnetic beads. When cells are placed in the MagniSort™ magnet, the undesired cells will be held in place by the magnetic field while the desired cells remain untouched and free in solution and can be isolated by decanting. For each kit, the biotinylated antibody cocktail and the magnetic beads have been pre-titrated and diluted to test size.

General Notes

Caution

The MagniSort™ Magnet, 5 mL, generates a strong magnetic field. Keep away from pacemakers, credit cards, magnetic I.D. cards, watches, computer monitors and hard disks to prevent damage to these devices.

Cell preparation

1. The MagniSort™ Enrichment Kits are optimized for use with single cell-suspensions of either mouse secondary lymphoid organs.
2. For mouse cells, removal of debris by passing through a 40 µm nylon filter is recommended for optimal performance of the kits.
3. Addition of EDTA to buffers will reduce cell clumping.

Use in sterile cultures

1. MagniSort™ Enrichment Antibody Cocktails and Negative Selection Beads contain small amounts of sodium azide as a preservative. This does not interfere with cellular functions when used in conjunction with sterile buffers that do not contain sodium azide. Performance in a given assay should be determined empirically.
2. For sorting sterile cells, perform all steps in a hood and use sterile polystyrene tubes with caps and sterile buffers.

Protocol:

Materials Provided

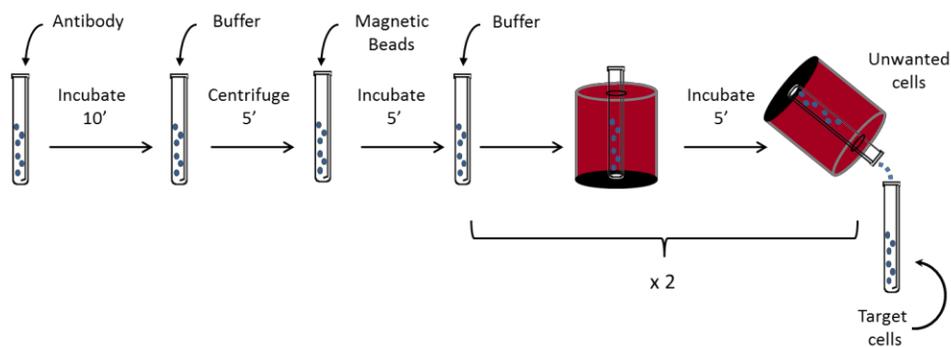
- MagniSort™ Enrichment Antibody Cocktail, 200 tests, 20 µL/test. Store at 2-8°C.
- MagniSort™ Negative Selection Beads, 4 mL. Store at 2-8°C.

Additional Materials Required

- Recommended buffer for cell separation: PBS or HBSS supplemented with 3% FBS and 10 mM EDTA. Store at 2-8°C.
Note: We do not recommend the use of tissue culture media, such as RPMI-1640 or DMEM, for use during cell separation.
- MagniSort™ Magnet, 5 mL
- 12 x 75 mm round bottom polystyrene tubes (5 mL, BD Falcon, cat. no. 352008, or equivalent)
- 15 mL conical tube (BD Falcon, cat. no. 352099, or equivalent)

Experiment Duration

- 40 minutes
- Work flow:



Experimental Procedure

1. Prepare a single-cell suspension of lymphocytes at a concentration of 1×10^7 cells/100 μL ($1 \times 10^8/\text{mL}$) in desired cell separation buffer.
Note: Cells must be in a single-cell suspension. Inspect sample and pulse vortex or pipet to remove clumps, if necessary, before proceeding.
2. Place desired number of cells but no more than 2×10^8 cells in a 12 x 75 mm, 5 mL tube.
3. Add 20 μL of MagniSort™ Enrichment Antibody Cocktail per 100 μL of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.
4. Wash cells by bringing the volume up to 4 mL with desired cell separation buffer and then centrifuge at $300 \times g$ for 5 minutes.
5. Discard the supernatant and thoroughly resuspend the cells to their original volume with desired cell separation buffer.
Note: Cells must be in a single-cell suspension. Inspect sample and pulse vortex or pipet to remove clumps, if necessary, before proceeding.
6. For cells from lymph nodes only, add 5 μL of MagniSort™ Negative Selection Beads per 100 μL of cells. For cells from spleen only, add 20 μL of MagniSort™ Negative Selection Beads per 100 μL of cells. For a mixture of cells from lymph nodes and spleens, add 10 μL of MagniSort™ Negative Selection Beads per 100 μL of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 5 minutes.
Note: The MagniSort™ Negative Selection Beads must be uniformly resuspended before adding to cells to ensure optimal performance. Thoroughly resuspend the beads by pipetting up and down 5 times with a P1000 pipette set to 1 mL or by vortexing.
7. Bring the volume up to 2.5 mL with desired cell separation buffer. Mix by pipetting up and down 3 times with a P1000 pipette set to 1 mL. Avoid vortexing.
8. Insert the tube into the magnet until the bottom of the tube is touching the bench top through the hole in the bottom of the magnet. Incubate at room temperature for 5 minutes.
9. Pick up the magnet, and in a continuous motion pour the supernatant into a 15 mL conical tube. Hold the inverted tube for 1 second and then return it to the upright position.
Note: Do not blot or shake the inverted tube as this may reduce the purity of the unbound cells.
10. Remove the tube containing bound cells from the magnet and repeat Step 7 to 9 one more time for a total of 2 separations. Pool the two fractions of unbound cells in the 15 mL conical tube.
11. Remove the tube containing bound cells from the magnet and discard. The untouched, negatively selected cells are ready to use in the 15 mL conical tube.

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