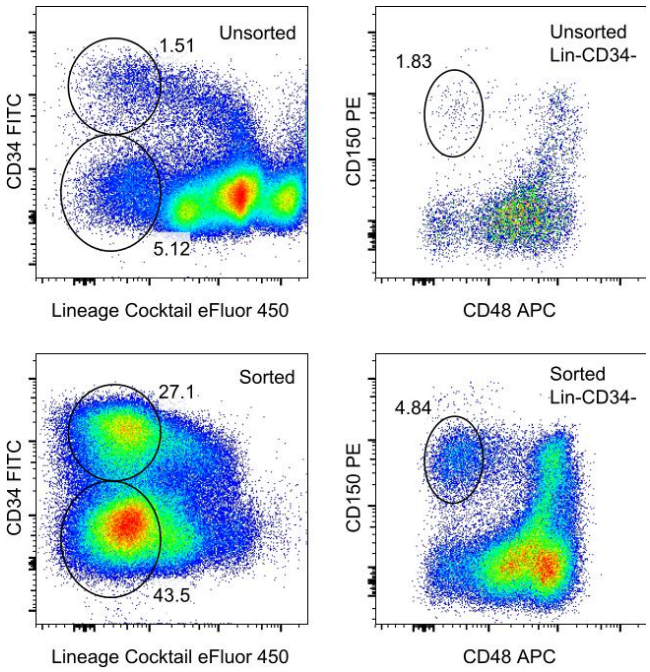
MagniSort™ Mouse Hematopoietic Lineage Depletion Kit

Catalog Number: 8804-6829

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



Mouse bone marrow cells were unsorted (top row) or sorted with the MagniSort® Mouse Hematopoietic Lineage Depletion Kit (bottom row) then stained with Mouse Hematopoietic Lineage eFluor® 450 Cocktail (cat. 88-7772), Anti-Mouse CD34 FITC (cat. 11-0341), Anti-Mouse CD48 APC (cat. 17-0481), and Anti-Mouse CD150 PE (cat. 12-1502). Viable cells were used for analysis of the hematopoietic progenitor cells (Lineage-CD34+) (left column). Viable, Lineage-CD34- cells were used for the analysis of long-term hematopoietic stem cells (CD48-CD150+) (right column).

Product Information

Contents: MagniSort™ Mouse Hematopoietic Lineage Depletion Kit
REF **Catalog Number:** 8804-6829
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.
LOT **Batch Code:** Refer to vial
Use By: Refer to vial
Contains sodium azide

Description

The MagniSort® Mouse Hematopoietic Lineage Depletion Kit is designed for the magnetic depletion of mouse hematopoietic cells with committed lineages from mouse bone marrow. It utilizes a cocktail of antibody specific for hematopoietic lineages and streptavidin-coated magnetic beads. Lineage committed cells are bound by antibody and then magnetic beads. When placed in a magnetic field, the lineage negative cells can be separated from lineage committed cells by decanting.

The MagniSort® Mouse Hematopoietic Lineage Depletion Antibody Cocktail contains the following antibodies:
Anti-Mouse CD2 Biotin
Anti-Mouse CD3 Biotin
Anti-Mouse CD5 Biotin
Anti-Mouse CD11b Biotin
Anti-Mouse CD19 Biotin
Anti-Mouse CD45R Biotin
Anti-Mouse Ly-6G (Gr-1) Biotin
Anti-Mouse TER-119 Biotin

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After depletion, the enrichment of multi-potent progenitor cells can be verified by staining with Anti-Mouse CD34 (clone RAM34), and the enrichment of CD34- hematopoietic stem cells can be verified by additional staining with Anti-Mouse CD150 (clone mShad150) and Anti-Mouse CD48 (clone HM48-1).

Components
MagniSort® Mouse Hematopoietic Lineage Antibody Cocktail (cat. MS22-6829): 200 tests, 20 µL/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 Hematopoietic Lineage Depletion Kit has been reported for use in magnetic cell separation.

Applications Tested
The MagniSort® Mouse Hematopoietic Lineage Depletion Kit has been tested by magnetic cell separation followed by flow cytometric analysis of mouse bone marrow cells. A test is defined as the amount of antibody or beads to be used to stain $1x10^7$ cells in 100 µL.

This MagniSort® kit can sort $2x10^9$ total cells.

Related Products
00-6993 eBioscience™ 7-AAD Viability Staining Solution
11-0341 eBioscience™ Anti-Mouse CD34 FITC (RAM34)
12-1502 eBioscience™ Anti-Mouse CD150 PE (mShad150)
17-0481 eBioscience™ Anti-Mouse CD48 APC (HM48-1)
88-7772 eBioscience™ Mouse Hematopoietic Lineage eFluor™ 450 Cocktail (17A2, RA3-6B2, M1/70, TER-119, RB6-8C5)
MAG-4902 MagniSort™ Magnet

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invitrogen

MagniSort™ Negative Selection Protocol V

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™ Enrichmment Kits are optimized for use with single cell-suspensions of either mouse secondary lymphoid organs or normal human peripheral blood mononuclear cells, unless otherwise noted.
2. For mouse cells, removal of debris by passing through a 40 µm nylon filter is recommended for optimal performance of the kits.
3. For preparation of normal human peripheral blood mononuclear cells, please refer to Best Protocols: Protocol D: Isolation of PBMC from whole blood located under the Resources Tab online. It is recommended to thoroughly wash the buffy coat cells to remove platelets for optimal performance in the MagniSort™ kits.
4. 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

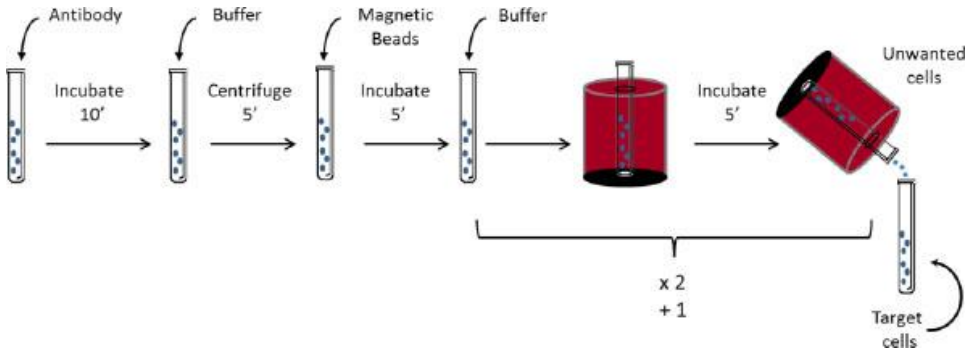
- MagniSort™ Enrichment Antibody Cocktail, 200 tests, 20 µL/test. Store at 2-8°C.
- MagniSort™ Negative Selection Beads, 4 mL. Store at 2-8°C.

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

- 45 minutes
- Work flow:



Experimental Procedure

1. Prepare a single-cell suspension of lymphocytes at a concentration of $1x10^7$ cells/100 µL ($1x10^8$/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 $2x10^8$ 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 x *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. Add 20 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 MagniSortTM 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 Steps 7-9 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. Centrifuge the unbound cells from Step 10 at 300 x *g* for 5 minutes. Discard the supernatant and add 2.5 mL of cell separation buffer. Mix the cells by pipetting, and transfer into a new 12 x 75 mm, 5 mL tube.
12. Repeat Steps 8-9 for an additional round of purification. Remove the tube containing bound cells from the magnet and discard. The untouched, negatively selected cells are ready to use in the new 15 mL conical tube.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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