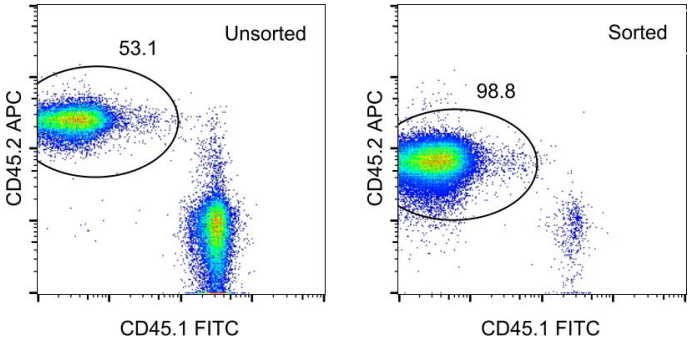
MagniSort™ Mouse CD45.2 Positive Selection Kit

Catalog Number: 8802-6849
RUO: For Research Use Only. Not for use in diagnostic procedures.



A mixture of C57Bl/6 and B6.SJL-Ptprca Pepcb/BoyJ splenocytes were unsorted (left) or sorted with the MagniSort® Mouse CD45.2 Positive Selection Kit (right) then stained with Anti-Mouse CD45.1 FITC (cat. 11-0453) and Anti-Mouse CD45.2 APC (cat. 17-0454). Viable cells in the lymphocyte gate were used for analysis.

Product Information

Contents: MagniSort™ Mouse CD45.2 Positive Selection Kit
REF **Catalog Number:** 8802-6849
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 CD45.2 Positive Selection Kit is designed for the magnetic separation of CD45.2+ cells by positive selection. It has been optimized for the isolation of CD45.2+ cells from mouse spleens or lymph nodes utilizing a biotinylated Anti-Mouse CD45.2 antibody and streptavidin-coated magnetic beads. CD45.2+ cells are bound by antibody and then magnetic beads. When placed in a magnetic field, the undesired cells can be separated from CD45.2+ cells by decanting.

After positive selection, the purity of selected cells can be verified by staining with Anti-Mouse CD45.2, clone 104.

Components

MagniSort® Anti-Mouse CD45.2 Biotin (cat. MS13-0454): 200 tests, 20 µL/test; store at 2-8°C.
MagniSort® Positive Selection Beads A (cat. PB-6003): 4 mL; store at 2-8°C.

Applications Reported

The MagniSort® Mouse CD45.2 Positive Selection Kit has been reported for use in magnetic cell separation.

Applications Tested

The MagniSort® Mouse CD45.2 Positive Selection 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 $1x10^7$ cells in 100 µL.

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

Special Notes

When the starting frequency of CD45.2 cells is greater than 75% of the total cells, the amount of MagniSort® Magnetic Positive Selection Beads to be used can be increased to 40 µL/test to maximize recovery.

Related Products

01-1234 123count™ eBeads Counting Beads

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11-0453 eBioscience™ Anti-Mouse CD45.1 FITC (A20)
17-0454 eBioscience™ Anti-Mouse CD45.2 APC (104)
MAG-4902 MagniSort™ Magnet

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MagniSort™ Positive Selection Protocol

Introduction

The following protocol is a general guideline for the MagniSort™ Positive Selection Kits. In positive selection, desired cells are labeled with biotinylated antibodies, followed by streptavidin-coated magnetic beads. When cells are placed in the MagniSort™ magnet, the desired cells will be held in place by the magnetic field while the undesired cells remain free in solution and can be removed by decanting. For each kit, the biotinylated antibody 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™ Positive Selection Kits have been 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™ Biotin Antibody and Positive Selection Beads contain small amounts of sodium azide as 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

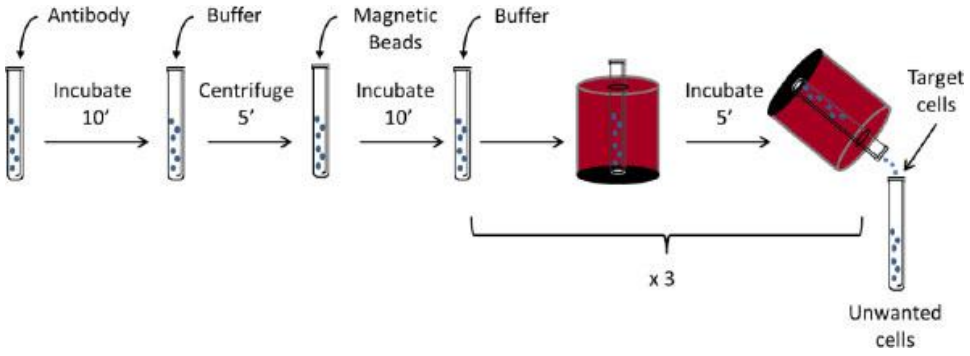
- MagniSort™ Positive Selection Antibody, 200 tests, 20 µL/test. Store at 2-8°C.
- MagniSort™ Positive Selection Beads, 4 mL. Store at 2-8°C.

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)

Experiment Duration

- 40 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™ Positive Selection Antibody 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™ Positive Selection Beads per 100 µL of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.
   Note: *The MagniSort™ Positive 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 waste or secondary receptacle; these are the undesired (unbound) cells. 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 recovery rate. The unbound cells can be collected and pooled, if needed.*
10. Remove the tube from the magnet and repeat Steps 7-9 two more times for a total of 3 washes.
11. Remove the tube containing target cells from the magnet and add 1 mL of desired cell separation buffer. Wash the sides of the tube by pipetting the buffer down the sides. The positively selected cells are ready to use.

Documentation and support

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

Limited product warranty

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23 January 2017

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