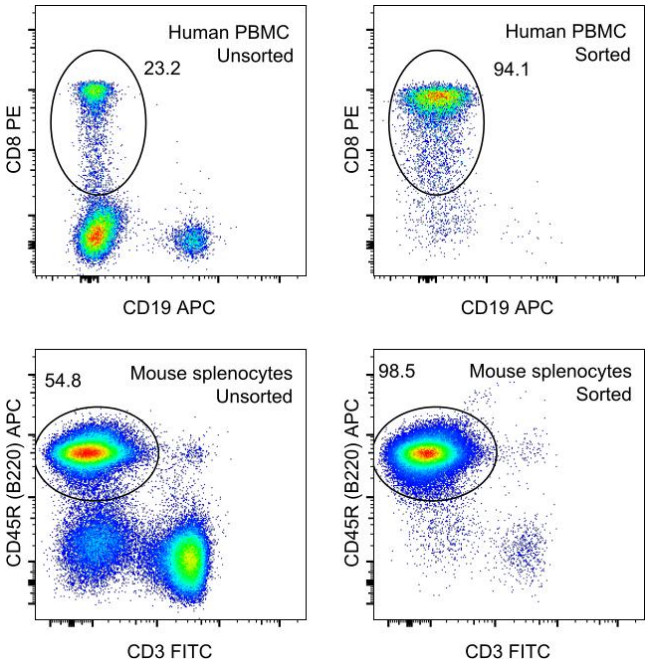
MagniSort™ Streptavidin Positive Selection Beads

Catalog Number: MSPB-6003

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



Top: Normal human peripheral blood mononuclear cells were unsorted (left) or sorted (right) with Anti-Human CD8a Biotin (cat. 13-0086) and the MagniSort® Streptavidin Positive Selection beads then stained with Anti-Human CD8a PE (cat. 12-0089) and Anti-Human CD19 APC (cat. 17-0198). Total viable cells were used for analysis.

Bottom: Mouse splenocytes were unsorted (left) or sorted (right) with Anti-Mouse CD19 Biotin (cat. 13-0191) and the MagniSort® Streptavidin Positive Selection Beads then stained with Anti-Human/Mouse CD45R (B220) APC (cat. 17-0452) and Anti-Mouse CD3 FITC (cat. 11-0032). Total viable cells were used for analysis.

Product Information

Contents: MagniSort™ Streptavidin Positive Selection Beads

REF **Catalog Number:** MSPB-6003

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

MagniSort® Streptavidin Positive Selection Beads are designed for the magnetic separation of cells by positive selection. Desired cells are bound by biotinylated antibody and then streptavidin-coated magnetic beads. When placed in a magnetic field, the desired cells are held in place and the undesired cells can be separated by decanting. The clone and concentration of biotinylated antibody to be used to label the desired cells, as well as the concentration of MagniSort® Streptavidin Positive Selection Beads must be determined empirically. Please refer to the protocol for details on how to optimize both the antibody and MagniSort® Streptavidin Positive Selection Beads.

The MagniSort® Streptavidin Positive Selection Beads may also be used to deplete undesired cells using a single antibody. For depletion of cells using multiple antibodies, we recommend the MagniSort® Streptavidin Negative Selection Beads (cat. MSNB-6002) and protocol.

Applications Reported

The MagniSort® Streptavidin Positive Selection Beads has been reported for use in magnetic cell separation.

Applications Tested

The MagniSort® Streptavidin Positive Selection Beads have been tested by magnetic cell separation followed by flow

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cytometric analysis of mouse lymphoid tissues and normal human peripheral blood mononuclear cells. Please refer to the protocol for details on how to optimize both the antibody and MagniSort® Streptavidin Positive Selection Beads.

Related Products
01-1234 123count™ eBeads Counting Beads
11-0032 eBioscience™ Anti-Mouse CD3 FITC (17A2)
12-0089 eBioscience™ Anti-Human CD8a PE (HIT8a)
13-0086 eBioscience™ Anti-Human CD8a Biotin (OKT8 (OKT-8))
13-0191 eBioscience™ Anti-Mouse CD19 Biotin (MB19-1)
17-0198 eBioscience™ Anti-Human CD19 APC (SJ25C1)
17-0452 eBioscience™ Anti-Human/Mouse CD45R (B220) APC (RA3-6B2)
MAG-4902 MagniSort™ Magnet

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invitrogen

MagniSort™ Streptavidin Positive Selection Beads Protocol

Introduction

The following protocol is a general guideline for the use of MagniSort™ Streptavidin Positive Selection Beads to sort cells that are first stained with biotinylated antibody. 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. The optimal concentration of biotinylated antibody to be used in sorting is dependent on the specificity and clone, and can be different from the optimal concentration used for flow cytometric analysis. The optimal concentration of MagniSort™ Streptavidin Positive Selection Beads to be used is dependent on the antigen density as well as target cell frequency in the unsorted sample. The optimal concentration of biotinylated antibody and Streptavidin Positive Selection Beads must be determined empirically. The MagniSort™ Streptavidin Positive Selection Beads may also be used to deplete undesired cells using a single antibody. For depletion of cells using multiple antibodies, we recommend the MagniSort™ Streptavidin Negative Selection Beads (cat. MSNB-6002) and protocol.

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. For mouse cells, removal of debris by passing through a 40 µm nylon filter is recommended for optimal performance of the kits.
2. 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.
3. Addition of EDTA to buffers will reduce cell clumping.

Use in sterile cultures

1. MagniSort™ Streptavidin 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

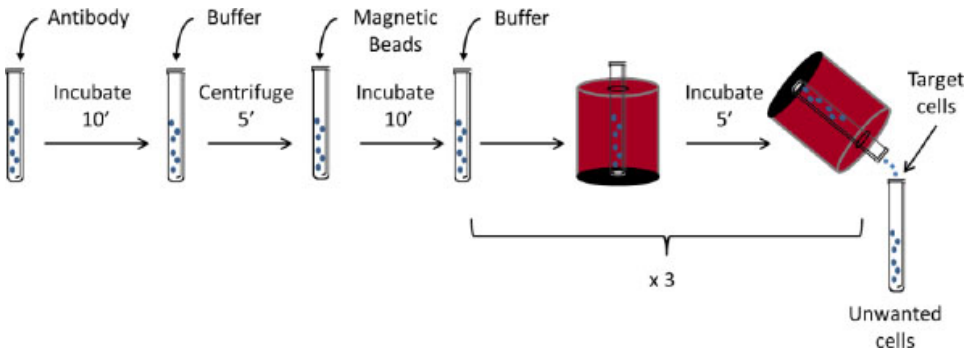
- MagniSort™ Streptavidin Positive Selection Beads (cat. MSPB-6003). Store at 2-8°C.

Additional Materials Required

- Biotinylated antibody
- 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

- Work flow:



Experimental Procedure

Step I: Optimizing the antibody concentration for magnetic cell separation:

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 ($1x10^7$ is recommended), but no more than $2x10^8$ cells, in several 12 x 75 mm, 5 mL tubes (minimum of 4 tubes is recommended).
3. Add biotinylated antibody in serial 2-fold dilution to the cells. Use the Technical Data Sheet (TDS) for the biotinylated antibody to identify the optimal concentration for flow cytometry and use that as the highest titration point. 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 recommended 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 recommended 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 the recommended amount of MagniSort™ Streptavidin Positive Selection Beads per 100 µL of cells (see below). Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.

   Recommended volume of MagniSort™ Streptavidin Positive Selection Beads

Desired cell starting frequency	*Recommended volume*
>50%	*40 µL*
10-50%	*20 µL*
<10%	*10 µL*

   Note: *The MagniSort™ Streptavidin 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 recommended 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; 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 magnet and add 1 mL of desired cell separation buffer. Wash the sides of the tube by pipetting the buffer down the sides.
12. Stain both unbound and bound fraction with surface antibody specific for the desired cells.
13. Choose an optimal concentration for the biotinylated antibody based on the desired purity and/or recovery rate.

    Note: *If you are using the MagniSort™ Streptavidin Positive Selection Beads to deplete cells with a single antibody, choose an optimal concentration of biotinylated antibody that sufficiently removes the undesired cells from the unbound fraction.*

Step II: Optimizing the MagniSort™ Streptavidin Positive Selection Beads:

1. If additional optimization is required after Step I, above, the concentration of MagniSort™ Streptavidin Positive Selection Beads used can be optimized as follows.
2. 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.*

3. Place desired number of cells ($1x10^7$ is recommended), but no more than $2x10^8$ cells, in several 12 x 75 mm, 5 mL tubes (minimum of 3 tubes is recommended).
4. To each tube, add biotinylated antibody at the optimal concentration, as determined in Step I, above. Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.
5. Wash cells by bringing the volume up to 4 mL with recommended cell separation buffer and then centrifuge at 300 x *g* for 5 minutes.
6. Discard the supernatant and thoroughly resuspend the cells to their original volume with recommended 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.*
7. Add MagniSort™ Streptavidin Positive Selection Beads at 2X, 1X, and 0.5X the amount used in Step I, 6, per 100 µL of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.
8. Bring the volume up to 2.5 mL with recommended cell separation buffer. Mix by pipetting up and down 3 times with a P1000 pipette set to 1 mL. Avoid vortexing.
9. 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.
10. Pick up the magnet and in a continuous motion pour the supernatant into a 15 mL conical tube; 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.*
11. Remove the tube from the magnet and repeat Steps 8-10 two more times for a total of 3 washes.
12. 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.
13. Stain both unbound and bound fraction with surface antibody specific for the desired cells.
14. Choose an optimal amount of MagniSort™ Streptavidin Positive Selection Beads based on the desired purity and/or recovery rate.

    Note: *If you are using the MagniSort™ Streptavidin Positive Selection Beads to deplete cells with a single antibody, choose an optimal concentration of beads that sufficiently removes the undesired cells from the unbound fraction.*

Step III: Positive selection of desired cells using the optimized conditions:

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. To each tube, add the optimal concentration of biotinylated antibody, as determined in Step I. 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 recommended 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 recommended 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 the optimal amount of MagniSort™ Streptavidin Positive Selection Beads per 100 µL of cells, as determined in Step II. Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.

   Note: *The MagniSort™ Streptavidin 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 recommended 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 recommended cell separation buffer. Wash the sides of the tube by pipetting the buffer down the sides. The positively selected cells are ready to use.

Troubleshooting Guide

Note: Staining the unbound cells to determine the amount of target cells left unsorted can provide useful information for further troubleshooting.

Problem	Possible Reasons	Solution
A. Low purity	1. Non-specific binding of undesired cells, if very few desired cells are left in the unbound fraction	1. Lower the amount of biotinylated antibody
	2. Insufficient binding of desired cells, if a large proportion of desired cells are left in the unbound fraction	2. Increase the amount of MagniSort™ Streptavidin Positive Selection Beads and/or increase the amount of biotinylated antibody
	3. Antibody is not optimal	3. Try using a different clone, if available
	4. Not enough washing steps	4. Add another washing step in Step III, 10
B. Low recovery rate (yield)	1. Insufficient binding of desired cells	1. Increase the amount of biotinylated antibody and/or MagniSort™ Streptavidin Positive Selection Beads
	2. Antibody is not optimal	2. Try using a different clone, if available
	3. Insufficient removal of excess biotinylated antibody	3. Add an additional wash step after the incubation with biotinylated antibody
	4. Use of tissue culture media as cell separation media	4. Do not use tissue culture media for cell separation. Wash cells thoroughly with recommended cell separation buffer before adding magnetic beads
	5. Too many wash steps	5. Decrease the number of washing steps in Step III, 10
	6. Loss of performance of the MagniSort™ Streptavidin Positive Selection Beads	6. Do not freeze the MagniSort™ Streptavidin Positive Selection Beads
C. High side scatter of desired cells	1. Too many beads bound	1. Decrease the amount of biotinylated antibody and/or MagniSort™ Streptavidin Positive Selection Beads
	2. Antibody is not optimal	2. Try using a different clone, if available
	3. Insufficient removal of excess biotinylated antibody	3. Add an additional wash step after the incubation with biotinylated antibody

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