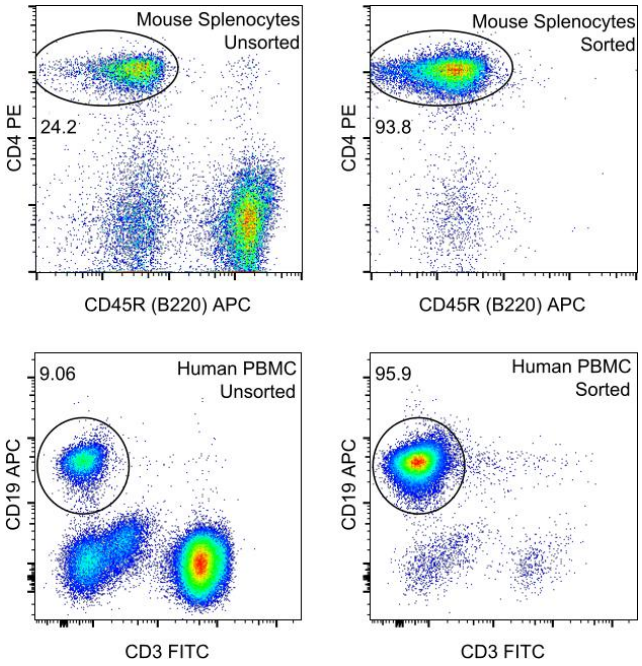
MagniSort™ Streptavidin Negative Selection Beads

Catalog Number: MSNB-6002

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



Top: Mouse splenocytes were unsorted (left) or sorted (right) with a cocktail of antibodies specific for non-CD4 cells and the MagniSort® Streptavidin Negative Selection Beads. Unbound cells were stained with Anti-Mouse CD4 PE (cat. 12-0042) and Anti-Human/Mouse CD45R (B220) APC (cat. 17-0452). Total viable cells were used for analysis.

Bottom: Normal human peripheral blood mononuclear cells were unsorted (left) or sorted (right) with a cocktail of antibodies specific for non-B cells and the MagniSort® Streptavidin Negative Selection Beads. Unbound cells were stained with Anti-Human CD19 APC (cat. 17-0198) and Anti-Human CD3 FITC (cat. 11-0037). Total viable cells were used for analysis.

Product Information

Contents: MagniSort™ Streptavidin Negative Selection Beads

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

For depletion of cells using a single antibody, we recoomend the MagniSort® Streptavidin Positive Selection Beads (cat. MSPB-6003) and protocol.

Applications Reported

The MagniSort® Streptavidin Negative Selection Beads have been reported for use in magnetic cell separation.

Applications Tested

The MagniSort® Streptavidin Negative Selection Beads have been tested by magnetic cell separation follow 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 or antibody cocktail and MagniSort® Streptavidin Negative Selection Beads.

Related Products
01-1234 123count™ eBeads Counting Beads
11-0037 eBioscience™ Anti-Human CD3 FITC (OKT3)
12-0042 eBioscience™ Anti-Mouse CD4 PE (RM4-5)
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 Negative Selection Beads Protocol

Introduction

The following protocol is a general guideline for the use of MagniSort™ Streptavidin Negative Selection Beads to deplete cells that are first stained with biotinylated antibodies. In negative selection, undesired cells are labeled with 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 free in solution and can be isolated by decanting. The optimal concentration of biotinylated antibody to be used in negative selection 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 Negative Selection Beads to be used is dependent on the frequency of the cells to be depleted in the unsorted sample. The optimal concentration of biotinylated antibody and Streptavidin Negative Selection Beads must be determined empirically. For depletion of cells using a single antibody, we recommend the MagniSort™ Streptavidin Positive Selection Beads (cat. MSPB-6003) 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 Negative 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

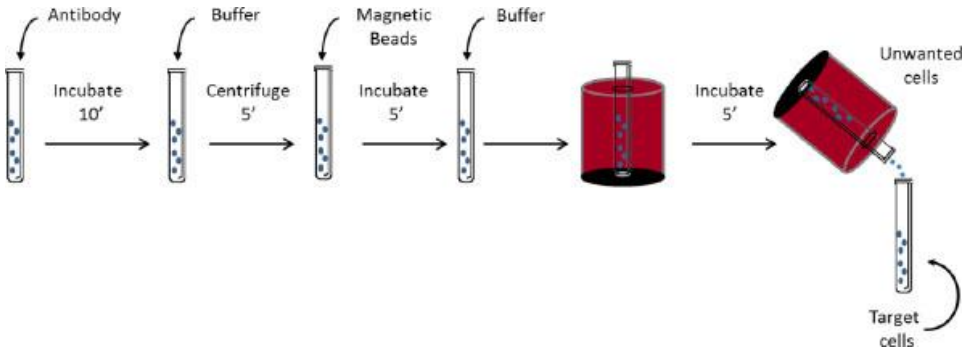
- MagniSort™ Streptavidin Negative Selection Beads (cat. MSNB-6002). Store at 2-8°C.

Additional Materials Required

- Biotinylated antibody
- Flow Cytometry Staining Buffer (cat. 00-4222)
- 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 individual antibody concentration for negative selection:

Note: *The optimal concentration of antibody to be used in negative selection in this step is assessed by flow cytometric analysis of unsorted cells. Please refer to Best Protocols: Staining Cell Surface Antigens for Flow Cytometry, Protocol A: Cell Suspensions located under the Resources Tab online for detailed instructions for staining cell surface antigens.*

1. Prepare a single-cell suspension of cells that will be used for magnetic separation.
2. For each biotinylated antibody to be used for negative selection, stain unsorted cells with each individual antibody with five points of a serial 2-fold titration. Use the Technical Data Sheet (TDS) for each biotinylated antibody to identify the optimal concentration for flow cytometry and use 2X that concentration as the highest titration point.
3. Wash cells two times with 3 mL of Flow Cytometry Staining Buffer, and then stain the cells with streptavidin conjugated to a fluorochrome of your choice, as well as a directly conjugated antibody (in a different fluorochrome than streptavidin) that will specifically recognize the desired cells. For example, if you are making a cocktail to isolate CD4 T cells by negative selection, then costain your samples with a CD4 antibody.
4. Wash cells two times with 3 mL of Flow Cytometry Staining Buffer, and then analyze samples by flow cytometry. The optimal concentration for each individual antibody is the concentration that has the brightest staining of the cells to be depleted and no background staining on the cells to be negatively selected.

   Note: *Desired cells should have similar fluorescence intensity as unstained cells.*
5. Combine all the antibodies to be used in the negative selection cocktail at their respective optimal concentration, as determined in Step I, 4.
6. Stain unsorted cells with the antibody cocktail, followed by fluorochrome-conjugated streptavidin and the costain, as performed in Step I, 2. Then analyze the cells by flow cytometry.
7. The cocktail should stain the majority of the cells to be depleted, and the desired cells should remain unstained.
8. If the antibody cocktail does not sufficiently stain all undesired cells, then additional antibodies may be optimized and added to the cocktail to improve removal of undesired cells. Optimize each additional antibody as described above.

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

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 ($1x10^7$ cells is recommended), but no more than $2x10^8$ cells, in several 12 x 75 mm, 5 mL tubes (minimum of 3 tubes is recommended).
3. Prepare a cocktail of biotinylated antibodies using the optimal concentrations, as determined in Step I.
4. Add the antibody cocktail to the cells. 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 2-fold serial dilutions of the MagniSort™ Streptavidin Negative Selection Beads to the tubes, starting with the highest concentration as recommended below. Mix well by pulse vortexing 5 times. Incubate at room temperature for 5 minutes.

Recommended volume of MagniSort™ Streptavidin Negative Selection Beads

Desired cell starting frequency	Recommended volume per 100 µL of cells
>60%	2.5 µL
30-60%	5 µL
10-30%	10 µL
<10%	20 µL

Note: *The MagniSort™ Streptavidin 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.*

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 new 12 x 75 mm, 5 mL tube, labeled "unbound fraction 1." 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.*
11. Remove the tube from the magnet and repeat Steps II, 8-10, pouring the supernatant into a new 12 x 75 mm, 5 mL tube, labeled "unbound fraction 2."
12. Discard the original tube containing the magnetically bound cells. Stain both unbound fraction 1 and fraction 2 with antibodies to assess purity and yield. Choose the optimal concentration of MagniSort™ Streptavidin Negative Selection Beads based on the desired purity and yield.

    Note: *It may be possible to combine unbound fraction 1 and fraction 2, depending on the purity and yield of each fraction.*

Step III: Negative 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. Prepare a cocktail of biotinylated antibodies using the optimal concentrations, as determined in Step I.
4. Add the antibody cocktail to the cells. 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 the optimized amount of MagniSort™ Streptavidin Negative Selection Beads per 100 µL of cells, as determined in Step II. Mix well by pulse vortexing 5 times. Incubate at room temperature for 5 minutes.

   Note: *The MagniSort™ Streptavidin 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.*
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 new 5 mL tube labeled Unbound. 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.*
11. [Optional] If pooling two fractions of unbound cells was determined to be the optimal protocol (see Step II, 12), then remove the tube from the magnet and repeat Steps III, 8-10, pouring the supernatant into the same collection tube from Step III, 10.
12. Discard the original tube containing the magnetically bound cells. The untouched, negatively selected cells are ready to use in the unbound collection tube from Step III, 10, for a single enrichment or from Step III, 11, if pooling the two unbound fractions.

Troubleshooting Guide ***Note:** Calculating the recovery rate (yield) of desired cells can provide useful information for further troubleshooting.*		
Problem	**Possible Reasons**	**Solution**
A. Low purity but high recovery rate	1. Insufficient removal of undesired cells	1a. Resuspend the unbound cells in a total volume of 2.5 mL and place back into the magnet for another round of separation
		1b. Add additional markers or additional clones for the same markers targeting the undesired cells to the cocktail
		1c. Increase the amount of antibodies used to deplete the undesired cells
		1d. Increase the amount of MagniSort™ Streptavidin Negative Selection Beads
	2. Use of tissue culture media as cell separation media	2. Do not use tissue culture media for cell separation. Wash cells thoroughly with recommended cell separation buffer before adding magnetic beads.
	3. Loss of performance of MagniSort™ Streptavidin Negative Selection Beads	3. Do not freeze MagniSort™ Streptavidin Negative Selection Beads
B. Low purity and low recovery rate	1. Excess non-specific removal of desired cells and insufficient removal of undesired cells	1a. Stain unsorted cells with cocktail and a costain for the desired cells to ensure that the cocktail is not staining the desired cells
		1b. Increase the amount of antibodies used to deplete the undesired cells
		1c. Add additional markers or additional clones for the same markers targeting the undesired cells to the cocktail
C. High purity but low recovery rate	1. Excess non-specific removal of desired cells	1a. Decrease the amount of MagniSort™ Streptavidin Negative Selection Beads
		1b. Pool multiple unbound fractions

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

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