MagniSort™ MagniSort Mouse CD11b Positive Selection Kit

Catalog Number 8802-6860-74

Pub. No. MAN0018688 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

The MagniSort™ MagniSort Mouse CD11b Positive Selection Kit is designed for the magnetic separation of CD11b+ cells by positive selection. It has been optimized for the isolation of CD11b+ cells from mouse spleens, bone marrow, or lymph nodes utilizing a biotinylated Anti-Mouse CD11b antibody and streptavidin-coated magnetic beads. CD11b+ cells are bound by antibody and then magnetic beads. When placed in a magnetic field, the undesired cells can be separated from CD11b+ cells by decanting.

The kit has been reported for use in magnetic cell separation, and 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 µL.

Contents and storage

Each kit contains enough reagent to perform 200 tests at 20 µL/test to sort a total of 2×10^9 cells.

Contents	Amount	Storage [1]
MagniSort™ Anti-Mouse CD11b Biotin	200 tests	2°C to 8°C. Do not freeze.
MagniSort™ Positive Selection Beads A	4 mL	

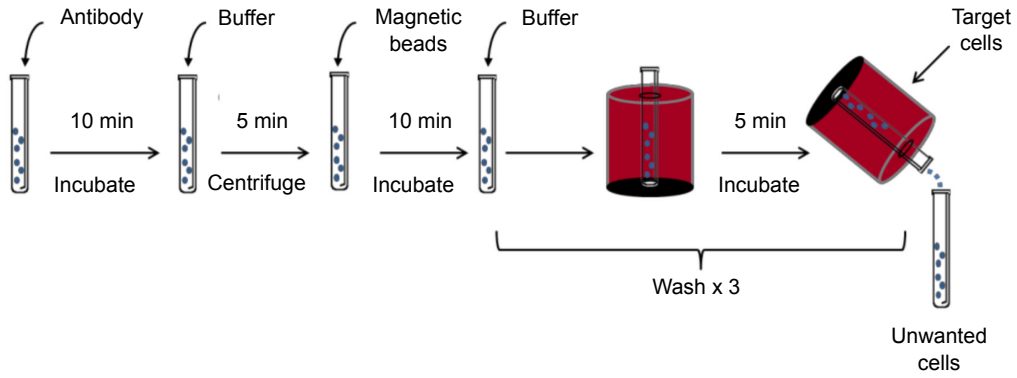
[1] When stored as directed, product is stable for at least 6 months.

Required materials not supplied

- Buffer for cell separation (e.g., PBS or HBSS supplemented with 3% FBS and 10 mM EDTA)
- MagniSort™ Magnet, 5 mL
- 12 × 75 mm round bottom polystyrene tubes (5 mL, BD Falcon, Cat. No. 352008, or equivalent)

Workflow

The following protocol is a general guideline for using 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. The total duration of the procedure is about 40 minutes.



Procedural guidelines

- Tissue culture media, such as RPMI-1640 or DMEM is not recommend for use as a cell separation buffer.
- 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.
- For sorting sterile cells, perform all steps in a hood and use sterile polystyrene tubes with caps and sterile buffers.

Guidelines for preparing cells

- The kit is optimized for use with single-cell suspensions of either mouse secondary lymphoid organs or normal human peripheral blood mononuclear cells, unless otherwise noted.
- Pass mouse cells through a 40 µm nylon filter to remove debris for optimal performance of the kits.
- For preparation of normal human peripheral blood mononuclear cells, 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 of the kit.
- Add EDTA to buffers in order to reduce cell clumping.

Perform positive selection

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.

1. Prepare a single-cell suspension of lymphocytes at a concentration of 1×10^7 cells/100 µL (1×10^8/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 the desired number of cells (≤2×10^8 cells, in a 12 × 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 × *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 Antibody 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.

Product list

Product	Cat. No.
123Count eBeads™ Counting Beads	01-1234
eBioscience™ Anti-Mouse CD3e FITC (145-2C11)	11-0031
eBioscience™ Anti-Mouse CD19 APC (eBio1D3 (1D3))	17-0193
MagniSort™ Magnet	MAG-4902

Limited product warranty

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