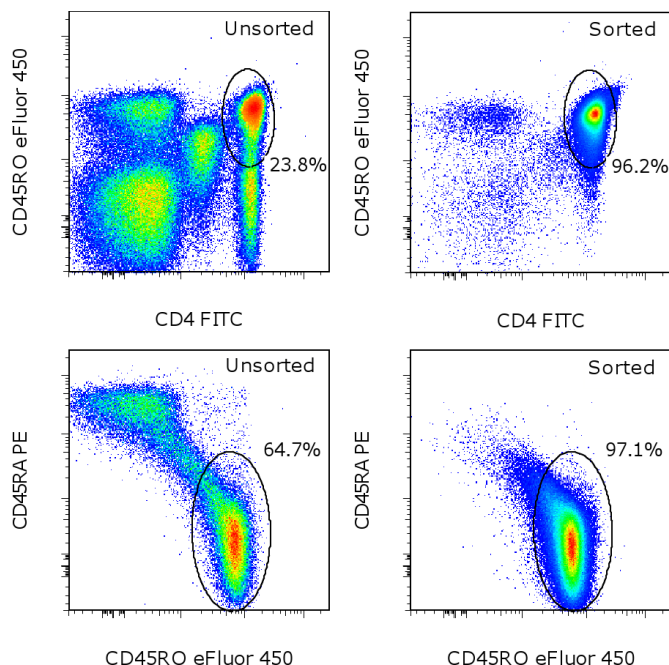


## MagniSort™ Human CD4 Memory T cell Enrichment Kit

Catalog Number: 8804-6813

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



Normal human peripheral blood mononuclear cells were unsorted (left) or sorted with the MagniSort® Human CD4 Memory T cell Enrichment Kit (right) then stained with Anti-Human CD4 FITC (cat. 11-0048), Anti-Human CD45RA PE (cat. 12-0458), and Anti-Human CD45RO eFluor® 450 (cat. 48-0457). Cells were gated on total viable cells (top) or viable CD4+ cells (bottom).

### Product Information

**Contents:** MagniSort™ Human CD4 Memory T cell Enrichment Kit

**Catalog Number:** 8804-6813

**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.

**Batch Code:** Refer to vial

**Use By:** Refer to vial

**Contains sodium azide**

### Description

The MagniSort® Human CD4 Memory T cell Enrichment Kit is designed for the magnetic separation of CD4 memory T cells by negative selection. It has been optimized for the isolation of human CD4 memory T cells from normal human peripheral blood mononuclear cells utilizing a biotinylated antibody cocktail and streptavidin-coated magnetic beads. Undesired cells are bound by antibody and then magnetic beads that, when placed in a magnetic field, leave CD4 memory T cells untouched and free in solution.

The MagniSort® Human CD4 Memory T cell Enrichment Antibody Cocktail contains the following antibodies:

- Anti-Human CD8 Biotin
- Anti-Human CD11b Biotin
- Anti-Human CD14 Biotin
- Anti-Human CD16 Biotin
- Anti-Human CD19 Biotin
- Anti-Human CD20 Biotin
- Anti-Human CD36 Biotin
- Anti-Human CD45RA Biotin
- Anti-Human CD56 Biotin

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## **MagniSort™ Human CD4 Memory T cell Enrichment Kit**

**Catalog Number:** 8804-6813

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Anti-Human CD123 Biotin

Anti-Human CD235a Biotin

Anti-Human  $\gamma\delta$  TCR Biotin

### **Components**

**MagniSort® Human CD4 Memory T cell Enrichment Antibody Cocktail** (cat. MS22-7795): 200 tests, 20  $\mu$ L/test; store at 2-8°C.

**MagniSort® Negative Selection Beads A** (cat. NB-6002): 4 mL; store at 2-8°C.

### **Applications Reported**

The MagniSort® Human CD4 Memory T cell Enrichment Kit has been reported for use in magnetic cell separation.

### **Applications Tested**

The MagniSort® Human CD4 Memory T cell Enrichment Kit has been tested by magnetic cell separation followed by flow cytometric analysis of normal human peripheral blood mononuclear cells. A test is defined as the amount of antibody or beads to be used to stain  $1 \times 10^7$  cells in 100  $\mu$ L.

This MagniSort® kit can sort  $2 \times 10^9$  total cells.

### **Related Products**

01-1234 123count™ eBeads Counting Beads

11-0048 eBioscience™ Anti-Human CD4 FITC (OKT4 (OKT-4))

12-0458 eBioscience™ Anti-Human CD45RA PE (HI100)

48-0457 eBioscience™ Anti-Human CD45RO eFluor™ 450 (UCHL1)

MAG-4902 MagniSort™ Magnet

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# MagniSort™ Negative Selection Protocol II

## 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™ Enrichment 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

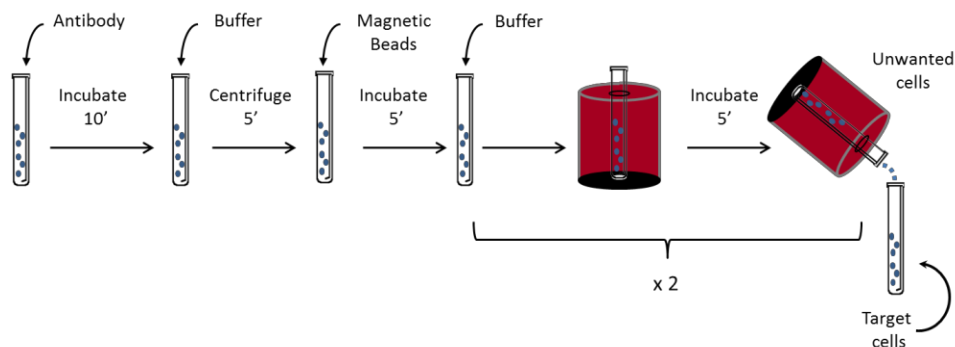
- MagniSort™ Enrichment Antibody Cocktail, 200 tests, 20 µL/test. Store at 2-8°C.
- MagniSort™ Negative Selection Beads, 4 mL. Store at 2-8°C.

### 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

- 40 minutes
- Work flow:



## Experimental Procedure

1. Prepare a single-cell suspension of lymphocytes at a concentration of  $1 \times 10^7$  cells/100  $\mu$ L ( $1 \times 10^8$ /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 desired number of cells but no more than  $2 \times 10^8$  cells in a 12 x 75 mm, 5 mL tube.
3. Add 20  $\mu$ L of MagniSort™ Enrichment Antibody Cocktail per 100  $\mu$ L 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  $300 \times g$  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  $\mu$ L of MagniSort™ Negative Selection Beads per 100  $\mu$ L of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 5 minutes.  
**Note:** The MagniSort™ 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 Step 7 to 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. The untouched, negatively selected cells are ready to use in the 15 mL conical tube.

## Documentation and support

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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.

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23 January 2017

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