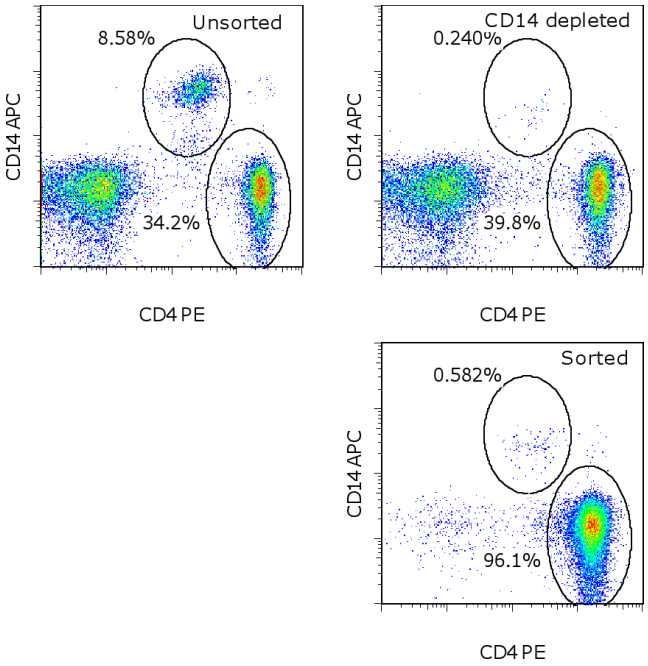
MagniSort™ Human CD4 T cell 2-Step Enrichment Kit

Catalog Number: 8802-6831

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



Normal human peripheral blood mononuclear cells were unsorted (top left), depleted of CD14+ cells (top right) then sorted for CD4+ T cells (bottom right). Cells were stained with Anti-Human CD4 PE (cat. 12-0048) and Anti-Human CD14 APC (cat. 17-0149). Total viable cells were used for analysis.

Product Information

Contents: MagniSort™ Human CD4 T cell 2-Step Enrichment Kit

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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

The MagniSort® Human CD4 2-Step Enrichment Kit is designed for the magnetic separation of CD4+ T cells by positive selection after first depleting monocytes. It has been optimized for the isolation of CD4+ T cells from human peripheral blood monocuclear cells, utilizing biotinylated Anti-Human CD14 and Anti-Human CD4 antibodies, as well as streptavidin-coated magnetic beads. In the first step, monocytes are bound by Anti-Human CD14 antibody and then magnetic beads. When placed in a magnetic field, the monocytes are held in the magnet while the remaining cells are decanted and used for the second step. In the second step, the monocyte-depleted cells are bound by Anti-Human CD4 antibody and then magnetic beads. Now, when placed in the magnetic field, the CD4+ T cells are held in the magnet while undesired cells can be decanted.

After enrichment, the purity of selected cells can be verified by staining with Anti-Human CD4, clones OKT4 or SK3.

Components

MagniSort® Anti-Human CD14 Biotin (cat. MS13-0149): 200 tests, 20 µL/test; store at 2-8°C.
MagniSort® Anti-Human CD4 Biotin (cat. MS13-0049): 200 tests, 20 µL/test; store at 2-8°C.
MagniSort® Positive Selection Beads A (cat. PB-6003): 2 x 4 mL; store at 2-8°C.

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Applications Reported
MagniSort® Human CD4 T cell 2-Step Enrichment Kit has been reported for use in magnetic cell separation.

Applications Tested
MagniSort® Human CD4 T cell 2-Step Enrichment Kit has been tested by magnetic cell separation followed by flow cytometric analysis of normal human peripheral blood cells. 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.

Related Products
01-1234 123count™ eBeads Counting Beads
12-0048 eBioscience™ Anti-Human CD4 PE (OKT4 (OKT-4))
17-0149 eBioscience™ Anti-Human CD14 APC (61D3)
MAG-4902 MagniSort™ Magnet

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invitrogen

MagniSort™ Human CD4 T cell 2-Step Enrichment Kit Protocol

Introduction

The following protocol is specifically for the MagniSort™ Human CD4 T cell 2-Step Enrichment Kit to enrich for CD4+ T cells from human peripheral blood mononuclear cells. Because human monocytes also express CD4, it is necessary to first deplete these cells before positively selecting for CD4-expressing T cells. In the first step of this protocol, monocytes are labeled with biotinylated anti-CD14 antibodies, followed by streptavidin-coated magnetic beads. When cells are placed in the MagniSort™ magnet, the monocytes will be held in place by the magnetic field and the cells decanted into a new tube will thus be depleted of the monocytes. In the second step of this protocol, CD4+ T cells are labeled with biotinylated anti-CD4 antibodies, followed by streptavidin-coated magnetic beads. When cells are placed in the MagniSort™ magnet this time, the desired CD4+ T cells will be held in place by the magnetic field, while the undesired cells will remain free in solution and can be removed by decanting. The biotinylated antibodies 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. 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.
2. Addition of 10 mM 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

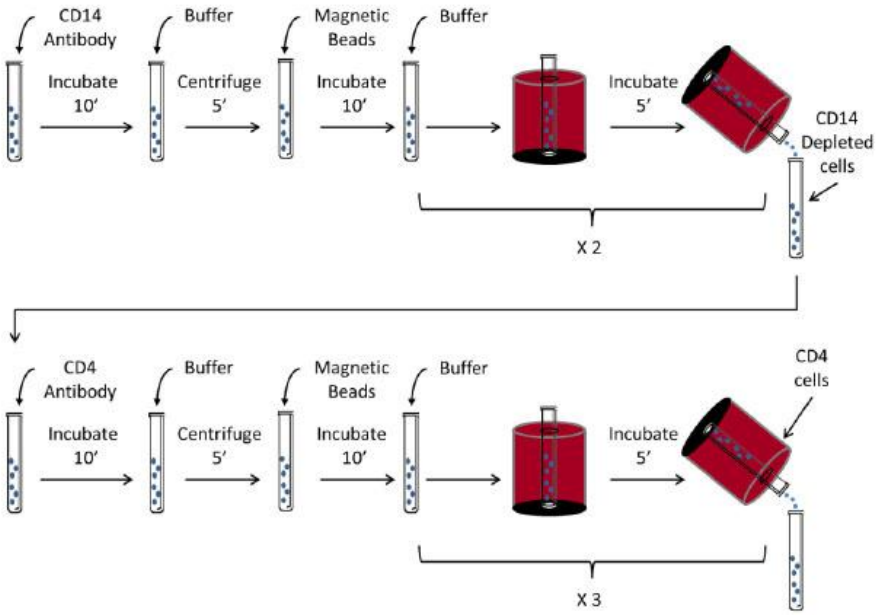
- MagniSort™ Anti-Human CD14 Biotin (cat. MS13-0149), 200 tests, 20 µL/test. Store at 2-8°C.
- MagniSort™ Anti-Human CD4 Biotin (cat. MS13-0049), 200 tests, 20 µL/test. Store at 2-8°C.
- MagniSort™ Positive Selection Beads A (cat. PB-6003), 2 x 4 mL. Store at 2-8°C.

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 centrifuge tube (BD Falcon, cat. no. 352099, or equivalent)

Experiment Duration

- 40 minutes
- Work flow:



Experimental Procedure

1. Prepare a single-cell suspension of human peripheral blood mononuclear cells 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™ Anti-Human CD14 Biotin 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 27 µL of MagniSort™ Positive Selection Beads A 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 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 recovery rate. The unbound cells can be collected and pooled, if needed.*
10. Remove the tube from the magnet and repeat Steps 7-9 one more time for a total of 2 washes, and pool the second decant with the first in the same 15 mL conical tube. Discard the 12 x 75 mm, 5 mL tube.
11. Count the CD14-depleted cells. Then, centrifuge the CD14-depleted cells at 300 x *g* for 5 minutes, and resuspend at a concentration of $1x10^7$ cells/100 µL ($1x10^8$/mL) in desired cell separation buffer. Transfer the CD14-depleted cells to a new, 12 x 75 mm, 5 mL tube.

    Note: *Cells must be in a single-cell suspension. Inspect sample and pulse vortex or pipet to remove clumps, if necessary, before proceeding.*
12. Add 20 µL of MagniSort™ Anti-Human CD4 Biotin per 100 µL of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.
13. 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.
14. Discard the supernatant and thoroughly resuspend the cells to the same volume used in Step 11 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.*

15. Add 20 µL of MagniSort™ Positive Selection Beads A per 100 µL of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 10 minutes.
16. 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.
17. 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.
18. 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.*
19. Remove the tube from the magnet and repeat Steps 16-18 two more times for a total of 3 washes.
20. Remove the tube containing CD4+ T 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 enriched human CD4 T cells are now ready to use.

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  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

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

Limited product warranty

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