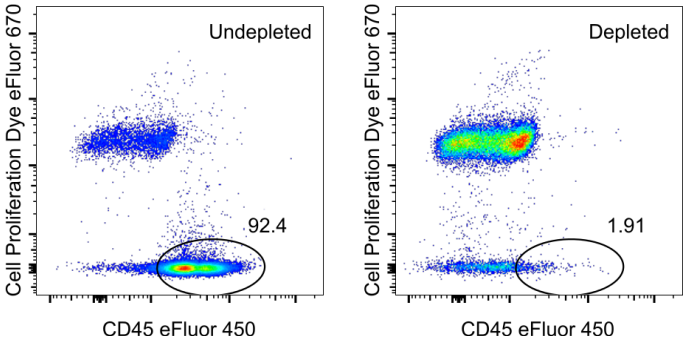
MagniSort™ Human CD45 Depletion Kit

Catalog Number: 8804-6802
RUO: For Research Use Only. Not for use in diagnostic procedures.



Human lysed whole blood was mixed with HeLa cells that were labeled with Cell Proliferation Dye eFluor® 670 (cat. 65-0840). Cells were either undepleted (left) or depleted with the MagniSort® Human CD45 Depletion Kit (right) and stained with Anti-Human CD45 eFluor® 450 (cat. 48-9459). Total viable cells were used for analysis.

Product Information

Contents: MagniSort™ Human CD45 Depletion Kit
REF **Catalog Number:** 8804-6802
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 CD45 Depletion Kit is designed to deplete CD45+ cells from human lysed whole blood or peripheral blood mononuclear cells, utilizing a biotinylated Anti-Human CD45 antibody and streptavidin-coated magnetic beads. CD45+ cells are bound by antibody and then magnetic beads. When placed in a magnetic field, the CD45- cells can be separated from CD45+ cells by decanting.

After depletion, the purity of selected cells can be verified by staining with Anti-Human CD45, clone 2D1.

Components

MagniSort® Human CD45 Biotin A (cat. MS22-6802): 200 tests, 20 µL/test; store at 2-8°C.
MagniSort® Negative Selection Beads B (cat. NB-6001): 4 mL; store at 2-8°C.

Applications Reported

The MagniSort® Human CD45 Depletion Kit has been reported for use in magnetic cell separation.

Applications Tested

The MagniSort® Human CD45 Depletion Kit has been tested by magnetic cell separation followed by flow cytometric analysis of human lysed whole blood or peripheral blood mononuclear 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

00-4300 eBioscience™ 10X RBC Lysis Buffer (Multi-species)
48-9459 eBioscience™ Anti-Human CD45 eFluor™ 450 (2D1)
65-0840 eBioscience™ Cell Proliferation Dye eFluor™ 670
MAG-4902 MagniSort™ Magnet

Tel: 888.999.1371 or 858.642.2058 • Fax: 858.642.2046 • thermofisher.com/ebioscience • info@ebioscience.com

invitrogen

MagniSort™ Human CD45 Depletion Protocol

Introduction

The following protocol is specifically for the MagniSort™ Human CD45 Depletion Kit to deplete CD45+ cells from lysed whole blood or peripheral blood mononuclear cells. CD45+ cells are labeled with biotinylated antibodies, followed by streptavidin-coated magnetic beads. When cells are placed in the MagniSort™ magnet, CD45+ cells are held in place by the magnetic field while CD45- cells remain free in solution and can be isolated by decanting.

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. For preparation of lysed whole blood, please refer to Best Protocols: Red Blood Cell Lysis Protocol located under the Resources Tab online.
3. Addition of EDTA to buffers will reduce cell clumping.

Use in sterile cultures

1. The MagniSort™ Human CD45 Biotin and 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

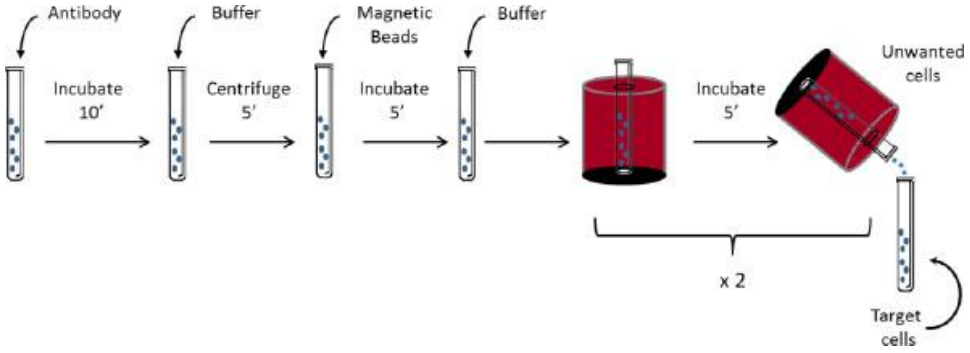
- MagniSort™ Human CD45 Biotin A (cat. MS22-6802), 200 tests, 20 µL/test. Store at 2-8°C.
- MagniSort™ Negative Selection Beads B (cat. NB-6001), 4 mL. Store at 2-8°C.

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)

Experiment Duration

- 40 minutes
- Work flow:



Experimental Procedure

1. Prepare a single-cell suspension of peripheral blood cells at a concentration of $1x10^7$ cells/100 µL ($1x10^8$/mL) in 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.*
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™ Human CD45 Biotin A 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 20 µL of MagniSort™ Negative Selection Beads B per 100 µL of cells. Mix well by pulse vortexing 5 times. Incubate at room temperature for 5 minutes.

   Note: *The MagniSortTM 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 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 new 12 x 75 mm, 5 mL 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 discard. Place the new 12 x 75 mm, 5 mL tube containing the unbound cells back into the magnet and incubate at room temperature for 5 minutes.
11. Pick up the magnet, and in a continuous motion pour the supernatant into a new 12 x 75 mm, 5 mL tube. Hold the inverted tube for 1 second and then return it to the upright position. Remove the tube containing bound cells from the magnet and discard. The untouched, negatively selected cells are ready to use in the final 12 x 75 mm, 5 mL tube.

Documentation and support

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - 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

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **thermofisher.com/support**.

Corporate entity: Life Technologies | Carlsbad, CA 92008 USA | Toll Free in USA 1.800.955.6288