MagCellect™ Mouse Memory CD4+ T Cell Isolation Kit

Catalog Number: MAGM206

INTENDED USE

The MagCellect™ Mouse Memory CD4+ T Cell Isolation Kit is designed to isolate Memory CD4+ T cells by negative selection. The resulting cell preparation is highly enriched with Memory CD4+ T cells ($CD3^+/CD4^+/CD62L^-/CD44^{high}$). Typical recoveries range from 5-35% and the purity of recovered cells is > 75%.

BACKGROUND

R&D Systems® MagCellect™ products are designed for the isolation of cells in a "liquid phase". MagCellect™ technology is based on the use of ferrofluids or magnetic nanoparticles that have no magnetic memory (superparamagnetic) and behave like colloidal particles. This feature allows the ferrofluids to remain in solution without the need for mixing and additionally allows for efficient diffusion kinetics during the binding reaction. The proprietary manufacturing technology of MagCellect™ Ferrofluids generates particles with higher ligand binding capacity per mass compared to many other larger diameter magnetic particles.

PRINCIPLE OF SELECTION

A mononuclear cell suspension is first incubated with the MagCellect™ Mouse Memory CD4+ T Cell Biotinylated Antibody Cocktail which targets unwanted cells. MagCellect™ Streptavidin Ferrofluid is added to the reaction and the streptavidin coated nanoparticles interact with the biotinylated antibody tagged cells. The tube containing the cell suspension is then placed within a magnetic field. Magnetically tagged cells will migrate toward the magnet (unwanted cell fraction), leaving the untagged cells or desired cell population in suspension. This population of cells can then be harvested by aspiration while the tube remains in the magnetic field. The enriched cell preparation is then available for a variety of applications including tissue culture, immune status monitoring, and flow cytometry.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date. **DO NOT FREEZE.**
The kit contains sufficient reagents to process $1x10^9$ total cells.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Mouse Memory CD4+ T Cell Biotinylated Antibody Cocktail	860046	1 mL of a phosphate buffered solution containing BSA and preservative.	May be stored 2-8 °C when handled aseptically.*
Streptavidin Ferrofluid	860127	1.25 mL of a solution containing BSA and preservative.	
10X Buffer	860040	10 mL of a 10X concentrated buffer.	May be stored for up to 24 hours at 2-8 °C after dilution.*

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- MagCellect™ Magnet (R&D Systems®, Catalog # MAG997)
- Mouse Erythrocyte Lysing Kit (R&D Systems®, Catalog # WL2000)
- 12 x 75 mm (5 mL) or 17 x 100 mm (15 mL) polystyrene round bottom tubes
- Sterile Pasteur pipettes or transfer pipettes (ThermoFisher, Catalog # 13-711-9B) or equivalent
- Sterile deionized or distilled water

PRECAUTION

Some components of this kit contain sodium azide, which may react with lead and copper plumbing to form explosive metallic azides. Flush with large volumes of water during disposal.

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REAGENT PREPARATION

Prepare 10 mL of 1X MagCellect™ Buffer for each 2 x 10^8 cells to be processed by mixing 1.0 mL of 10X Buffer with 9.0 mL sterile deionized or distilled water. **The buffer must be kept cold (2-8 °C) for the following procedures.**

CELL PREPARATION

1. Gently tease apart the mouse spleen(s) in order to generate a single cell suspension in Hanks' BSS (or other preferred media) supplemented with 10% bovine serum. To remove cell clumps and/or debris pass the suspended cells through a 40-70 µm nylon cell strainer.
2. Wash the cells once by filling a 15 or 50 mL centrifuge tube with Hanks' BSS + 10% serum and spinning the cells for 10 minutes at 200 x g (use a 50 mL tube when processing more than 2 spleens).
3. Decant the supernatant, disrupt the cell pellet by "racking" the tube, resuspend the cells in M-Lyse Buffer from R&D Systems®' Mouse Erythrocyte Lysing Kit (Catalog # WL2000) that has been diluted to 1X strength with sterile deionized or distilled water and quickly vortex the tube (using 2 mL of 1X M-Lyse Buffer per processed spleen is recommended).
4. Incubate the cells for 10 minutes at room temperature and then fill the tube with 1X Wash Buffer from the Lysing kit. **Note:** *The wash buffer must also be diluted with sterile water to 1X strength prior to use.*
5. Spin the cells for 10 minutes at 200 x g and then resuspend the cells in a small volume of cold 1X MagCellect™ Buffer.
6. Perform a cell count and then adjust the cell concentration to 2 x 10^8 cells per mL with cold 1X MagCellect™ Buffer.
7. Continue the cell selection by referring to step # 1 of the cell selection procedure.

CELL SELECTION PROCEDURE

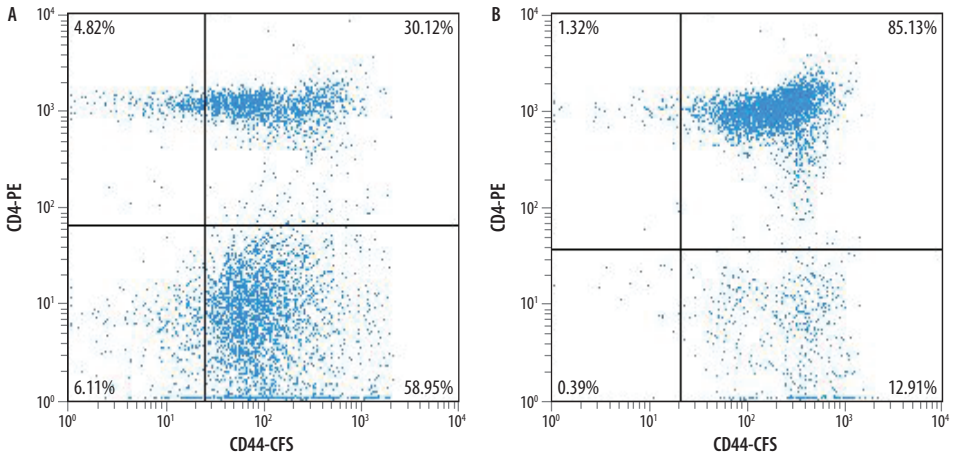
This procedure is for processing 2 x 10^8 total cells using 5 mL tubes and the MagCellect™ Magnet. For processing other cell numbers please refer to the Technical Hints section of this insert. Cells and reagents should be kept cold using an ice bath or a refrigerator. **Reaction incubations must be carried out at 2-8 °C in a refrigerator and not in an ice bath in order to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

1. Prepare a single cell suspension of mouse leukocytes by traditional methods or by following the instructions outlined above in the Cell Preparation section of this insert. Cells must be suspended in **cold** 1X MagCellect™ Buffer prior to beginning the procedure and be at a cell density of 2 x 10^8 cells/mL.
2. Transfer 2 x 10^8 cells (1.0 mL volume) into a 5 mL polystyrene tube and then add 200 µL of Mouse Memory $CD4^+$ T Cell Biotinylated Antibody Cocktail. Gently mix the cell-antibody suspension, avoiding bubble formation, and incubate at 2-8 °C in a refrigerator for 15 minutes.
3. Add 250 µL of Streptavidin Ferrofluid to the cell suspension, mix gently and incubate at 2-8 °C in a refrigerator for 15 minutes.
4. At the end of the incubation period bring the volume of the reaction in the tube to 3 mL by adding 1.6 mL of 1X MagCellect™ Buffer. Mix gently to ensure that all reactants in the tube are in suspension.
5. Place the reaction tube in the MagCellect™ Magnet that has been positioned horizontally to accommodate 5 mL tubes and incubate for 8 minutes at room temperature. Magnetically tagged cells will migrate toward the magnet (these are the unwanted cells), leaving the untouched desired cells in suspension.
6. Recovery of desired cells is achieved as follows: While the tube is in the magnet, using a sterile Pasteur pipette or transfer pipette, **carefully aspirate** all of the reaction suspension and place it in a new 5 mL tube. Remove the tube containing the magnetically trapped cells from the magnet, and discard.
7. To ensure that all of the magnetic nanoparticles have been removed, repeat the magnetic depletion (steps 5 and 6) with the new tube containing the recovered cells. The suspension obtained at the end of these steps is the final depleted cell fraction containing the desired enriched Memory $CD4^+$ T Cells. The cells are now ready for counting and further downstream applications.

TECHNICAL HINTS

- If sterile cells are required following cell selection, the entire procedure should be carried out in a laminar flow hood to maintain sterile conditions. Use sterile equipment when pipetting reagents that will be reused at a later date.
- Avoid antibody capping on cell surfaces and non-specific cell tagging by working quickly, keeping cells and solutions cold through the use of pre-cooled solutions and by adhering to the incubation times and temperatures specified in the protocol. Increased temperature and prolonged incubation times may lead to non-specific cell labeling thus lowering cell purity and yield.
- When processing different numbers of cells observe the following guidelines: keep the antibody cocktail and ferrofluid incubation times and temperatures the same; keep the cell density at 2 x 10^8 cells/mL; add 10 µL of the antibody cocktail per 1 x 10^7 cells being processed; add 12.5 µL of Streptavidin Ferrofluid per 1 x 10^7 cells being processed.
- When processing 2 x 10^8 cells or fewer, use the 12 x 75 mm (5 mL) tubes with the MagCellect™ Magnet horizontally positioned to accommodate up to six 5 mL tubes. **Do not process more than 2 x 10^8 cells in each 5 mL tube and do not exceed a total reaction volume of 3 mL in each tube. Reaction volume adjustments must be made using 1X MagCellect™ Buffer just prior to the magnetic separation step.**
- When processing greater than 2 x 10^8 cells, use the 17 x 100 mm (15 mL) tubes with the MagCellect™ Magnet vertically positioned to accommodate up to two 15 mL tubes. **Do not process more than 6 x 10^8 cells in each 15 mL tube and do not exceed a total reaction volume of 9 mL in each tube.** When using this larger tube, increase the reaction volume before the magnetic separation step according to the following formula: 3 mL for each 2 x 10^8 cells processed. Also increase the magnetic incubation time described in step #5 to 8 minutes. **Reaction volume adjustments must be made using 1X MagCellect™ Buffer just prior to the magnetic separation step.**

DATA EXAMPLE



Mouse splenocytes before **(A)** and after **(B)** isolation of Memory CD4$^+$ T cells using the MagCellect™ Mouse Memory CD4$^+$ T Cell Isolation Kit. Dot plots reflect double staining of all viable cells with CD44-CFS and CD4-PE (R&D Systems®, Catalog # FAB554P).