MagCellect™ Human CD8+ T Cell Isolation Kit

Catalog Number: MAGH112

INTENDED USE

The MagCellect Human CD8+ T Cell Isolation Kit is designed to isolate CD8+ T cells via a negative selection principle. The resulting cell preparation is highly enriched with CD8+ T cells. Typical purity of recovered CD8+ T cells ranges from 75-87%.

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 cell suspension is first incubated with the MagCellect Human CD8+ 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 to 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 FREEZE.**
This kit contains sufficient reagents to process 1 x 10^9 total cells; up to 25 isolations.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Human CD8+ T Cell Biotinylated Antibody Cocktail	860204	1.0 mL of a phosphate buffered solution containing BSA.	May be stored at 2-8 °C when handled aseptically.*
Streptavidin Ferrofluid	860129	2.0 mL of a solution containing BSA and preservatives.	
10X Buffer	860040	10 mL of a 10-fold concentrated buffer.	

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

OTHER SUPPLIES REQUIRED

- MagCellect Magnet (R&D Systems, Catalog # MAG997) or equivalent
- Human Erythrocyte Lysing Kit (R&D Systems, Catalog # WL1000)
- 12 x 75 mm (5 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.

REAGENT PREPARATION

Prepare 10 mL of 1X MagCellect Buffer for each 1 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 procedure.**

CELL PREPARATION

1. Process cells on a density gradient, like Ficoll Hypaque to enrich for mononuclear cells.
2. Recover the "buffy coat" containing the mononuclear cells and wash the cells two times with excess PBS to remove any residual separation media. This can be done by spinning the cells for 10 minutes at 200 x g.
3. After the second wash step, disrupt the cell pellet by "racking" the tube, resuspend the cells in H-Lyse Buffer from R&D Systems' Human Erythrocyte Lysing Kit (Catalog # WL1000) that has been diluted to 1X strength with sterile distilled water. Quickly vortex the tube (10 mL of 1X H Lyse solution per 250 million cells 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 1X MagCellect Buffer.
6. Perform a cell count and then adjust the cell concentration to 1 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 1 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 to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

1. Prepare a single cell suspension of human leukocytes by traditional methods or by following the instructions outlined 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 1 x 10^8 cells/mL.
2. Transfer 1 x 10^8 cells (1.0 mL volume) into a 5 mL polystyrene tube. Add 100 µL of MagCellect Human $CD8^+$ 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 200 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 2 mL by adding 0.7 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 6 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 firmly held in the magnet, using a sterile Pasteur pipette or transfer pipette, **carefully and slowly** 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 $CD8^+$ T cells. The cells are now ready for counting and further downstream applications.

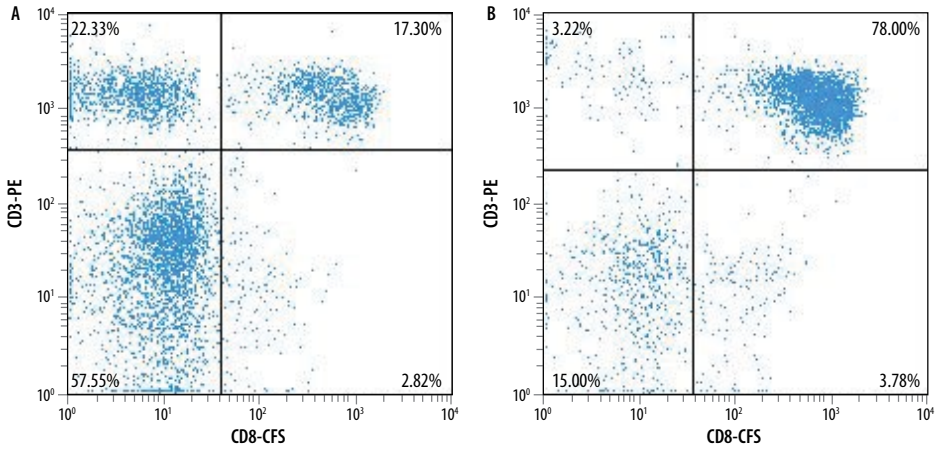
TECHNICAL HINTS

- 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 fast, 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, follow the recommendations in the table below or observe the following guidelines: keep the antibody cocktail and Ferrofluid incubation times and temperatures the same; keep the cell density at 1 x 10^8 cells/mL.
- A minimum of 125 µL of Streptavidin Ferrofluid per isolation is recommended. We do not recommend using less than 5 x 10^7. Reaction volume adjustments must be made using 1X MagCellect Buffer just prior to the magnetic separation step.

Recommended quantities to be used in steps 4-5 of the Cell Selection Procedure (1 X 10^8 is recommended).

Number of Cells in Starting Preparation	5 x 10^7	1 x 10^8
Reaction Volume	0.5 mL	**1 mL**
Human CD8$^+$ T Cell Biotinylated Antibody Cocktail	50 µL	**100 µL**
Streptavidin Ferrofluid	125 µL	**200 µL**

DATA EXAMPLES



Ficolled human PBMCs before **(A)** and after **(B)** isolation of CD8$^+$ T cells using the MagCellect Human CD8$^+$ T Cell Isolation Kit. Dot plots reflect double-staining of all viable cells with CD8-CFS (R&D Systems, Catalog # FAB1509F)/CD3-PE (R&D Systems, Catalog # FAB100P).