MagCellect Human Naive CD8⁺ T Cell Isolation Kit*

Catalog Number: MAGH113

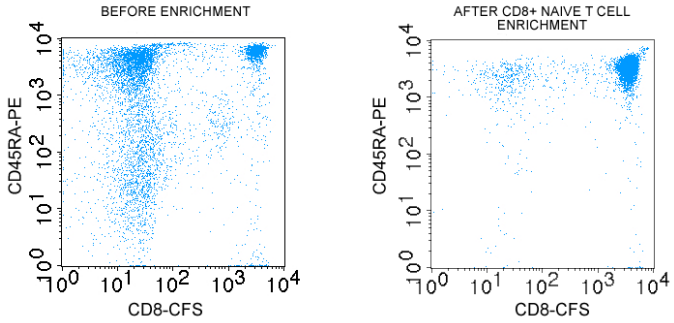
Kit Contents

- **MagCellect Human Naive CD8⁺ T Cell Biotinylated Antibody Cocktail** (Part 860208) 1 mL in a phosphate buffered solution containing BSA.
- **MagCellect Streptavidin Ferrofluid** (Part 860127) 2 mL in a solution containing BSA and preservative.
- **MagCellect 10X Buffer** (Part 860040) 10 mL of a 10X concentrated buffer.

Storage

Store all reagents at 2 - 8° C. **DO NOT FREEZE.**

The kit contains sufficient reagents to process 1 x 10^9 total cells.



Ficolled human PBMCs before and after isolation of Naive CD8⁺ T cells using the MagCellect Naive CD8⁺ T Cell Isolation Kit. Dot plots reflect double staining of all viable cells with CD8-CFS (R&D Systems, Catalog # FAB1509F) and CD45RA-PE.

Other Required Supplies

- MagCellect Magnet (R&D Systems, Catalog # MAG997) or equivalent[1]
- 12 x 75 mm (5 mL) or 17 x 100 mm (15 mL) polystyrene round bottom tubes
- Sterile Pasteur pipettes or transfer pipettes

[1] MagCellect Cell Isolation kits have been tested with other supplier's magnets with excellent performance results.

Intended Use

The MagCellect Human Naive CD8⁺ T Cell Isolation Kit is designed to isolate Naive CD8⁺ T cells via a negative selection principle. The resulting cell preparation is highly enriched with Naive CD8⁺ T cells. Typical purity of recovered Naive CD8⁺ T cells ranges between 75% and 85%.

Background

R&D Systems MagCellect products are designed for the isolation of cells in a "liquid phase". R&D Systems MagCellect technology is based on the use of Ferrofluids or magnetic nanoparticles that have no magnetic memory (superparamagnetic). These Ferrofluids have a diameter of ~150 nm and as a result 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 Antibody Cocktail which targets the unwanted cells. MagCellect Streptavidin Ferrofluid is next added to the reaction which allows the streptavidin coated nanoparticles to interact with 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.

Cell Preparation

- Process cells on a density gradient, like Ficoll Hypaque to enrich for mononuclear cells.
- 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.
- After the second washing step, disrupt the cell pellet by "racking" the tube, resuspend the cells in R&D Systems' H-Lyse buffer (Catalog # WL1000) that has been diluted to 1X strength with sterile distilled water and quickly vortex the tube (10 mL of 1X H-Lyse solution per 250 million cells is recommended).
- Incubate the cells for 10 minutes at room temperature and then fill the tube with 1X Wash Buffer from the Lysing kit. Note that the Wash Buffer must also be diluted with sterile water to 1X strength prior to use.
- Spin the cells for 10 minutes at 200 x g and then resuspend the cells in a small volume of 1X MagCellect Buffer.
- Perform a cell count and then adjust the cell concentration to 100 x 10^6 cells per mL with cold 1X MagCellect Buffer.
- Continue the cell selection by referring to step 1 of the Cell Selection Procedure.

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Cell Selection Procedure

This procedure is for the processing of 100 x 10^6 total cells using 5 mL tubes and the MagCellect Magnet. For processing other cell numbers please refer to the Technical Hints section on 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 on ice baths to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

1. Prepare 10 mL of 1X MagCellect Buffer for each 100 x 10^6 cells to be processed by mixing 1.0 mL of MagCellect 10X Buffer with 9.0 mL sterile deionized or distilled water. The 1X buffer should be kept on ice or refrigerated and used within 1 month.
2. Prepare a single cell suspension of human leukocytes by traditional methods or by following the instructions outlined in the Cell Preparation section on this insert. Cells must be suspended in cold 1X MagCellect Buffer prior to beginning the procedure and be at a cell density of 200 x 10^6 cells/mL.
3. Transfer 100 x 10^6 cells (2.0 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.
4. Add 200 µL of MagCellect Streptavidin Ferrofluid to the cell suspension, mix gently and incubate at 2 - 8° C in a refrigerator for 15 minutes.
5. At the end of the incubation period bring the volume of the reaction in the tube to 3 mL by adding 0.7 mL of 1X MagCellect Buffer. Mix gently to ensure that all reactants in the tube are in suspension.
6. 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 (18 - 25° C). Magnetically tagged cells will migrate toward the magnet (these are the unwanted cells), leaving the untouched desired cells in suspension in the supernatant.
7. 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 supernatant and place it in a new 5 mL tube. Remove the tube containing the magnetically trapped cells from the magnet, and discard.
8. To ensure that all of the magnetic nanoparticles have been removed, repeat the magnetic depletion (steps 6 and 7) with the new tube containing the recovered cells. The supernatant 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 the 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 Table 1 or observe the following guidelines: keep antibody cocktail and Ferrofluid incubation times and temperatures the same; keep the cell density at 200 x 10^6 cells/mL; add 10 µL of the antibody cocktail per 10 x 10^6 cells being processed; add 20 µL of Streptavidin Ferrofluid per 10 x 10^6 cells being processed.
- A minimum of 75 µL of Streptavidin Ferrofluid per isolation is recommended. We do not recommend using less than 50 x 10^6 or more than 200 x 10^6 total mononuclear cells per isolation. **Reaction volume adjustments must be made using 1X MagCellect Buffer just prior to the magnetic separation step.**

Table 1: Recommended quantities to be used in steps 3 - 4 of the Cell Selection Procedure.

Number of cells in starting preparation	**50 x 10^6**	**100 x 10^6 (†)**	**200 x 10^6**
Reaction Volume	1 mL	2 mL	3 mL
MagCellect Human Naïve $CD8^+$ T Cell Biotinylated Antibody Cocktail	50 µL	100 µL	200 µL
MagCellect Streptavidin Ferrofluid	100 µL	200 µL	400 µL

(†) Recommended setup