*MagCellect™ Rat CD8⁺ T Cell Isolation Kit**

Catalog Number: MAGR305

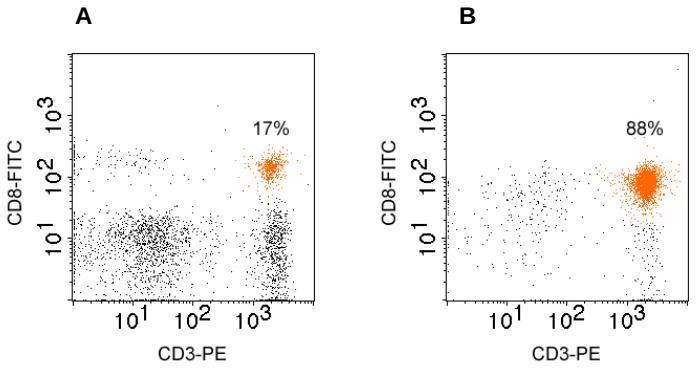
Kit Contents

- **MagCellect Rat CD8⁺ T Cell Antibody Cocktail** (Part 860302): 1 mL in a phosphate buffered solution containing BSA.
- **MagCellect Goat Anti-Mouse IgG Ferrofluid** (Part 860130): 1.25 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.



Rat splenocytes before (A) and after (B) isolation of CD8⁺ T cells using the MagCellect Rat CD8⁺ T Cell Isolation Kit. Dot plots reflect double staining of all viable cells with CD8-FITC/CD3-PE.

Other Required Supplies

- MagCellect Magnet (R&D Systems, Catalog # MAG997)
- 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)

Intended Use

This MagCellect Rat 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 between 80 and 90%.

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 (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 that targets the unwanted cells. MagCellect Goat Anti Mouse (GAM) Ferrofluid is next added to the reaction allowing the GAM coated nanoparticles to interact with the cells tagged with the monoclonal antibodies. 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 Selection Procedure

This procedure is for the processing of 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 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 in an ice bath to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

1. Prepare 10 mL of 1X MagCellect Buffer for each 2 x 10^8 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 24 hours.
2. Prepare a single cell suspension of rat 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 1 x 10^8 cells/mL.

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3. Transfer 2 x 10^8 cells (2.0 mL volume) into a 5 mL polystyrene tube. Add 200 µL of MagCellect Rat $CD8^+$ T Cell 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 250 µL of MagCellect GAM Ferrofluid to the cell suspension, mix gently and incubate for 15 minutes in a refrigerator at 2 - 8° C.
5. At the end of the incubation period bring the volume of the reaction in the tube to 3 mL by adding 0.55 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 10 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 in the magnet, using a sterile Pasteur pipette or transfer pipette, carefully 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 observe the following guidelines: keep 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 GAM 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 20 x 10^7 cells in each 5 mL tube and do not exceed a total reaction volume of 3 mL in each tube**. A reaction volume of 2 mL is recommended for processing 1 x 10^8 cells. A reaction volume of 1 mL is recommended when processing 5 x 10^7 or fewer cells. **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 #6 to 12 minutes. **Reaction volume adjustments must be made using 1X MagCellect Buffer just prior to the magnetic separation step.**

Cell Preparation

- Gently tease apart the rat 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. Transfer the single cell suspension generated from one rat spleen into two 50 mL centrifuge tubes.
- Wash the cells once by filling each 50 mL centrifuge tube with Hanks' BSS + 10% serum and spinning the cells for 10 minutes at 200 x g.
- Decant the supernatant, disrupt the cell pellet by "racking" the tube, resuspend the cells in R&D Systems' Mouse Erythrocyte Lysing Kit (Catalog # WL2000) that has been diluted to 1X strength with sterile distilled water and quickly vortex the tube (*using 10 mL of 1X M-Lyse Buffer per centrifuge tube which contains one half of the total number spleen cells is recommended*).
- Incubate the cells for 10 minutes at room temperature and then fill each 50 mL 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 cold 1X MagCellect Buffer.
- Perform a cell count and then adjust the cell concentration to 2 x 10^8 cells per mL with cold 1X MagCellect Buffer.
- Continue the cell selection by referring to step #1 of the cell selection procedure.