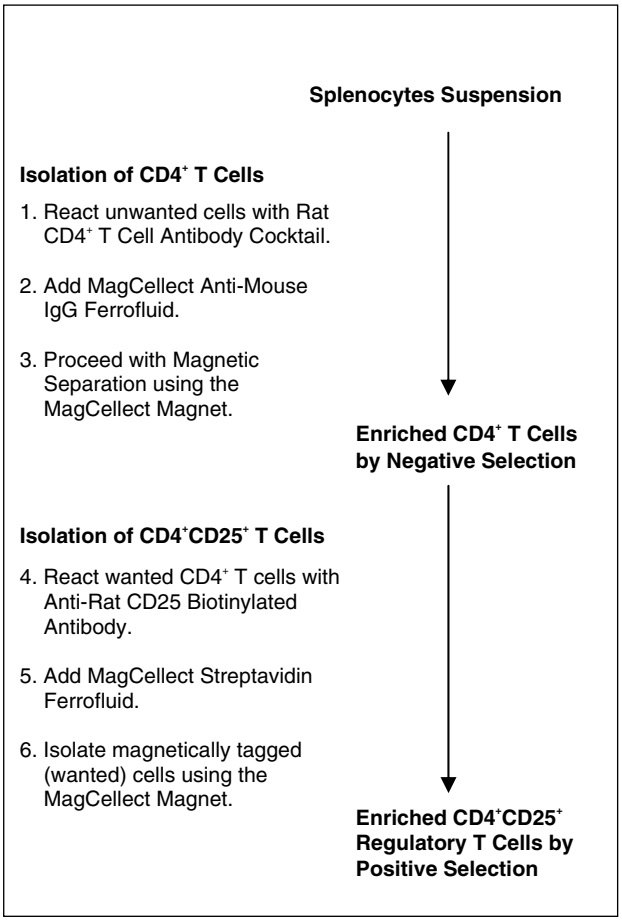
MagCellect Rat CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit*

Catalog Number: MAGR304

Kit Contents

- **MagCellect Rat CD4$^+$ T Cell Antibody Cocktail -** 1 mL in a phosphate buffered solution containing BSA. To be used only in the negative selection step to isolate CD4$^+$ T cells.
- **MagCellect Anti-Mouse IgG Ferrofluid -** 1.25 mL in a solution containing BSA and preservative. To be used in the negative selection step to enrich for CD4$^+$ T cells.
- **MagCellect Streptavidin Ferrofluid -** 0.25 mL in a solution containing BSA and preservative. To be used in the positive selection step to isolate CD4$^+$CD25$^+$ regulatory T cells.
- **Anti-Rat CD25 Biotinylated Antibody -** 0.25 mL in a phosphate buffered solution containing BSA. To be used only in the positive selection step to isolate CD4$^+$CD25$^+$ regulatory T cells.
- **MagCellect 10X Buffer -** 25 mL of a 10X concentrated buffer.

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



Storage

Store all reagents at 2 - 8° C. **Do not freeze.**

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.
- 15 mL conical centrifuge tubes and benchtop centrifuge.
- Sterile Pasteur pipettes or transfer pipettes.

Intended Use

The MagCellect Rat CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit is designed to isolate Rat CD4$^+$CD25$^+$ regulatory T cells using a two-step procedure that combines both negative and positive selection techniques. The resulting cell preparation is highly enriched with CD4$^+$CD25$^+$ T cells with a purity of recovered cells ranging between 75 - 85%.

Principle of Selection

The isolation of rat CD4$^+$CD25$^+$ regulatory T cells is done using a two-step procedure. CD4$^+$ T cells are initially isolated by negative selection, and CD25$^+$ T cells are then isolated by positive selection from the CD4$^+$ T cell fraction.

Isolation of CD4$^+$ T cells by negative selection is done in a test tube and is achieved by tagging unwanted cells with the MagCellect Rat CD4$^+$ T Cell Antibody Cocktail followed by the addition of MagCellect Goat Anti-Mouse Ferrofluid (GAM-FF). The tube with the cell suspension is then placed in the MagCellect Magnet (R&D Systems, Catalog # MAG997). Magnetically tagged cells will migrate toward the tube wall on the magnet side (unwanted cell fraction), leaving the untagged cells or desired cell population in suspension ready to be harvested by aspiration while the tube remains in the magnet.

Isolation of CD4$^+$CD25$^+$ regulatory T cells from the CD4$^+$ T cell isolated fraction, is done by positive selection in a test tube by tagging the cells of interest with Anti-Rat CD25 Biotinylated Antibody followed by the addition of the MagCellect Streptavidin Ferrofluid (SAV-FF). The tube with the cell suspension is placed in the magnet, and magnetically tagged cells will migrate toward the tube wall on the magnet side (desired cell population), leaving the untagged (unwanted) cells in suspension. Unwanted cells are removed by aspiration while the tube remains in the magnet. The tube containing the magnetically trapped (wanted) cells is removed from the magnet and the cells resuspended in 1X MagCellect Buffer or media.

R&D Systems, Inc.
1-800-343-7475

Cell Selection Procedure

This procedure describes 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 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 on ice baths to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

Prepare 40 mL of 1X MagCellect Buffer for each 2 x 10^8 cells to be processed by mixing 4.0 mL of MagCellect 10X Buffer with 36.0 mL sterile deionized or distilled water. The 1X buffer should be kept on ice or refrigerated and used within 24 hours.

Isolation of $CD4^+$ T Cells By Negative Selection

1. Prepare a single cell suspension of Rat 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 2 x 10^8 cells (2.0 mL volume) into a 5 mL polystyrene tube. Add 200 µL of MagCellect Rat $CD4^+$ 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.
3. Add 250 µL of MagCellect Anti-Mouse IgG 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 0.55 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 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.
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 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.
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 supernatant obtained at the end of these steps is the final depleted cell fraction containing the desired enriched $CD4^+$ cells. The cells are now ready for counting.

Isolation of $CD4^+CD25^+$ Cells by Positive Selection

8. After counting, cells are transferred to a 15 mL conical centrifuge tube and washed by filling the tube to the 15 mL mark with 1X cold MagCellect Buffer. Centrifuge at 300 x g for 8 minutes.
9. Remove the supernatant **completely** and resuspend cell pellet with 100 µL of cold 1X MagCellect Buffer per 1 x 10^7 cells and transfer the cells to a 5 mL tube.
10. Add 10 µL of Anti-Rat CD25 Biotinylated Antibody per each 1 x 10^7 cells and incubate at 2 - 8° C in a refrigerator for 15 minutes.
11. Add 10 µL of MagCellect Streptavidin Ferrofluid per 1 x 10^7 cells and incubate at 2 - 8° C in a refrigerator for 15 minutes.
12. Bring volume to 1 mL by adding cold 1X MagCellect Buffer. Mix gently to ensure that all reactants in the tube are in suspension.
13. 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 **wanted** cells), leaving the untagged unwanted cells in suspension in the supernatant.
14. Removal of unwanted 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 discard.
15. Remove the tube containing the magnetically trapped (**wanted**) cells from the magnet, and resuspend cells by adding 1 mL of cold 1X MagCellect Buffer.
16. To complete the cell isolation procedure repeat steps #13 and #14 one more time with the resuspended cell fraction.
17. Remove the tube containing the magnetically trapped (**wanted**) cells from the magnet, and resuspend cells by adding 0.5 - 1.0 mL of 1X MagCellect Buffer or tissue culture media. This final magnetically isolated fraction contains the desired enriched $CD4^+$ $CD25^+$ cells. The cells are now ready to be used in other downstream applications.

Technical Hints

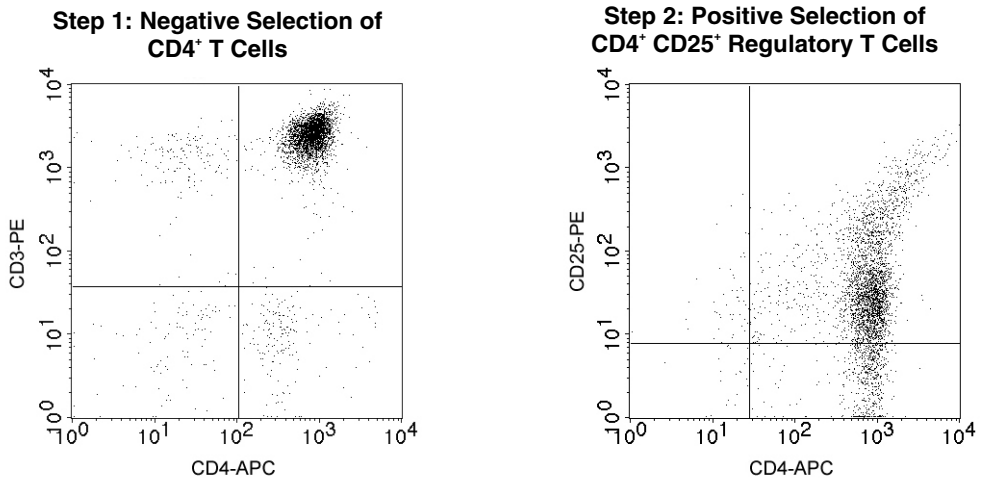
- If sterile cells are required following the cell selection, the entire procedure should be carried out in a laminar flow hood to maintain sterile conditions. Use sterile equipment when pipetting reagents that will be reused at a later date.
- Avoid antibody capping on cell surfaces and non-specific 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 10 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**. 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 0.5 x 10^8 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. **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 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' M-Lyse buffer (Catalog # WL2000) that has been diluted to 1X strength with sterile deionized or distilled water and quickly vortex the tube. The use of 10 mL of 1X M-Lyse solution 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.
- 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 1 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.

Typical Data



Enrichment of $CD4^+$ T cells (Step 1) and $CD4^+CD25^+$ Regulatory T cells (Step 2) from rat splenocytes using this MagCellect Rat $CD4^+CD25^+$ Regulatory T Cell Kit. $CD25^+$ T cells are typically a heterogeneous population that expresses different levels of CD25. Both $CD25^{dim}$ and $CD25^{high}$ cells are isolated with this kit.

***This product utilizes and/or contains technology licensed from Veridex, LLC, Raritan, NJ 08869, USA, which is covered by one or more claims of United States and International patents and/or pending patent applications.**