MagCellect™ Mouse CD4+ CD25+T Cell Isolation Kit

Catalog Number: MAGM208

INTENDED USE

MagCellect™ Mouse CD4+ CD25+ Regulatory T Cell Isolation Kit is designed to isolate mouse 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 > 60%.

PRINCIPLE OF SELECTION

Isolation of mouse CD4+ CD25+ regulatory T cells is done using a two step procedure. CD4+ T cells are initially isolated by negative selection and then CD25+ T cells are 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™ Mouse CD4+ T Cell Biotinylated Antibody Cocktail followed by the addition of MagCellect™ Streptavidin Ferrofluid (SAV 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 an anti-mouse CD25 biotinylated antibody followed by the addition of the MagCellect™ SAV-FF. The tube with the cell suspension is placed in the magnet. 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; next the tube containing the magnetically trapped (wanted) cells is removed from the magnet and the cells are resuspended in reaction buffer or media.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. **DO NOT FREEZE.**
This kit contains sufficient reagents to process up to 1 x 10^9 total cells.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Mouse CD4+ T Cell Biotinylated Antibody Cocktail	860044	1 mL of a phosphate buffered solution containing BSA and preservative.	May be stored for up to 3 months at 2-8 ºC.*
Mouse CD25 Biotinylated Antibody	860126	0.5 mL in a phosphate buffered solution containing BSA and preservative.	
Streptavidin Ferrofluid	860128	1.5 mL of a solution containing BSA and preservative.	
10X Buffer	860125	25 mL of a 10-fold concentrated buffer.	May be stored for up to 3 months at 2-8 ºC.* Use diluted buffer within 24 hours.

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

OTHER SUPPLIES REQUIRED

- 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
- Sterile deionized or distilled water
- Anti-Mouse CD4-FITC Mab (R&D Systems®, Catalog # FAB554F)
- Anti-Mouse IL-2 Rα/CD25-PE Mab (R&D Systems®, Catalog # FAB2438P)

Global bio-techne.com info@bio-techne.com TEL +1 612 379 2956 **USA** TEL 800 343 7475 **Canada** TEL 855 668 8722
Europe | Middle East | Africa TEL +44 (0)1235 529449 **China** TEL +86 (21) 52380373

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.

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 efficiently, 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 consistent.
- When processing 2 x 10^8 or fewer cells, use 12 x 75 mm (5 mL) tubes with the MagCellect™ Magnet positioned horizontally 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 17 x 100 mm (15 mL) tubes with the MagCellect™ magnet positioned vertically 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 incubation time in the magnet described in step #5 to 8 minutes. **Reaction volume adjustments must be made using 1X MagCellect™ Buffer just prior to the magnetic separation step.**

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 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 that 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 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 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.**

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 mL sterile deionized or distilled water. The 1X buffer should be kept on ice or refrigerated and used within 24 hours.

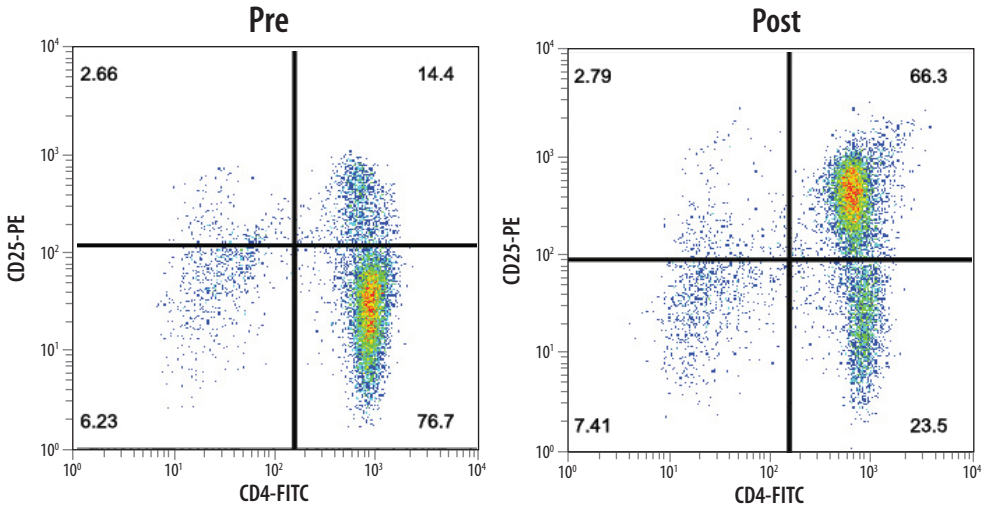
Isolation of $CD4^+$ T cells By Negative Selection

1. Prepare a single cell suspension of mouse 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 2 x 10^8 cells/mL.
2. Transfer 2 x 10^8 cells (1 mL volume) into a 5 mL polystyrene tube. Add 200 µL of MagCellect™ Mouse $CD4^+$ 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 200 µL of MagCellect™ 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 5.0 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 in the supernatant.
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 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, transfer the cells to a 15 mL conical centrifuge tube and wash 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**, resuspend the 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 Mouse 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 the 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 8 minutes at room temperature. 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 the 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 the cells by adding 0.5-1 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. The purity of the recovered cells can be assessed by staining with anti-mouse CD4-FITC and anti-mouse CD25-PE.

DATA EXAMPLE



Isolation of Mouse CD4$^+$ CD25$^+$ T Cells from Spleen using R&D Systems® MagCellect™ Mouse CD4$^+$CD25$^+$ Regulatory T Cell Isolation Kit. Mouse splenocytes were prepared as described in the Cell Preparation Section. Cells were stained with Rat Anti-Mouse CD4-FITC Monoclonal Antibody (R&D Systems®, Catalog # FAB554F) and Rat Anti-Mouse CD25-PE Monoclonal Antibody (R&D Systems®, Catalog # FAB2438P) either before (Pre) or after (Post) isolation of CD4$^+$ CD25$^+$ cells.