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MagCellect™ Human CD4+CD25+ Regulatory T Cell Isolation Kit

Catalog Number: MAGH104

INTENDED USE

The MagCellect™ Human CD4+CD25+ Regulatory T Cell Isolation Kit is designed to isolate human 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 for CD4+CD25+ T cells with a purity of recovered cells ranging between 85-95%.

PRINCIPLE OF SELECTION

Isolation of human CD4+CD25+ regulatory T cells is done using a two-step procedure. CD4+ T cells are initially isolated by negative selection followed by isolation of CD25+ T cells from the CD4+ T cell fraction by positive selection.

Isolation of CD4+ T cells by negative selection is done in a test tube and is achieved by tagging unwanted cells with the MagCellect Human CD4+ T Cell Biotinylated Antibody Cocktail followed by addition of the 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. These cells can then 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-human CD25 biotinylated antibody followed by 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. The tube containing the magnetically trapped (wanted) cells is then removed from the magnet and the cells are resuspended in 1X MagCellect Buffer.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date. **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
Human CD4+ T Cell Biotinylated Antibody Cocktail	860052	1 mL of a phosphate buffered solution containing BSA. *To be used only in the negative selection step to isolate CD4+ T cells.*	May be stored at 2-8 °C when handled aseptically.*
Anti-Human CD25 Biotinylated Antibody (Clone 24238)	860306	0.5 mL of a solution containing BSA and preservative. *To be used only in the positive selection step to isolate CD4+CD25+ regulatory T cells.*	
Human CD4+CD25+ Regulatory T Cell Staining Reagent	860054	1 mL contains a mixture of anti-human CD4 FITC (Clone 11830, Mouse IgG_{2A}) and anti-human CD25 PE (Clone 24212, Mouse IgG_{2A}) sufficient to perform 50 tests at 20 µL per test.	
Streptavidin Ferrofluid	860129	2.0 mL of a solution containing BSA and preservative. *To be used both in the negative selection step to isolate CD4+ T cells, and in the positive selection step to isolate CD4+CD25+ regulatory T cells.*	
10X Buffer	860125	25 mL of a 10X concentrated buffer.	May be stored for up to 24 hours at 2-8 °C.*

* 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
- Sterile Pasteur pipettes or transfer pipettes (ThermoFisher, Catalog # 13-711-9B) or equivalent

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Bio-Techne is a trading name for R&D Systems

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 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 1 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 Streptavidin Ferrofluid per additional 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 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 #5 to 8 minutes. **Reaction volume adjustments must be made using 1X MagCellect Buffer just prior to the magnetic separation step.**

REAGENT PREPARATION

1X MagCellect Buffer - For each 2 x 10^8 cells to be processed, dilute 4.0 mL of 10X Buffer with 36.0 mL of sterile deionized or distilled water to prepare 40 mL of 1X MagCellect Buffer.

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 washing step, disrupt the cell pellet by "racking" the tube, resuspend the cells in R&D Systems' Human Erythrocyte Lysing Kit (Catalog # WL1000) that has been diluted to 1X strength with sterile distilled water and quickly vortex the tube (using 10 mL of 1X H-Lyse Buffer 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 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 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 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.**

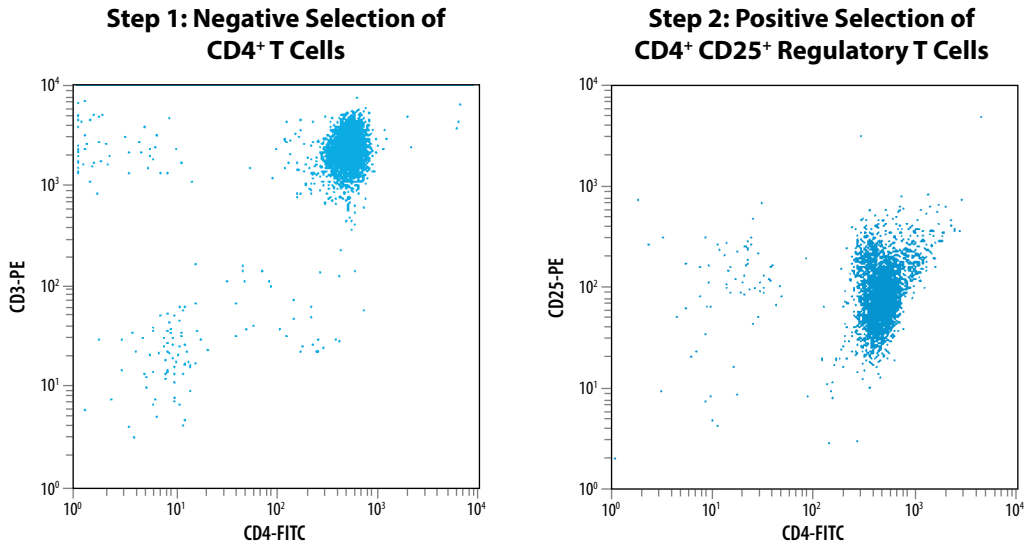
Isolation of $CD4^+$ T Cells by Negative Selection

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 2 x 10^8 cells (2.0 mL volume) into a 5 mL polystyrene tube. Add 200 µL of Human $CD4^+$ 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 250 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 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 6 minutes at room temperature. Magnetically tagged cells will migrate toward the magnet (these are the unwanted cells), leaving the desired $CD4^+$ T cells in suspension in the supernatant.
6. Recovery of the desired cells is achieved as follows: While the tube is in the magnet, use a sterile Pasteur pipette or transfer pipette to 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 $CD4^+$ T cells. The cells are now ready for counting.

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

8. Counted cells are transferred to a 15 mL conical centrifuge tube and washed by filling the tube to the 15 mL mark with **cold** 1X MagCellect Buffer. Centrifuge at 300 x g for 8 minutes.
9. Remove the supernatant **completely** and resuspend the cell pellet with 100 µL of **cold** 1X MagCellect Buffer per 1 x 10^7 cells. Transfer the cell suspension to a 5 mL tube.
10. Add 10 µL of Anti-Human CD25 Biotinylated Antibody per 1 x 10^7 cells and incubate at 2-8 °C in a refrigerator for 15 minutes.
11. Add 25 µL of Streptavidin Ferrofluid per 1 x 10^7 cells and incubate at 2-8 °C in a refrigerator for 15 minutes.
12. At the end of the incubation period bring the cell 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. Magnetically tagged cells will migrate toward the magnet (these are the **wanted** $CD4^+CD25^+$ T 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, use a sterile Pasteur pipette or transfer pipette to 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 13-14 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 ready to be used in other downstream applications. The purity of the recovered cells can be assessed by staining with the two color staining reagent provided in the kit, using 20 µL of reagent for each 1-5 x 10^5 cells.

DATA EXAMPLES



Enrichment of $CD4^+$ T cells (Step 1) and $CD4^+CD25^+$ Regulatory T cells (Step 2) from human PBMCs using this MagCellect Human $CD4^+CD25^+$ Regulatory T Cell Isolation Kit. Cells were stained using the conjugated antibodies provided in this kit. The additional reagent used was CD3-PE (R&D Systems Cat.# FAB100P).