*MagCellect™ Human Blood Myeloid Dendritic Cell Isolation Kit**

Catalog Number: MAGH120

Kit Contents

- **MagCellect Human DC Enrichment Antibody Cocktail 1** (Part 860200) - 1 mL in a phosphate buffered solution containing BSA.
- **MagCellect Human DC Enrichment Antibody Cocktail 2** (Part 860201) - 1 mL in a phosphate buffered solution containing BSA.
- **MagCellect Streptavidin Ferrofluid** (Part 860165) - 3.0 mL in a solution containing BSA and preservative.
- **MagCellect 10X Buffer** (Part 860040) - 10 mL of a 10X concentrated buffer.
- **MagCellect Human Blocking Reagent** (Part 860061) - 0.2 mL of human IgG.

This kit contains sufficient reagents to process 1 x 10^9 total cells; up to 20 isolations.

Storage

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

Other Required Supplies

- MagCellect Magnet (R&D Systems, Catalog # MAG997 or equivalent) [1]
- 12 x 75 mm (5 mL) polystyrene round bottom tubes
- Sterile Pasteur pipettes or transfer pipettes (ThermoFisher, Catalog # 13-711-9B or equivalent)

If using the Cell Preparation instructions listed below, the following is also required:

- Human Erythrocyte Lysing Kit (R&D Systems, Catalog # WL1000 or equivalent)

Intended Use

This MagCellect Human Blood Myeloid Dendritic Cell Isolation Kit is designed to isolate myeloid dendritic cells (mDCs) from human blood samples via a two-step negative selection principle. The resulting cell preparation is highly enriched with all reported mDC subpopulations. The phenotype of the recovered mDCs show donor variation, but are typically 7 - 31% CD1c (BDCA1)$^+$/CD11c$^+$ cells, 10 - 38% CD141 (BDCA3)$^+$/CD11c$^+$ cells, 15 - 31% CD16$^+$/CD11c$^+$ cells, and 10 - 24% CD56$^+$/MHCII$^+$ cells. BDCA2$^+$/CD45$^+$ plasmacytoid DCs are not enriched.

Principle of Selection

A mononuclear cell suspension is incubated with two MagCellect Antibody Cocktails (1 and 2) that target the unwanted cells. MagCellect Streptavidin Ferrofluid is 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

Use this procedure or traditional methods to prepare a single cell suspension of mouse leukocytes.

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 wash step, disrupt the cell pellet by “racking” the tube, resuspend the cells in the H-Lyse Buffer found in the Human Erythrocyte Lysing Kit (R&D Systems, 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).
4. Incubate the cells for 10 minutes at room temperature and 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. Prepare 1X MagCellect Buffer. Spin the cells for 10 minutes at 200 x g and resuspend the cells in a small volume of 1X MagCellect Buffer.
6. Perform a cell count and adjust the cell concentration to 110 x 10^6 cells per mL with cold 1X MagCellect Buffer.
7. Continue the cell selection by referring to step #1 of the Cell Selection Procedure.

[1] This MagCellect Kit has been tested with Miltenyi MidiMACS™ and Stem Cell Technologies EasySep® magnets with excellent performance results.

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1-800-343-7475

Cell Selection Procedure

This procedure is for the processing of 100 x 10^6 total cells per sample 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 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 24 hours.
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 110 x 10^6 cells/mL.
3. Transfer 100 x 10^6 cells (0.9 mL volume) into a 5 mL polystyrene tube. Add 10 µL of MagCellect Human Blocking Reagent and 100 µL of MagCellect Human Myeloid DC Enrichment Antibody Cocktail 1. Gently mix the cell-antibody suspension, avoiding bubble formation, and incubate at 2 - 8° C for 15 minutes.
4. Add 150 µL of MagCellect Streptavidin Ferrofluid to the cell suspension, mix gently, and incubate at 2 - 8° for 15 minutes.
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 (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 the 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 initially depleted cell fraction containing the desired DCs and some unwanted cells. These cells are now ready for a second negative selection.
8. Add 100 µL of MagCellect Human Myeloid DC Enrichment Antibody Cocktail 2 to the 1 mL supernatant from step 7. Gently mix the cell-antibody suspension, avoiding bubble formation, and incubate at 2 - 8° C for 15 minutes.
9. Add 150 µL of MagCellect Streptavidin Ferrofluid to the cell suspension, mix gently, and incubate at 2 - 8° C for 15 minutes.
10. 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 DCs in suspension in the final supernatant.
11. 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.
12. To ensure that all of the magnetic nanoparticles have been removed, repeat the magnetic depletion (steps 10 and 11) 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 DCs. 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 110 x 10^6 cells/mL. If using 50 x 10^6 cells/sample, add 50 µL of the antibody cocktail and 75 µL of Streptavidin Ferrofluid.
- Phenotype of isolated cells might vary due to donor-to-donor variation.
- **Do not process more than 100 x 10^6 cells in each 5 mL tube**. **Reaction volume adjustments must be made using 1X MagCellect Buffer just prior to the magnetic separation step.**

Typical Data

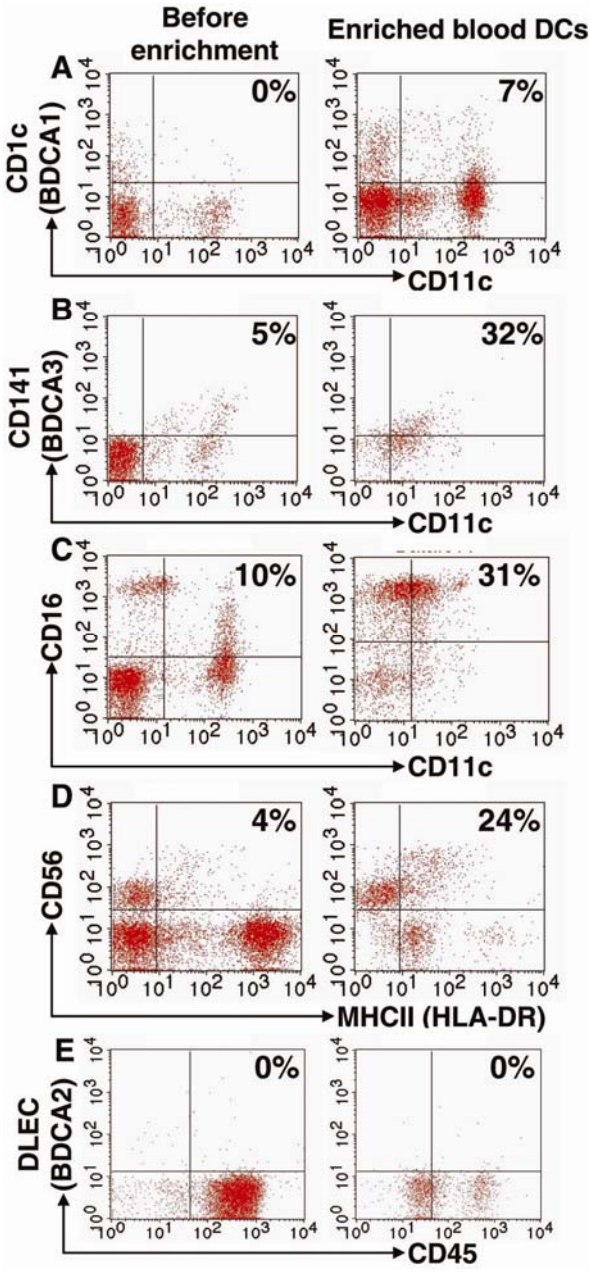

Figure 1: Example of enrichment of myeloid dendritic cells (mDCs) from human blood using the MagCellect Human Blood Myeloid Dendritic Cell Isolation Kit. Cells before enrichment (left column) and the enriched blood mDCs (right column) were stained with the indicated antibodies to specifically label mDC subpopulations. Phenotypical profile of isolated cells might vary due to donor-to-donor variation.

A. The CD1c (BDCA1)$^+$/CD11c$^+$ mDC subpopulation before and after enrichment.

B. The CD141 (BDCA3)$^+$/CD11c$^+$ mDC subpopulation before and after enrichment.

C. The CD16$^+$/CD11c$^+$ mDC subpopulation before and after enrichment.

D. The CD56$^+$/MHCII$^+$ mDC subpopulation before and after enrichment.

E. No enrichment of plasmacytoid DCs (CD45$^+$/BDCA2$^+$).

Troubleshooting Guide

Most difficulties arise from the following three areas:

- **Quality of the Cell Preparation:** Dead cells and debris might interfere with isolation and detection, affecting both the purity and recovery of intended cells. Cell aggregation (very common in some cell types) might also affect the enrichment performance. The presence of large numbers of granulocytes in the preparation could also interfere with an efficient isolation.
- **Sample Variations:** For less-abundant cells (*i.e.* NK, dendritic, endothelial cells, etc.), sample (donor, patient, etc.) variation can affect the efficiency of the isolation.
- **Few Expected Target Cells:** If less than 50,000 - 100,000 expected target cells are present, recovery and/or purity of the isolation is compromised. This will typically be a lesser problem when isolating abundant populations (*i.e.* T cells, B cells, epithelial cells from solid tumor, etc.), but it could be a problem when isolating rare populations (*i.e.* dendritic cells, NK subpopulations, tumor initiating cells, etc.).

Issue	Possible Cause	Possible Solution
Low yield of isolated cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris will affect the isolation efficiency. **Make sure your cell preparation contains a minimal amount of dead cells or cell debris.** Test a small sample of cells with a vital dye before performing the Cell Selection Procedure. The presence of cell debris is also easily identified in the FSS/SSC flow cytometry analysis.
	Cell aggregates	Cell aggregates will interfere with the cell selection. **Make sure you have a single-cell suspension before performing the cell selection procedure.** A small sample of cells can be tested with a vital dye before performing the cell selection procedure to ensure a healthy single-cell suspension.
	Few expected cell targets	If the cell fraction to be isolated contains less than ~250,000 cells or represents less than 1% of the total cell preparation, the recovery could be affected. For a better yield, **increase the number of cells in your starting population**, if possible, or consider performing a pre-enrichment step by removing undesirable cells in steps (R&D Systems has MagCellect or PlusCellect™ kits for negative-selection of undesirable cells).
	Poor magnetic selection	When removing unwanted cells during the Cell Selection Procedure, **make sure the tube in the magnet does not move.** If the tube is allowed to move or shift, positive cells that should be magnetically attached to the magnet might become loose. If the placement of the tube in the magnet is not tight, immobilize it with adhesive tape. Also, **be sure to aspirate the supernatant very carefully** when removing the unwanted cells. Strong pipetting might release positive cells from the magnet.
Low purity of isolated cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris will affect the isolation efficiency. **Make sure your cell preparation contains minimal amount of dead cells or cell debris.** Test a small sample of your cells with a vital dye before performing the cell selection procedure. The presence of cell debris could also be easily identified in the FSS/SSC flow cytometry analysis.
	Poor cell preparation or too many granulocytes	An excessive presence of granulocytes in your cell preparation might affect the purity of the enriched fraction. If the presence of large amounts of granulocytes in the cell preparation is unavoidable, consider a pre-depletion using biotinylated anti-CD66b (CEACAM-8) antibody (R&D Systems, Catalog # BAF4246).
	Few positive cell targets	If the cell fraction to be isolated contains less than ~250,000 cells or represents less than 1% of the total cell preparation, the purity of the isolated cells could be affected. For a better purity and yield, **increase the number of cells in the sample**, if possible, or consider performing a pre-enrichment step by removing undesirable cells in steps (R&D Systems has MagCellect or PlusCellect kits for negative-selection of specific cells).
	Enriched cells not washed well	**Extra washes** can be performed subjecting the cells to an extra step of magnetic migration (steps 5 - 6 of the Cell Selection Procedure). Additional magnetic selection steps could increase cell purity (typically ~5% increase) of the target population. Keep in mind that with every added step, a reduced yield can be expected.
No cells recovered	Insufficient cell targets	If the cell fraction to be isolated represents a very small fraction of the total cell preparation, recovery could be significantly reduced. For a better yield, **increase the number of cells in your starting population** and/or consider performing a pre-enrichment step by removing undesirable cells in steps (R&D Systems has MagCellect or PlusCellect kits for negative-selection of undesirable cells).