MagCellect™ Human CD14+ Cell Isolation Kit

Catalog Number: MAGH105

INTENDED USE

MagCellect Human CD14+ Cell Isolation Kit is designed to isolate CD14+ cells via a positive selection principle. The resulting cell preparation is highly enriched for CD14+ cells. Typical recovery ranges from 45-75% and the purity of recovered CD14+ cells ranges from 90-97%.

CD14 is highly expressed in most monocytes and macrophages and weakly expressed on neutrophils. Monocytes isolated using the MagCellect CD14+ Cell Isolation Kit can be used for various downstream applications including the *ex vivo* generation of dendritic cells.

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 or magnetic nanoparticles that have no magnetic memory (superparamagnetic), and 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

Isolation of CD14+ cells is done by positive selection in a test tube by tagging the cells of interest with an anti-human CD14 biotinylated antibody followed by the addition of Streptavidin Ferrofluid. 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 (desired cell population), leaving the untagged (unwanted) cells in suspension. Unwanted cells are first removed by aspiration while the tube remains in the magnet; next the tube containing the magnetically selected (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 use past kit expiration date. **DO NOT FREEZE.**
The kit contains sufficient reagents to process $1x10^9$ total cells.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Anti-Human CD14 (Clone: 134620) Biotinylated Antibody	963641	1 mL of biotinylated antibody cocktail in a phosphate buffered solution containing BSA and preservative.	May be stored 2-8 °C when handled aseptically.*
Streptavidin Ferrofluid	860038	1 mL of streptavidin-coated nanoparticles in a solution containing BSA and preservative.	
10X Buffer	860125	25 mL of a 10X concentrated buffer.	

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

OTHER SUPPLIES REQUIRED

- MagCellect Magnet (R&D Systems, Catalog # MAG997)
- Human Erythrocyte Lysing Kit (R&D Systems, Catalog # WL1000)
- 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
- Sterile deionized or distilled water
- Hanks' BSS or equivalent
- Bovine serum

REAGENT PREPARATION

Prepare 25 mL of 1X MagCellect Buffer for each 1 x 10^8 cells to be processed by mixing 2.5 mL of 10X Buffer with 22.5 mL sterile deionized or distilled water. **Must be kept at cold (2-8 °C) for the following procedure.**

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 H-Lyse Buffer from 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:** 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 1 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 in order to avoid excessively low temperatures that can slow the kinetics of the optimized reactions.**

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 1 x 10^8 cells (1.0 mL volume) into a 15 mL conical centrifuge tube. Add 50 µL of Anti-human CD14 Biotinylated Antibody. Gently mix the cell-antibody suspension, avoiding bubble formation, and incubate at 2-8 °C in a refrigerator for 15 minutes.
3. At the end of the incubation period, wash the cell suspension by filling the tube up to the 15 mL mark with cold 1X MagCellect Buffer and centrifuge at 300 x g for 8 minutes. Remove the supernatant **completely** and resuspend the cell pellet by pipetting gently into the tube 1 mL of cold 1X MagCellect Buffer.
4. Add 100 µL of Streptavidin Ferrofluid to the cell suspension, mix gently and incubate at 2-8 °C in a refrigerator for 15 minutes.
5. At the end of the incubation period wash the cell suspension by filling the tube up to the 15 mL mark with 1X cold MagCellect Buffer and centrifuge at 300 x g for 8 minutes. Remove the supernatant completely and resuspend the cell pellet by pipetting gently into the tube 2 mL of cold 1X MagCellect Buffer.
6. Transfer the cell suspension to a 5 mL tube.
7. 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 cells), leaving the untagged unwanted cells in suspension in the supernatant.
8. 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.
9. Remove the tube containing the magnetically selected (wanted) cells from the magnet, and resuspend cells by adding 2.0 mL of cold 1X MagCellect Buffer.
10. To complete the cell isolation procedure repeat steps #6 and #7 one more time with the resuspended cell fraction.
11. Remove the tube containing the magnetically selected (wanted) cells from the magnet, and resuspend cells by adding 2.0 mL of 1X MagCellect Buffer or tissue culture media. This final magnetically isolated fraction contains the desired isolated $CD14^+$ cells. The cells are now ready to be counted, stained and 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 1 x 10^8 cells/mL; add 5 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 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 incubation time in the magnet described in step #6 to 8 minutes. **Reaction volume adjustments must be made using 1X MagCellect Buffer just prior to the magnetic separation step.**

DATA EXAMPLE

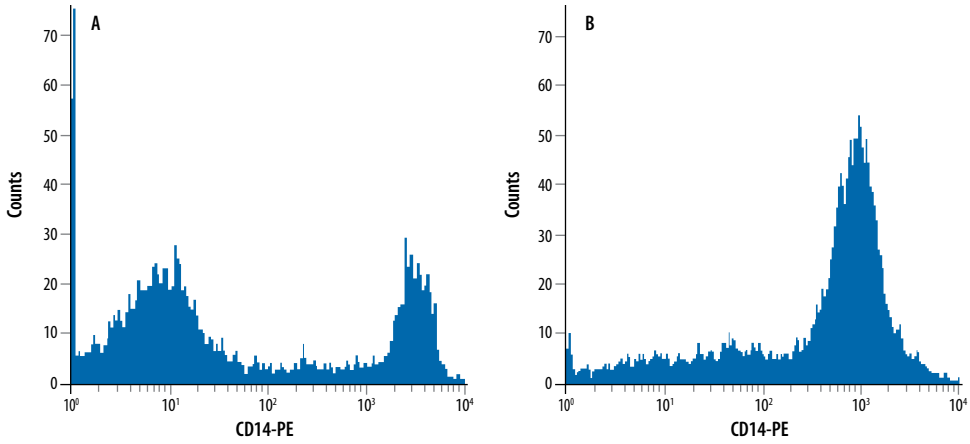

Figure 1: Isolation of human CD14$^+$ cells from ficolled PBMCs using R&D Systems MagCellect Human CD14$^+$ Cell Isolation Kit. Histograms show cell before **(A)** and **(B)** after isolation. Histograms reflect all viable cells stained with Anti-Human CD14-PE (R&D Systems, Catalog # FAB3832P).