R&D SYSTEMS
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MagCellect™ Plus Human CD44+ Cell Isolation Kit

Catalog Number: MAGH122

INTENDED USE

The MagCellect™ Human CD44+ Cell Isolation Kit is designed to isolate CD44 expressing cells via a positive selection principle. The resulting cell preparation is highly enriched with CD44+ cells. Typical purity of recovered CD44+ cells ranges from 80-95%.

BACKGROUND

R&D Systems® MagCellect™ products are designed for the isolation of cells in a "liquid phase". 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

A mononuclear cell suspension is first incubated with the Human CD44 Biotinylated Antibody that targets the desired cells. MagCellect™ Streptavidin (SA) Ferrofluid is next added to the reaction that allows the SA coated nanoparticles to interact with the cells tagged with the monoclonal antibodies. The tube containing the cell suspension is then placed within a magnetic field. Magnetically tagged cells will migrate toward the magnet (desired cell fraction), leaving the untagged cells (unwanted cell population) in suspension to be harvested by aspiration while the tube remains in the magnetic field. The tube containing the magnetically selected (desired) cells is then removed from the magnet and the cells are resuspended in MagCellect™ Plus Buffer or tissue culture media. The enriched cell preparation is then available for a variety of applications including tissue culture, immune status monitoring and flow cytometry.

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 1 x 10^9 total cells.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Human CD44 Biotinylated Antibody	965901	625 µL of Human CD44 biotinylated antibody in a phosphate buffered solution containing BSA and preservative.	Handle aseptically and store at 2-8 ºC.*
Human CD44 AlexaFluor® 647 Antibody	968420	125 µL of AlexaFluor® 647-conjugated Mouse anti-human CD44 detection antibody	
Streptavidin Ferrofluid	860127	1.25 mL of SA-coated nanoparticles in a solution containing BSA and preservative.	
Plus (10X) Buffer	895921	2 bottles (50 mL/bottle) of a 10X concentrated buffer.	Stored at 2-8 ºC.* Use diluted buffer within 24 hours.

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

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.

bio-techne.com
info@bio-techne.com
techsupport@bio-techne.com

North America
TEL 800 343 7475

Europe • Middle East • Africa
TEL +44 (0)1235 529449

China
info.cn@bio-techne.com
TEL +86 (21) 52380373

Rest of World
bio-techne.com/find-us/distributors
TEL +1 612 379 2956

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

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
- 15 mL conical centrifuge tubes
- Sterile deionized or distilled water
- Hank's BSS or equivalent
- Bovine Serum

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 2 x 10^8 cells or fewer, use the 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 2x10^8 cells in each 5 mL tube and do not exceed a total reaction volume of 3 mL in each tube.** A reaction volume of 3 mL is recommended when processing 2 x 10^8 cells. A reaction volume of 1 mL is recommended when processing 5 x 10^7 or fewer cells.
- When processing greater than 2 x10^8 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 6x10^8 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. Increase the magnetic incubation time described in step #6 to 10 minutes.
- **Reaction volume adjustments must be made using 1X MagCellect™ Plus Buffer just prior to the magnetic separation step.**

When processing different numbers of cells, observe the following guidelines:

- Keep the biotinylated antibody and ferrofluid incubation times the same
- Keep the cell density at 1 x 10^7 cells/mL
- If blocking, add 100 µg of human IgG per 10^7 cells being processed
- Add 5 µL of biotinylated antibody per additional 10^7 cells being processed.
- Add 10 µL of Streptavidin Ferrofluid per additional 10^7 cells being processed.

REAGENT PREPARATION

Prepare 27 mL of 1X MagCellect™ Plus Buffer for each 1 x 10^7 cells to be processed by mixing 2.7 mL of Plus (10X) Buffer with 24.3 mL sterile deionized or distilled water. **The buffer must be kept cold (2-8 °C) for the following procedures.**

CELL PREPARATION

MagCellect™ Plus kits work with a single, cell suspension preparation. Cell suspensions can be prepared by traditional methods or by following the instructions below.

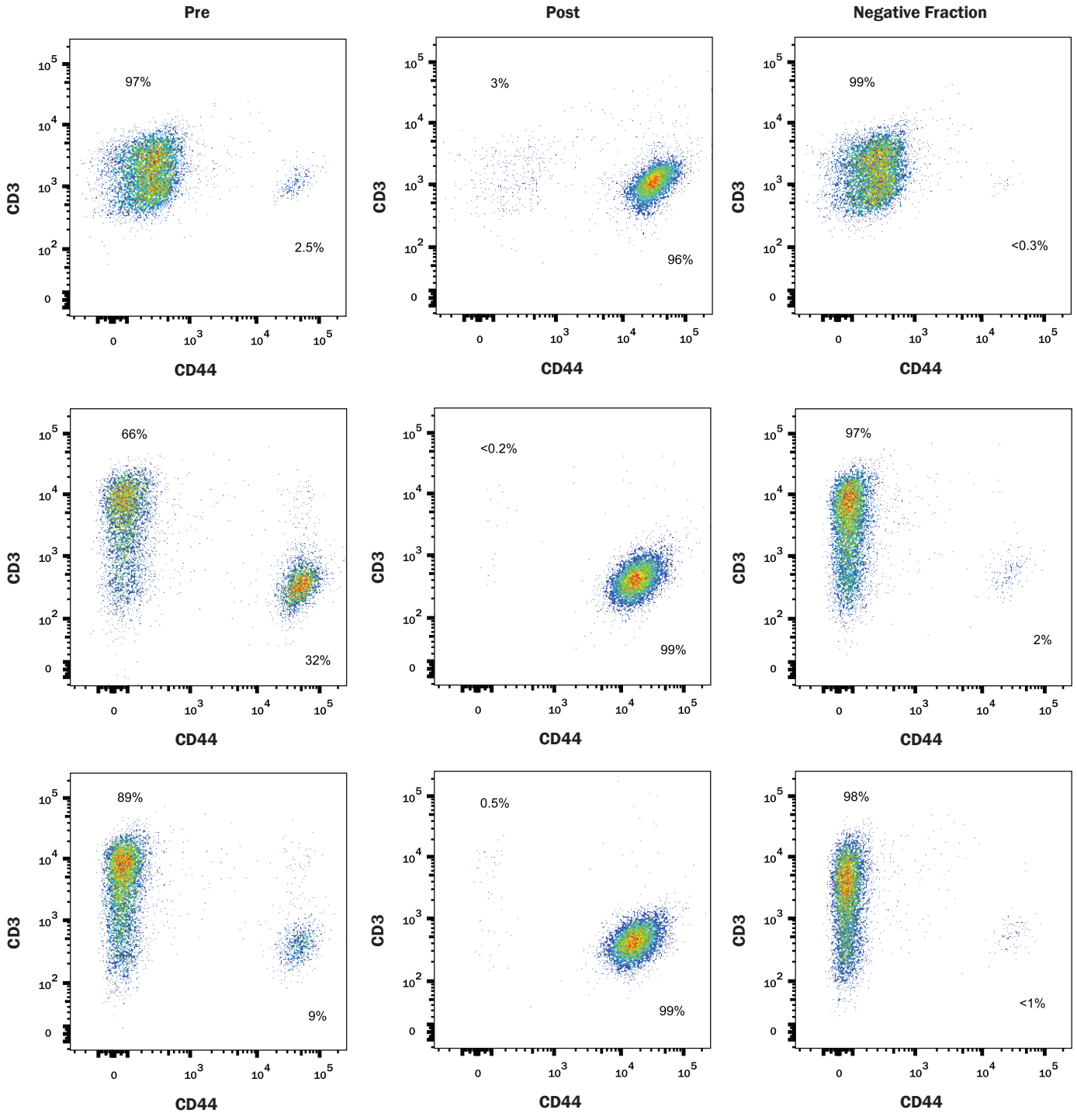
1. Process cells on a density gradient (*i.e.* Ficoll Hypaque) or any other method to enrich for mononuclear cells.
2. Recover the "buffy coat" containing the mononuclear cells and wash 2X by centrifuging for 10 minutes at 200 x g with PBS to remove any residual separation media.
3. Red blood cell lyse **(recommended)**:
    a. After washing, resuspend pellet in H-Lyse Buffer (Human Erythrocyte Lysing Kit; R&D Systems®, Catalog # WL1000 or equivalent) that has been diluted to 1X strength with sterile distilled water and quickly vortex the tube. Using 10 mL of 1X H-Lyse solution per 250 million cells is recommended.
    b. Incubate the cells for 5-10 minutes at room temperature until red cell lysis is complete, wash the cells by adding 1X Wash Buffer to the cells, vortex and centrifuge for 5 minutes at 250-500 x g.
4. Resuspend the cells in a small volume of 1X MagCellect™ Plus Buffer and perform a cell count. Adjust the cell concentration to 1 x 10^7 cells per mL with cold 1X MagCellect™ Plus Buffer and continue with Cell Selection Procedure.

CELL SELECTION PROCEDURE

This procedure is for processing 1 x 10^7 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 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 cells 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™ Plus Buffer prior to beginning the procedure and be at a cell density of 1x10^7 cells/mL. **Note:** If necessary, block Fc receptor sites by adding 100 µg of human IgG per 10^7 cells processed.
2. Transfer 1x10^7 cells (1.0 mL volume) into a 15 mL conical centrifuge tube and then add 25 µL of Human CD44 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 adding 9 mL of cold 1X MagCellect™ Plus Buffer and centrifuge at 300 x g for 8 minutes. Completely remove the supernatant and resuspend the cell pellet by gently pipetting 1 mL of cold 1X Magcellect™ Plus Buffer into the tube.
4. Add 50 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 adding 9 mL of cold 1X MagCellect™ Plus Buffer and centrifuge at 300 x g for 8 minutes. Completely remove the supernatant and resuspend the cell pellet by gently pipetting 3 mL of cold 1X MagCellect™ Plus Buffer into the tube. Transfer the cell suspension to a 5 mL polystyrene round bottom tube.
6. Place the 5 mL reaction tube with your cells 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 desired cells).
7. 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 suspension (unwanted cells) and discard. Remove the tube containing the magnetically selected cells from the magnet and resuspend in 3.0 mL cold 1X MagCellect™ Plus Buffer.
8. Repeat steps 6-7 at least once more with the resuspended cell fraction. If purity of the cell selection is critical, repeat this step several times.
9. Remove the tube containing the magnetically selected cells from the magnet and resuspend by adding 1-2 mL of 1X MagCellect™ Plus Buffer or tissue culture media. This final magnetically isolated fraction contains the desired $CD44^+$ cells. The cells are now ready for counting, staining and further downstream applications.
10. If the isolated $CD44^+$ cells are to be visualized by flow cytometry, resuspend the appropriate amount of selected cells in 100 µL of 1X MagCellect™ Plus Buffer and stain them using 5 µL of Human CD44 AlexaFluor® 647 Detection Antibody. Proceed as usual with standard staining procedures.

DATA EXAMPLES



Isolation of human CD44⁺ cells from Jurkat cells using the MagCellect™ Plus Human CD44⁺ Cell Isolation Kit. Jurkat cells were prepared as described in the Cell Preparation Section. Jurkat cells were spiked with U937 cells, either 3% (top row), or 30% (middle row). Cells were stained with Mouse Anti-Human CD44 AlexaFluor® 647 Monoclonal Antibody (included in the kit) and Mouse Anti-Human CD3 epsilon PE Conjugated Monoclonal Antibody (R&D Systems, Catalog # FAB100P) either before (first column "Pre") or after (middle column "Post") isolation of CD44⁺ cells. The third column shows staining of cells in the negative fraction. Typical staining is also shown for a 5X scale up isolation using a larger starting population of cells as indicated in the Technical Hints (bottom row).