MagCellect™

Human $CD24^{-}CD44^{+}$ Breast Cancer Stem Cells Isolation Kit

Catalog Number MAGH111

For the isolation of $CD24^{-}CD44^{+}$ expressing cells via a positive selection principle.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTENDED USE

The MagCellect Human $CD24^{-}CD44^{+}$ Breast Cancer Stem Cells Isolation Kit is designed to isolate putative human breast cancer stem cells via a positive selection principle. The resulting cell preparation is highly enriched for $CD24^{low/-}CD44^{+}$ cells. Purity of recovered $CD24^{low/-}CD44^{+}$ cells varies depending on the preparation.

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 THE ASSAY

Cell isolation is done by magnetic selection in two steps:

Step 1: Negative selection of $CD24^{+}$ cells. $CD24^{+}$ cells are removed by tagging cells with a biotinylated anti-human CD24 antibody followed by the addition of Streptavidin-conjugated magnetic particles (MagCellect* Streptavidin Ferrofluid). The tube with the cell suspension is then placed in a magnet. Magnetically tagged cells ($CD24^{+}$) will migrate toward the tube wall on the magnet side, leaving the untagged cells ($CD24^{low/-}$) in suspension.

Step 2: Positive selection of $CD44^{+}$ cells. The untagged $CD24^{low/-}$ cells are subsequently labeled with a biotinylated anti-human CD44 antibody and magnetically tagged with MagCellect Streptavidin Ferrofluid to select the $CD44^{+}$ cells.

To assess the efficiency of enrichment, recovered cells may be stained with the conjugated anti-human CD24 and anti-human CD44 antibodies provided.

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 for 20 tests (up to 2 x 10^8 total cells).

PART	PART #	DESCRIPTION	STORAGE OF OPENED/DILUTED MATERIAL
Human CD24 Biotinylated Antibody	860214	0.5 mL of biotinylated mouse anti-human CD24 antibody.	May be stored 2-8 °C when handled aseptically.*
Human CD44 Biotinylated Antibody	860215	0.2 mL of biotinylated mouse anti-human CD44 antibody.	
Human CD24 Detection Antibody	860216	0.2 mL of APC-conjugated mouse anti-human CD24 antibody.	
Human CD44 Detection Antibody	860217	0.2 mL of PE-conjugated mouse anti-human CD44 antibody.	
Streptavidin Ferrofluid	860129	2 mL of a solution containing BSA and preservative.	
Plus Buffer (10X)	895072	2 vials (25 mL/vial) of a 10X concentrated buffer.	May be stored for up to 24 hours at 2-8 °C after dilution.*

* 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 (Falcon, Catalog # 352008, 352006, or equivalent)
- Sterile Pasteur pipettes or transfer pipettes
- Benchtop centrifuge
- 2-8 °C refrigerator
- Deionized or distilled water

PRECAUTION

The PE- and APC-conjugated detection antibodies provided in 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.

REAGENT PREPARATION

1X MagCellect Plus Buffer - Prepare 25 mL of 1X MagCellect Plus Buffer for each sample to be processed by mixing 2.5 mL of 10X MagCellect Plus Buffer with 22.5 mL of sterile deionized or distilled water. The 1X MagCellect Plus Buffer should be kept on ice or refrigerated.

CELL SELECTION PROCEDURE

Cells and reagents should be kept at 2-8 °C. Incubations should be performed in a 2-8 °C refrigerator. Do not perform incubations in an ice bath. Excessively low temperatures can slow the kinetics of the optimized reactions.

Step 1: Removal of $CD24^+$ Cells by Negative Selection

Note: *This procedure describes the processing of 5 x 10^6 total cells using 5 mL tubes. Please refer to the Technical Hints section for processing other cell numbers.*

1. Prepare a single-cell suspension of your preparation containing $CD24^{low/-}CD44^+$ target cells. Cells must be suspended in cold 1X MagCellect Plus Buffer at a density of 1 x 10^7 cells/mL prior to beginning the procedure.
2. Place 5 x 10^6 cells (0.5 mL) into a 5 mL round bottom tube.
3. Add 25 µL of Human CD24 Biotinylated Antibody. Gently mix the cell/antibody suspension, avoiding bubble formation, and incubate for 15 minutes at 2-8 °C in a refrigerator. At the end of the incubation period, wash the cell suspension by adding 3 mL of cold 1X MagCellect Plus Buffer, and centrifuge at 300 x g for 8 minutes or 1 minute in a single-speed clinical benchtop centrifuge. **Completely** remove the supernatant and resuspend the cell pellet by gently pipetting 0.5 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 for 15 minutes at 2-8 °C in a refrigerator.

   Note: *If using a magnetic selection system other than MagCellect, this part of the procedure will need to be adapted according to the supplier's instructions.*
5. At the end of the incubation period, wash the cell suspension by adding 3 mL of cold 1X MagCellect Plus Buffer and centrifuge at 300 x g for 8 minutes or 1 minute in a single-speed clinical benchtop centrifuge. **Completely** remove the supernatant and resuspend the cell pellet by gently pipetting 2 mL of cold 1X MagCellect Plus Buffer into the tube.
6. Place the reaction tube in the MagCellect magnet (or equivalent) that has been positioned horizontally to accommodate 5 mL tubes, and incubate for 6 minutes at room temperature. Magnetically tagged $CD24^+$ cells will migrate toward the magnet, leaving the untagged $CD24^{low/-}$ cells in suspension in the supernatant.
7. While the tube is **firmly held** in the magnet, **collect** the wanted $CD24^{low/-}$ cells by **slowly and carefully** aspirating the supernatant with a sterile Pasteur pipette or transfer pipette, and place them in a new tube. Discard or set aside the tube with the magnet-bound $CD24^+$ cells. They could be used as a control of the depletion efficacy.
8. To complete the removal of $CD24^+$ cells, repeat steps 6-7 at least once more with the collected $CD24^{low/-}$ cell fraction.

   Note: *If necessary, this step could be repeated one or two more times.*
9. The collected $CD24^{low/-}$ cells can now be used in the next step.

CELL SELECTION PROCEDURE *CONTINUED*

Step 2: Positive Selection of $CD24^{low/-}CD44^{+}$ Cells

1. Concentrate $CD24^{low/-}$ cells obtained in Step 1 by centrifuging at 300 x g for 8 minutes or 1 minute in a single-speed clinical benchtop centrifuge. Resuspend the pellet in 0.5 mL of 1X MagCellect Plus Buffer.

2. Add 10 µL of Human CD44 Biotinylated Antibody. Gently mix the cell/antibody suspension, avoiding bubble formation, and incubate for 15 minutes at 2-8 °C in a refrigerator. At the end of the incubation period, wash the cell suspension by adding 3 mL of cold 1X MagCellect Plus Buffer and centrifuge at 300 x g for 8 minutes or 1 minute in a single-speed clinical benchtop centrifuge. **Completely** remove the supernatant and resuspend the cell pellet by gently pipetting 0.5 mL of cold 1X MagCellect Plus Buffer into the tube.

3. Add 50 µL of Streptavidin Ferrofluid to the cell suspension. Mix gently and incubate for 15 minutes at 2-8 °C in a refrigerator.

   Note: *If using a magnetic selection system other than MagCellect, this part of the procedure will need to be adapted according to the supplier's instructions.*

4. At the end of the incubation period, wash the cell suspension by adding 3 mL of cold 1X MagCellect Plus Buffer and centrifuge at 300 x g for 8 minutes or 1 minute in a single-speed clinical benchtop centrifuge. **Completely** remove the supernatant and resuspend the cell pellet by gently pipetting 2 mL of cold 1X MagCellect Plus Buffer into the tube.

5. Place the reaction tube in the MagCellect magnet (or equivalent) that has been positioned horizontally to accommodate 5 mL tubes and incubate for 6 minutes at room temperature. Magnetically tagged $CD44^{+}$ cells will migrate toward the magnet leaving the untagged $CD44^{-}$ cells in suspension in the supernatant.

6. While the tube is **firmly held** in the magnet, remove the unwanted ($CD44^{-}$) cells by **slowly and carefully** aspirating the supernatant with a sterile Pasteur pipette or transfer pipette. Discard or set aside the supernatant containing the $CD44^{-}$ cells. They could be used as a control of the cell separation efficacy.

7. Remove the tube containing the magnetically selected cells from the magnet and resuspend cells by adding 2.0 mL of cold 1X MagCellect Plus Buffer.

8. To complete the cell isolation procedure, repeat steps 5-6 at least once more with the resuspended cell fraction.

9. Remove the tube containing the magnetically selected cells from the magnet and resuspend the cells by adding 100-500 µL of 1X MagCellect Plus Buffer or tissue culture media. This final magnetically isolated fraction contains the desired isolated $CD24^{low/-}$ $CD44^{+}$ cells. The cells are now ready to be counted, stained, and used in other downstream applications.

10. If isolated $CD24^{low/-}CD44^{+}$ 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 10 µL of both Human CD24 Detection Antibody and Human CD44 Detection Antibody. Proceed as usual with standard staining procedures.

CELL PREPARATION

This kit works with any single-cell suspension preparation. Isolation and suspension of single cells from breast tumor or other samples can be performed according to standard and published procedures. Examples of these are:

1. Breast Tumor-Initiating Cells Isolated from Patient Core Biopsies for Study of Hormone Action. Marsden *et al. Methods in Molecular Biology* (2009) Vol. 590 pp. 363-375.
2. Phenotypic and Functional Characterization *In Vitro* of a multipotent Epithelial Cell Present in the Normal Adult Human Breast. *Differentiation* (2004) Vol. 62 pp. 201-213.
3. *Cell Biology Protocols*, eds. J. Robin Harris et al. (2006) John Wiley & Sons.

CELL STAINING PROCEDURE

After successfully selecting the desired cell population, cells can be stained by traditional methods or by following the instructions below.

1. Add 100 µL of the positively selected cells to a 5 mL tube.
2. Add 7 µL of the APC-conjugated Human CD24 Detection Antibody and 7 µL of the PE-conjugated Human CD44 Detection Antibody.
3. Incubate for 30-40 minutes at 2-8 °C.
4. Following this incubation, remove the unreacted antibody by washing the cells twice in 2 mL of 1X MagCellect Plus Buffer or PBS.
5. Resuspend the cells in 200-400 µL of 1X MagCellect Plus Buffer or PBS for final flow cytometric analysis.

TECHNICAL HINTS

- If sterile cells are required following 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 quickly, by keeping cells and solutions cold through the use of pre-cooled solutions, and by adhering to the incubation times and temperatures specified in the procedure. Increased temperature and prolonged incubation times may lead to non-specific cell labeling, which may result in lower cell purity and yield.
- When processing different numbers of cells, observe the following guidelines:
  - **Using less than 5 x 10^6 total cells or more than 5 x 10^7 total cells per initial isolation (Step 1) is not recommended. For better results, the expected target $CD24^{low/-}CD44^+$ population should be between 1-25% of the starting cell population. If the expected fraction is below 1%, consider using 1-5 x 10^7 starting cells.**
  - Keep the biotinylated antibody and ferrofluid incubation times the same.
  - Add 5 µL of the biotinylated antibody per 10^6 cells being processed **to a maximum of 100 µL.**
  - Add 10 µL of Streptavidin Ferrofluid per 10^6 cells being processed **to a maximum of 150 µL.**

DATA EXAMPLES

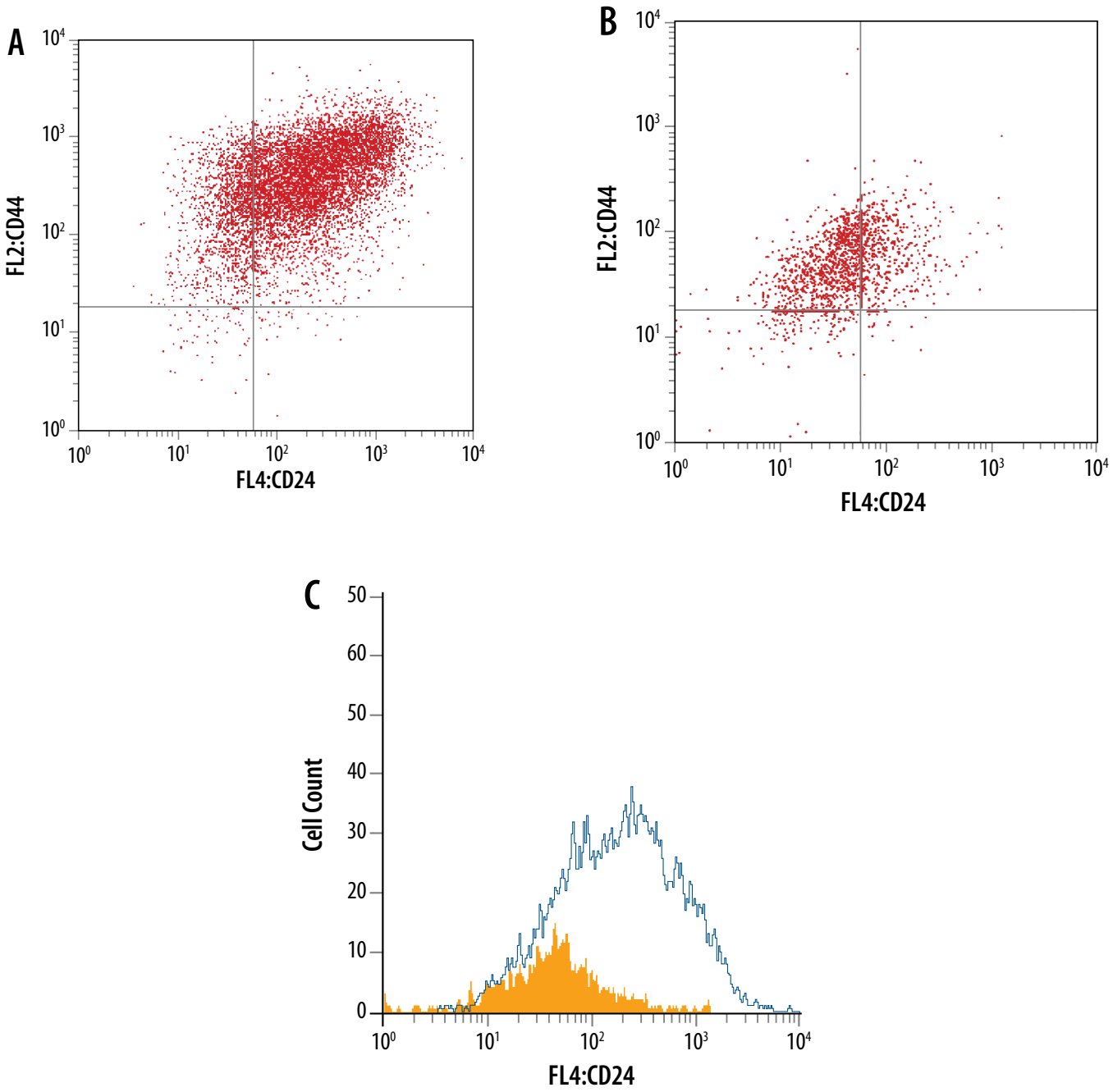

Figure 1: Example of isolation of a putative breast cancer stem cell population from MCF-7 human breast cancer cells. A small $CD24^{low/-}CD44^{+}$ population (A, upper-left quadrant) was isolated using this MagCellect Human Breast Cancer Stem Cells Isolation Kit (B). A histogram profiling the enrichment of $CD24^{low/-}CD44^{+}$ cells (shaded histogram) is shown (C).