

PlusCollect™

Mouse E-Cadherin⁺ Cancer/Stem Cells Kit

Catalog Number PLS748

For the isolation of epithelial, stem, or other cells expressing E-Cadherin via a positive selection principle.

This kit contains sufficient reagents for 20 tests (up to 1×10^9 total cells).

This package insert must be read in its entirety before using this product.

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**FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.**

TABLE OF CONTENTS

Contents	Page
INTRODUCTION	2
PRINCIPLE OF THE TEST	2
INTENDED USE	3
MATERIALS PROVIDED.....	3
STORAGE	3
OTHER MATERIALS REQUIRED.....	3
PRECAUTION	4
REAGENT PREPARATION.....	4
CELL PREPARATION	4
CELL SELECTION PROCEDURE.....	5
CELL STAINING PROCEDURE	6
TECHNICAL HINTS.....	6
TYPICAL DATA	7
REFERENCES	8
TROUBLESHOOTING GUIDE	9

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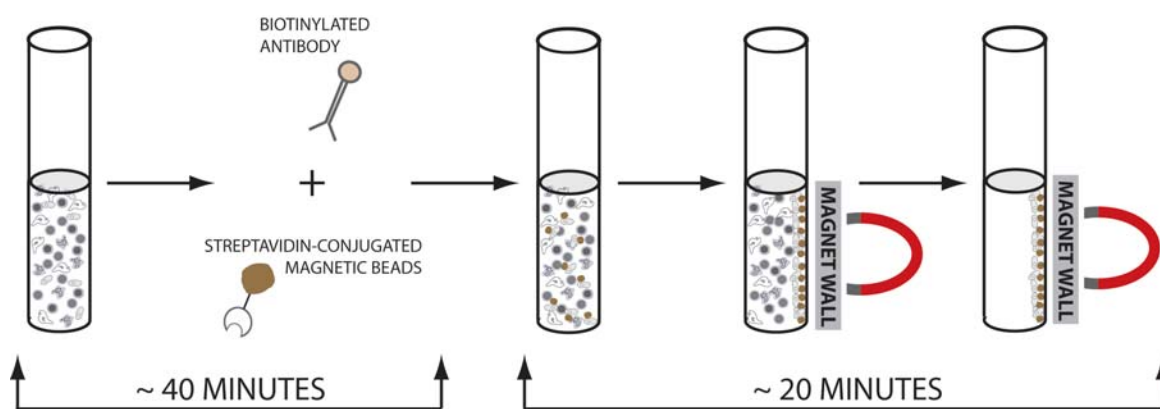
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INTRODUCTION

Mouse E-Cadherin, also known as Cadherin-1 and CD324, is a type I transmembrane protein belonging to the Cadherin family of calcium-dependent cell adhesion molecules. Cadherins preferentially interact with themselves in a homophilic manner in connecting cells, directly stimulating differentiation into certain types of tissues (1). E-cadherin is expressed in epithelial and embryonic stem (ES) cells (1, 2). It is expressed during early mouse embryonic development in all cell types, and it becomes inactivated in mesodermal cells at the gastrulation stage (1). The differentiation of mouse embryonic stem cells is associated with an E- to N-Cadherin switch, typically known as the “Cadherin switch” (3). The Cadherin switch is part of the “epithelial-to-mesenchymal” transition (EMT), a process in which epithelial cells lose their characteristic polarity, disassemble cell-cell junctions, and become more migratory. EMT is proposed to occur not only in early developmental processes but also during tumor metastasis (3).

PRINCIPLE OF THE TEST

Cell isolation is done by positive selection in a test tube by tagging the cells of interest with a biotinylated antibody followed by the addition of Streptavidin-conjugated magnetic particles (MagCollect™ Streptavidin Ferrofluid or equivalent). The tube with the cell suspension is then placed in a 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 first removed by aspiration while the tube remains in the magnet. The tube containing the magnetically selected (desired) cells is then removed from the magnet, and the cells are resuspended in PlusCollect Buffer or tissue culture media. To detect the presence of positively-selected cells or to assess the efficiency of enrichment, selected cells may be stained with the PE-conjugated antibody provided.



PlusCollect kits work with any single-cell suspension preparation. Cell suspensions can be stained by traditional methods or by following the instructions outlined on page 6.

INTENDED USE

The Mouse E-Cadherin⁺ PlusCelect Kit is designed to directly isolate epithelial, stem, or other E-Cadherin⁺ cells via a positive selection principle. The resulting cell preparation is highly enriched for E-Cadherin⁺ cells. Purity of recovered E-Cadherin⁺ cells typically ranges between 75 - 90%.

MATERIALS PROVIDED

Mouse E-Cadherin Selection Antibody (Part 965617) - 625 μ L of biotinylated goat anti-mouse E-Cadherin antibody.

Mouse E-Cadherin Detection Antibody (Part 965618) - 250 μ L of PE-conjugated rat anti-mouse E-Cadherin antibody.

10X PlusCelect Buffer (Part 895921) - 50 mL of a proprietary formulation.

STORAGE

Reagents are stable for 12 months from the date of receipt when stored **in the dark at 2 - 8° C. DO NOT FREEZE.**

OTHER MATERIALS REQUIRED

- MagCelect Streptavidin Ferrofluid* (R&D Systems, Catalog # MAG999 or equivalent)
- MagCelect Magnet* (R&D Systems, Catalog # MAG997 or equivalent)
- 12 x 75 mm (5 mL) or 17 x 100 mm (15 mL) polystyrene round bottom tubes (Falcon, Catalog # 352008, 352006, or equivalent)
- 15 mL conical centrifuge tubes (Corning Costar, Catalog # 3375 or equivalent)
- Sterile Pasteur pipettes or transfer pipettes (Fisher Scientific, Catalog # 13-711-9B or equivalent)
- Phosphate-Buffered Saline (PBS)
- Benchtop centrifuge
- 2 - 8° C refrigerator
- Deionized water

**While optimized for R&D Systems' reagents and supplies, the PlusCelect kit was tested in combination with EasySep™ (StemCell Technologies), iMag™ (Becton Dickinson), and Streptavidin Microbeads™ (Miltenyi Biotec) magnetic beads and magnets. When using other supplier's magnetic selection systems, the protocol may need to be adapted according to the supplier's directions for optimal performance.*

Please note that PlusCelect kits only work with streptavidin-based magnetic beads.

PRECAUTION

The PE-conjugated detection antibody provided in this kit contains 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 PlusCollect Buffer - Prepare 25 mL of 1X PlusCollect Buffer for each sample to be processed by mixing 2.5 mL of 10X PlusCollect Buffer with 22.5 mL of sterile deionized or distilled water. The 1X PlusCollect Buffer should be kept on ice or refrigerated.

CELL PREPARATION

PlusCollect kits work with any single-cell suspension preparation. Cell suspensions can be prepared by traditional methods.

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.

Note: This procedure describes the processing of less than 1×10^7 total cells using 5 mL tubes. Please refer to the Technical Hints section for processing other cell numbers.

1. Prepare a single-cell suspension by traditional methods. Cells must be suspended in cold 1X PlusCollect Buffer at a density of approximately 1×10^7 cells/mL prior to beginning the procedure.
2. Place 1×10^7 cells (1.0 mL) into a 15 mL conical centrifuge tube.
Note: If necessary, block Fc receptor sites by adding 100 μ g of mouse IgG in a volume not exceeding 100 μ L. Incubate for 10 minutes in a refrigerator at 2 - 8° C.
3. Add 25 μ L of Mouse E-Cadherin Selection 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 9 mL of cold 1X PlusCollect 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 PlusCollect Buffer into the tube.
4. Add 50 μ L of MagCollect Streptavidin Ferrofluid magnetic beads (or equivalent) 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 MagCollect, 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 9 mL of cold 1X PlusCollect Buffer and centrifuge at 300 x g for 8 minutes. **Completely** remove the supernatant and resuspend the cell pellet by gently pipetting 2 mL of cold 1X PlusCollect Buffer into the tube. Transfer the cell suspension to a 5 mL reaction tube.
6. Place the reaction tube in the MagCollect magnet (or equivalent) that has been positioned horizontally to accommodate 5 mL tubes and incubate for 6 minutes at room temperature (18 - 25° C). Magnetically tagged (**desired**) cells will migrate toward the magnet, leaving the untagged (unwanted) cells in suspension in the supernatant.
7. While the tube is still in the magnet, remove unwanted cells by carefully aspirating all of the reaction supernatant with a sterile Pasteur pipette or transfer pipette. Discard the supernatant.
8. Remove the tube containing the magnetically selected cells from the magnet and resuspend cells by adding 2.0 mL of cold 1X PlusCollect Buffer.
9. To complete the cell isolation procedure, repeat steps 6 - 7 at least once more with the resuspended cell fraction.
Note: If purity of the cell selection is critical, repeat this step one or two more times.
10. Remove the tube containing the magnetically selected cells from the magnet and resuspend the cells by adding 1 - 2 mL of 1X PlusCollect Buffer or tissue culture media. This final magnetically isolated fraction contains the desired isolated E-Cadherin⁺ cells. The cells are now ready to be counted, stained, and used in other downstream applications.
11. If the isolated E-Cadherin⁺ cells are to be visualized by flow cytometry, resuspend the appropriate amount of selected cells in 100 μ L of 1X PlusCollect Buffer and stain them using 10 μ L of Mouse E-Cadherin Detection Antibody. Proceed as usual with standard staining procedures.

CELL STAINING PROCEDURE

1. Add 100 μL of the positively selected cells to a 5 mL tube.
2. Add 10 μL of Mouse E-Cadherin Detection Antibody.
3. Incubate for 30 - 45 minutes at 2 - 8° C.
4. Following this incubation, remove the unreacted antibody by washing the cells twice in 2 mL of 1X PlusCelect Buffer or PBS.
5. Resuspend the cells in 200 - 400 μL of 1X PlusCelect Buffer or PBS for final flow cytometric analysis.

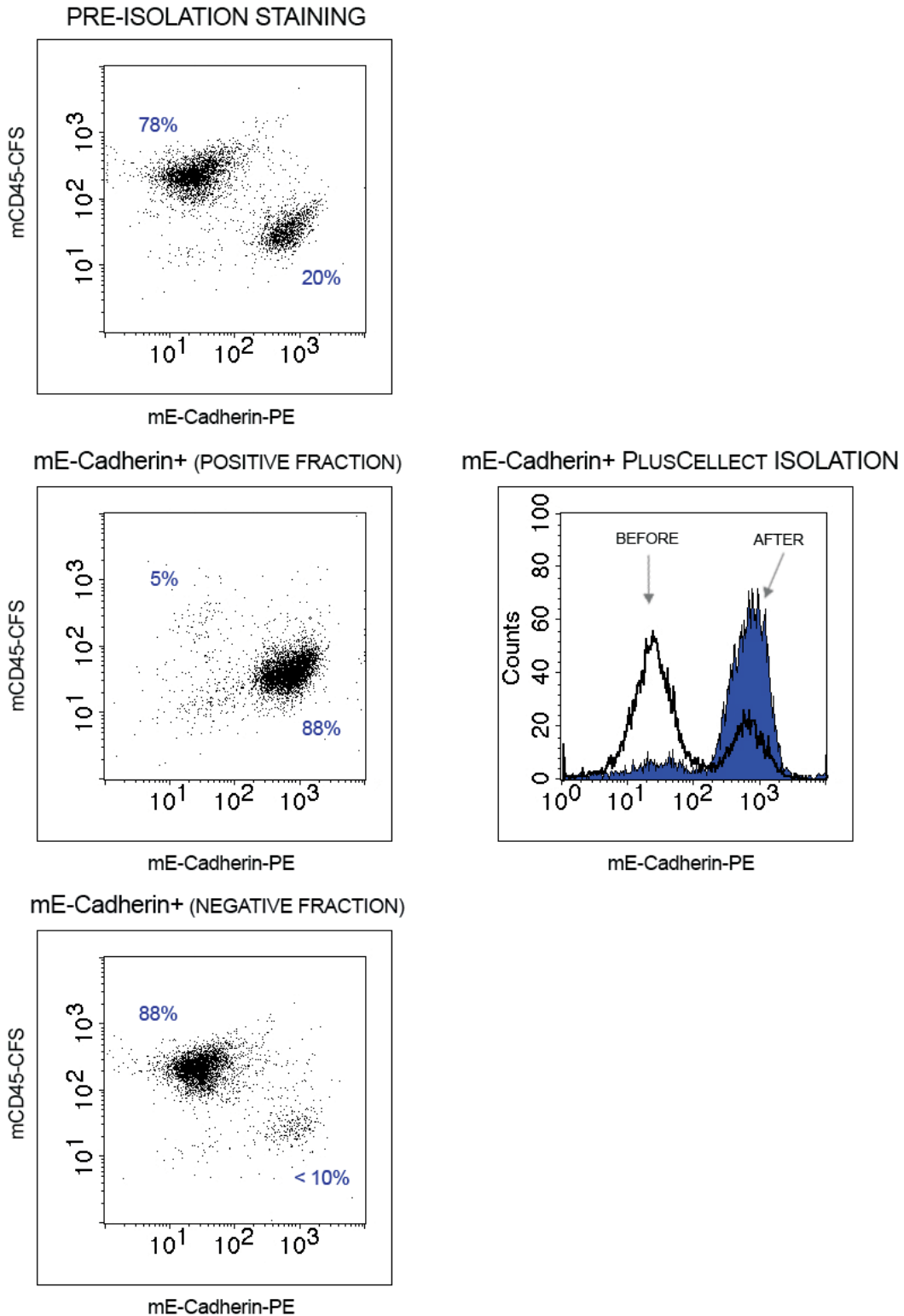
TECHNICAL HINTS

- 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 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 lowered cell purity and yield.
- 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 1×10^7 cells/mL.
 - If blocking, add 100 μg of mouse IgG per 10^7 cells being processed.
 - Add 5 μL of the biotinylated antibody per additional 10^7 cells being processed.
 - Add 10 μL of Streptavidin Ferrofluid per additional 10^7 cells being processed **to a maximum of 125 μL .**
- When processing 2×10^8 cells or fewer, use the 12 x 75 mm (5 mL) tubes with the MagCelect magnet horizontally positioned to accommodate up to six 5 mL tubes. **Do not process more than 2×10^8 cells in each 5 mL tube, and do not exceed a total reaction volume of 3 mL in each tube.** A reaction volume of 3 mL is recommended when processing 2×10^8 cells. A reaction volume of 1 mL is recommended when processing 5×10^7 or fewer cells. **Reaction volume adjustments must be made using 1X PlusCelect Buffer** just prior to the magnetic separation step.
- When processing greater than 2×10^8 cells, use 17 x 100 mm (15 mL) tubes with the MagCelect magnet vertically positioned to accommodate up to two 15 mL tubes. **Do not process more than 6×10^8 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 2×10^8 cells processed. Increase the magnetic incubation time (step 6 of the Cell Selection Procedure) to 8 minutes. Reaction volume adjustments must be made using 1X PlusCelect Buffer just prior to the magnetic separation step.

TYPICAL DATA

For the isolation of mouse stem cells using the PlusCelect Mouse E-Cadherin⁺ Cancer/Stem Cells Kit, murine splenocytes were spiked with mouse D3 embryonic stem cells. Samples were stained with Mouse E-Cadherin Detection Antibody and anti-mouse CD45-CFS (R&D Systems, Catalog # FAB114F) to assess isolation efficiency.

PlusCelect Isolation of Mouse E-Cadherin⁺ Stem Cells



REFERENCES

1. Larue, L. *et al.* (1996) *Development* **122**:3185.
2. Spencer, H.L. *et al.* (2007) *Mol. Biol. Cell* **18**:2838.
3. Wheelock, M.J. *et al.* (2008) *J. Cell. Sci.* **121**:727.

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Possible Solution
Low yield of positively selected cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris are very sticky and will preferentially bind to the magnetic beads. If the cell preparation contains too many dead cells or debris, your magnetically selected fraction will be contaminated. Make sure the 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 both cell selection and detection. Make sure the cell preparation is 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 positive cell targets	If the cell fraction to be isolated contains less than ~50,000 cells, and it represents less than 1% of the total cell preparation, 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 (R&D Systems has MagCollect or PlusCollect kits for negative selection of undesirable cells).
	Poor magnetic selection	When removing unwanted cells in step 7 of 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 an 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 positively selected cells	Poor cell preparation, too many dead cells, or cell debris	Dead cells and cell debris are very sticky and will preferentially bind to the magnetic beads. If the cell preparation contains too many dead cells or debris, your magnetically selected fraction will be contaminated. Make sure the 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.
	Few positive cell targets	If the cell fraction to be isolated contains less than ~50,000 cells, and it represents less than 1% of the total cell preparation, 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 (R&D Systems has MagCollect or PlusCollect kits for negative selection of undesirable cells).

Problem	Possible Cause	Possible Solution
Low purity of positively selected cells	Positive cells not washed well	Extra washes can be performed subjecting the cells to an extra step of magnetic migration (steps 7 and 8 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 recovered cells	Insufficient positive cell targets	If the cell fraction to be isolated contains very few target cells or the positive cells represent a very small fraction of the total cell preparation, recovery could be significantly reduced and positive cells could be lost in the procedure. For a better yield, increase the number of cells in the starting population and/or consider performing a pre-enrichment step by removing undesirable cells (R&D Systems has MagCollect or PlusCollect kits for negative selection of undesirable cells).
	Cells lost in the process	If the cell fraction to be isolated contains very few target cells, there will be a low number of positively selected cells attached to the magnet, which may easily be lost during washes in steps 8 and 9 of the Cell Selection Procedure. If dealing with very few expected target cells, purity may need to be sacrificed for the sake of recovery. Consider skipping steps 8 and 9 to avoid losing target cells. Additionally, make sure that cells are not lost during magnetic selection in step 7 of the Cell Selection Procedure. Please refer to Poor Magnetic Selection on the first page of the Troubleshooting Guide for more details.
Biologically inactive recovered cells	The antibody or magnetic bead surface could interfere with biological processes	If the attached antibody/magnetic bead complex on the cell surface could be a problem, culture the cells in the appropriate media for a few hours or overnight to solve the issue. When in culture, and particularly while remaking the cell membrane during cell division, the antibody/magnetic bead complexes on the cell surface should be lost. Make sure the cells are cultured long enough to allow this process to occur.
Unusual staining of positive cells in flow cytometry	The antibody or magnetic bead surface could affect flow cytometric analysis	No correction needed. There are two primary reasons that this would occur. Magnetic beads on the cell surface could slightly change the cells' scattering characteristics, and the presence of the selection antibody/magnetic bead complex on the cell surface might slightly quench the fluorescence of the conjugated detection antibody. In both cases, the shift should be minor.

NOTES

EasySep™ is a trademark of StemCell Technologies
iMag™ is a trademark of Becton Dickinson
Microbeads™ is a trademark of Miltenyi Biotec