

# PlusCollect™

## Human Nectin-4 Kit

Catalog Number PLS2659

**For the isolation of Nectin-4 expressing cells via a positive selection principle.**

**This kit contains sufficient reagents for 25 tests (up to  $1 \times 10^9$  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.**

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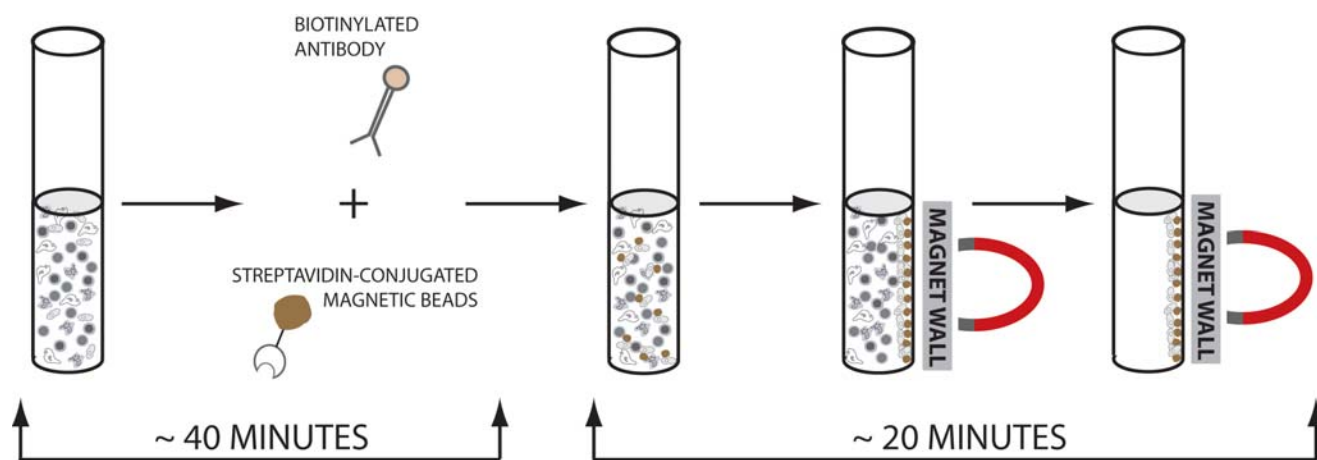
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## PRINCIPLE OF THE TEST

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 also offers an alternate method for the enrichment of positively selected cells by depletion of unwanted cells via negative selection using the CD45 PlusCollect Kit (R&D Systems, Catalog # PLS1430) before positive selection with the specific PlusCollect kit. This is an attractive option when isolating a small population of cells and/or a greater purity of the selected cells is of particular importance.

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

## INTENDED USE

The Human Nectin-4 PlusCollect Kit is designed to isolate cells via a positive selection principle. The resulting cell preparation is highly enriched for Nectin-4<sup>+</sup> cells. Purity of recovered Nectin-4<sup>+</sup> cells typically ranges between 80 - 96%.

## STORAGE

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

## MATERIALS PROVIDED

**Human Nectin-4 Selection Antibody** (Part 965606) - 625  $\mu$ L of biotinylated goat anti-human Nectin-4 antibody.

**Human Nectin-4 Detection Antibody** (Part 965607) - 250  $\mu$ L (25 tests) of PE-conjugated mouse anti-human Nectin-4 antibody.

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

## OTHER MATERIALS REQUIRED

- MagCollect Streptavidin Ferrofluid\* (R&D Systems, Catalog # MAG999 or equivalent)
- MagCollect 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)
- Human IgG for Fc receptor blocking (if applicable, see the Technical Hints section for additional information; R&D Systems, Catalog # 1-001-A or equivalent)
- Benchtop centrifuge
- 2 - 8° C refrigerator

## 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.

*\*While optimized for R&D Systems' reagents and supplies, the PlusCollect 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 PlusCollect kits only work with streptavidin-based magnetic beads.***

## 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  $1 \times 10^7$  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 or by following the instructions outlined in the Cell Preparation section. Cells must be suspended in cold 1X PlusCollect Buffer at a density of  $1 \times 10^7$  cells/mL prior to beginning the procedure.
2. Place  $1 \times 10^7$  cells (1.0 mL) into a 15 mL conical centrifuge tube.  
**Note:** If necessary, block Fc receptor sites by adding 100  $\mu$ g of human IgG in a volume not exceeding 100  $\mu$ L. Incubate for 10 minutes in a refrigerator at 2 - 8° C.
3. Add 25  $\mu$ L of Human Nectin-4 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  $\mu$ 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 Nectin-4<sup>+</sup> cells. The cells are now ready to be counted, stained, and used in other downstream applications.
11. If the isolated Nectin-4<sup>+</sup> cells are to be visualized by flow cytometry, resuspend the appropriate amount of selected cells in 100  $\mu$ L of 1X PlusCollect Buffer and stain them using 10  $\mu$ L of Human Nectin-4 Detection Antibody. Proceed as usual with standard staining procedures.

## TECHNICAL HINTS

- Fc receptor blocking (step 2 of the Cell Selection Procedure) can be enhanced by also adding 25  $\mu\text{L}$  of autologous plasma per  $10^7$  cells being processed.
- 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  $1 \times 10^7$  cells/mL.
  - If blocking, add 100  $\mu\text{g}$  of human IgG per  $10^7$  cells being processed.
  - Add 5  $\mu\text{L}$  of the biotinylated antibody per additional  $10^7$  cells being processed.
  - Add 10  $\mu\text{L}$  of Streptavidin Ferrofluid per additional  $10^7$  cells being processed **to a maximum of 125  $\mu\text{L}$ .**
- When processing  $2 \times 10^8$  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  $2 \times 10^8$  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  $2 \times 10^8$  cells. A reaction volume of 1 mL is recommended when processing  $5 \times 10^7$  or fewer cells. **Reaction volume adjustments must be made using 1X PlusCelect Buffer** just prior to the magnetic separation step.
- When processing greater than  $2 \times 10^8$  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  $6 \times 10^8$  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  $2 \times 10^8$  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.

## CELL PREPARATION

PlusCelect kits work with any 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 the cells two times by centrifuging for 10 minutes at 200 x g with excess PBS to remove any residual separation media.
3. Optional red cell lyse (recommended).
  - a. After the second washing step, disrupt the cell pellet by “racking” the tube. Resuspend the cells 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 10 - 12 minutes at room temperature and fill the tube with 1X Wash Buffer from the Lysing Kit. Centrifuge for 10 minutes at 200 x g.
4. After the second washing step (or red cell lysis), disrupt the cell pellet by “racking” the tube. Resuspend the cells in a small volume of 1X PlusCelect Buffer, and perform a cell count. Adjust the cell concentration to  $1 \times 10^7$  cells per mL with cold 1X PlusCelect Buffer.
5. Continue with the Cell Selection Procedure (page 4).

## 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  $\mu$ L of the positively selected cells to a 5 mL tube.
2. Add 10  $\mu$ L of Human Nectin-4 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 PlusCelect Buffer or PBS.
5. Resuspend the cells in 200 - 400  $\mu$ L of PlusCelect Buffer or PBS for final flow cytometric analysis.

*EasySep™ is a trademark of StemCell Technologies*

*iMag™ is a trademark of Becton Dickinson*

*Microbeads™ is a trademark of Miltenyi Biotec*

## TYPICAL DATA

Isolation of Nectin-4<sup>+</sup> cells from leukocytes using this Nectin-4 PlusCollect kit. Peripheral blood leukocytes ( $1 \times 10^7$  cells) were prepared as described in the Cell Preparation section and spiked with breast carcinoma MCF-7 cells ( $\sim 5 \times 10^5$  cells). Samples were stained with Human Nectin-4 Detection Antibody and human CD45-APC.

### PlusCollect™ Isolation of Nectin-4+ cells from MCF-7 spiked peripheral blood leukocytes

