PlusCellect™

Human Endoglin/CD105 Kit

Catalog Number PLS1097

For the isolation of Endoglin expressing cells via a positive selection principle.

This kit contains sufficient reagents to process up to 1 x 10^9 total cells.

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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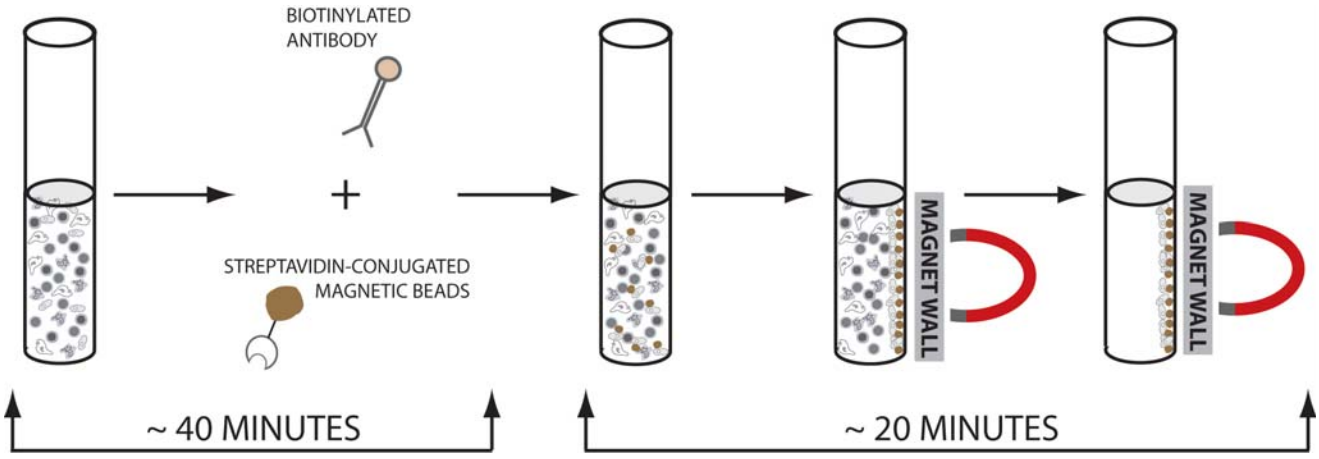
PRINCIPLE OF THE TEST

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 (MagCellect™ 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 PlusCellect 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.



PlusCellect also offers an alternate method for the enrichment of positively selected cells by depletion of unwanted cells via negative selection using the CD45 PlusCellect Kit (R&D Systems, Catalog # PLS1430) before positive selection with the specific PlusCellect 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.

PlusCellect 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 Endoglin/CD105 PlusCellect Kit is designed to isolate cells via a positive selection principle. The resulting cell preparation is highly enriched for Endoglin$^+$ cells. Purity of recovered Endoglin$^+$ cells typically ranges between 60 - 85%.

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 Endoglin/CD105 Selection Antibody (Part 965778) - 625 µL of biotinylated mouse anti-human Endoglin antibody.

Human Endoglin/CD105 Detection Antibody (Part 965779) - 250 µL (25 tests) of PE-conjugated mouse anti-human Endoglin antibody.

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

OTHER MATERIALS REQUIRED

- MagCellect Streptavidin Ferrofluid* (R&D Systems, Catalog # MAG999 or equivalent)
- MagCellect 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 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 PlusCellect Buffer - Prepare 25 mL of 1X PlusCellect Buffer for each sample to be processed by mixing 2.5 mL of 10X PlusCellect Buffer with 22.5 mL of sterile deionized or distilled water. The 1X PlusCellect Buffer should be kept on ice or refrigerated.

**While optimized for R&D Systems' reagents and supplies, the PlusCellect 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 PlusCellect 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 x 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 of cells by traditional methods or by following the instructions outlined in the Cell Preparation section. Cells must be suspended in cold 1X PlusCellect Buffer at a density of 1 x 10^7 cells/mL prior to beginning the procedure.
2. Place 1 x 10^7 cells (1.0 mL) into a 15 mL conical centrifuge tube.

   Note: *If necessary, block Fc receptor sites by adding 100 μg of human IgG in a volume not exceeding 100 μL. Incubate for 10 minutes in a refrigerator at 2 - 8° C.*
3. Add 25 μL of Human Endoglin Selection Antibody. Gently mix the cell/antibody suspension, avoiding bubble formation, and incubate for 15 minutes at 2 - 8° C in a refrigerator.
4. Add 50 μL of MagCellect 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 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 9 mL of cold 1X PlusCellect 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 PlusCellect Buffer into the tube. Transfer the cell suspension to a 5 mL reaction 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 (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 PlusCellect 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 PlusCellect Buffer or tissue culture media. This final magnetically isolated fraction contains the desired isolated Endoglin$^+$ cells. The cells are now ready to be counted, stained, and used in other downstream applications.
11. If the isolated Endoglin$^+$ cells are to be visualized by flow cytometry, resuspend the appropriate amount of selected cells in 100 μL of 1X PlusCellect Buffer and stain them using 10 μL of Human Endoglin 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 μL 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 x 10^7 cells/mL.
  - If blocking, add 100 μg of human IgG per 10^7 cells being processed.
  - Add 5 μL of the biotinylated antibody per 10^7 cells being processed.
  - Add 10 μL of Streptavidin Ferrofluid per 10^7 cells being processed **to a maximum of 125 μL.**
- 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 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. **Reaction volume adjustments must be made using 1X PlusCellect Buffer** just prior to the magnetic separation step.
- When processing greater than 2 x 10^8 cells, use 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. Increase the magnetic incubation time (step 6 of the Cell Selection Procedure) to 8 minutes. Reaction volume adjustments must be made using 1X PlusCellect Buffer just prior to the magnetic separation step.

CELL PREPARATION

PlusCellect 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 PlusCellect Buffer, and perform a cell count. Adjust the cell concentration to 1 x 10^7 cells per mL with cold 1X PlusCellect 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 µL of the positively selected cells to a 5 mL tube.
2. Add 10 µL of Human Endoglin 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 PlusCellect Buffer or PBS.
5. Resuspend the cells in 200 - 400 µL of 1X PlusCellect Buffer or PBS for final flow cytometric analysis.

TYPICAL DATA

Isolation of Endoglin/CD105^{+} cells from whole blood using the Human Endoglin/CD105 PlusCellect kit. White blood cells were spiked with human umbilical vein endothelial cells (HUVECs). Cells were stained with the Human Endoglin/CD105 Detection Antibody and with anti-CD45-APC antibody. HUVECs are Endoglin/CD105^{+} and CD45^{-}. A fraction of the monocytes are Endoglin/CD105^{+} and CD45^{+}. That is why they are positively selected with the kit (*). Dead cells and debris were excluded from the analysis.