

PlusCollect™

Human SSEA-4⁺ Pluripotent Stem Cells Isolation Kit

Catalog Number PLS1435

For the isolation or depletion of SSEA-4 expressing cells.

This kit contains sufficient reagents for 25 tests (up to 1×10^9 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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MANUFACTURED AND DISTRIBUTED BY:

R&D Systems, Inc.
614 McKinley Place NE
Minneapolis, MN 55413
United States of America

TELEPHONE: (800) 343-7475
(612) 379-2956
FAX: (612) 656-4400
E-MAIL: info@RnDSystems.com

DISTRIBUTED BY:

R&D Systems Europe, Ltd.
19 Barton Lane
Abingdon Science Park
Abingdon, OX14 3NB
United Kingdom

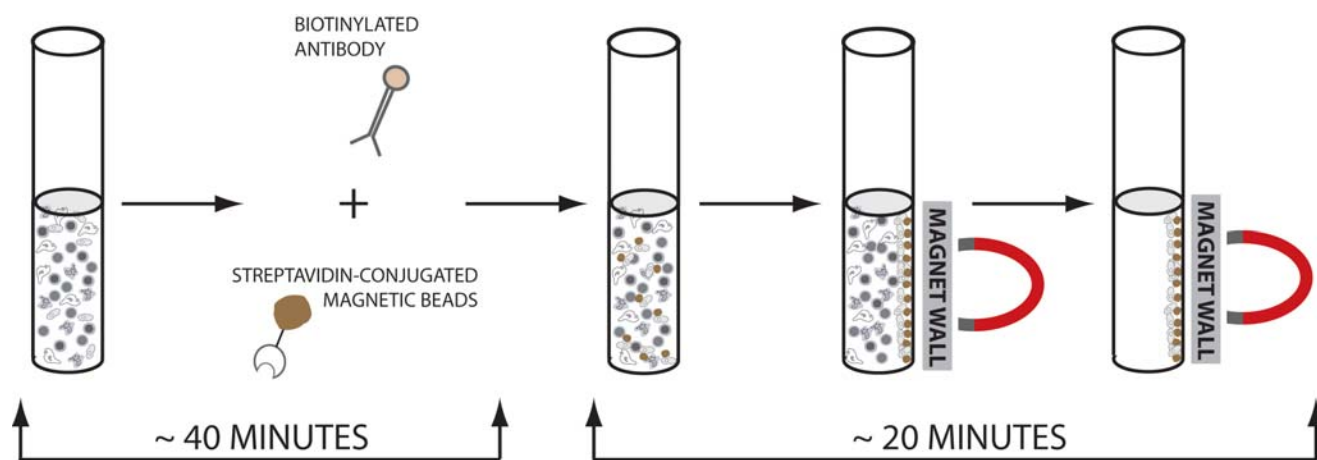
TELEPHONE: +44 (0)1235 529449
FAX: +44 (0)1235 533420
E-MAIL: info@RnDSystems.co.uk

R&D Systems China Co. Ltd.
24A1 Hua Min Empire Plaza
726 West Yan An Road
Shanghai PRC 200050

TELEPHONE: +86 (21) 52380373
FAX: +86 (21) 52371001
E-MAIL: info@RnDSystemsChina.com.cn

PRINCIPLE OF THE TEST

Cell isolation is done by magnetic selection in a test tube by tagging the cells of interest with a biotinylated antibody followed by the addition of Streptavidin-conjugated magnetic particles (MagCelect™ Streptavidin Ferrofluid or equivalent). The tube with the cell suspension is then placed in a magnet. Magnetically tagged cells (SSEA-4⁺) will migrate toward the tube wall on the magnet side, leaving the untagged cells (SSEA-4⁻) in suspension. SSEA-4⁻ cells are first removed by aspiration while the tube remains in the magnet. The tube containing the magnetically selected SSEA-4⁺ cells is then removed from the magnet, and the cells are resuspended in PlusCelect Buffer or tissue culture media. To assess the efficiency of enrichment or depletion, obtained cells may be stained with the PE-conjugated antibody provided.



PlusCelect 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 SSEA-4⁺ Pluripotent Stem Cell PlusCelect Kit is designed to isolate human pluripotent stem cells via a positive selection principle. The resulting cell preparation is highly enriched for SSEA-4⁺ cells. Purity of recovered SSEA-4⁺ cells typically ranges between 85 - 95%.

This kit can also be used for the depletion of SSEA-4-expressing pluripotent cells via the same principle. Purity of SSEA-4-depleted cells typically ranges between 92 - 99%.

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 SSEA-4 Selection Antibody (Part 965718) - 625 μ L of biotinylated mouse anti-human SSEA-4 antibody.

Human SSEA-4 Detection Antibody (Part 965719) - 250 μ L (25 tests) of PE-conjugated mouse anti-human SSEA-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)
- Sterile Pasteur pipettes or transfer pipettes
- Benchtop centrifuge
- 2 - 8° C refrigerator
- Deionized or distilled water

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 15 mL of 1X PlusCollect Buffer for each sample to be processed by mixing 1.5 mL of 10X PlusCollect Buffer with 13.5 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, PlusCollect kits were 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.

EasySep™ is a trademark of StemCell Technologies

iMag™ is a trademark of Becton Dickinson

Microbeads™ is a trademark of Miltenyi Biotec

POSITIVE SELECTION OF SSEA-4⁺ CELLS

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 5×10^6 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 SSEA-4⁺ target cells. Cells must be suspended in cold 1X PlusCelect Buffer at a density of 1×10^7 cells/mL prior to beginning the procedure.
2. Place 5×10^6 cells (0.5 mL) into a 5 mL round bottom tube.
3. Add 10 μ L of Human SSEA-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 3 mL of cold 1X PlusCelect 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 PlusCelect Buffer into the tube.
4. Add 50 μ L of MagCelect 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 MagCelect, 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 PlusCelect 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 PlusCelect Buffer into the tube.
6. Place the reaction tube in the MagCelect 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 cells will migrate toward the magnet, leaving the untagged cells in suspension in the supernatant.
7. While the tube is **firmly held** in the magnet, remove unwanted cells by **slowly and 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 PlusCelect 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 0.5 - 1 mL of 1X PlusCelect Buffer or tissue culture media. This final magnetically isolated fraction contains the desired isolated SSEA-4⁺ cells. The cells are now ready to be counted, stained, and used in other downstream applications.
11. If the isolated SSEA-4⁺ cells are to be visualized by flow cytometry, resuspend the appropriate amount of selected cells in 100 μ L of 1X PlusCelect Buffer and stain them using 10 μ L of Human SSEA-4 Detection Antibody. Proceed as usual with standard staining procedures.

REMOVAL OF SSEA-4⁺ CELLS BY NEGATIVE SELECTION

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 5×10^6 total cells using 5 mL tubes. Please refer to the Technical Hints section for processing other cell numbers.

1. Follow steps 1 - 6 of the Positive Selection of SSEA-4⁺ Cells section.
2. While the tube is **firmly held** in the magnet, obtain the wanted cells by **slowly and carefully** aspirating the supernatant with a sterile Pasteur pipette or transfer pipette, and place them in a new tube. Set the tube with the magnetically selected cells aside (unwanted positive fraction). They could be used as a control of the depletion efficacy.
3. To complete the cell isolation procedure, repeat step 2 at least once more with the obtained cell fraction.
4. If the depletion of SSEA-4⁺ cells is to be confirmed by flow cytometry, resuspend the appropriate amount of the negatively selected cells in 100 µL of 1X PlusCelect Buffer and stain them using 10 µL of Human SSEA-4 Detection Antibody. The positively selected fraction obtained in step 2 can be used as a control.

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, follow the recommendations in Table 1 or observe the following guidelines:
 - **Using less than 2.5×10^6 or more than 1×10^8 cells is not recommended.**
 - Keep the biotinylated antibody and ferrofluid incubation times the same.
 - Add 2 µL of the biotinylated antibody per 1×10^6 cells being processed, to a **maximum of 75 µL.**
 - Add 50 µL of Streptavidin Ferrofluid per 1×10^7 cells being processed. A **minimum of 50 µL and a maximum of 125 µL** of ferrofluid should be used.
- A reaction volume of 0.5 mL is recommended when processing less than 5×10^7 cells. A reaction volume of 1 mL is recommended when processing 5×10^7 or more cells. **Reaction volume adjustments must be made using 1X PlusCelect Buffer** just prior to the magnetic separation step.

Table 1: Recommended quantities for steps 3 - 4 of the Cell Selection Procedure

Number of Cells in Starting Preparation	5×10^6 (*)	1×10^7	1×10^8
Reaction Volume	0.5 mL	0.5 mL	1 mL
PlusCelect SSEA-4 Biotinylated Antibody	10 µL	20 µL	50 µL
Streptavidin Ferrofluid	50 µL	50 µL	100 µL

(*) Recommended Setup

CELL PREPARATION

PlusCollect kits work with any single-cell suspension preparation.

CELL STAINING PROCEDURE

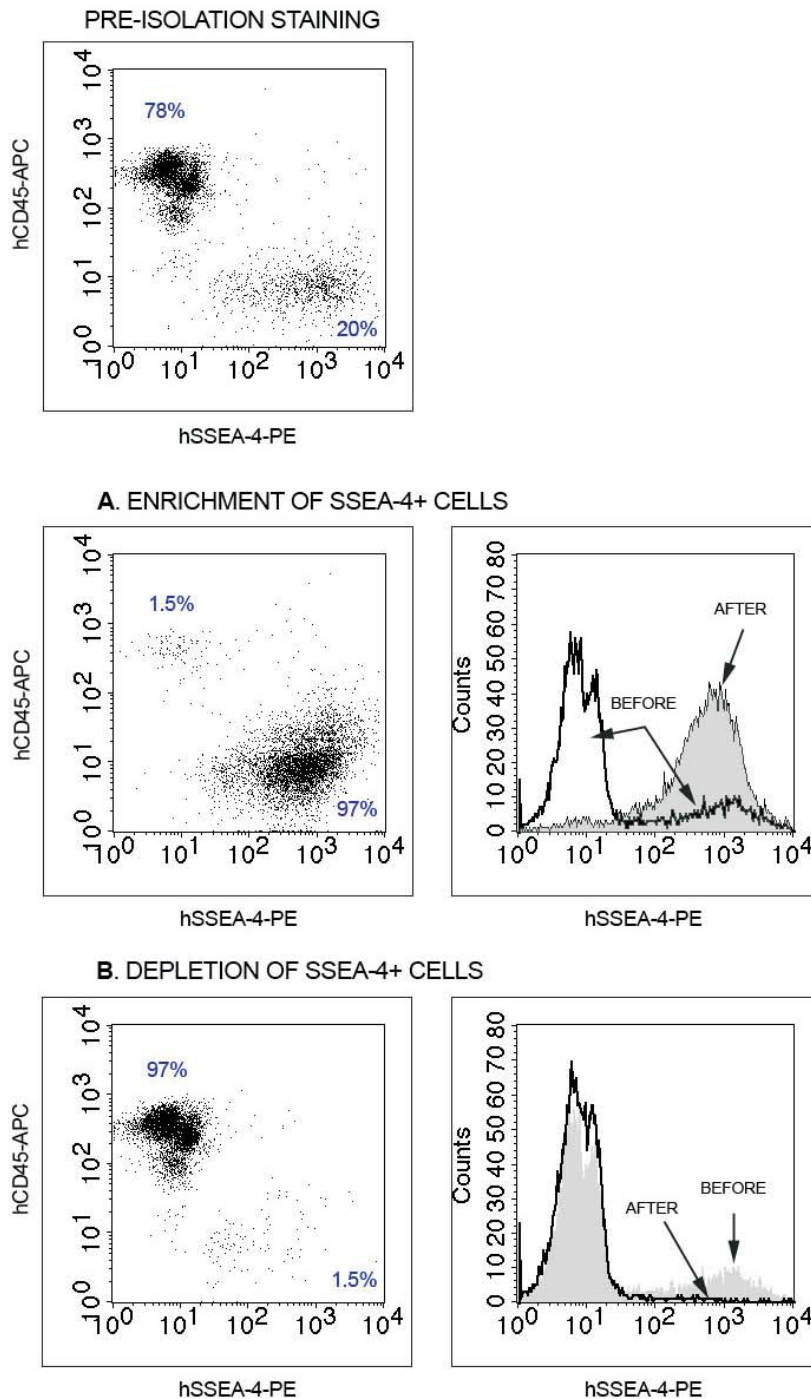
After successfully selecting the desired cell population, cells can be stained by traditional methods or by following the instructions below.

1. Add 100 μ L of the positively selected cells to a 5 mL tube.
2. Add 10 μ L of Human SSEA-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 1X PlusCollect Buffer or PBS.
5. Resuspend the cells in 200 - 400 μ L of 1X PlusCollect Buffer for final flow cytometric analysis.

TYPICAL DATA

PBMCs were spiked with human embryonic stem cells (BG01V). Enrichment (Panel A) or depletion (Panel B) of BG01V cells is shown. Samples were stained with Human SSEA-4 Detection Antibody and with anti-human CD45-APC to assess the isolation efficiency.

PlusCollect™ Isolation of Human SSEA-4+ Pluripotent Stem Cells



BG01V cells are licensed from Novocell, Inc.

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