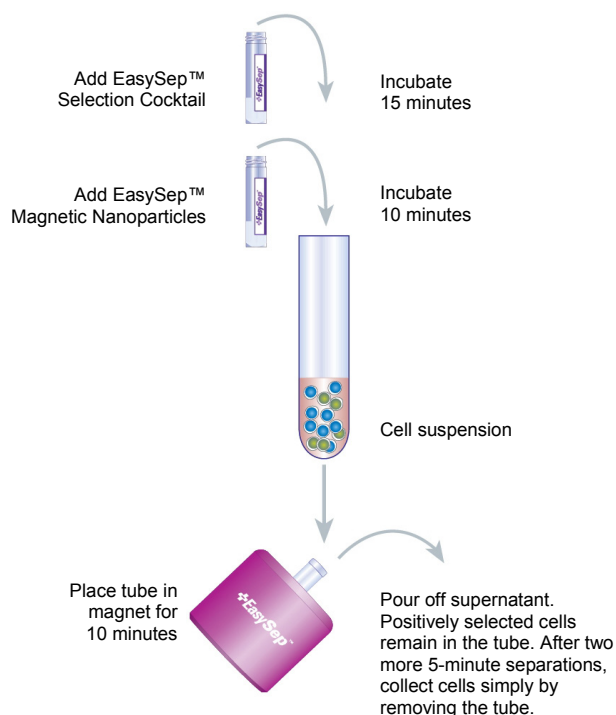




THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE PURPLE EASYSEP™ MAGNET (CATALOG #18000).

MANUAL EASYSEP™ PROTOCOL DIAGRAM



MANUAL EASYSEP™ PROTOCOL USING THE PURPLE EASYSEP™ MAGNET (CATALOG #18000)

This procedure is used for processing **100 - 500 µL** of sample (up to 5×10^7 cells).

1. Prepare cell suspension at a concentration of 1×10^8 cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the EasySep™ Magnet. For samples containing 10^7 cells or fewer, resuspend in 100 µL.
Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD Biosciences, Catalog #352058) are recommended.
2. Add the EasySep™ hESC/hiPSC SSEA-4 Positive Selection Cocktail at **100 µL/mL of cells** (e.g. for 100 µL of cells, add 10 µL of cocktail). Mix well and incubate at 2 - 8°C for **15 minutes**.
3. Mix the EasySep™ Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting up and down more than 5 times. Vortexing is not recommended.
4. Add the EasySep™ Magnetic Nanoparticles at **50 µL/mL of cells** (e.g. for 100 µL of cells, add 5 µL of magnetic particles). Mix well and incubate at 2 - 8°C for **10 minutes**.
5. Bring the cell suspension up to a total volume of **2.5 mL** by adding recommended medium. Mix the cells in the tube by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **10 minutes** at room temperature (15 - 25°C).
6. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled cells will remain inside the tube, held by the magnetic field of the EasySep™ Magnet. Leave the magnet and tube in inverted position for 2 - 3 seconds, then return to upright position. *Do not shake or blot off any drops that may remain hanging from the mouth of the tube.*
7. Remove the tube from the magnet and add **2.5 mL** recommended medium. Mix the cell suspension by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.
8. Repeat Steps 6 and 7, and then Step 6 once more, for a total of 1 x 10-minute and 2 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired cell culture medium (such as mTesR™1 or TeSR™2), if the cells are to be cultured further. Be sure to collect any cells that may be stuck to the sides of the tube. The positively selected cells are now ready for use.

Note: SSEA-4+ purity can be improved by performing an additional 1 x 5-minute separation in the magnet for a total of 1 x 10-minute plus 3 x 5-minute separations. Cell recovery may be decreased though with increased purity.

RELATED PRODUCTS

PRODUCT	CATALOG #
Anti-Oct 3/4 antibody	01550/01551
Anti-Mouse SSEA-1 Antibody, Clone MC-480	60060
Anti-Mouse SSEA-1 Antibody, Clone MC-480, Alexa Fluor® 488	60060AD
Anti-Mouse SSEA-1 Antibody, Clone MC-480, PE	60060PE
Anti-Mouse SSEA-3 Antibody, Clone MC-631	60061
Anti-Mouse SSEA-3 Antibody, Clone MC-631, Alexa Fluor® 488	600601AD
Anti-Mouse SSEA-3 Antibody, Clone MC-631, PE	600601PE
Anti-Human SSEA-4 Antibody, Clone MC-813-70	60062
Anti-Human SSEA-4 Antibody, Clone MC-813-70, Alexa Fluor® 488	60062AD
Anti-Human SSEA-4 Antibody, Clone MC-813-70, PE	60062PE
Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R	60064
Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R, Alexa Fluor® 488	60064AD
Anti-Human TRA-1-60 Antibody, Clone TRA-1-60R, PE	60064PE
Anti-Human TRA-1-81 Antibody, Clone TRA-1-81	60065
Anti-Human TRA-1-81 Antibody, Clone TRA-1-81, Alexa Fluor® 488	60065AD
Anti-Human TRA-1-81 Antibody, Clone TRA-1-81, PE	60065PE
ACCUTASE™	07920

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VERSION 2.2.0

DOCUMENT #29974

Components:

- EasySep™ hESC/hiPSC SSEA-4 Positive Selection Cocktail 1 mL
- EasySep™ Magnetic Nanoparticles Positive Selection 1 mL



POSITIVE SELECTION

REQUIRED EQUIPMENT:

EasySep™ Magnet (Catalog #18000).

PRODUCT DESCRIPTION AND APPLICATIONS:

The EasySep™ hESC/hiPSC SSEA-4 Positive Selection Kit is designed to isolate human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) expressing SSEA-4 by positive selection. Desired cells are targeted with an antibody complex recognizing SSEA-4 and dextran-coated magnetic particles. Labeled cells are separated using an EasySep™ magnet without the use of columns. Cells of interest remain in the tube while unwanted cells are poured off. The SSEA-4 antigen is expressed on undifferentiated hESCs and hiPSCs.

NOTES AND TIPS:**PREPARING THE CELL SUSPENSION**

ACCUTASE™ (Catalog #07920) is recommended for preparation of a single-cell suspension containing hESCs or hiPSCs. Rinse cells once with phosphate-buffered saline (PBS), add ACCUTASE™ to the cells (e.g. 1 - 2 mL ACCUTASE™ per well of a 6-well plate), and incubate at 37°C for 5 - 10 minutes. Use a pipette to break up any remaining clumps, and transfer the cell suspension to a new tube. Rinse the well with PBS and add this solution into the same tube. Centrifuge, and resuspend the cell suspension in PBS 2% FBS.

RECOMMENDED MEDIUM

The recommended medium is phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS; Catalog #07905), and 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free.

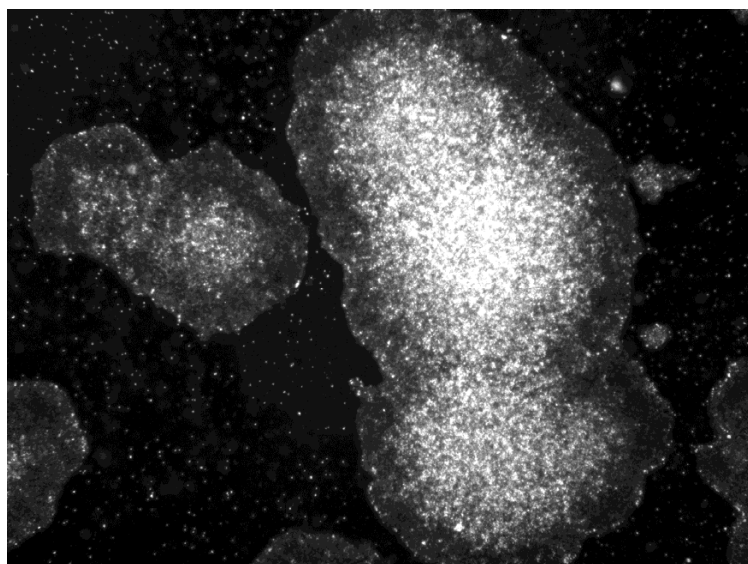
ASSESSING PURITY

The purity of the isolated hESCs or hiPSCs can be measured by flow cytometry after labeling with a fluorochrome-conjugated anti-SSEA-4 antibody, clone MC-813-70, as blocking by the EasySep™ SSEA-4 Positive Selection Cocktail is minimal.

Note: Flow cytometry analysis of the isolated cells may show slightly increased side scatter relative to the start sample.

SSEA4+ CELL DEPLETION

The EasySep™ hESC/hiPSC SSEA-4 Positive Selection Kit can also be used to deplete SSEA-4+ cells. Please refer to the depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf or contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com to receive a copy of the protocol.

CULTURE OF SELECTED CELLS

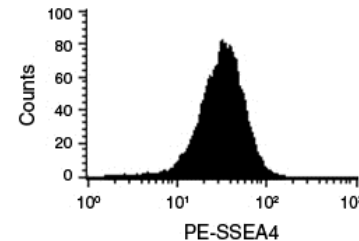
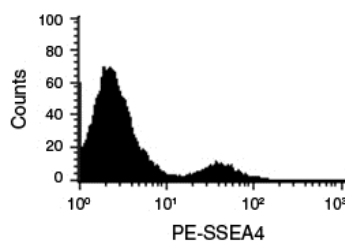
SSEA-4+ selected cells grown in mTeSR 1 (Catalog #05850/05870/05875/05857), on BD Matrigel™ (BD Biosciences, Catalog #354277) for 3 passages, utilizing dispase (Catalog #07923) and passaging procedures outlined in "Maintenance of hESCs and hiPSCs in mTeSR™1 and TeSR™2" Technical Manual (Document #29106).

Matrigel™ is a trademark of Becton, Dickinson and Company.

TYPICAL EASYSEP™ SSEA-4 POSITIVE SELECTION PROFILE:

Start 11.85% SSEA-4+ cells

Enriched 98.84% SSEA-4+ cells



Starting with a single cell suspension containing approximately 10% human ES cells or human iPS cells, the SSEA-4+ cell content of the enriched fraction typically ranges from 85 - 99%.

COMPONENT DESCRIPTIONS:**EASYSEP™ hESC/hiPSC SSEA-4 POSITIVE SELECTION COCKTAIL**

CODE # 18165C.1

This cocktail contains a combination of monoclonal antibodies purified from hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. The anti-SSEA-4 antibody was raised in mice and is of subclass IgG3, κ. This cocktail is supplied in phosphate buffered saline. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

EASYSEP™ MAGNETIC NANOPARTICLES POSITIVE SELECTION CODE #18150

A suspension of dextran-coated magnetic iron particles in water.

STABILITY AND STORAGE:**EASYSEP™ hESC/hiPSC SSEA-4 POSITIVE SELECTION COCKTAIL**

CODE # 18165C.1

EASYSEP™ MAGNETIC NANOPARTICLES POSITIVE SELECTION CODE #18150

Products stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze these products. These products may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

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