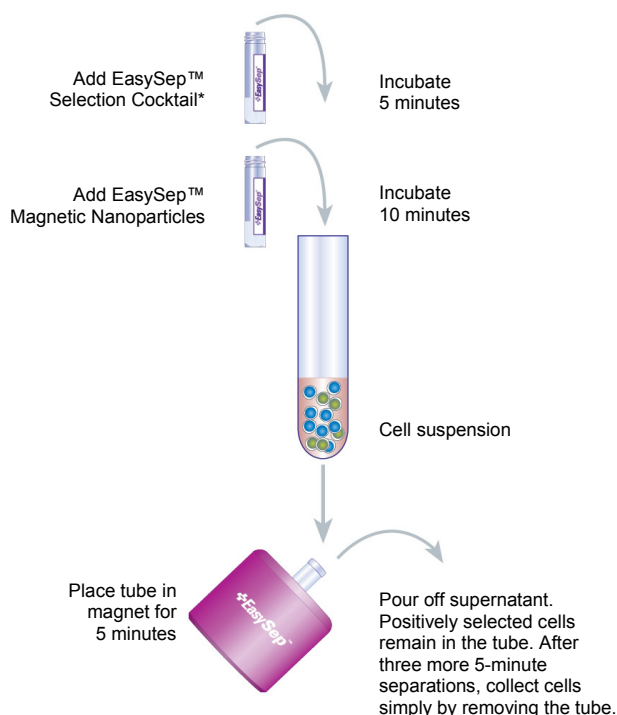




THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE PURPLE EASYSEP™ MAGNET (CATALOG #18000).

MANUAL EASYSEP™ PROTOCOL DIAGRAM



* The cocktail is prepared by mixing equal volumes of EasySep™ TRA-1-60 Positive Selection Component A and EasySep™ TRA-1-60 Positive Selection Component B, and incubating it at room temperature (15 - 25°C) for at least 5 minutes before use.

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. Prepare the required amount of EasySep™ TRA-1-60 Positive Selection Cocktail by mixing equal volumes of EasySep™ TRA-1-60 Positive Selection Component A and EasySep™ TRA-1-60 Positive Selection Component B in a tube. For each 100 µL of cells, prepare 5 µL of cocktail (i.e. mix 2.5 µL of Component A with 2.5 µL of Component B).
3. Incubate the EasySep™ TRA-1-60 Positive Selection Cocktail at room temperature (15 - 25°C) **for at least 5 minutes** before use. The prepared cocktail is stable for up to two weeks when stored at 2 - 8°C.
4. Add the EasySep™ TRA-1-60 Positive Selection Cocktail at **50 µL/mL of cells** (e.g. for 100 µL of cells, add 5 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. 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.
6. 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 room temperature (15 - 25°C) for **10 minutes**.
7. 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 **5 minutes**.
8. 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.
9. 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**.
10. Repeat Steps 8 and 9 two more times, and then Step 8 once more, for a total of four 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). 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.

RELATED PRODUCTS

PRODUCT	CATALOG #
AggreWell™400	27845/27945
Anti-Oct 3/4 antibody	01550/01551
Anti-SSEA-1 antibody	01552
Anti-SSEA-3 antibody	01553
Anti-SSEA-4 antibody	01554
Anti-TRA-1-60 antibody	01559
Anti-TRA-1-81 antibody	01556
RoboSep™ Buffer 2	20164
mTeSR™1	05850/05870/05875/05857
TeSR™2	05860/05880
ACCUTASE™	07920

FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.



TOLL-FREE T. 1 800 667 0322 • T. +1 604 877 0713 • TOLL-FREE F. 1 800 567 2899 • F. +1 604 877 0704

ORDERS@STEMCELL.COM • INFO@STEMCELL.COM • FOR FULL CONTACT DETAILS WORLDWIDE VISIT WWW.STEMCELL.COM

VERSION 1.0.0

DOCUMENT #29262

Components:

- | | |
|--|---------|
| • EasySep™ TRA-1-60 Positive Selection Component A | 0.25 mL |
| • EasySep™ TRA-1-60 Positive Selection Component B | 0.25 mL |
| • EasySep™ Magnetic Nanoparticles Positive Selection | 1 mL |



POSITIVE SELECTION

REQUIRED EQUIPMENT:

EasySep™ Magnet (Catalog #18000).

PRODUCT DESCRIPTION AND APPLICATIONS:

The EasySep™ Human ES/iPS Cell TRA-1-60 Positive Selection Kit is designed to isolate TRA-1-60+ cells by positive selection. Desired cells are targeted with an antibody complex recognizing TRA-1-60 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 TRA-1-60 antigen is expressed on undifferentiated human embryonic stem (ES) cells and human induced pluripotent stem (iPS) cells.

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

ACCUTASE™ (Catalog #07920) is recommended for preparation of a single-cell suspension containing human ES or iPS cells. 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.

RECOMMENDED MEDIUM

The recommended medium is PBS containing 0.5% bovine albumin and 2 mM EDTA, e.g. RoboSep™ Buffer 2 (Catalog #20164). Medium should be Ca⁺⁺ and Mg⁺⁺ free. Other compatible media include mTeSR™1 (Catalog #05850/05857/05870/05875) and TeSR™2 (Catalog #05860/05880).

ASSESSING PURITY

The purity of the isolated human ES or iPS cells can be measured by flow cytometry after labeling with a fluorochrome-conjugated anti-TRA-1-60 antibody, as blocking by the EasySep™ TRA-1-60 Positive Selection Cocktail is minimal.

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

TRA-1-60+ CELL DEPLETION

The EasySep™ Human ES/iPS Cell TRA-1-60 Positive Selection Kit can also be used to deplete TRA-1-60+ cells. Please refer to the depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf or contact STEMCELL Technologies's Technical Support at techsupport@stemcell.com to receive a copy of the protocol.

COMPONENT DESCRIPTIONS:

EASYSEP™ TRA-1-60 POSITIVE SELECTION COMPONENT A CODE # 18168C

EASYSEP™ TRA-1-60 POSITIVE SELECTION COMPONENT B CODE # 18169C

These components contain monoclonal antibodies purified by affinity chromatography using Protein A or Protein G Sepharose. The anti-TRA-1-60 antibody was raised in mice and is of subclass IgM, κ. The components are supplied in PBS. It should be noted that these products are biological reagents, 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™ TRA-1-60 POSITIVE SELECTION COMPONENT A CODE # 18168C

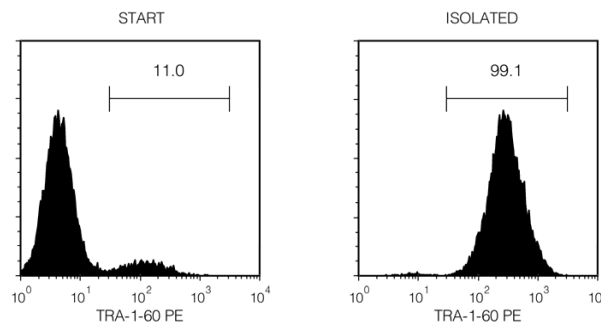
EASYSEP™ TRA-1-60 POSITIVE SELECTION COMPONENT B CODE # 18169C

EASYSEP™ MAGNETIC NANOPARTICLES POSITIVE SELECTION CODE #18150

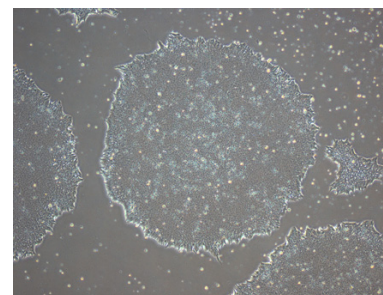
Products stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. Do not freeze these products. These products may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

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

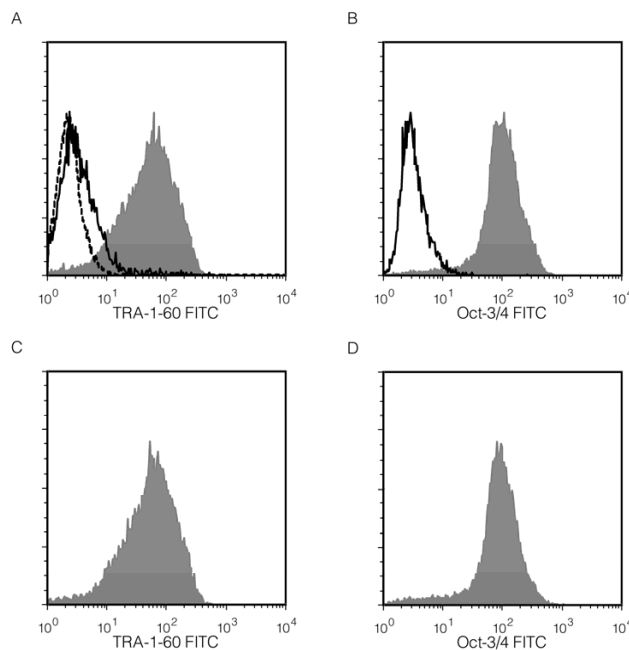
FOR RESEARCH USE ONLY. NOT FOR THERAPEUTIC OR DIAGNOSTIC USE.

TYPICAL EASYSEP™ TRA-1-60 POSITIVE SELECTION PROFILE:

Starting with a single-cell suspension, the TRA-1-60+ cell content of the isolated fraction typically ranges from 96 - 99%.

CULTURE OF CELLS ISOLATED BY TRA-1-60 POSITIVE SELECTION

Human ES cells isolated from a start sample containing 12% TRA-1-60+ cells were cultured in mTeSR™1 on BD Matrigel™ hESC-qualified Matrix (BD, Catalog #354277) for 4 passages. Procedures outlined in the Technical Manual: Maintenance of hPSCs in mTeSR™1 and TeSR™2 (Document #29106, available on our website at www.stemcell.com) were used except that all cells were harvested and plated at each passage.

MAINTENANCE OF PLURIPOTENCY MARKERS BY ISOLATED CELLS

Human ES cells isolated by TRA-1-60 positive selection (A, B) and control human ES cells (C, D) were cultured for 4 passages, as described above. The cells were then analyzed for expression of TRA-1-60 (A, C) and Oct-3/4 (B, D) (filled histograms). Empty histograms show labeling of negative control cells (fibroblasts: solid line) or an IgM, κ isotype control antibody (dashed line).