PROCEDURE

EasySep®

POSITIVE SELECTION

V 1.0.2

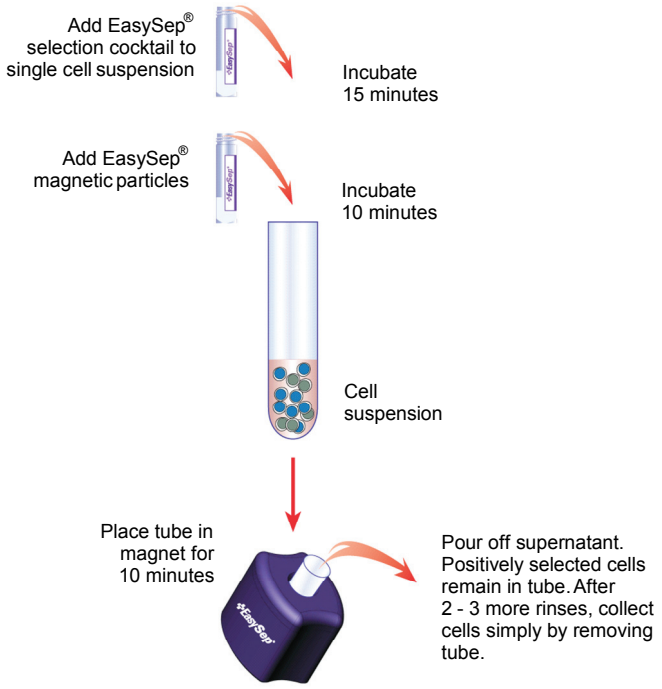
STEMCELL TECHNOLOGIES

hESC-DERIVED CD34 POSITIVE SELECTION KIT

CATALOG #18167

THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH THE PURPLE EASYSEP• MAGNET. THIS PRODUCT IS NOT COMPATIBLE WITH ROBOSEP• – THE FULLY AUTOMATED CELL SEPARATOR.

MANUAL EASYSEP• PROTOCOL DIAGRAM



A) MANUAL EASYSEP• PROTOCOL USING PURPLE EASYSEP• MAGNET (CATALOG #18000).

This procedure is used for processing **8 x 10^6 – 5 x 10^8 cells.**

1. Prepare a single cell suspension in the recommended medium (see Notes and Tips, reverse side).
   - If starting with < 10^7 total cells, suspend cells in 100 µL of the recommended medium.
   - If starting with 10^7 - 10^8 total cells, suspend cells at 1 x 10^8 cells/mL in the recommended medium.
   - If starting with 2 - 5 x 10^8 total cells, suspend cells in 1 mL of medium.

   Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the EasySep® Magnet.

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.
2. Add EasySep® Positive Selection Cocktail at **100 µL/mL** cells (e.g. for 100 µL of cells, add 10 µL of cocktail). Mix well and incubate at room temperature (15 – 25°C) for **15** minutes.
3. Mix 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. Add the particles at **50 µL/mL** cells (e.g. for 100 µL of cells, add 5 µL of nanoparticles). Mix well and incubate at room temperature (15 – 25°C) for **10** minutes.
4. Bring the cell suspension 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.
5. Pick up the 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.*
6. 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 **10** minutes.
7. Repeat Steps 5 and 6, and then Step 5 once more, for a total of **3 x 10**-minute separations in the magnet.
8. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

RELATED PRODUCTS

PRODUCT	CATALOG #
AggreWell™400 plates for the reproducible formation of uniformly sized embryoid bodies	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	01555
Anti-TRA-1-81 antibody	01556
FITC-conjugated goat anti-mouse IgG	10210
FITC-conjugated goat anti-mouse IgM	10211
APC-conjugated goat anti-rat IgM	10215
mTeSR™1 medium for the maintenance of hESCs and hiPSCs	05850 05870
ACCUTASE®	07920
Dispase (1 mg/mL)	07923

CATALOG #18167 For labeling 5 x 10^9 total cells

Components:

- EasySep® hESC-Derived CD34 Positive Selection Cocktail 1.0 mL
- EasySep® Magnetic Nanoparticles 1.0 mL

EasySep® POSITIVE SELECTION

REQUIRED EQUIPMENT:

EasySep® Magnet (Catalog #18000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® hESC-derived CD34 Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label $CD34^+$ cells for magnetic separation. These positive selection reagents are designed to positively select $CD34^+$ cells (cells expressing the CD34 antigen) from differentiated human embryonic stem and induced pluripotent (hESC and hiPSC) cell cultures. If isolating $CD34^+$ cells from fresh or previously frozen mobilized peripheral blood or bone marrow mononuclear cells, or from previously frozen cord blood mononuclear cells, we recommend using EasySep® Human CD34 Selection Kit (Catalog #18056). If isolating $CD34^+$ cells from fresh cord blood, we recommend using the EasySep® Human Cord Blood CD34 Selection Kit (Catalog #18096). If isolating $CD34^+$ cells from fresh whole blood or buffy coat, we recommend using the EasySep® Whole Blood / Buffy Coat CD34 Selection Kit (Catalog #18086).

EASYSEP® LABELING OF HUMAN CELLS:

Desired cells are specifically labeled with dextran-coated magnetic nanoparticles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the cell surface antigen expressed on the unwanted cell (Figure 1). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep® procedure (reverse side).

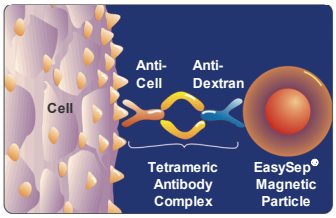


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING A SINGLE CELL SUSPENSION. To prepare a single cell suspension of hESC or hiPSC-derived cells we recommend using Accutase™ (Catalog #07920).

For hESC or hiPSC differentiation cultures containing embryoid bodies (EBs), transfer EBs to a 15 mL conical tube and allow to sediment. Remove medium and replace with 1 mL of Accutase™. Incubate EBs in Accutase™ for 10 mins at 37°C, then gently pipette up and down 15 times using a blue tip. Add 10 mL of PBS containing 2% FBS and centrifuge. Discard supernatant, and resuspend pellet in recommended medium.

For differentiation protocols using co-culture on adherent stromal layers, remove the non-adherent cells first, and transfer to a 15 mL conical tube. Then apply 1 mL of Accutase™ to the plate and incubate for 10 mins at 37°C. After this incubation period, extract the dissociated cells from the plate by pipetting and transfer to the same 15 mL conical tube. Wash the plate with 10 mL of PBS containing 2% FBS and transfer to the same tube. Centrifuge, discard supernatant, and resuspend pellet in recommended medium.

If clumps remain, we recommend incubating the cells with 100 µg/mL DNase I (Catalog #07900) in buffer without EDTA for at least 15 minutes at room temperature (15 – 25°C) prior to labeling and separation. Filter clumpy suspensions through a 70 µm mesh nylon strainer for optimal results.

RECOMMENDED MEDIUM. The recommended medium is PBS containing 2% FBS (Catalog #07905) and 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

$CD34^+$ CELL DEPLETION. The EasySep® CD34 Positive Selection Cocktail can also be used to deplete $CD34^+$ cells. Please refer to depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf.

ASSESSING PURITY. The CD34 Positive Selection Cocktail uses the anti-CD34 antibody clone QBend10. Alternate clones for CD34 positive selection area available as custom kits. Contact STEMCELL Technologies for details. QBend10 is a class II antibody and may block some class I and II anti-CD34 antibody clones used to assess purity by flow cytometry. We recommend using class III anti-CD34 clones such as 8G12 (HPCA-2, Catalog #10513), 581, AC136, or Birma K3 to assess purity by flow cytometry.

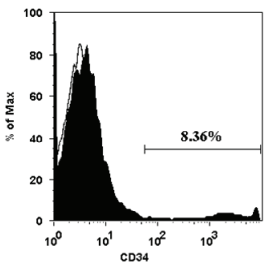
Note: Flow cytometry analysis of the positively selected cells may show slightly increased side scatter relative to the start sample.

OPTIMIZING CELL PURITY. The $CD34^+$ cell purity of the enriched fraction may be improved by performing an additional round of separation in the magnet. The improvement in purity is typically up to an additional 4%. Please note that recovery will decrease with each additional round of separation.

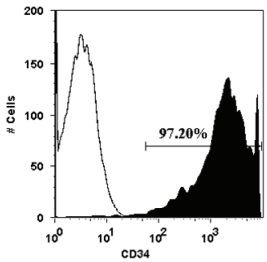
OPTIMIZING CELL RECOVERY. $CD34^+$ cell recovery can be improved adding magnetic nanoparticles at an increased concentration of 100 µL/mL of cells.

TYPICAL EASYSEP® hESC-DERIVED CD34 POSITIVE SELECTION PROFILE:

Start: 8.36% $CD34^+$ Cells



Selected: 97.20% $CD34^+$ Cells



Starting with a differentiated population containing at least 5% CD34+ cells, the $CD34^+$ cell content of the enriched fraction typically ranges from 84 - 99%.

Data courtesy of D. Kaufman, University of Minnesota.

COMPONENT DESCRIPTIONS:

EASYSEP® hESC-DERIVED CD34 POSITIVE SELECTION COCKTAIL **CODE #18167C**

This cocktail contains a combination of monoclonal antibodies purified from hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are bound in bispecific tetrameric antibody complexes (TAC) which are directed against CD34 and dextran. The mouse monoclonal antibody subclass is IgG_1. This cocktail is supplied in phosphate buffered saline and contains an antibody against human Fc receptor. 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 **CODE #18150**

A suspension of magnetic dextran iron particles in water.

STABILITY AND STORAGE:

EASYSEP® hESC-DERIVED CD34 POSITIVE SELECTION COCKTAIL

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

EASYSEP® MAGNETIC NANOPARTICLES

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

IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899 T. 1 604 877 0713 F. 1 604 877 0704 E. INFO@STEMCELL.COM
IN EUROPE T. +33 (0)4 76 04 75 30 F. +33 (0)4 76 18 99 63 E. INFO.EU@STEMCELL.COM
IN AUSTRALIA TOLL-FREE T./F. 1 800 060 350 T. 07 5474 5042 E. INFO.AUS@STEMCELL.COM

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