

Positive Selection


EasySep

Human CD34 Selection Kit

PROCEDURE

Version 2.1.1

CATALOG #18056



This Product Information Sheet is provided for use with RoboSep® (section A), the purple EasySep® magnet (section B) or the silver “The Big Easy” EasySep® magnet (section C).

A) Fully Automated Protocol Using RoboSep® (Catalog #20000).

This procedure is used for processing **250 µL - 4 mL** of sample (up to 2×10^9 cells).

1. Prepare a mononuclear cell suspension in RoboSep® Buffer (Catalog #20104).
 - If starting with $<5 \times 10^7$ total cells, suspend cells in 250 µL of buffer.
 - If starting with $5 \times 10^7 - 2 \times 10^8$ total cells, suspend cells at 2×10^8 cells/mL.
 - If starting with $2 - 5 \times 10^8$ total cells, suspend cells in 1 mL of buffer.
 - If starting with $5 \times 10^8 - 2 \times 10^9$ total cells, suspend cells at 5×10^8 cells/mL.

Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel.

Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352057) are recommended.

2. Select the appropriate RoboSep® protocol:

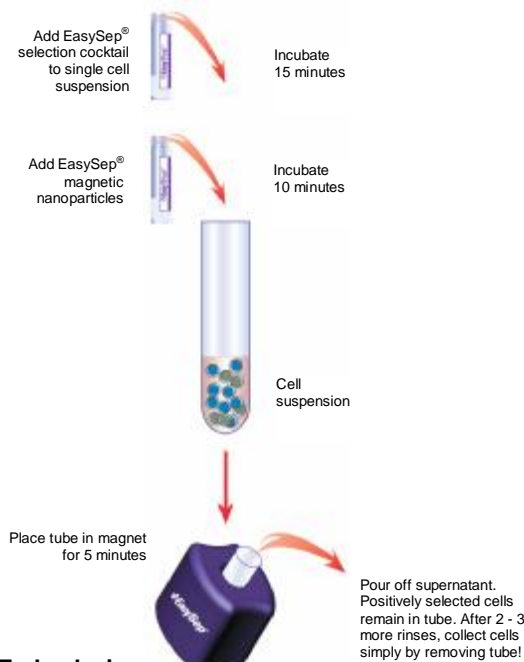
RoboSep® protocols can be optimized for high CD34⁺ cell purity or high CD34⁺ cell recovery. Select one of the protocols listed below, as appropriate.

- “Human CD34 Positive Selection 18056-high purity”.
- “Human CD34 Positive Selection 18056-high recovery”.

If a modified RoboSep® protocol is required, please contact StemCell Technologies’ Technical Support at techsupport@stemcell.com.

3. Load the RoboSep® carousel as directed by the on-screen prompts. Mix EasySep Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. When all desired quadrants are loaded, press the green “Run” button. All cell labeling and separation steps will be performed by RoboSep®.
4. When cell separation is complete, remove the tube containing the isolated cells from the magnet. The positively selected cells are now ready for use.

Manual EasySep® Protocol Diagram



B) Manual EasySep® Protocol Using Purple EasySep® Magnet (Catalog #18000).

This procedure is used for processing **100 µL - 1 mL** of sample (up to 5×10^8 cells).

1. Prepare a mononuclear cell suspension in recommended medium (see Notes and Tips, reverse side).
 - If starting with $<2 \times 10^7$ total cells, suspend cells in 100 µL of medium.
 - If starting with $2 \times 10^7 - 2 \times 10^8$ total cells, suspend cells at 2×10^8 cells/mL.
 - If starting with $2 - 5 \times 10^8$ 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 (Becton Dickinson, Catalog #352058) are recommended.

2. Add EasySep® Positive Selection Cocktail at **100 µL/mL** cells (e.g. for 1 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature 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 1 mL of cells, add 50 µL of nanoparticles). Mix well and incubate at room temperature 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 **5** 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 **5** minutes.
7. Repeat Steps 5 and 6 three times, and then Step 5 once more, for a total of 5 x 5-minute separations in the magnet. 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.

C) Manual EasySep® Protocol Using Silver “The Big Easy” EasySep® Magnet (Catalog #18001).

This procedure is used for processing **250 µL - 4 mL** of sample (up to 2×10^9 cells).

1. Prepare a mononuclear cell suspension in recommended medium (see Notes and Tips, reverse side).
 - If starting with $<5 \times 10^7$ total cells, suspend cells in 250 µL of medium.
 - If starting with $5 \times 10^7 - 2 \times 10^8$ total cells, suspend cells at 2×10^8 cells/mL.
 - If starting with $2 - 5 \times 10^8$ total cells, suspend cells in 1 mL of medium.
 - If starting with $5 \times 10^8 - 2 \times 10^9$ total cells, suspend cells at 5×10^8 cells/mL.

Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the silver magnet.

Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352057) are recommended.

2. Add EasySep® Positive Selection Cocktail at **100 µL/mL** cells (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature for **15** minutes.
3. Mix EasySep® Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. Vortexing is not recommended. Add the particles at **50 µL/mL** cells (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature for **10** minutes.
4. Bring the cell suspension to a **total volume** of 5.0 mL (for $<5 \times 10^8$ cells) or 10 mL (for $5 \times 10^8 - 2 \times 10^9$ cells) 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.
5. 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.*
6. Remove the tube from the magnet and add 5.0 mL (for $<5 \times 10^8$ cells) or 10 mL (for $5 \times 10^8 - 2 \times 10^9$ cells) 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.
7. Repeat Steps 5 and 6 twice, then Step 5 once more, for a total of 4 x 5-minute separations in the magnet. 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.

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Catalog #18056For labeling up to 5 x 10⁹ total cells**Components:**

- EasySep[®] Human CD34 Positive Selection Cocktail 1.0 mL
- EasySep[®] Magnetic Nanoparticles 1.0 mL

**Product Information Sheet****REQUIRED EQUIPMENT:**

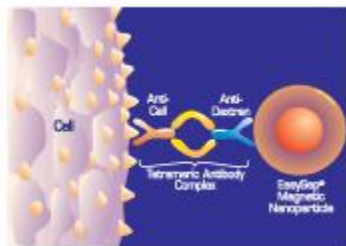
EasySep[®] Magnet (Catalog #18000), or "The Big Easy" EasySep[®] Magnet (Catalog #18001), or RoboSep[®] (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] Human 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 fresh or previously frozen mobilized peripheral blood or bone marrow mononuclear cells, or from previously frozen cord blood mononuclear cells. 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:

Target cells are specifically labeled with dextran-coated magnetic nanoparticles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the target cell surface antigen (Figure 1). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells, and does not interfere with subsequent FACS analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep[®] procedure (reverse side).

**Figure 1.**

Schematic Drawing of EasySep[®] TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Mononuclear Cell Suspension. Prepare a mononuclear cell suspension from whole bone marrow, or mobilized peripheral blood by Ficoll-Paque™ density separation (Catalog #07957). Older samples may require a longer (i.e. 30 minute) Ficoll™ centrifugation step in order to reduce contamination by hypodense granulocytes. Alternatively, the RosetteSep[®] Human Granulocyte Depletion Cocktail (Catalog #15624/15664) can be used to deplete total granulocytes prior to EasySep[®] magnetic separation.

For previously frozen mononuclear cells, 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 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.

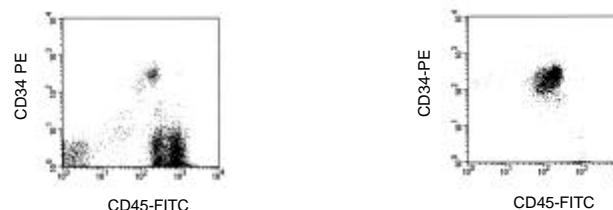
CD34⁺ Cell Depletion. The EasySep[®] CD34 Positive Selection Cocktail can also be used to deplete CD34⁺ cells. Please refer to depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf.

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 by performing a total of 4 x 5-minute (purple magnet) or 3 x 5-minute (silver magnet) separations in the magnet, and / or by adding magnetic nanoparticles at an increased concentration of 100 µL/mL of cells.

Selection from Samples with High CD34⁺ Cell Content (>5%). Please contact us for protocols to further purify CD34⁺ cells from samples that have been previously enriched for CD34⁺ cells.

TYPICAL EASYSEP[®] CD34 SELECTION PROFILE:Start: 1.0% CD34⁺ CellsSelected: 96.0% CD34⁺ Cells

Starting with previously frozen cord blood or fresh mobilized peripheral blood mononuclear cells, the CD34⁺ cell content of the enriched fraction typically ranges from 84 - 99% (reported as a percentage of viable CD45⁺ cells).

COMPONENT DESCRIPTIONS:**EasySep[®] Human CD34 Positive Selection Cocktail code #18056C.1**

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 IgG₁. 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[®] Human CD34 Positive Selection Cocktail**

Stable at 4°C for 2 years. Do not freeze this product. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

EasySep[®] Magnetic Nanoparticles

Stable at 4°C for 2 years. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

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