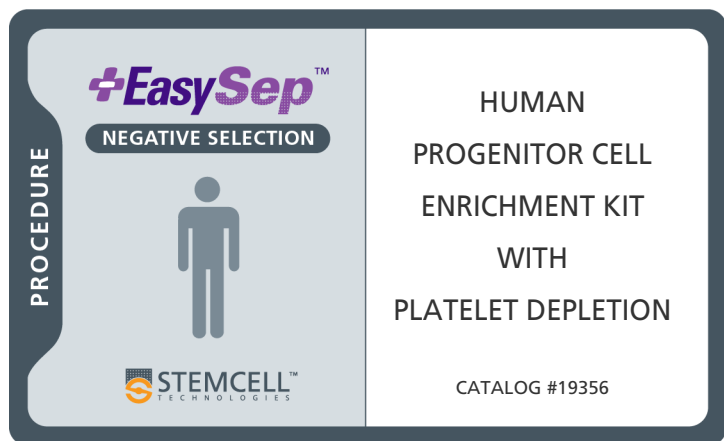




THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSep™ (SECTION A), THE PURPLE EASYSEP™ MAGNET (SECTION B) OR "THE BIG EASY" SILVER EASYSEP™ MAGNET (SECTION C).

If using other EasySep™ Magnets, please visit www.stemcell.com to download the magnet-specific Product Information Sheet or contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

A) FULLY AUTOMATED PROTOCOL USING ROBOSep™

This procedure is used for processing **500 µL - 8.5 mL** of sample (up to 4.25×10^8 cells).

1. Prepare cell suspension at a concentration of 5×10^7 cells/mL in Modified RoboSep™ Buffer (Catalog #20164) (see Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep™ carousel.

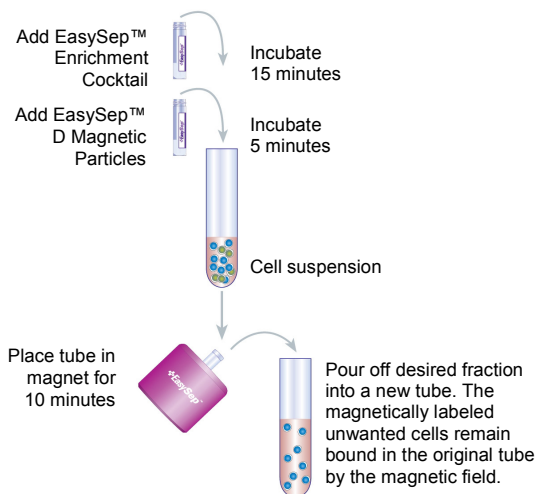
Falcon® 14 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352057) are recommended.

2. Select the appropriate RoboSep™ protocol:
 - Human Progenitor Negative Selection with Platelet Depletion 19356

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. Vortex the EasySep™ D Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates. 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 enriched cells in the 50 mL tube located to the left of the tip rack. The isolated cells in the new tube are now ready for use.

MANUAL EASYSEP™ PROTOCOL DIAGRAM



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

This procedure is used for processing **250 µL - 2.0 mL** of sample (up to 1.0×10^8 cells).

1. Prepare cell suspension at a concentration of 5×10^7 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 Purple EasySep™ Magnet.

Falcon® 5 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352058) are recommended.

2. Add the EasySep™ Human Progenitor Cell Enrichment Cocktail with Platelet Depletion at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
3. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the EasySep™ D Magnetic Particles at **100 µL/mL of cells** (e.g. for 2 mL of cells, add 200 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **5 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**.
6. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 5 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original 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. The isolated cells in the new tube are now ready for use.

C) MANUAL EASYSEP™ PROTOCOL USING "THE BIG EASY" SILVER EASYSEP™ MAGNET (CATALOG #18001)

This procedure is used for processing **2.0 mL - 8.5 mL** of sample (up to 4.25×10^8 cells).

1. Prepare cell suspension at a concentration of 5×10^7 cells/mL in recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep™ Magnet.

Falcon® 14 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352057) are recommended.

2. Add the EasySep™ Human Progenitor Cell Enrichment Cocktail with Platelet Depletion at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
3. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the EasySep™ D Magnetic Particles at **100 µL/mL of cells** (e.g. for 2 mL of cells, add 200 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **5 minutes**.
5. Bring the cell suspension up to a total volume of **10 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**.
6. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 14 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original 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. The isolated cells in the new tube are now ready for use.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485 MEDICAL DEVICE STANDARDS.
FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.



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VERSION 1.1.1

DOCUMENT #29667

Components:

- EasySep™ Human Progenitor Cell Enrichment Cocktail With Platelet Depletion 1.0 mL
- EasySep™ D Magnetic Particles 2 x 1.0 mL



NEGATIVE SELECTION

REQUIRED EQUIPMENT:

EasySep™ Magnet (Catalog #18000), or "The Big Easy" EasySep™ Magnet (Catalog #18001), or RoboSep™.

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Human Progenitor Cell Enrichment Cocktail with Platelet Depletion and EasySep™ D Magnetic Particles label non-progenitor cells for magnetic separation. These reagents are designed to enrich hematopoietic progenitor cells from fresh or previously frozen cord blood and other cell preparations that contain large numbers of platelets by depletion of non-progenitor cells. For applications in which depletion of platelets, megakaryocytes and megakaryocytic progenitor cells is not desired, we recommend the EasySep™ Human Progenitor Cell Enrichment Kit (Catalog #19056), which does not contain anti-CD61.

EASYSEP™ LABELING OF HUMAN CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles using bispecific Tetrameric Antibody Complexes (TACs). These complexes recognize both dextran and the unwanted cell surface antigen (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep™ procedure (reverse side).

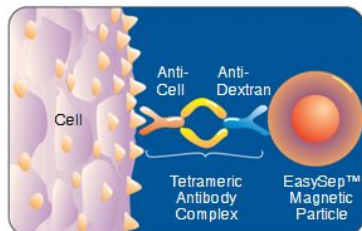


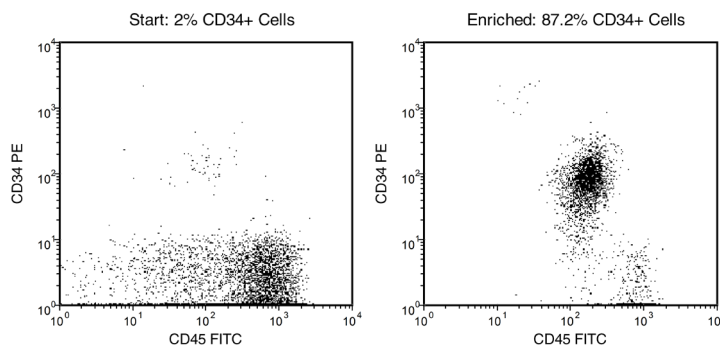
Figure 1.
Schematic Drawing of EasySep™
TAC Magnetic Labeling of Human
Cells.

NOTES AND TIPS:**PREPARING THE CELL SUSPENSION****FROM WHOLE PERIPHERAL BLOOD**

Prepare a nucleated cell suspension from whole cord blood by density gradient centrifugation, or alternatively, remove red blood cells by lysis using Ammonium Chloride Solution (Catalog #07800). **For previously frozen mononuclear cells, we recommend incubating the cells with DNase I (Catalog #07900) at a concentration of 100 µg/mL for at least 15 minutes at room temperature (15 - 25°C) prior to labeling and separation.** Filter clumpy suspensions through a 30 µm mesh nylon strainer for optimal results.

RECOMMENDED MEDIUM. The recommended medium is Modified RoboSep™ Buffer (Catalog #20164), or phosphate-buffered saline (PBS) + 0.5% bovine serum albumin (BSA) with 2 mM EDTA. Medium should be Ca^{2+} and Mg^{2+} free.

ASSESSING PURITY. The frequency of CD34+ cells can be measured by flow cytometry after labeling with fluorescently-conjugated antibodies against CD34 and, optionally, CD45 (e.g., anti-CD34 PE, Catalog #60013, and anti-CD45 FITC, Catalog #60018). Purity of CD34+ cells is typically expressed as a percentage of viable CD45+ cells. Viability is measured by exclusion of propidium iodide or 7-aminoactinomycin D (7-AAD). The frequency of erythroid (BFU-E), myeloid (CFU-GM) and multilineage (CFU-GEMM) progenitor cells can be assessed in colony-forming cell assays in semi-solid culture media using MethoCult™ H4034 Optimum (Catalog #04034).

TYPICAL EASYSEP™ HUMAN PROGENITOR CELL ENRICHMENT PROFILE WITH PLATELET DEPLETION:

Starting with nucleated cells, the CD34+ cell content of the enriched fraction typically ranges from 50 - 75% depending on the quality of the start sample.

Note: CD34+ enrichment is dependent on the frequency of CD34+ cells in the start sample, which is variable between cord blood samples. Use of poor quality cord blood or frozen samples may result in lower CD34+ cell content of the enriched fraction.

COMPONENT DESCRIPTIONS:**EASYSEP™ HUMAN PROGENITOR CELL ENRICHMENT COCKTAIL WITH PLATELET DEPLETION****CODE #19356C**

This cocktail contains a combination of monoclonal antibodies bound in bispecific TACs which are directed against cell surface antigens on human blood cells (CD2, CD3, CD11b, CD11c, CD14, CD16, CD19, CD24, CD56, CD61, CD66b, glycoprotein A) and dextran. The mouse monoclonal antibody subclass is IgG₁. 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™ D MAGNETIC PARTICLES**CODE #19250**

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:**EASYSEP™ HUMAN PROGENITOR CELL ENRICHMENT COCKTAIL WITH PLATELET DEPLETION****EASYSEP™ D MAGNETIC PARTICLES**

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.