



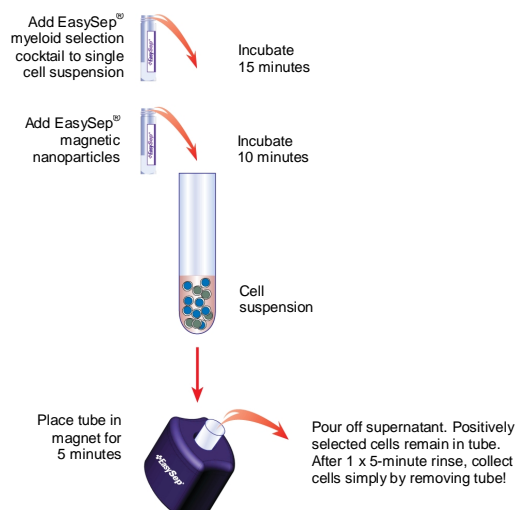
THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP® (SECTION A), THE PURPLE EASYSEP® MAGNET (SECTION B) OR "THE BIG EASY" SILVER EASYSEP® MAGNET (SECTION C).

A) FULLY AUTOMATED PROTOCOL USING ROBOSEP® (CATALOG #20000).

This procedure is used for processing **250 µL – 8.0 mL** of sample (up to 8.0×10^8 cells).

1. Prepare a mononuclear cell suspension at a concentration of 1×10^8 cells/mL in RoboSep® Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 µL.
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
2. Select the appropriate RoboSep® protocol:
For most normal samples, select the protocol entitled "Human Myeloid Positive Selection 18653-high purity".
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 for processing **100 µL – 2.5 mL** of sample (up to 2.5×10^8 cells).

1. Prepare a mononuclear 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 Purple EasySep® Magnet. For samples containing 10^7 cells or fewer, resuspend in 100 µL.
Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.
Note: The EasySep® Myeloid Positive Selection Cocktail can also be used to deplete myeloid cells. Please refer to the depletion procedure at <http://www.stemcell.com/technical/EasySepDepletion.pdf>
2. Add EasySep® Myeloid 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 (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 **100 µL/mL** cells (e.g. for 2 mL of cells, add 200 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 **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 2.5 mL of 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 Step 5 for a total of 2 x 5-minute separations in the magnet. Remove tube from magnet and resuspend cells in an appropriate amount of medium. The positively selected cells are now ready for use.

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

This procedure is for processing **250 µL – 8.5 mL** of sample (up to 8.5×10^8 cells).

1. Prepare a mononuclear 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 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep® magnet. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 µL.
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
Note: The EasySep® Myeloid Positive Selection Cocktail can also be used to deplete myeloid cells. Please refer to the depletion procedure at <http://www.stemcell.com/technical/EasySepDepletion.pdf>
2. Add EasySep® Myeloid 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 (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 **100 µL/mL** cells (e.g. for 2 mL of cells, add 200 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 5.0 mL (for $<4 \times 10^8$ cells) or 10.0 mL (for $4 - 8.5 \times 10^8$ 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 $<4 \times 10^8$ cells) or 10.0 mL (for $4 - 8.5 \times 10^8$ cells) of 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 Step 5 for a total of 2 x 5-minute separations in the magnet. Remove tube from magnet and resuspend cells in an appropriate amount of medium. The positively selected cells are now ready for use.

Components:

• EasySep [®] Human Myeloid Positive Selection Cocktail	1.0 mL
• EasySep [®] Magnetic Nanoparticles	1.0 mL
• SpinSep [®] Density Medium	100 mL



POSITIVE SELECTION

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] Human Myeloid Positive Selection Cocktail and EasySep[®] Magnetic Nanoparticles label myeloid cells for magnetic separation. These positive selection reagents are designed to positively select CD33⁺ and CD66b⁺ cells (cells expressing the CD33 and CD66b antigens) from ammonium chloride lysed blood or bone marrow, or from nucleated cells prepared by density centrifugation with SpinSep[®] Density Medium (Catalog #17531).

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).

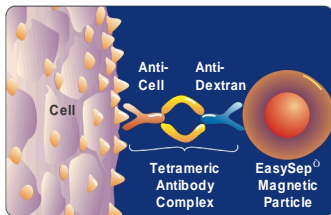


Figure 1.
Schematic Drawing of EasySep[®] TAC
Magnetic Labeling of Human Cells.

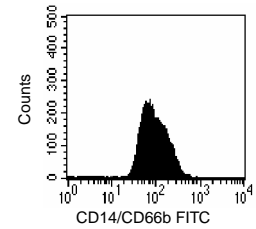
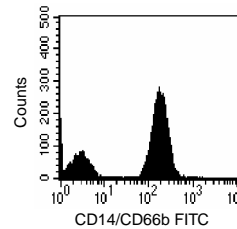
NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION. Cells can be prepared from blood or bone marrow by ammonium chloride lysis. Alternatively, cells can be prepared by density separation with SpinSep[®] Density Medium (Catalog #17531, supplied). Please note that centrifugation with other density media, such as Ficoll-Paque[™] PLUS, may result in lower recovery of CD66b⁺ granulocytes.

RECOMMENDED MEDIUM. The recommended medium is RoboSep[®] Buffer (Catalog #20104), or 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 Myeloid Positive Selection Cocktail uses the CD33 antibody clone p67.6, which may block some anti-CD33 antibody clones used to assess purity by flow cytometry, and the anti-CD66b clone BIRMA17C. We recommend using a combination of FITC-conjugated anti-CD14 and anti-CD66b antibodies (Catalog #10406 and #10419 respectively). One of the following methods can also be used to assess purity:

1. Add PE-labeled antibodies at the same time as the cocktail: Add the fluorochrome-conjugated anti-CD33 antibody at a concentration of 0.4 µg/mL immediately after adding the cocktail to provide a strong detection signal without affecting separation performance. **This method labels the positive cells in the entire sample.**
2. Use a secondary fluorochrome-conjugated antibody, such as FITC-labeled sheep anti-mouse IgG, to detect the primary anti-CD33 and anti-CD66b antibodies.

TYPICAL EASYSEP[®] MYELOID SELECTION PROFILE:Start: 73.8% CD14⁺/CD66b⁺ CellsSelected: 98.9% CD14⁺/CD66b⁺ Cells

Starting with fresh peripheral blood mononuclear cells, the myeloid cell content of the enriched fraction typically ranges from 96.2 - 99.0%.

COMPONENT DESCRIPTIONS:

EASYSEP[®] HUMAN MYELOID
POSITIVE SELECTION COCKTAIL

CODE #18653C

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 dextran and CD33 or CD66b. The mouse monoclonal antibody subclass is IgG. 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

CODE #18150

A suspension of magnetic dextran iron particles in water.

SPINSEP[®] DENSITY MEDIUM

CODE #17531

A density separation medium used to prepare nucleated cell suspensions.

STABILITY AND STORAGE:

EASYSEP[®] HUMAN MYELOID 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.

SPINSEP[®] DENSITY MEDIUM.

Stable at room temperature (15 - 25°C) for 1 year from date of manufacture as indicated on label.

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