PROCEDURE

Positive Selection

EasySep®

Human Whole Blood Myeloid Selection Kit

Version 1.1.1

CATALOG #18683

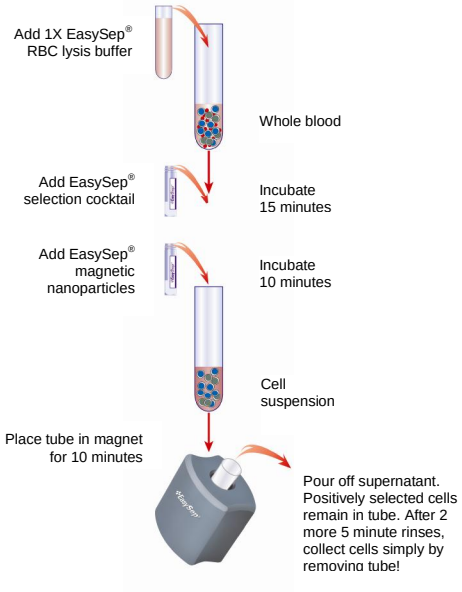
This Product Information Sheet is provided for use with RoboSep® (section A) or "The Big Easy" Silver EasySep® magnet (section B).

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

This procedure is used for processing **up to 4.5 mL** of whole blood per separation.

1. Collect whole blood in a heparinized blood collection tube. Transfer a maximum of 4.5 mL whole blood to a 14 mL (17 x 100 mm) polystyrene tube. Cells must be placed in a 14 mL polystyrene tube to properly fit into the RoboSep® carousel.
   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352057) are recommended.
2. Add 1X EasySep® RBC Lysis Buffer (see Notes and Tips, reverse side) at a ratio of 1 part lysis buffer to 1 part whole blood. Mix well.
3. Select the appropriate RoboSep® protocol:
   For most normal samples, select the protocol entitled "Human Myeloid WB Positive Selection 18683-high purity".
   If a modified RoboSep® protocol is required, please contact *StemCell Technologies'* Technical Support at techsupport@stemcell.com.
4. 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®.
5. 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 "The Big Easy" Silver EasySep® Magnet (Catalog #18001).

This procedure is used for processing **up to 4.5 mL** of whole blood per separation.

1. Collect whole blood in a heparinized blood collection tube. Transfer a maximum of 4.5 mL whole blood to a 14 mL (17 x 100 mm) polystyrene tube. Cells must be placed in a 14 mL polystyrene tube to properly fit into the EasySep® Magnet.
   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352057) are recommended.
2. Add 1X EasySep® RBC Lysis Buffer (see Notes and Tips, reverse side) at a ratio of 1 part lysis buffer to 1 part whole blood. Mix well.
3. Add EasySep® Positive Selection Cocktail at **25 µL/mL** whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/lysis buffer mixture add 50 µL of cocktail). Mix well and incubate at room temperature for **15** minutes.
4. Mix EasySep® Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously more than 5 times. Vortexing is not recommended. Add the nanoparticles at **25 µL/mL** whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/lysis buffer mixture add 50 µL of nanoparticles). Mix well and incubate at room temperature for **10** minutes.
5. If total volume is less than 2.5 mL, add recommended medium to 5 mL, otherwise add recommended medium to 10 mL (see Notes and Tips, reverse side). 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 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 inverted 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.***
7. Remove the tube from the magnet and add either 5 mL or 10 mL of recommended medium (as in Step 5). 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.
8. Repeat Steps 6 and 7, and then Step 6 once more, for a total of 1 x 10-minute and 2 x 5-minute separations in the magnet. Remove tube from magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

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Printed on recycled paper.

Catalog #18683 For labeling 60 mL whole blood

Components:

- EasySep® Human Whole Blood Myeloid Positive Selection Cocktail 3 x 1.0 mL
- EasySep® Whole Blood Magnetic Nanoparticles 3 x 1.0 mL
- EasySep® RBC Lysis Buffer 10X Concentrate 10 mL

EasySep® Positive Selection

Product Information Sheet

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Human Whole Blood Myeloid Positive Selection Cocktail and EasySep® Whole Blood Magnetic Nanoparticles label $CD33^+$ and $CD66b^+$ 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 fresh whole blood.

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 flow cytometric analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep® procedure (reverse side).

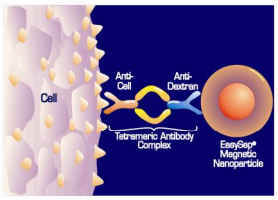


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

EasySep® RBC Lysis Buffer. Lysis buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

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.

Donor Variability. Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic nanoparticles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots during flow cytometry analysis of the enriched fraction. This population consists solely of particles, with no cells or platelets present, as determined by staining with fluorescently-labeled antibodies against dextran, CD41 and CD45.

Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in the recommended medium, and centrifuge at 300 x *g* for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend sample to original volume with recommended medium before beginning the separation procedure.

If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

Optimizing Purity. The myeloid 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.

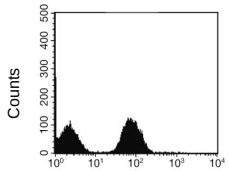
Optimizing Recovery. The myeloid cell recovery of the enriched fraction may be improved by increasing the length of separation time in the magnet from 5 minutes to 10 minutes.

Assessing Purity. The myeloid positive selection cocktail uses the anti-CD33 antibody clone D3H260.251, 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).

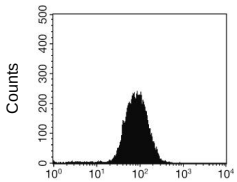
A secondary fluorochrome-conjugated antibody, such as FITC-labeled sheep anti-mouse IgG, can also be used to assess purity.

TYPICAL EASYSEP® MYELOID CELL SELECTION PROFILE:

Start*: 51.8% $CD14^+/CD66b^+$ Cells Selected: 98.4% $CD14^+/CD66b^+$Cells



CD14/CD66b FITC



CD14/CD66b FITC

Starting with fresh whole blood, the $CD14^+/CD66b^+$ cell content of the enriched fraction typically ranges from 94.3 - 99.1%.

*Red blood cells were removed by lysis prior to flow cytometry.

COMPONENT DESCRIPTIONS:

EasySep® Human Whole Blood Myeloid Positive Selection Cocktail **code #18683C**

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 CD33 or CD66b and dextran. The mouse monoclonal antibody subclass is IgG_1. 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® Whole Blood Magnetic Nanoparticles **code #18180**

A suspension of magnetic dextran iron particles in water.

EasySep® RBC Lysis Buffer 10X Concentrate **code #20110**

Concentrated buffer used to lyse red blood cells prior to cell labeling and separation.

STABILITY AND STORAGE:

EasySep® Human Whole Blood Myeloid 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® Whole Blood 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.

EasySep® RBC Lysis Buffer 10X Concentrate

10X concentrate is stable at room temperature for 2 years. Store at room temperature. 1X Lysis Buffer is stable at 4°C for 3 months. Store at 2 - 8°C. Do not freeze.

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