PROCEDURE

Negative Selection

EasySep®

Human Neutrophil Enrichment Kit

Version 1.0.0

CATALOG #19257

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).

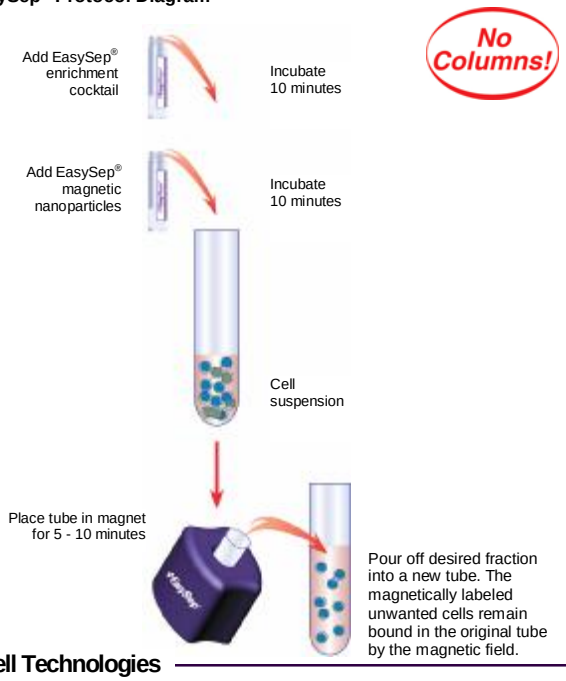
This procedure is used for processing **0.5 - 6.5 mL** of HetaSep™-treated blood (up to 3.25 x 10^8 cells).

1. Prepare nucleated cell suspension (see Notes and Tips, reverse side) at a concentration of 5 x 10^7 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.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352057) are recommended.
2. Select the appropriate RoboSep® protocol:
   - For most normal samples, select the protocol entitled "Human Neutrophil Negative Selection 19257-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® Nanoparticles before loading to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. Vortexing is not recommended. 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, collect the enriched cells in the 14 mL tube located to the left of the magnet in the second quadrant of the 2-quadrant protocol. The enriched cells 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 **0.5 - 2 mL** of HetaSep™-treated blood (up to 1 x 10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 5 x 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 (Becton Dickinson, Catalog #352058) are recommended.
2. Add EasySep® Human Neutrophil Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature or 4°C for **10** minutes.
3. Mix EasySep® Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. Vortexing is not recommended. Add the nanoparticles at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of nanoparticles). Mix well and incubate at room temperature or 4°C for **10** minutes.
4. Bring the cell suspension to a **total volume** of 2.5 mL by adding recommended medium. Thoroughly mix the cells to disrupt any red blood cell aggregates by gently pipetting up and down. 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 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 magnet. Leave the magnet and the 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 empty tube from the EasySep® magnet and place the new tube containing the supernatant fraction into the magnet. Set aside for **5** minutes.
7. Repeat Step 5. The negatively selected enriched 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 **0.5 - 6.5 mL** of HetaSep™-treated blood (up to 3.25 x 10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 5 x 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 (Catalog #352057) are recommended.
2. Add EasySep® Human Neutrophil Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature or 4°C for **10** minutes.
3. Mix EasySep® Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously 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 or 4°C for **10** minutes.
4. Bring the cell suspension to a **total volume** of 5.0 mL (for <1.5 x 10^8 cells) or 10 mL (for 1.5 – 3.25 x 10^8 cells) by adding recommended medium. Thoroughly mix the cells to disrupt any red blood cell aggregates by gently pipetting up and down. Place the tube (without cap) into the magnet. Set aside for **10** minutes.
5. 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 magnet. Leave the magnet and the 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 empty tube from the EasySep® magnet and place the new tube containing the supernatant fraction into the magnet. Set aside for **10** minutes.
7. Repeat Step 5 for a total of 2 x 10-minute separations in the magnet. The negatively selected enriched cells in the new tube are now ready for use. *Note: this second separation step is optional It may be preferable to stop the procedure after Step 5 and perform only 1 x 10-minute separation. Performing only 1 round of magnetic separation will increase cell recovery, but may reduce purity.*

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FOR RESEARCH USE ONLY

#29125

Catalog #19257 — For labeling 10^9 total cells

Components:

• EasySep® Human Neutrophil Enrichment Cocktail	1.0 mL
• EasySep® Magnetic Nanoparticles	3 x 1.0 mL

EasySep® Negative Selection

Product Information Sheet

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

The EasySep® Human Neutrophil Enrichment Kit is designed to enrich human neutrophils by depleting non-neutrophils. Start cell samples are prepared by performing a HetaSep™ sedimentation of the peripheral blood (see Notes and Tips, below).

EASYSEP® LABELING OF HUMAN CELLS:

Unwanted 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 cells (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).

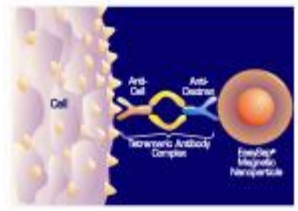


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Nucleated Cell Suspension for Isolation of Neutrophils Using HetaSep™ Red Blood Cell Sedimentation (preferred for faster, lysis-free, sample processing).

Collect whole blood in a blood collection tube containing heparin or other anticoagulant. Add 1 part HetaSep™ (Catalog #07906) to 5 parts blood and mix well. Use the minimum sized tube for the total volume of HetaSep™: blood sample. Centrifuge for 6 minutes at 110 x *g* at room temperature with the brake off. Remove tube from centrifuge and let sit until the plasma layer represents 40 - 50% of the total volume (a maximum of 15 minutes). Carefully remove all of the plasma containing the nucleated cells (everything above the red blood cell (RBC) pellet) and wash this fraction, using 4 parts recommended medium to 1 part recovered plasma/cells. Centrifuge for 10 minutes at 500 x *g* with the brake set to low. Discard supernatant and wash a second time to remove excess platelets, centrifuging for 10 minutes at 120 x *g* with the brake off. Discard supernatant and resuspend at 5 x 10^7 cells/mL in recommended medium.

Do not use dextran sedimentation to prepare cells.

Preparing a Polymorphonuclear Cell (PMNC) Suspension for Isolation of Neutrophils Using Ficoll™ with Red Blood Cell Lysis (preferred for slightly higher purity).

Collect whole blood in a blood collection tube containing heparin or another anticoagulant. Carefully perform a standard Ficoll-Paque™ PLUS density separation procedure (Catalog #07957). Remove and discard the plasma layer, the band of mononuclear cells and the Ficoll™ leaving the RBC pellet intact. At this point the pellet may be transferred to a new tube in order to avoid contamination by remaining mononuclear cells left in the tube. Lyse the RBC pellet with Ammonium Chloride Solution (Catalog #07800) for 10 minutes on ice. (If hypotonic RBC lysis is preferred, please contact *StemCell Technologies'* Technical Support at techsupport@stemcell.com for a suggested protocol). Centrifuge for 10 minutes at 500 x *g* with the brake set to low. Discard supernatant and wash a second time to remove platelets, centrifuging for 10 minutes at 120 x *g* with the brake off. Discard supernatant and resuspend cells in recommended medium at 5 x 10^7 cells/mL.

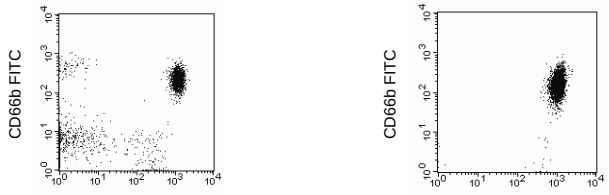
Optimal Cell Number. The use of fewer than 5 x 10^7 cells per separation may result in sub-optimal performance.

Recommended Medium. The recommended medium is RoboSep® Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) and 1 mM EDTA. Media should be Ca^{++} and Mg^{++} free.

Assessing Purity. Purity of neutrophils can be measured by flow cytometry after simultaneously staining with fluorochrome-conjugated anti-CD66b (e.g. anti-CD66b FITC, Catalog #10419), and anti-CD16 (e.g. CD16 PE, Catalog #10508) antibodies. Neutrophils are defined as $CD66b^+CD16^+$. Alternatively, purity may be assessed by performing a cytospin on the enriched cells followed by Wright's or May-Grünwald-Giemsa staining (e.g. Sigma-Aldrich Catalog #MG500 or #W546).

TYPICAL EASYSEP® NEUTROPHIL ENRICHMENT PROFILE:

Start: 55% $CD66b^+CD16^+$ — Enriched: 99% $CD66b^+CD16^+$



Starting with fresh HetaSep™-treated blood, the neutrophil content of the enriched fraction typically ranges from 98 - 99%.

COMPONENT DESCRIPTIONS:

EasySep® Negative Selection Human Neutrophil Enrichment Cocktail — **code #19257C**

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 cell surface antigens on human blood cells (CD2, CD3, CD9, CD19, CD36, CD56, glycophorin A) and dextran. The mouse monoclonal antibody subclass is IgG_1. 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 #19150.1**

A suspension of magnetic dextran iron particles in water.

STABILITY AND STORAGE:

EasySep® Human Neutrophil Enrichment 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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