PROCEDURE

Negative Selection

EasySep®

HLA B Cell Enrichment: Complete Processing Kit for Whole Blood

Version 1.0.0

CATALOG #19954HLA

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).

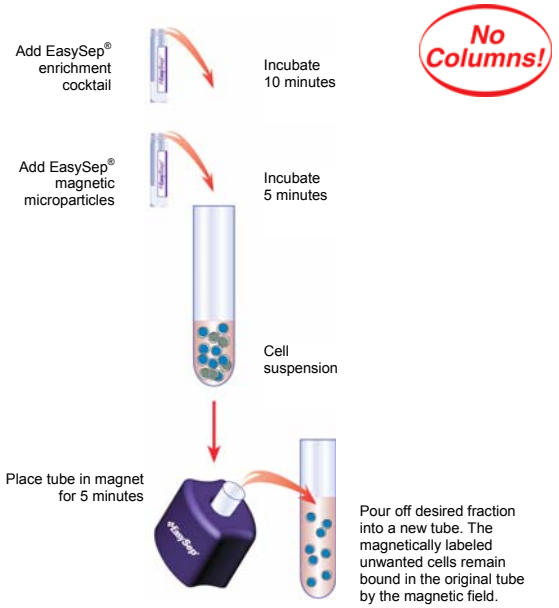
This procedure is used for processing **up to 5 mL** of sample (equivalent to 50 mL of whole blood).

1. Prepare nucleated cell suspension in RoboSep® Buffer (Catalog #20104) using HetaSep™ (Catalog #07806: 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 (Becton Dickinson, Catalog #352057) are recommended.
2. Select the appropriate RoboSep® protocol:
   - For most samples, select the protocol entitled "Human B Cell Negative Selection from WB 19954HLA".

   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® Magnetic Microparticles 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 from the RoboSep® carousel in the 50 mL tube located to the left of the tip rack.

The enriched cells are now ready for use. The cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application. Any residual red blood cells can be removed by lysis using ammonium chloride (Catalog #07800) if desired.

Manual EasySep® Protocol Diagram



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

This procedure is used for processing **up to 2 mL** of sample (equivalent to 20 mL of whole blood).

1. Prepare nucleated cell suspension in recommended medium using HetaSep™ (Catalog #07806: 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 EasySep® Magnet.
   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352058) are recommended.
2. Add EasySep® HLA B Cell 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 for **10** minutes.
3. Vortex EasySep® Magnetic Microparticles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the microparticles at **200 µL/mL cells** (e.g. for 2 mL of cells, add 400 µL of particles). Mix well and incubate at room temperature 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 **5** 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 or 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 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.* The negatively selected, enriched cells in the new tube are now ready for use. The cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application. Any residual red blood cells can be removed by lysis using ammonium chloride (Catalog #07800) if desired.

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

This procedure is used for processing **up to 8 mL** of sample (equivalent to 80 mL of whole blood).

1. Prepare nucleated cell suspension in recommended medium using HetaSep™ (Catalog #07806: 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 magnet.
   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352057) are recommended.
2. Add EasySep® HLA B Cell 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 for **10** minutes.
3. Vortex EasySep® Magnetic Microparticles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the microparticles at **200 µL/ mL cells** (e.g. for 2 mL of cells, add 400 µL of particles). Mix well and incubate at room temperature for **5** minutes.
5. Bring the cell suspension to a **total volume** of 5 mL (for samples of up to 5 mL) or 10 mL (for samples of 5 - 8 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.
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 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 negatively selected, enriched cells in the new tube are now ready for use. The cells are immediately compatible with flow cytometric crossmatch analysis and any other downstream application. Any residual red blood cells can be removed by lysis using ammonium chloride (Catalog #07800) if desired.

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FOR RESEARCH USE ONLY

#29104

Catalog #19954HLA For processing 200 mL of whole blood

Components:

• EasySep® HLA B Cell Enrichment Cocktail	1.0 mL
• EasySep® Magnetic Microparticles	4 x 1.0 mL
• HetaSep™	2 x 20 mL

EasySep® Positive Selection

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:

The EasySep® HLA B Cell Enrichment Cocktail and EasySep® Magnetic Microparticles label non-B cells for magnetic separation. These reagents are designed to enrich B cells from HetaSep™-treated whole blood by depletion of non-B cells.

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 cell (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).

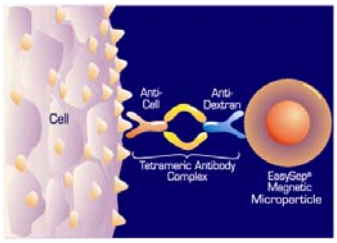

Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Nucleated Cell Suspension.

1. Collect whole blood in a blood collection tube containing heparin or ACD. Using EDTA as an anticoagulant is not recommended. Add 1 part HetaSep™ (Catalog #07806) to 5 parts blood and mix well. Use the minimum sized tube for the total volume of HetaSep™: blood.
2. Centrifuge at room temperature at 50 x *g* for **5** minutes with the brake off. Remove tube from the centrifuge and let sit for **10** minutes or until the red blood cell interface is at approximately 40% of the total volume,
   OR
   Place sample in 37°C incubator and allow to settle until the red blood cell interface is at approximately 40% of the total volume (recommended for older blood samples).
3. Harvest the supernatant containing the nucleated cells.
4. Wash cells in recommended medium and centrifuge at room temperature at 120 x *g* for **10** minutes with the brake off. This step may be repeated if necessary to remove excess platelets.
5. Resuspend cells in recommended medium at 1/10th of the original starting volume (e.g. resuspend the cells recovered from 10 mL of whole blood in 1 mL of recommended medium).

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

Assessing Purity. Purity of B cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD19 antibody (e.g. PE anti-CD19, Catalog #10509).

TYPICAL EASYSEP® B CELL ENRICHMENT PROFILE:

Start: 3.8% $CD19^+$ Cells Selected: 99.7% $CD19^+$ Cells

Starting with HetaSep™-treated whole blood, the $CD19^+$ cell content of the enriched fraction typically ranges from 81.5 - 99.7% (gated on $CD45^+$ cells).

Starting with 0.5 mL of HetaSep™-treated whole blood (equivalent to 5 mL of whole blood), 1.2 - 9.1 x 10^5 $CD19^+$ cells are typically recovered.

COMPONENT DESCRIPTIONS:

EasySep® HLA B Cell Enrichment Cocktail **code #19954HC**

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, CD14, CD16, CD33, CD36, CD41, CD43, CD56, CD66b, 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 Microparticles **code #19250**

A suspension of magnetic dextran iron particles in TRIS buffer.

HetaSep™ **code #07806**

Hetastarch solution used for the separation of nucleated cells from red blood cells in peripheral blood and cord blood.

STABILITY AND STORAGE:

EasySep® HLA B Cell 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 Microparticles

Stable at 4°C for 1 year. Contents sterile in unopened tube. This product may be shipped at room temperature, and should be refrigerated upon receipt.

HetaSep™

Stable for at least two years at room temperature. Contents guaranteed sterile if seal is not tampered with.

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