


**PROCEDURE** **Positive Selection**  
**EasySep**

**HLA  
 Buffy Coat or  
 Whole Blood  
 Lymphoid  
 Selection Kit**

**CATALOG #18684HLA**

Version 1.0.0



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 buffy coat or whole blood per separation.

1. Collect whole blood in a blood collection tube containing heparin or ACD. Lymphoid cells can be positively selected directly from unprocessed whole blood, or from buffy coat if preferred. If a buffy coat is required, process the collected blood as directed (see Notes and Tips, reverse side). Transfer a maximum of 4.5 mL buffy coat or unprocessed 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.*

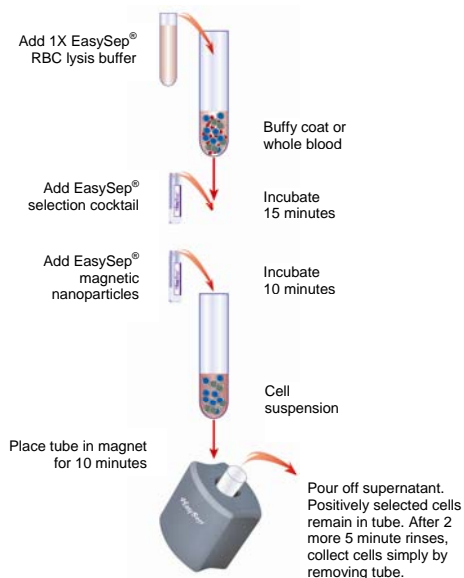
2. Add 1X EasySep® RBC Lysis Buffer (see Notes and Tips, reverse side) at a ratio of 1 part lysis buffer to 1 part sample. Mix well.
3. Select the appropriate RoboSep® protocol:

For most normal samples, select the protocol entitled "Human Lymphoid WB Positive Selection 18684-high purity".

If a modified RoboSep® protocol is required, please contact *StemCell Technologies' Technical Support* at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

4. Load the RoboSep® carousel as directed by the on-screen prompts. 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 and resuspend cells in an appropriate amount of desired medium. Be sure to collect any cells that may be stuck to the sides of the tube. 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 buffy coat or whole blood per separation.

1. Collect whole blood in a blood collection tube containing heparin or ACD. Lymphoid cells can be positively selected directly from unprocessed whole blood, or from buffy coat if preferred. If a buffy coat is required, process the collected blood as directed (see Notes and Tips, reverse side). Transfer a maximum of 4.5 mL buffy coat or unprocessed 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 sample. Mix well.
3. Add EasySep® Positive Selection Cocktail at 25 µL/mL sample/lysis buffer mixture (e.g. for 2 mL of sample/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 sample/lysis buffer mixture (e.g. for 2 mL of sample/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. Be sure to collect any cells that may be stuck to the sides of the tube. The positively selected cells are now ready for use.

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September 2007

**FOR RESEARCH USE ONLY**

**#29099**

**Catalog #18684HLA**

For labeling 60 mL buffy coat or whole blood

**Components:**

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

**Product Information Sheet****REQUIRED EQUIPMENT:**

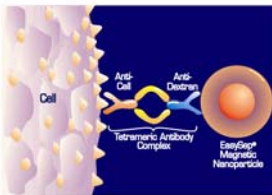
"The Big Easy" EasySep® Magnet (Catalog #18001) or RoboSep® (Catalog #20000).

**PRODUCT DESCRIPTION AND APPLICATIONS:**

EasySep® HLA Whole Blood Lymphoid Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label CD3<sup>+</sup> and CD19<sup>+</sup> cells for magnetic separation. These positive selection reagents are designed to positively select cells expressing the CD3 and CD19 antigens from fresh buffy coat or 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 cytometry 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.

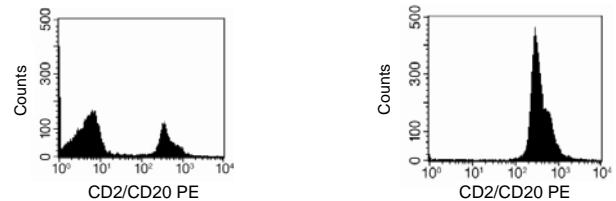
**Recommended Medium.** The recommended medium is RoboSep® Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) containing 2% Fetal Bovine Serum (FBS, Catalog #07905) and 1 mM EDTA. Medium should be Ca<sup>++</sup> and Mg<sup>++</sup> free.

**Preparing a Buffy Coat.** Positive selection of Lymphoid cells from buffy coat uses less reagent per mL of blood and reduces donor variability (see below). Add 1 part recommended medium to 1 part whole blood. Centrifuge at room temperature at 200 x g for 10 minutes with the brake off. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells, to a 14 mL polystyrene tube. The purpose of this step is to concentrate leukocytes approximately 5-fold while maintaining the same hematocrit.

**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 preparing a buffy coat before cell separation (see above), or by washing the blood after collection (contact techsupport@stemcell.com for a suggested protocol).

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.

**Assessing Purity.** The lymphoid positive selection cocktail uses the anti-CD3 antibody UCHT-1 and the anti-CD19 antibody AE1. Both may block some anti-CD3 and anti-CD19 clones used to assess purity by flow cytometry. We recommend using a combination of PE-conjugated anti-CD2 and anti-CD20 antibodies (Catalog #10501 and #10510 respectively). A secondary fluorochrome-conjugated antibody, such as FITC-labeled sheep anti-mouse IgG, can also be used to assess purity.

**TYPICAL EASYSEP® LYMPHOID CELL SELECTION PROFILE:**Start\*: 33.1% CD2<sup>+</sup>/CD20<sup>+</sup> CellsSelected: 90.0% CD2<sup>+</sup>/CD20<sup>+</sup> Cells

Starting with fresh whole blood, the CD2<sup>+</sup>/CD20<sup>+</sup> cell content of the enriched fraction typically ranges from 97.9 - 99.9%.

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

**COMPONENT DESCRIPTIONS:****EasySep® HLA Whole Blood Lymphoid Positive Selection Cocktail**

code #18684HC

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 CD3 or CD19. The mouse monoclonal antibody subclass is IgG<sub>1</sub>. This cocktail is supplied in phosphate buffered saline and contains an antibody against human Fc receptor. 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.

**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® HLA Lymphoid 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® 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. 1X Lysis Buffer is stable at 4°C for 3 months. Do not freeze.

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