PROCEDURE

EasySep™

POSITIVE SELECTION

STEMCELL™ TECHNOLOGIES

HLA BUFFY COAT OR WHOLE BLOOD CD2 POSITIVE SELECTION KIT

CATALOG #18687HLA

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™.

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 heparinized blood collection tube. CD2+ 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 whole blood or buffy coat 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 (Corning® 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:

   - Human CD2 WB Positive Selection 18687HLA

   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

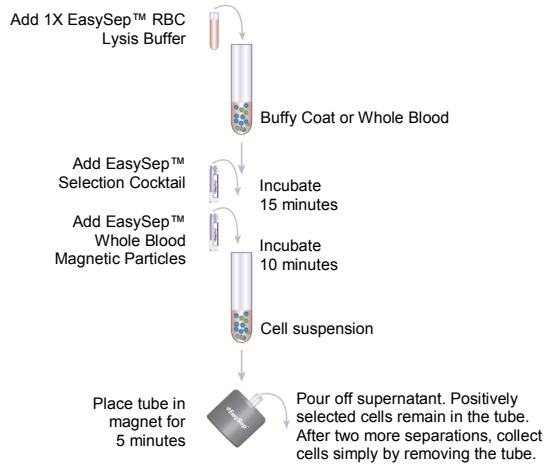
4. Mix the EasySep™ Whole Blood Magnetic Particles to ensure that they are in a uniform suspension by vigorously pipetting up and down more than 5 times. Vortexing is not recommended.

5. 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™.

6. 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.

7. If proceeding to flow cytometric crossmatch analysis, add 200 µL of EasySep™ HLA FCXM Blocking Solution to the resuspended cells.

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 heparinized blood collection tube. CD2+ 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 whole blood or buffy coat 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 Big Easy" EasySep™ Magnet.

   Falcon® 14 mL Polystyrene Round-Bottom Tubes (Corning® 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 the EasySep™ HLA WB CD2 Positive Selection Cocktail at **25 µL/mL of sample/lysis buffer mixture** (e.g. for 2 mL of sample/lysis buffer, add 50 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes.**

4. Mix the EasySep™ Whole Blood Magnetic Particles to ensure that they are in a uniform suspension by vigorously pipetting up and down more than 5 times. Vortexing is not recommended.

5. Add the EasySep™ Whole Blood Magnetic Particles at **25 µL/mL of sample/lysis buffer mixture** (e.g. for 2 mL of sample/lysis buffer mixture, add 50 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes.**

6. If total volume is less than 2.5 mL, add recommended medium to **5 mL,** otherwise add recommended medium to **10 mL**. 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.**

7. 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.

8. Remove the tube from the magnet and add **5 mL** or **10 mL** (as in Step 6) 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**.

9. Repeat Step 7 and 8, and then Step 7 once more for a total of 3 x 5-minute separations in the magnet. Remove the tube 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.

10. If proceeding to flow cytometric crossmatch analysis, add 200 µL of EasySep™ HLA FCXM Blocking Solution to the resuspended cells.

CATALOG #18687HLA For labeling 60 mL buffy coat or whole blood

Components:

- EasySep™ HLA WB CD2 Positive Selection Cocktail 3 x 1.0 mL
- EasySep™ Whole Blood Magnetic Particles 3 x 1.0 mL
- EasySep™ HLA FCXM Blocking Solution 5 x 2.0 mL
- EasySep™ 10X RBC Lysis Buffer 10 mL

EasySep™ POSITIVE SELECTION

REQUIRED EQUIPMENT:

"The Big Easy" EasySep™ Magnet (Catalog #18001), or RoboSep™.

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ HLA WB CD2 Positive Selection Cocktail and EasySep™ Whole Blood Magnetic Particles label CD2+ cells for magnetic separation. These reagents are designed to positively select CD2+ cells from fresh whole blood. CD2 is expressed on T cells and NK cells. Positively selected cells are compatible with flow cytometric crossmatch analysis (when used with EasySep™ HLA FCXM Blocking Solution) and other downstream assays.

EASYSEP™ LABELING OF HUMAN CELLS:

Target cells are specifically labeled with dextran-coated magnetic particles using bispecific Tetrameric Antibody Complexes (TACs). These complexes recognize both dextran and the target cell surface antigen (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep™ procedure (reverse side).

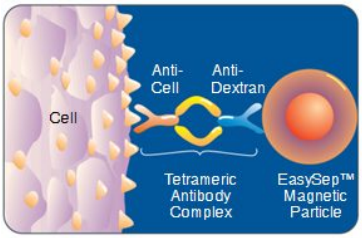


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

EASYSEP™ 10X 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), EasySep™ Buffer (Catalog #20144), or phosphate-buffered saline (PBS) + 2% fetal bovine serum (FBS) (Catalog #07905) with 1 mM EDTA. Medium should be Ca^{2+} and Mg^{2+} free.

PREPARING A BUFFY COAT Positive selection of CD2+ 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 200 x *g* for 10 minutes at room temperature (15 - 25°C) with the break off. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells, and transfer 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 labeling with fluorescently-conjugated antibodies against dextran, CD41 and CD45.

Potential aggregation can be avoided by preparing a buffy coat before cell separation (see above), or 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.

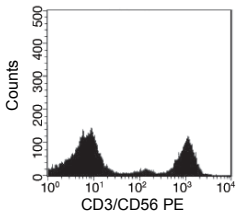
If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

ASSESSING PURITY The EasySep™ HLA WB CD2 Positive Selection Cocktail uses the anti-CD2 antibody clone MT910. To our knowledge this clone blocks all anti-CD2 antibody clones used to assess purity by flow cytometry. One of the following methods can be used to assess purity:

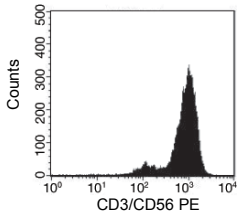
1. Use alternative markers after separation such as anti-CD3 or anti-CD56 antibodies.
2. Use a secondary fluorochrome-conjugated antibody, such as FITC-labeled sheep anti-mouse IgG.

TYPICAL EASYSEP™ HLA CD2+ CELL SELECTION PROFILE:

Start: 36.2% CD2+ Cells



Selected: 99.2% CD2+ Cells



Starting with fresh whole blood, the CD2+ cell content of the isolated fraction (as assessed by anti-CD3 and anti-CD56 staining) typically ranges from 95.7 - 99.6%.

COMPONENT DESCRIPTIONS:

EASYSEP™ HLA WB CD2 POSITIVE SELECTION COCKTAIL CODE #18687HC

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 TACs which are directed against CD2 and dextran. The mouse monoclonal antibody subclass is IgG_1. This cocktail is supplied in PBS 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™ WHOLE BLOOD MAGNETIC PARTICLES CODE #18180H

A suspension of magnetic dextran iron particles in water.

EASYSEP™ HLA FCXM BLOCKING SOLUTION CODE #18210HC

A blocking solution required for flow cytometric crossmatch analysis following cell isolation with EasySep™ or RoboSep™.

EASYSEP™ 10X RBC LYSIS BUFFER CODE #20110

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

STABILITY AND STORAGE:

EASYSEP™ HLA WB CD2 POSITIVE SELECTION COCKTAIL

EASYSEP™ WHOLE BLOOD MAGNETIC PARTICLES

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™ HLA FCXM BLOCKING SOLUTION

Stable at room temperature (15 - 25°C) until expiry date as indicated on label. Please note that repeated exposure to air may cause some crystallization to occur around the edge of the tube. This crystallization does not affect the performance of the blocking solution in flow cytometric crossmatch analysis.

EASYSEP™ 10X RBC LYSIS BUFFER

Product stable at room temperature (15 - 25°C) until expiry date as indicated on label. Once diluted 1X Lysis Buffer is stable at 2 - 8°C for 3 months. Do not freeze.