PROCEDURE

EasySep™

POSITIVE SELECTION

STEMCELL™ TECHNOLOGIES

HLA BUFFY COAT OR WHOLE BLOOD CD33 POSITIVE SELECTION KIT

CATALOG #18287HLA

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 (BD Biosciences, 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:

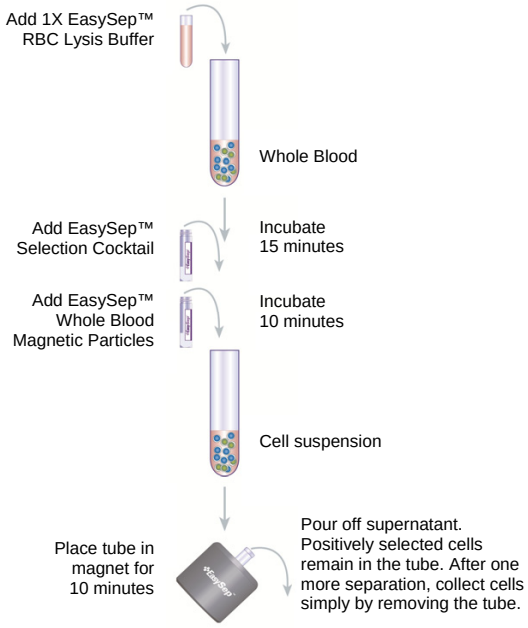
   - Human CD33 WB Positive Selection 18287HLA

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

4. Load the RoboSep™ carousel as directed by the on-screen prompts. 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. 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 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 Big Easy" EasySep™ Magnet.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD Biosciences, 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 the EasySep™ HLA WB CD33 Positive Selection Cocktail at **25 µL/mL of whole blood/lysis buffer mixture** (e.g. for 2 mL of whole blood/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 whole blood/lysis buffer mixture** (e.g. for 2 mL of whole blood/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 **10 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 **10 mL** 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 **15 minutes**.

9. Repeat Step 7 for a total of 1 x 10-minute and 1 x 15-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.

CATALOG #18287HLA For labeling 60 mL buffy coat or whole blood

Components:

- EasySep™ HLA WB CD33 Positive Selection Cocktail 3 x 1.0 mL
- EasySep™ Whole Blood Magnetic Particles 3 x 1.0 mL
- EasySep™ 10X RBC Lysis Buffer 10 mL

EasySep™ POSITIVE SELECTION

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ HLA WB CD33 Positive Selection Cocktail and EasySep™ Whole Blood Magnetic Particles label CD33+ cells for magnetic separation. These reagents are designed to positively select CD33+ cells from fresh whole blood.

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).

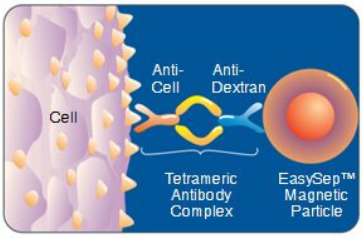


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

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), or EasySep™ Buffer (Catalog #20144), or phosphate-buffered saline (PBS) + 2% fetal bovine serum (FBS) (Catalog #07905) with 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 labeling with fluorescently-conjugated antibodies against dextran, CD41 and CD45.

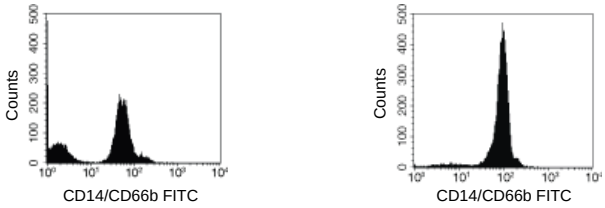
Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in the recommended medium, and centrifuge at 300 x *g* for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend sample to original volume with recommended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

ASSESSING PURITY The EasySep™ HLA WB CD33 Positive Selection Cocktail uses the anti-CD33 antibody clone p67-6, which may block some anti-CD33 antibody clones used to assess purity by flow cytometry. We recommend using a combination of FITC-conjugated anti-CD14 (Catalog #10406) and anti-CD66b (Catalog #10419) antibodies. Alternatively, a secondary fluorochrome-conjugated antibody, such as FITC-labeled goat anti-mouse IgG (Catalog #12010), can also be used to assess purity.

TYPICAL EASYSEP™ HLA CD33+ CELL SELECTION PROFILE:

Start: 54.4% CD14+/CD66b+ Cells Selected: 93.6% CD14+/CD66b+ Cells



Starting with fresh whole blood, the CD33+ cell content of the isolated fraction (as assessed by anti-CD14 and anti-CD66b antibody staining) typically ranges from 82 to 97%.

COMPONENT DESCRIPTIONS:

EASYSEP™ HLA WB CD33 POSITIVE SELECTION COCKTAIL CODE #18287HC.1

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 CD33 and dextran. The mouse monoclonal antibody subclass is IgG_1. This cocktail is supplied in PBS. 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™ 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 CD33 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™ 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.