PROCEDURE

EasySep™

POSITIVE SELECTION

STEMCELL™ TECHNOLOGIES

HLA BUFFY COAT CD56 SELECTION KIT

CATALOG #18085HLA

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 per separation.

1. Collect whole blood in a heparinized blood collection tube. Process the collected blood to obtain a buffy coat as directed (see Notes and Tips, reverse side). Transfer a maximum of 4.5 mL 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 (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 buffy coat. Mix well.

3. Select the appropriate RoboSep™ protocol:

   - Human CD56 Buffy Coat Positive Selection 18085HLA

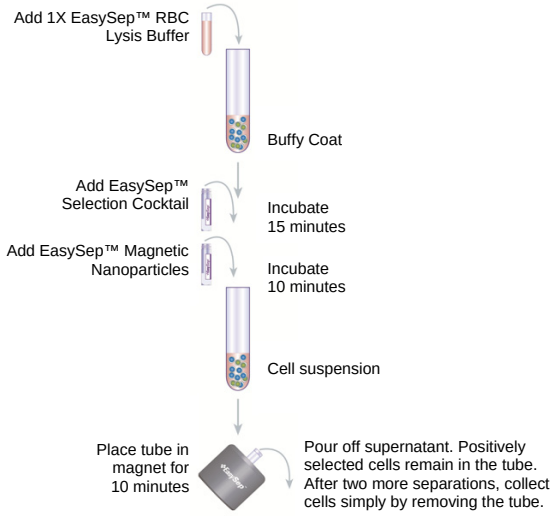
   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

4. Mix the EasySep™ Magnetic Nanoparticles 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.

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 per separation.

1. Collect whole blood in a heparinized blood collection tube. Process the collected blood to obtain a buffy coat as directed (see Notes and Tips, reverse side). Transfer a maximum of 4.5 mL 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 (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 buffy coat. Mix well.

3. Add the EasySep™ HLA BC CD56 Positive Selection Cocktail at **50 µL/mL of buffy coat/lysis buffer mixture** (e.g. for 2 mL of buffy coat/lysis buffer, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes.**

4. Mix the EasySep™ Magnetic Nanoparticles 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™ Magnetic Nanoparticles at **50 µL/mL of buffy coat/lysis buffer mixture** (e.g. for 2 mL of buffy coat/lysis buffer mixture, add 100 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 **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 1 x 10-minute and 2 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.

CATALOG #18085HLA For labeling 30 mL buffy coat

Components:

- EasySep™ HLA BC CD56 Positive Selection Cocktail 3 x 1.0 mL
- EasySep™ Magnetic Nanoparticles Positive Selection 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 Buffy Coat CD56 Positive Selection Cocktail and EasySep™ Magnetic Nanoparticles label CD56+ cells for magnetic separation. These reagents are designed to positively select CD56+ cells from freshly prepared buffy coat.

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).

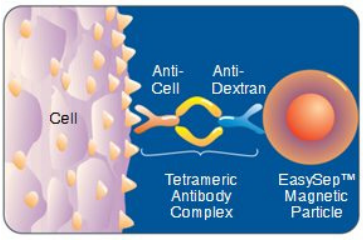


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

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 phosphate-buffered saline (PBS) + 2% fetal bovine serum (FBS) (Catalog #07905) with 1 mM EDTA. Medium should be Ca++ and Mg++ free.

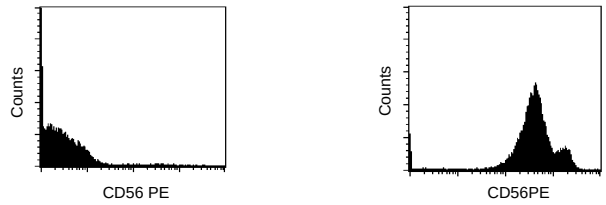
PREPARING A BUFFY COAT Collect whole blood in a heparinized blood collection tube. Add 1 part recommended medium to 1 part whole blood. Centrifuge at 200 x *g* for 10 minutes at room temperature (15 - 25°C) with the break off. Remove the concentrated leukocyte band (this is the buffy coat), plus a small 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.

ASSESSING PURITY The EasySep™ HLA BC CD56 Positive Selection Cocktail uses the anti-CD56 antibody clone B159. Purity can be assessed using one of the following methods:

- Use the anti-CD56 clones HCD56 or CMSSB or NCAM16.2. These clones may be partially blocked but will still label CD56 positive cells.
- Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG, to detect the anti-CD56 primary antibody.

TYPICAL EASYSEP™ HLA CD56+ CELL SELECTION PROFILE:

Start*: 3.9% CD56+ Cells Selected: 99.4% CD56+ Cells



Starting with buffy coat, the CD56+ cell content of the isolated fraction typically ranges from 89.7 - 99.8%.

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

COMPONENT DESCRIPTIONS:

EASYSEP™ HLA BC CD56 POSITIVE SELECTION COCKTAIL CODE #18085HC

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 CD56 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™ MAGNETIC NANOPARTICLES POSITIVE SELECTION CODE #18150H

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 BC CD56 POSITIVE SELECTION COCKTAIL

EASYSEP™ MAGNETIC NANOPARTICLES POSITIVE SELECTION

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.

STEMCELL™ TECHNOLOGIES

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