

**+** Positive Selection


**+** EasySep®

**PROCEDURE**

**HLA  
Buffy Coat  
CD56  
Selection Kit**

**CATALOG #18085HLA**

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 per separation.

1. Collect whole blood in a blood collection tube containing heparin or ACD. Process the collected blood 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 (Becton Dickinson, Catalog #352057) are recommended.*

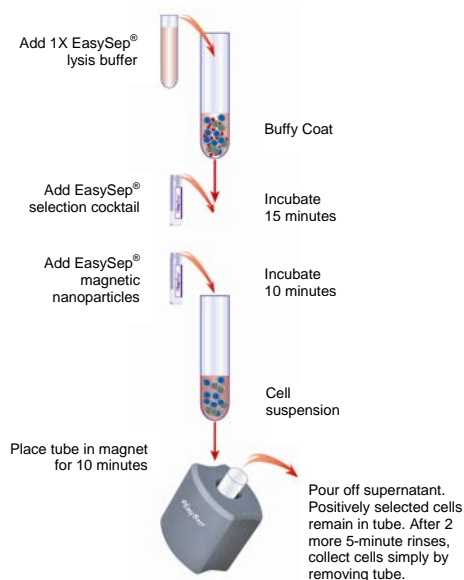
2. Add 1X EasySep® RBC Lysis Buffer (see Notes and Tips, reverse side) at a ratio of 1 part lysis buffer to 1 part sample. Mix well.
3. Select the appropriate RoboSep® protocol:

For most normal samples, select the protocol entitled “Human CD56 BC Positive Selection 18085-high purity”.

If a modified RoboSep® protocol is required, please contact StemCell Technologies’ Technical Support at techsupport@stemcell.com.

4. Load the RoboSep® carousel as directed by the on-screen prompts. Mix EasySep® Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by pipetting vigorously 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 buffy coat per separation.

1. Collect whole blood in a blood collection tube containing heparin or ACD. Process the collected blood 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 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 buffy coat. Mix well.
3. Add EasySep® Positive Selection Cocktail at **50 µL/mL** buffy coat/lysis buffer mixture (e.g. for 2 mL of buffy coat/lysis buffer mixture, add 100 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 **50 µL/mL** buffy coat/lysis buffer mixture (e.g. for 2 mL of buffy coat/lysis buffer mixture add 100 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 a total volume of 5 mL, otherwise add recommended medium to a total volume of 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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**FOR RESEARCH USE ONLY**

**#29097**

**Catalog #18085HLA**

For labeling 30 mL of Buffy Coat

**Components:**

- |  |            |
|--|------------|
| • EasySep <sup>®</sup> HLA Buffy Coat CD56 Positive Selection Cocktail | 3 x 1.0 mL |
| • EasySep <sup>®</sup> Magnetic Nanoparticles                          | 3 x 1.0 mL |
| • EasySep <sup>®</sup> RBC Lysis Buffer 10X Concentrate                | 10 mL      |

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

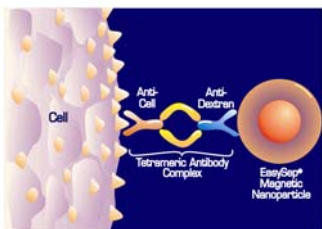
"The Big Easy" EasySep<sup>®</sup> Magnet (Catalog #18001) or RoboSep<sup>®</sup> (Catalog #20000).

**PRODUCT DESCRIPTION AND APPLICATIONS:**

EasySep<sup>®</sup> HLA Buffy Coat CD56 Positive Selection Cocktail and EasySep<sup>®</sup> Magnetic Nanoparticles label CD56<sup>+</sup> cells for magnetic separation. These positive selection reagents are designed to positively select CD56<sup>+</sup> cells (cells expressing the CD56 antigen) from freshly prepared buffy coat.

**EASYSEP<sup>®</sup> 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 cytometric analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep<sup>®</sup> procedure (reverse side).



**Figure 1.**  
Schematic Drawing of EasySep<sup>®</sup> TAC Magnetic Labeling of Human Cells.

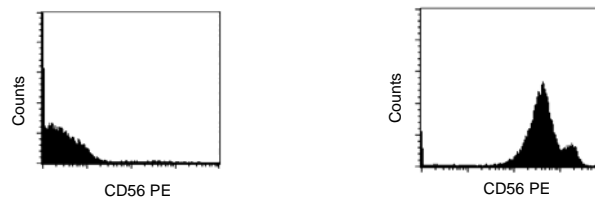
**NOTES AND TIPS:**

**Buffy Coat Preparation.** Collect whole blood in a heparinized blood collection tube. Add 1 part recommended medium to 1 part fresh whole blood (the sample must be washed before use to remove donor-specific soluble serum factor(s) that can cause cross-linking with magnetic nanoparticles). Spin sample at 200 x g for 10 minutes at room temperature with the brake off. Remove the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated RBCs. The target is to concentrate leukocytes approximately 5-fold while maintaining the same hematocrit. Transfer a maximum of 4.5 mL of buffy coat to a 14 mL polystyrene tube.

**EasySep<sup>®</sup> 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<sup>®</sup> 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.

**Assessing Purity.** The CD56 Positive Selection Cocktail uses the anti-CD56 antibody clone B159. We recommend the clone NCAM16.2 to assess purity by flow cytometry. A fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG, can also be used to assess purity.

**TYPICAL EASYSEP<sup>®</sup> CD56 SELECTION PROFILE:**Start\*: 3.9% CD56<sup>+</sup> CellsSelected: 99.4% CD56<sup>+</sup> Cells

Starting with buffy coat, the CD56<sup>+</sup> cell content of the enriched fraction typically ranges from 89.7 - 99.8%.

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

**COMPONENT DESCRIPTIONS:**

**EasySep<sup>®</sup> HLA Buffy Coat 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 tetrameric antibody complexes (TAC) which are directed against CD56 and dextran. The mouse monoclonal antibody subclass is IgG<sub>1</sub>. This cocktail is supplied in PBS. Also contains an antibody directed against mouse CD16/32 (Fcγ III/II 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<sup>®</sup> Magnetic Nanoparticles** code #18150  
A suspension of magnetic dextran iron particles in water.

**EasySep<sup>®</sup> 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<sup>®</sup> HLA Buffy Coat CD56 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<sup>®</sup> 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<sup>®</sup> RBC Lysis Buffer 10X Concentrate**

10X Concentrate is stable at room temperature for 2 years. Store at room temperature. 1X Lysis Buffer is stable at 4°C for 3 months. Store at 2 - 8°C. Do not freeze.

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