

**THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP® (SECTION A) OR THE SILVER "THE BIG EASY" 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. CD3<sup>+</sup> 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 (BD, Catalog #352057) are recommended.*

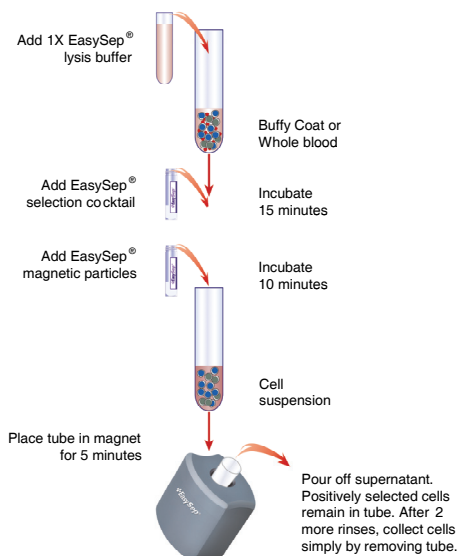
2. Add 1X EasySep® RBC Lysis Buffer (see Notes and Tips) at a ratio of 1 part lysis buffer to 1 part sample. Mix well.

3. Select the appropriate RoboSep® protocol:
  - For most samples, select the protocol entitled "Human CD3 WB Positive Selection 18081HLA".

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.
6. If proceeding to flow cytometric crossmatch analysis, add 200 µL of EasySep® HLA FCXM Blocking Solution (Catalog #18210HC) to the resuspended cells. The positively selected cells are now ready for use.

**MANUAL EASYSEP® PROTOCOL DIAGRAM**



**B) MANUAL EASYSEP® PROTOCOL USING THE SILVER "THE BIG EASY" 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. CD3<sup>+</sup> 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 (BD, Catalog #352057) are recommended.*

2. Add 1X EasySep® RBC Lysis Buffer (see Notes and Tips) 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 (15 - 25°C) for 15 minutes.

4. Mix EasySep® Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. Vortexing is not recommended. Add the particles 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 (15 - 25°C) for 10 minutes.

5. If total volume is less than 2.5 mL, add the recommended medium (see Notes and Tips) to 5 mL, otherwise add the 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.

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 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.*

7. Remove the tube from the magnet and add 10 mL of the 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.

8. Repeat Steps 6 and 7, and then Step 6 once more, for a total of three 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.

9. If proceeding to flow cytometric crossmatch analysis, add 200 µL of EasySep® HLA FCXM Blocking Solution (Catalog #18210HC) to the resuspended cells. The positively selected cells are now ready for use.

## Components:

• EasySep® HLA Whole Blood CD3 Positive Selection Cocktail	3 x 1.0 mL
• EasySep® Magnetic Nanoparticles	3 x 1.0 mL
• EasySep® HLA FCXM Blocking Solution	5 x 2.0 mL
• EasySep® RBC Lysis Buffer 10X Concentrate	10 mL




POSITIVE SELECTION

## REQUIRED EQUIPMENT:

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

## PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® HLA Whole Blood CD3 Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label CD3<sup>+</sup> cells for magnetic separation. These reagents are designed to positively select CD3<sup>+</sup> cells (cells expressing the CD3 antigen) from fresh buffy coat or whole blood. Positively selected cells are compatible with flow cytometric crossmatch analysis (when used with EasySep® HLA FCXM Blocking Solution) and any other downstream assay.

## 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 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 FACS analysis. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep® procedure (reverse side).

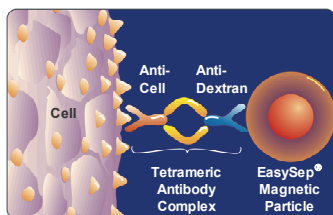


Figure 1.

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 one 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 PBS containing 2% 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 CD3<sup>+</sup> 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.

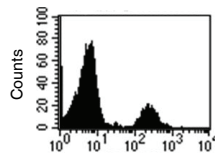
**DONOR VARIABILITY.** Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic nanoparticles. This may result in visible aggregates in the enriched cell fraction following positive selection directly from whole blood. These aggregates may appear as a distinct, 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.

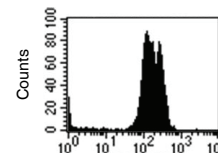
**ASSESSING PURITY.** The CD3 Positive Selection Cocktail uses the anti-CD3 antibody clone UCHT-1. To our knowledge the cocktail blocks all anti-CD3 antibody clones used to assess purity by flow cytometry. We recommend one of the following methods to assess purity:

1. Add PE-labeled antibodies at the same time as the cocktail: Add the fluorochrome-conjugated anti-CD3 (Catalog #10502) antibody at a concentration of 0.04 µg/mL immediately after adding the cocktail to provide a strong detection signal without affecting separation performance. **This method labels the positive cells in the entire sample.**
2. Use alternative markers after separation: detect CD5<sup>+</sup>CD20<sup>-</sup> or CD2<sup>+</sup> cells.
3. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG, to detect the anti-CD3 TAC.

## TYPICAL EASYSEP® HUMAN CD3 POSITIVE SELECTION PROFILE:

Start\*: 14.8% CD3<sup>+</sup> CellsSelected: 99.7% CD3<sup>+</sup> Cells

CD3 FITC



CD3 FITC

Starting with fresh whole blood, the CD3<sup>+</sup> cell content of the enriched fraction typically ranges from 93 - 99%.

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

## COMPONENT DESCRIPTIONS:

## EASYSEP® HLA WHOLE BLOOD CD3 POSITIVE SELECTION COCKTAIL

CODE #18081HC.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 Tetrameric Antibody Complexes which are directed against CD3 and dextran. 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 #18150H

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® 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 WHOLE BLOOD CD3 SELECTION COCKTAIL

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® MAGNETIC NANOPARTICLES

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® RBC LYSIS BUFFER 10X CONCENTRATE

10X concentrate stable at room temperature (15 - 25°C) for 2 years from date of manufacture as indicated on label. Store at room temperature (15 - 25°C). 1X Lysis Buffer is stable at 2 - 8°C for 3 months. Store at 2 - 8°C. Do not freeze.

## EASYSEP® HLA FCXM BLOCKING SOLUTION

Product stable at room temperature (15 - 25°C) until expiry date as indicated on label. Contents have been sterility tested. 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.

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