

## HUMAN WHOLE BLOOD CD3 POSITIVE SELECTION KIT

CATALOG #18081

**THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP® (SECTION A) OR THE SILVER "THE BIG EASY" EASYSEP® MAGNET (SECTION B - NORMAL SAMPLES OR SECTION C - ABNORMAL SAMPLES).**

### 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 magnet).

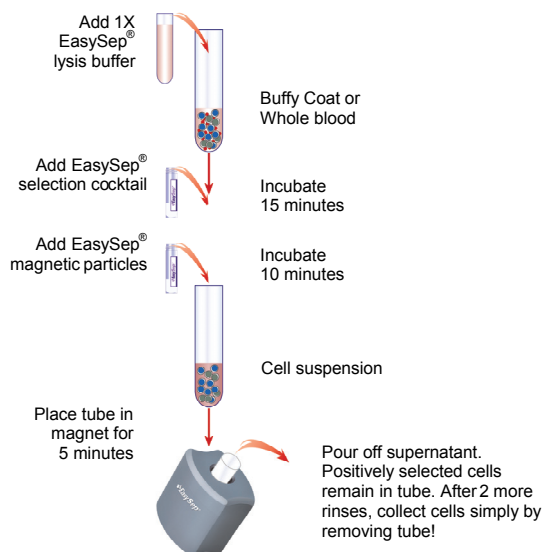
*Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.*

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:
  - For most normal samples, select the protocol entitled "**Human CD3 WB Positive Selection 18081-high purity**".
  - For samples with an abnormally low proportion of CD3<sup>+</sup> cells, or in which T cells express CD3 at a low level (e.g. for chimerism analysis following bone marrow transplantation), select the protocol entitled "**Human CD3 WB Positive Selection 18081-chimerism**".

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. 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) WITH NORMAL SAMPLES.

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 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, reverse side) at a ratio of 1 part lysis buffer to 1 part whole blood. Mix well.
3. Add EasySep® Positive Selection Cocktail at **25 µL/mL** whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/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** whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/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 recommended medium (see Notes and Tips) 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**.
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 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 3 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

### C) MANUAL EASYSEP® PROTOCOL USING THE SILVER "THE BIG EASY" EASYSEP® MAGNET (CATALOG #18001) FOR SAMPLES WITH AN ABNORMALLY LOW PROPORTION OF CD3<sup>+</sup> CELLS, OR IN WHICH T CELLS EXPRESS CD3 AT A LOW LEVEL (E.G. FOR CHIMERISM ANALYSIS FOLLOWING BONE MARROW TRANSPLANTATION).

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 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, reverse side) at a ratio of 1 part lysis buffer to 1 part whole blood. Mix well.
3. Add EasySep® Positive Selection Cocktail at **25 µL/mL** whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/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** whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/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 recommended medium (see Notes and Tips) 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 **8 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** 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 twice, and then Step 6 once more, for a total of 1 x 8-minute and 3 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

## Components:

• EasySep® Human Whole Blood CD3 Positive Selection Cocktail	3 x 1.0 mL
• EasySep® Whole Blood Magnetic Nanoparticles	3 x 1.0 mL
• EasySep® RBC Lysis Buffer 10X Concentrate	10 mL



POSITIVE SELECTION

**REQUIRED EQUIPMENT:**

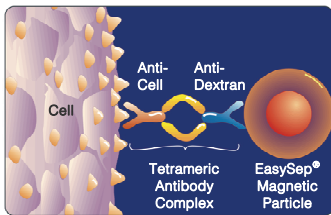
"The Big Easy" EasySep® Magnet (Catalog #18001), or RoboSep® (Catalog #20000).

**PRODUCT DESCRIPTION AND APPLICATIONS:**

EasySep® Human Whole Blood CD3 Positive Selection Cocktail and EasySep® Whole Blood 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 whole blood.

**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).

**NOTES AND TIPS:**

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

**EASYSEP® 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 PBS containing 2% FBS (Catalog #07905) and 1 mM EDTA. Media should be Ca<sup>++</sup> and Mg<sup>++</sup> free.

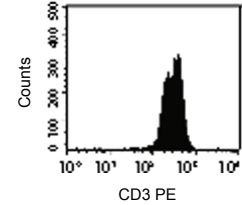
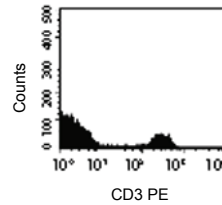
**DONOR VARIABILITY.** Certain donors express 1 or more soluble serum factors that can cause cross-linking with magnetic particles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population 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 staining with fluorescently-labeled 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 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 for CD3<sup>+</sup> selection.
3. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG.

**TYPICAL EASYSEP® HUMAN CD3 POSITIVE SELECTION PROFILE:**Start\*: 14.8% CD3<sup>+</sup> CellsSelected: 99.7% CD3<sup>+</sup> Cells

Starting with fresh whole blood, the CD3<sup>+</sup> cell content of the enriched fraction typically ranges from 98.2 - 99.8%.

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

**COMPONENT DESCRIPTIONS:****EASYSEP® HUMAN WHOLE BLOOD CD3 POSITIVE SELECTION COCKTAIL**

CODE #18081C.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 #18150

A suspension of magnetic dextran iron particles in water.

**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® HUMAN 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.

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