



THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP® (SECTION A), THE PURPLE EASYSEP® MAGNET (SECTION B) OR "THE BIG EASY" SILVER EASYSEP® MAGNET (SECTION C).

A) FULLY AUTOMATED PROTOCOL USING ROBOSEP® (CATALOG #20000).

This procedure is used for processing 250 μ L – 8.5 mL of sample (up to 8.5×10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^8 cells/mL in RoboSep® Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 μ L.

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

2. Select the appropriate RoboSep® Protocol:

- For most samples, select the protocol entitled "Human CD3 Positive Selection 18051HLA".

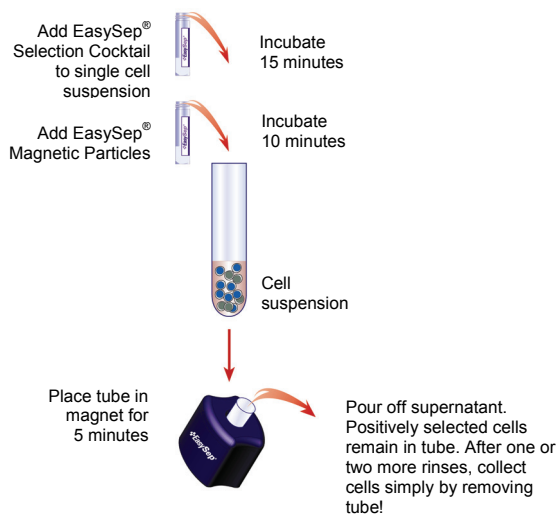
If a modified RoboSep® protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

3. 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®.

4. 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.

5. If proceeding to flow cytometric crossmatch analysis, add 200 μ L 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 PURPLE EASYSEP® MAGNET (CATALOG #18000).

This procedure is used for processing 100 μ L – 2.5 mL of sample (up to 2.5×10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^8 cells/mL in recommended medium (See Notes and Tips, reverse side). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the EasySep® Magnet. For samples containing 10^7 cells or fewer, resuspend in 100 μ L.

Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.

2. Add EasySep® Positive Selection Cocktail at 100 μ L/mL cells (e.g. for 2 mL of cells add 200 μ L of cocktail). Mix well and incubate at room temperature (15 – 25°C) for 15 minutes.

3. 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 50 μ L/mL cells (e.g. for 2 mL of cells add 100 μ L of nanoparticles). Mix well and incubate at room temperature (15 – 25°C) for 10 minutes.

4. Bring the cell suspension to a **total volume** of 2.5 mL by adding recommended medium. 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.

5. 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.

6. Remove the tube from the magnet and add 2.5 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.

7. Repeat Steps 5 and 6, and then Step 5 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.

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

C) MANUAL EASYSEP® PROTOCOL USING "THE BIG EASY" SILVER EASYSEP® MAGNET (CATALOG #18001).

This procedure is used for processing 250 μ L – 8 mL of sample (up to 8×10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^8 cells/mL in recommended medium (See Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the magnet. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 μ L.

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

2. Add EasySep® Positive Selection Cocktail at 100 μ L/mL cells (e.g. for 2 mL of cells add 200 μ L of cocktail). Mix well and incubate at room temperature (15 – 25°C) for 15 minutes.

3. 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 50 μ L/mL cells (e.g. for 2 mL of cells add 100 μ L of nanoparticles). Mix well and incubate at room temperature (15 – 25°C) for 10 minutes.

4. Bring the cell suspension to a **total volume** of 5 mL (for $<10^8$ cells) or 10 mL (for $1 - 8 \times 10^8$ cells) by adding recommended medium. 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.

5. 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.

6. Remove the tube from the magnet and add 5 mL (for $<10^8$ cells) or 10 mL (for $1 - 8 \times 10^8$ cells) 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.

7. Repeat Steps 5 and 6, and then Step 5 once more, for a total of three 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.

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

Components:

• EasySep [®] HLA CD3 Positive Selection Cocktail	1.0 mL
• EasySep [®] Magnetic Nanoparticles	1.0 mL
• EasySep [®] HLA FCXM Blocking Solution	5 x 2.0 mL



POSITIVE SELECTION

REQUIRED EQUIPMENT:

EasySep[®] Magnet (Catalog #18000), or "The Big Easy" EasySep[®] Magnet (Catalog #18001), or RoboSep[®] (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] HLA CD3 Positive Selection Cocktail and EasySep[®] Magnetic Nanoparticles label CD3⁺ cells for magnetic separation. These reagents are designed to positively select CD3⁺ cells (cells expressing the CD3 antigen) from fresh or previously frozen peripheral blood mononuclear cells. 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:

Unwanted 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 expressed on the unwanted cell (Figure 1). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep[®] procedure (reverse side).

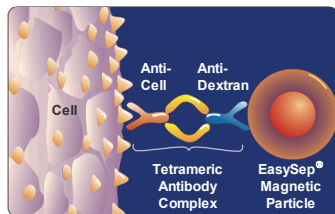


Figure 1.
Schematic Drawing of EasySep[®] TAC
Magnetic Labeling of Human Cells.

NOTES AND TIPS:

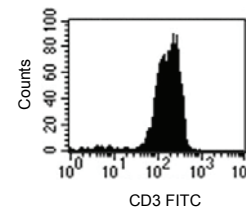
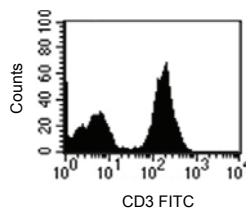
PREPARING A MONONUCLEAR CELL SUSPENSION. Prepare a mononuclear cell suspension from whole peripheral blood by Ficoll-Paque™ density separation (Catalog #07957). For previously frozen mononuclear cells, we recommend incubating the cells with 100 µg/mL DNase I (Catalog #07900) for at least 15 minutes at room temperature (15 – 25°C) prior to labeling and separation. Filter clumpy suspensions through a 30 µm mesh nylon strainer for optimal results.

RECOMMENDED MEDIUM. The recommended medium is PBS containing 2% FBS (Catalog #07905) and 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free.

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 0.4 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 T cell markers after separation: detect CD5⁺/20⁻ or CD2⁺ cells.
3. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG, to detect the anti-CD3 TAC.

CD3⁺ CELL DEPLETION. The EasySep[®] HLA CD3 Positive Selection Cocktail can also be used to deplete CD3⁺ cells. Please refer to depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf.

TYPICAL EASYSEP[®] CD3 HLA SELECTION PROFILE:Start: 59.3% CD3⁺ CellsSelected: 98.3% CD3⁺ Cells

Starting with peripheral blood mononuclear cells, the CD3⁺ cell content of the enriched fraction typically ranges from 94.6 - 99.6%.

COMPONENT DESCRIPTIONS:**EASYSEP[®] HLA CD3 POSITIVE SELECTION COCKTAIL** CODE #18051HC.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 IgG₁. 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[®] HLA FCXM BLOCKING SOLUTION CODE #18210HC

A blocking solution required for flow cytometric crossmatch analysis following cell isolation with EasySep[®] or RoboSep[®].

STABILITY AND STORAGE:**EASYSEP[®] HLA CD3 POSITIVE 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[®] HLA FCXM BLOCKING SOLUTION

Product stable at room temperature (15 - 25°C) for 1 year from date of manufacture 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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