

PROCEDURE **Positive Selection**

+EasySep

Human CD4 Selection Kit

CATALOG #18052

Version 2.1.1



B) Manual EasySep[®] Protocol Using the Purple EasySep[®] Magnet (Catalog #18000).

This procedure is used for processing **100 µL - 2.5 mL** of sample ($\leq 2.5 \times 10^8$ cells)

1. Prepare mononuclear cell suspension at a concentration of 1×10^6 cells/mL in recommended medium (See Notes and Tips, reverse side). **Please see Notes and Tips for recommendations on depleting CD4⁺ monocytes prior to selection.** Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the Purple EasySep[®] Magnet. For samples containing 10^7 cells or fewer, resuspend in 100 µL.

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

2. Add EasySep[®] Positive Selection Cocktail at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature for **15 minutes**.
3. Mix magnetic nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. Vortexing is not recommended. Add the nanoparticles at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature 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 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.**
6. Remove the tube from the magnet and add 2.5 mL of 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 3 x 5-minute separations in the magnet. Remove tube from 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 Big Easy” Silver EasySep[®] Magnet (Catalog #18001).

This procedure is used for processing **250 µL - 8.5 mL** of sample ($\leq 8.5 \times 10^8$ cells)

1. Prepare mononuclear cell suspension at a concentration of 1×10^6 cells/mL in recommended medium (See Notes and Tips, reverse side). **Please see Notes and Tips for recommendations on depleting CD4⁺ monocytes prior to selection.** Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep[®] magnet. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 µL.

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

2. Add EasySep[®] Positive Selection Cocktail at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature for **15 minutes**.
3. Mix magnetic nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously more than 5 times. Vortexing is not recommended. Add the particles at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature for **10 minutes**.
4. Bring the cell suspension to a **total volume** of 5.0 mL (for $<10^8$ cells) or 10 mL (for $1 - 8.5 \times 10^8$ 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 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.**
6. Remove the tube from the magnet and add 5.0 mL (for $<10^8$ cells) or 10 mL (for $1 - 8.5 \times 10^8$ cells) of 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, then Step 5 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.

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 µL - 8.5 mL** of sample ($\leq 8.5 \times 10^8$ cells)

1. Prepare mononuclear cell suspension at a concentration of 1×10^6 cells/mL in RoboSep[®] Buffer (Catalog #20104). **Please see Notes and Tips for recommendations on depleting CD4⁺ monocytes prior to selection.** 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 µL.

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

2. Select the appropriate RoboSep[®] protocol:

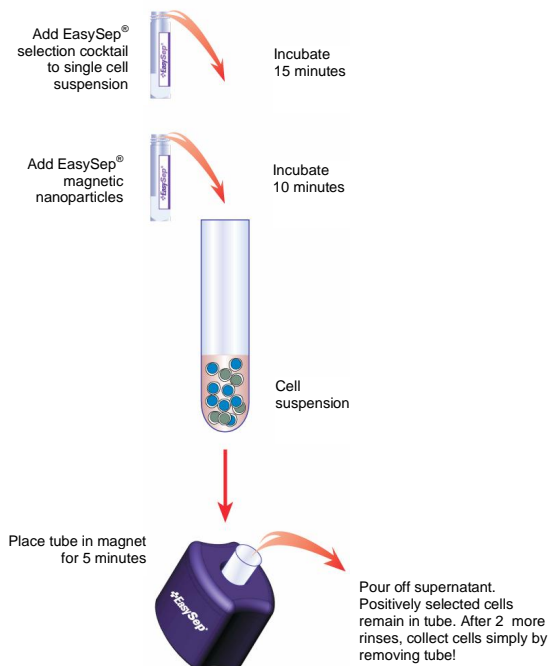
RoboSep[®] protocols can be optimized modified for high CD14⁺ cell purity or high CD4⁺ cell recovery. Select one of the protocols listed below, as appropriate.

- “Human CD4 Positive Selection 18052-high purity”.
- “Human CD4 Positive Selection 18052-high recovery”.

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. Mix EasySep[®] Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. 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. The positively selected cells are now ready for use.

Manual EasySep[®] Protocol Diagram



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October 2007

FOR RESEARCH USE ONLY

#28383

Catalog #18052	For labeling 10 ⁹ total cells
Components:	
• EasySep [®] Human CD4 Positive Selection Cocktail	1.0 mL
• EasySep [®] Magnetic Nanoparticles	1.0 mL

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] Human CD4 Positive Selection Cocktail and EasySep[®] Magnetic Nanoparticles label CD4⁺ cells for magnetic separation. These positive selection reagents are designed to positively select CD4⁺ cells (cells expressing the CD4 antigen) from fresh or previously frozen peripheral blood mononuclear cells.

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

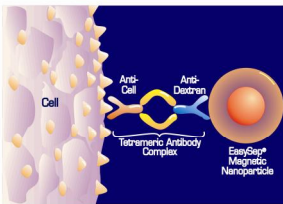


Figure 1.
Schematic Drawing of EasySep[®] TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Mononuclear Cell Suspension (without monocyte depletion). 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 DNase I (Catalog #07900) at a concentration of 100 µg/mL for at least 15 minutes at room temperature prior to labeling and separation. Filter clumpy suspensions through a 30 µm mesh nylon strainer for optimal results. If monocyte-depleted CD4⁺ T cells are desired, see below.

Monocyte Depletion and CD4⁺ T Cell Isolation. Monocyte depletion is required to isolate a pure population of CD4⁺ T cells because monocytes express the CD4 antigen. A monocyte-depleted mononuclear cell (MNC) suspension can be prepared from whole blood by Ficoll-Paque™ density separation using the RosetteSep[®] Monocyte (CD36) Depletion Cocktail (Catalog #15628). This procedure is quicker and more convenient than a magnetic separation because it combines the MNC preparation and monocyte depletion into one protocol. Monocytes can be depleted from previously frozen MNC suspensions using the StemSep[®] Human CD14 Positive Selection Cocktail (Catalog #14758).

Recommended Medium. RoboSep[®] Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) containing 2% Fetal Bovine Serum (FBS) (Catalog #07905) and 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free.

Assessing Purity. The CD4 Positive Selection Cocktail uses the anti-CD4 antibody clone QS4120 and blocks the following anti-CD4 antibody clones: RFT4, SK3, SK4, RPA.T4, 13B8.2. We recommend the clone L120 to assess purity by flow cytometry.

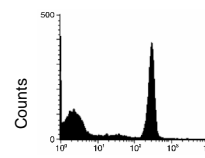
One of the following methods can also be used to assess purity:

1. Add PE-labeled antibodies at the same time as the cocktail: Add the fluorochrome-conjugated anti-CD4 antibody (Catalog #10435) 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 alternate markers after separation: detect CD3⁺/8⁻ cells.
3. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG, to detect the primary anti-CD4 antibody.

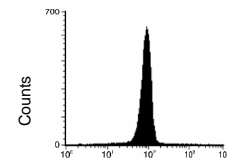
TYPICAL EASYSEP[®] CD4 SELECTION PROFILE:

Start: 47.0% CD4⁺ Cells

Selected: 98.8% CD4⁺ Cells



CD4 PE



CD4 PE

Starting with monocyte-depleted fresh peripheral blood mononuclear cells, the CD4⁺ cell content of the enriched fraction typically ranges from 97.4 - 99.5%.

COMPONENT DESCRIPTIONS:

EasySep[®] Human CD4 Positive Selection Cocktail code #18052C.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 (TAC) which are directed against CD4 and dextran. The mouse monoclonal antibody subclass is IgG₁. This cocktail is supplied in phosphate buffered saline. 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.

STABILITY AND STORAGE:

EasySep[®] Human CD4 Positive Selection Cocktail

Stable at 4°C for 2 years. Do not freeze this product. This product may be shipped at room temperature, and should be refrigerated upon receipt.

EasySep[®] Magnetic Nanoparticles

Stable at 4°C for 1 year. This product may be shipped at room temperature, and should be refrigerated upon receipt.

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