


**PROCEDURE** **Negative Selection**  
**EasySep**  
**Human Naïve CD4<sup>+</sup> T Cell Enrichment Kit**  
**CATALOG #19155**  
 Version 1.0.1



This Product Information Sheet is provided for use with RoboSep<sup>®</sup> (section A), the purple EasySep<sup>®</sup> magnet (section B) or "The Big Easy" Silver EasySep<sup>®</sup> magnet (section C).

**A) Fully Automated Protocol Using RoboSep<sup>®</sup> (Catalog #20000).**

This procedure is used for processing **500 µL - 8.5 mL** of sample ( $\leq 4.25 \times 10^8$  cells).

1. Prepare mononuclear cell suspension at a concentration of  $5 \times 10^7$  cells/mL in RoboSep<sup>®</sup> Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep<sup>®</sup> carousel.

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

2. Select the appropriate RoboSep<sup>®</sup> protocol:

RoboSep<sup>®</sup> protocols can be optimized for high naïve CD4<sup>+</sup> T cell purity or high naïve CD4<sup>+</sup> T cell recovery. Select one of the protocols listed below, as appropriate.

- "Human Naïve CD4<sup>+</sup> T Cell Negative Selection 19155-high purity".
- "Human Naïve CD4<sup>+</sup> T Cell Negative Selection 19155-high recovery".

If a modified RoboSep<sup>®</sup> protocol is required, please contact *StemCell Technologies* Technical Support at [techsupport@stemcell.com](mailto:techsupport@stemcell.com).

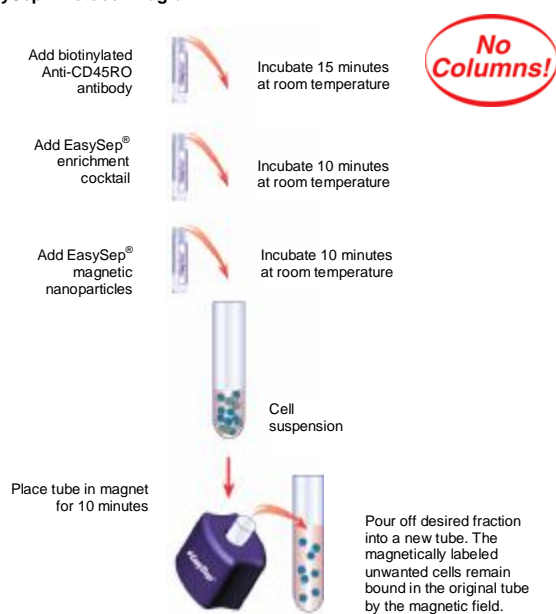
3. Load the RoboSep<sup>®</sup> carousel as directed by the on-screen prompts. Mix EasySep<sup>®</sup> Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. Do not vortex. When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep<sup>®</sup>.

4. When cell separation is complete, remove the tube containing the enriched cells from the RoboSep<sup>®</sup> carousel:

- After the 2-quadrant high purity protocol, collect the enriched cells in the 14 mL tube located to the left of the magnet in the second quadrant.
- After the 1-quadrant high recovery protocol, collect the enriched cells in the 50 mL tube located to the left of the tip rack.

The enriched cells are now ready for use.

**Manual EasySep<sup>®</sup> Protocol Diagram**



**B) Manual EasySep<sup>®</sup> Protocol Using Purple EasySep<sup>®</sup> Magnet (Catalog #18000).**

This procedure is used for processing **250 µL - 2 mL** of sample ( $\leq 1 \times 10^8$  cells).

1. Prepare mononuclear cell suspension at  $5 \times 10^7$  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 Purple EasySep<sup>®</sup> Magnet.

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

2. Add Anti-CD45RO Antibody at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of antibody). Mix well and incubate at room temperature for **15 minutes**.
3. Add EasySep<sup>®</sup> Negative Selection Human Naïve CD4<sup>+</sup> T Cell Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature for **10 minutes**.
4. Mix EasySep<sup>®</sup> Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. Do not vortex. Add the nanoparticles at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of nanoparticles). Mix well and incubate at room temperature for **10 minutes**.
5. Bring the sample to a **total volume** of 2.5 mL with recommended medium. Mix the cells by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet for **10 minutes**.
6. Pick up the EasySep<sup>®</sup> Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 5 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the EasySep<sup>®</sup> magnet. Leave the magnet and the 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 original tube containing the labeled unwanted cells from the EasySep<sup>®</sup> Magnet and place the new tube containing the desired cells inside the magnet to perform a second round of magnetic separation. Set aside for **10 minutes** and repeat Step 6. The enriched cells are now ready for use.

**Additional Notes:**

- I. Performing only a single round of magnetic separation (i.e. stopping the procedure after Step 6) will improve recovery, but may reduce purity.
- II. Some of the desired cells may be left behind in the original tube in Step 6. These cells may be recovered by resuspending the magnetically labeled cells in 2.5 mL of recommended medium and performing an additional round of magnetic separation. The supernatant of this separation step can be combined with the primary desired fraction. This will improve recovery, but may decrease purity. Purity assessment prior to pooling is therefore recommended, especially for cell populations with lower start percentages.

**C) Manual EasySep<sup>®</sup> Protocol Using "The Big Easy" Silver EasySep<sup>®</sup> Magnet (Catalog #18001).**

This procedure is used for processing **500 µL - 8.5 mL** of sample ( $\leq 4.25 \times 10^8$  cells).

1. Prepare mononuclear cell suspension at  $5 \times 10^7$  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 Silver EasySep<sup>®</sup> magnet.

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

2. Add Anti-CD45RO Antibody at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of antibody). Mix well and incubate at room temperature for **15 minutes**.
3. Add EasySep<sup>®</sup> Negative Selection Human Naïve CD4<sup>+</sup> T Cell Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature for **10 minutes**.
4. Mix EasySep<sup>®</sup> Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting more than 5 times. Do not vortex. Add the particles at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of nanoparticles). Mix well and incubate at room temperature for **10 minutes**.
5. Bring the sample to a **total volume** of 5.0 mL (for  $<10^8$  cells) or 10 mL (for  $1 - 4.25 \times 10^8$  cells) with recommended medium. Mix the cells by gently pipetting 2 - 3 times. Place the tube (without cap) into the magnet for **10 minutes**.
6. Pick up the EasySep<sup>®</sup> Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 14 mL tube. The magnetically labeled unwanted cells will remain bound inside the original tube, held by the magnetic field of the EasySep<sup>®</sup> 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 original tube containing the labeled unwanted cells from the EasySep<sup>®</sup> Magnet and place the new tube containing the desired cells inside the magnet to perform a second round of magnetic separation. Set aside for **10 minutes** and repeat Step 6. The enriched cells are now ready for use.

**Additional Notes:**

- I. Performing only a single round of magnetic separation (i.e. stopping the procedure after Step 6) will improve recovery, but may reduce purity.
- II. Some of the desired cells may be left behind in the original tube in Step 6. These cells may be recovered by resuspending the magnetically labeled cells in 5 mL (for  $<10^8$  cells) or 10 mL (for  $1 - 4.25 \times 10^8$  cells) of recommended medium and performing an additional round of magnetic separation. The supernatant of this separation step can be combined with the primary desired fraction. This will improve recovery, but may decrease purity. Purity assessment prior to pooling is therefore recommended, especially for cell populations with lower start percentages.

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**FOR RESEARCH USE ONLY**

**#28925**

**Catalog #19155**For labeling  $10^9$  total cells**Components:**

- EasySep<sup>®</sup> Negative Selection Human Naïve CD4<sup>+</sup> T Cell Enrichment Cocktail 1.0 mL
- Biotinylated Anti-CD45RO Antibody 1.0 mL
- EasySep<sup>®</sup> Magnetic Nanoparticles 3 x 1.0 mL

**REQUIRED EQUIPMENT:**

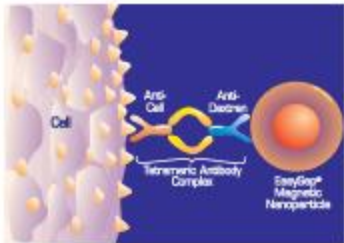
EasySep<sup>®</sup> Magnet (Catalog #18000), or "The Big Easy" EasySep<sup>®</sup> Magnet (Catalog #18001), or RoboSep<sup>®</sup> (Catalog #20000).

**PRODUCT DESCRIPTION AND APPLICATIONS:**

The Biotinylated Anti-CD45RO Antibody, EasySep<sup>®</sup> Negative Selection Human Naïve CD4<sup>+</sup> T Cell Enrichment Cocktail and EasySep<sup>®</sup> Magnetic Nanoparticles label non-CD4<sup>+</sup> T cells and memory CD4<sup>+</sup> T cells for magnetic separation. These reagents are designed to enrich Naïve CD4<sup>+</sup> T cells from fresh or previously frozen peripheral blood mononuclear cells by depletion of non-CD4<sup>+</sup> T cells and memory CD4<sup>+</sup> T cells.

**EASYSEP<sup>®</sup> 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 cells (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<sup>®</sup> procedure (reverse side).



**Figure 1.**  
Schematic Drawing of EasySep<sup>®</sup> 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™ PLUS 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.

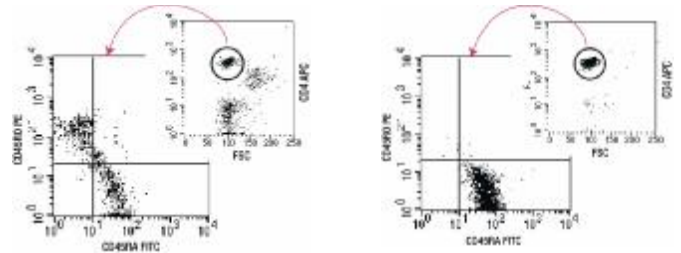
**Optimal Cell Number.** The use of fewer than  $5 \times 10^7$  cells per separation may result in sub-optimal performance.

**Recommended Medium.** The recommended medium is RoboSep<sup>®</sup> Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) containing 2% FBS (Catalog #07905). Medium should be Ca<sup>++</sup> and Mg<sup>++</sup> free.

**Assessing Purity.** Purity of Naïve CD4<sup>+</sup> T cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD4 antibody (e.g. anti-CD4 APC), anti-CD45RO antibody (e.g. anti-CD45RO PE, Catalog #10524) and anti-CD45RA antibody (e.g. anti-CD45RA FITC, Catalog #10418).

**TYPICAL EASYSEP<sup>®</sup> NAÏVE CD4<sup>+</sup> T CELL ENRICHMENT PROFILE:**

Start: 15% CD4<sup>+</sup>CD45RA<sup>+</sup>CD45RO<sup>-</sup> Cells Enriched: 94% CD4<sup>+</sup>CD45RA<sup>+</sup>CD45RO<sup>-</sup> Cells



Starting with previously frozen mononuclear cells, the Naïve CD4<sup>+</sup> T cell content of the enriched fraction typically ranges from 91 - 95%.

**COMPONENT DESCRIPTIONS:**

**EasySep<sup>®</sup> Negative Selection Human Naïve CD4<sup>+</sup> T Cell Enrichment Cocktail** code #19155C

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 cell surface antigens on human blood cells (CD8, CD14, CD16, CD19, CD20, CD36, CD56, CD123, TCRγ/δ, glycophorin A) and dextran. A biotinylated anti-CD45RO antibody is supplied in a separate vial. A bispecific TAC (against biotin and dextran) is included in the enrichment cocktail. The mouse monoclonal antibody subclass is IgG<sub>1</sub>. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

**Biotinylated Anti-CD45RO Antibody** code #19156C

Biotinylated monoclonal antibody against human CD45RO. The monoclonal antibody is purified by affinity chromatography using Protein A or Protein G Sepharose. This antibody is supplied in PBS. 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 #19150.1

A suspension of magnetic dextran iron particles in water.

**STABILITY AND STORAGE:**

**EasySep<sup>®</sup> Negative Selection Human Naïve CD4<sup>+</sup> T Cell Enrichment 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.

**Biotinylated Anti-CD45RO Antibody**

Stable at 4°C for 1 year. 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.

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