PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

HUMAN CD4+CD127LOW T CELL PRE-ENRICHMENT KIT

CATALOG #19231

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).

If using other EasySep™ Magnets, please visit www.stemcell.com to download the magnet-specific Product Information Sheet or contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

A) FULLY AUTOMATED PROTOCOL USING ROBOSEP™

This procedure is used for processing **500 µL - 8.5 mL** of sample (up to 4.25 x 10^8 cells).

1. Prepare cell suspension at a concentration of 5 x 10^7 cells/mL in RoboSep™ Buffer (Catalog #20104) (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 RoboSep™ carousel.

   Falcon® 14 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352057) are recommended.

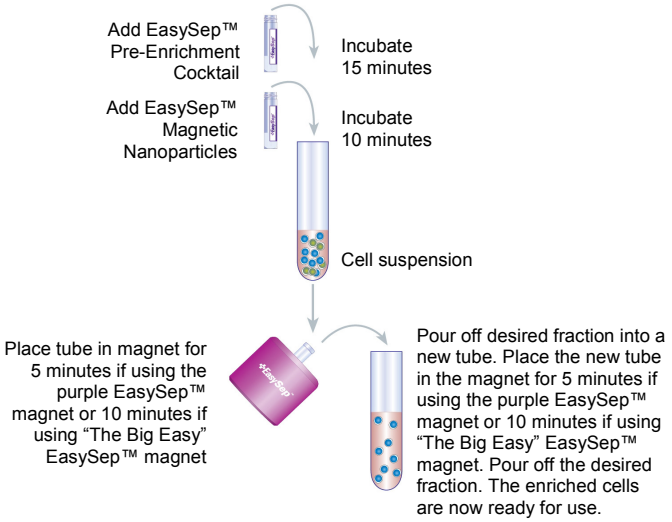
2. Select the appropriate RoboSep™ protocol:
   - Human $CD4^+CD127^{low}$ T Cell Negative Selection 19231-high purity

   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 vigorously pipetting up and down more than 5 times. Vortexing is not recommended. 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 enriched cells in the 14 mL tube located to the left of the magnet. The isolated cells in the new tube are now ready for use.

MANUAL EASYSEP™ PROTOCOL DIAGRAM



B) MANUAL EASYSEP™ PROTOCOL USING THE PURPLE EASYSEP™ MAGNET (CATALOG #18000)

This procedure is used for processing **250 µL - 2 mL** of sample (up to 1 x 10^8 cells).

1. Prepare cell suspension at a concentration of 5 x 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™ Magnet.

   Falcon® 5 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352058) are recommended.

2. Add the EasySep™ Human $CD4^+CD127^{low}$ T Cell Pre-Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.

3. Mix the EasySep™ Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting up and down more than 5 times. Vortexing is not recommended

4. Add the EasySep™ Magnetic Nanoparticles at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**. (For an alternative high-purity protocol see Notes and Tips, reverse side).

5. Bring the cell suspension up 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**.

6. Pick up the EasySep™ 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™ 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 first tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet. Set aside for **5 minutes**. The isolated cells in the new tube are now ready for use.

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

This procedure is used for processing **500 µL - 8.5 mL** of sample (up to 4.25 x 10^8 cells).

1. Prepare cell suspension at a concentration of 5 x 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™ Magnet.

   Falcon® 14 mL Polystyrene Round-Bottom Tubes (Corning® Catalog #352057) are recommended.

2. Add the EasySep™ Human $CD4^+CD127^{low}$ T Cell Pre-Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.

3. Mix the EasySep™ Magnetic Nanoparticles to ensure that they are in a uniform suspension by vigorously pipetting up and down more than 5 times. Vortexing is not recommended

4. Add the EasySep™ Magnetic Nanoparticles at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**. (For an alternative high-purity protocol see Notes and Tips, reverse side).

5. Bring the cell suspension up to a total volume of **5 mL** (for < 10^8 cells) or **10 mL** (for 1 - 4.25 x 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 **10 minutes**.

6. Pick up the EasySep™ Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired fraction into a new 14 mL polystyrene tube. The magnetically labeled unwanted cells will remain bound inside the original 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 first tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet. Set aside for **10 minutes**. The isolated cells in the new tube are now ready for use.

CATALOG #19231 For labeling up to 10^9 total cells

Components:

- EasySep™ Human $CD4^+CD127^{low}$ T Cell Pre-Enrichment Cocktail 1.0 mL
- EasySep™ Magnetic Nanoparticles 2 x 1.0 mL

EasySep™ NEGATIVE SELECTION

REQUIRED EQUIPMENT:

EasySep™ Magnet (Catalog #18000), or "The Big Easy" EasySep™ Magnet (Catalog #18001), or RoboSep™.

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Human $CD4^+CD127^{low}$ T Cell Pre-Enrichment Cocktail and EasySep™ Magnetic Nanoparticles label non-CD4+ T cells and $CD127^{high}$ cells for magnetic separation. These reagents are designed to enrich $CD4^+CD127^{low}$ T cells from fresh or previously frozen peripheral blood mononuclear cells by depletion of non-CD4+ T cells and $CD127^{high}$ cells as a pre-enrichment step prior to fluorescence-activated cell sorting.

EASYSEP™ LABELING OF HUMAN CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles using bispecific Tetrameric Antibody Complexes (TACs). These complexes recognize both dextran and the unwanted cell surface antigen (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep™ procedure (reverse side).

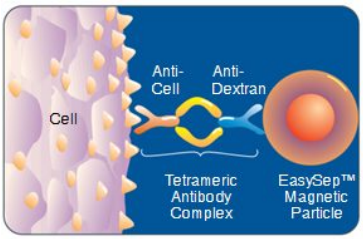


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION. Prepare a mononuclear cell suspension from whole peripheral blood by density gradient centrifugation. **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 (15 - 25°C) prior to labeling and separation. Filter clumpy suspensions through a 30 µm mesh nylon strainer for optimal results.

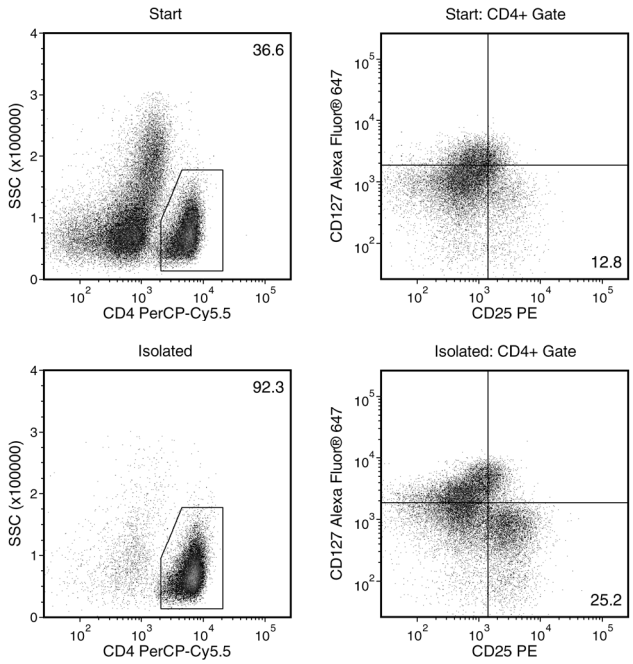
OPTIMAL CELL NUMBER. The use of fewer than 2.5 x 10^7 cells per separation may result in sub-optimal performance.

RECOMMENDED MEDIUM. The recommended medium is EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or phosphate-buffered saline (PBS) + 2% fetal bovine serum (FBS; Catalog #07905) with 1 mM EDTA. Medium should be Ca^{2+} and Mg^{2+} free.

ASSESSING PURITY. Purity of $CD4^+CD127^{low}$ T cells can be measured by flow cytometry after labeling with a fluorochrome-conjugated anti-CD4 (Catalog #60016) and anti-CD127 antibodies. For CD127 labeling, we recommend clone hIL-7R-M21 since it is not blocked by the anti-CD127 TAC present in the selection cocktail.

HIGH PURITY PROTOCOL. The purity of $CD4^+CD127^{low}CD25^+$ T cells can be increased by using 100 µL/mL of EasySep™ Magnetic Nanoparticles in step 4 of the manual protocol. The increased purity will also result in reduced recovery.

TYPICAL EASYSEP™ HUMAN $CD4^+CD127^{LOW}$ T CELL PRE-ENRICHMENT PROFILE:



Starting with human peripheral blood mononuclear cells, the regulatory T cell ($CD4^+CD127^{low}CD25^+$) content of the enriched fraction typically ranges from 14.9 to 25.2%. In the example above, the final purities of the start and isolated fractions are 4.7% and 23.2%, respectively.

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN $CD4^+CD127^{LOW}$ T CELL PRE-ENRICHMENT COCKTAIL **CODE #19231C.1**

This cocktail contains a combination of monoclonal antibodies bound in bispecific TACs which are directed against cell surface antigens on human blood cells and dextran. The mouse monoclonal antibody subclass is IgG_1. 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 #19150.1**

A suspension of magnetic dextran iron particles in water.

STABILITY AND STORAGE:

EASYSEP™ HUMAN $CD4^+CD127^{LOW}$ T CELL PRE-ENRICHMENT COCKTAIL

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.

STEMCELL™ TECHNOLOGIES

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