PROCEDURE

Positive Selection

EasySep®

Human CD14 Selection Kit

Version 3.2.1

CATALOG #18058

This Product Information Sheet is provided for use with RoboSep® (section A), the purple EasySep® magnet (section B) or the silver "The Big Easy" 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 (up to 8.5 x 10^8 cells).

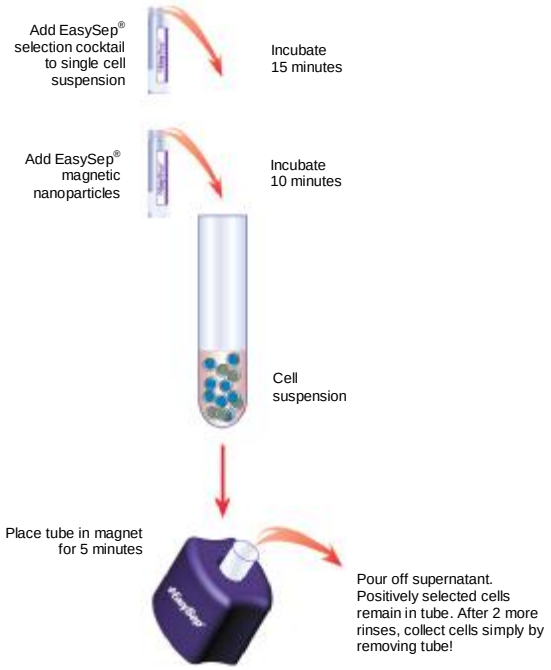
1. Prepare mononuclear cell suspension at a concentration of 1 x 10^8 cells/mL in RoboSep® Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep® carousel. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
2. Select the appropriate RoboSep® protocol:

   RoboSep® protocols can be optimized for high $CD14^+$ cell purity or high $CD14^+$ cell recovery. Select one of the protocols listed below, as appropriate.
   - "Human CD14 Positive Selection 18058-high purity".
   - "Human CD14 Positive Selection 18058-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



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

This procedure is used for processing **100 µL - 2.5 mL** of sample (up to **2.5 x 10^8** cells).

1. Prepare mononuclear cell suspension at a concentration of 1 x 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 µL.

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, 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 (15 - 25°C) for **15** minutes.
3. Mix 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. 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 (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 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 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 Big Easy" Silver EasySep® Magnet (Catalog #18001).

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

1. Prepare mononuclear cell suspension at a concentration of 1 x 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 silver magnet. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, 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 (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 µL/ mL** cells (e.g. for 2 mL of cells, add 100 µL 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 5.0 mL (for <10^8 cells) or 10 mL (for 1 - 8 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 **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 5.0 mL (for <10^8 cells) or 10 mL (for 1 - 8 x 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, 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.

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May 2008

#28510

Catalog #18058 For labeling 10^9 total cells

Components:

- EasySep® Human CD14 Positive Selection Cocktail 1.0 mL
- EasySep® Magnetic Nanoparticles 1.0 mL

EasySep® Positive Selection

Product Information Sheet

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Human CD14 Positive Selection Cocktail and EasySep® Magnetic Nanoparticles label $CD14^+$ cells for magnetic separation. These positive selection reagents are designed to positively select $CD14^+$ cells (cells expressing the CD14 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).

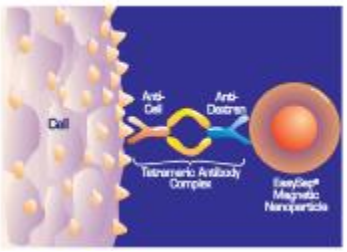


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

Preparing a Mononuclear Cell Suspension. Prepare a mononuclear cell suspension from whole peripheral blood by Ficoll-Paque™ density separation (Catalog #07957). Following density centrifugation, platelets should be removed by resuspending the cells in recommended medium and centrifugation for 10 minutes at 120 *x g* at room temperature (15 - 25°C) with the brake off. Carefully remove the supernatant, which contains the platelets, and resuspend the cell pellet in fresh buffer. Repeat for a total of 2 slow washes.

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.

$CD14^+$ Cell Depletion. The EasySep® CD14 Positive Selection Cocktail can also be used to deplete $CD14^+$ cells. Please refer to depletion procedure at www.stemcell.com/technical/EasySepDepletion.pdf.

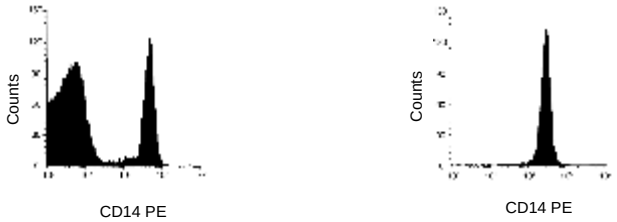
Optimizing Cell Recovery. $CD14^+$ cell recovery can be improved by performing a total of 2 x 5-minute separations in the magnet rather than 3, and by adding magnetic nanoparticles at an increased concentration of 100 µL/mL of cells.

Assessing Purity. The CD14 Positive Selection Cocktail uses the αCD14 antibody clone BA-8. We recommend one of the following clones to assess purity by flow cytometry: UCHM1 (no blocking) or M0P9 (no blocking). 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-CD14 antibody (catalog #10506) 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 an alternative marker such as fluorochrome-conjugated anti-CD36.
3. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG.

TYPICAL EASYSEP® CD14 SELECTION PROFILE:

Start: 27.0% $CD14^+$ Cells Selected: 99.6% $CD14^+$ Cells



Starting with fresh peripheral blood mononuclear cells, the $CD14^+$ cell content of the enriched fraction typically ranges from 97.8 - 99.7%.

COMPONENT DESCRIPTIONS:

EasySep® Human CD14 Positive Selection Cocktail code #18058C.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 CD14 and dextran. The mouse monoclonal antibody subclass is IgG_1. 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 CD14 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. Contents sterile in unopened tube. 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.

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