

Positive Selection


EasySep[®]

PROCEDURE

Human Whole Blood CD15 Selection

Version 1.1.3

CATALOG #18681



This Product Information Sheet is provided for use with RoboSep[®] (section A) or "The Big Easy" silver EasySep[®] magnet (section B).

A) Fully Automated Protocol Using RoboSep[®] (Catalog #20000).

This procedure is used for processing up to 4.5 mL of whole blood per separation.

1. Collect whole blood in a heparinized blood collection tube. Transfer a maximum of 4.5 mL whole blood to a 14 mL (17 x 100 mm) polystyrene tube. (Cells must be placed in a 14 mL polystyrene tube to properly fit into the RoboSep[®] carousel).

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

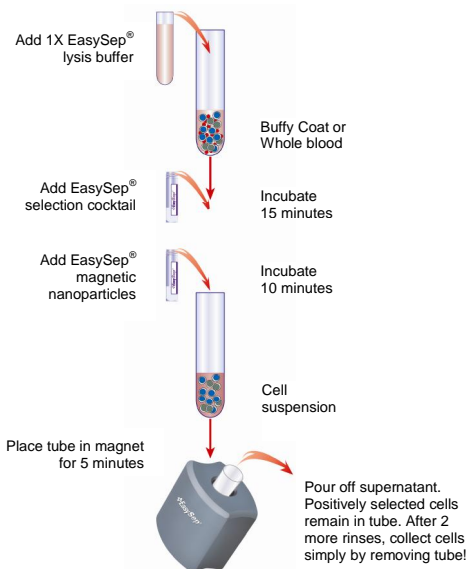
2. Add 1X EasySep[®] RBC Lysis Buffer (see Notes and Tips, reverse side) at a ratio of 1 part lysis buffer to 1 part sample. Mix well.
3. Select the appropriate RoboSep[®] protocol:

For most normal samples, select the protocol entitled "Human CD15 WB Positive Selection 18681-high purity".

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

4. 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[®].
5. 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 Big Easy" Silver EasySep[®] Magnet (Catalog #18001).

This procedure is used for processing up to 4.5 mL of whole blood per separation.

1. Collect whole blood in a heparinized blood collection tube. Transfer a maximum of 4.5 mL whole blood to a 14 mL (17 x 100 mm) polystyrene tube. (Cells must be placed in a 14 mL polystyrene tube to properly fit into the EasySep[®] Magnet).

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

2. Add 1X EasySep[®] RBC Lysis Buffer (see Notes and Tips, reverse side) at a ratio of 1 part lysis buffer to 1 part whole blood. Mix well.
3. Add EasySep[®] Positive Selection Cocktail at 25 $\mu\text{L/mL}$ of whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/lysis buffer mixture add 50 μL of cocktail). Mix well and incubate at room temperature for 15 minutes.
4. Mix EasySep[®] Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously more than 5 times. Vortexing is not recommended. Add the nanoparticles at 25 $\mu\text{L/mL}$ of whole blood/lysis buffer mixture (e.g. for 2 mL of whole blood/lysis buffer mixture add 50 μL of nanoparticles). Mix well and incubate at room temperature for 10 minutes.
5. If total volume is less than 2.5 mL, add recommended medium to 5 mL, otherwise add recommended medium to 10 mL (see Notes and Tips, reverse side). 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 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.**
7. Remove the tube from the magnet and add either 5 mL or 10 mL of recommended medium (as in Step 5). 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.
8. Repeat Steps 6 and 7, and then Step 6 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.

StemCell Technologies

In North America

Tel: 1.604.877.0713
 Fax: 1.604.877.0704
 Toll Free Tel: 1.800.667.0322
 Toll Free Fax: 1.800.567.2899
 e-mail: info@stemcell.com
www.stemcell.com

In the United Kingdom

Tel: +44.(0).20.7691.3561
 Fax: +33.(0).4.76.18.99.63
 Toll Free within United Kingdom:
 Tel: 0800.731.27.14
 Fax: 0800.731.27.13
 e-mail: info@stemcellgb.com

In Europe

Tel: +33.(0).4.76.04.75.30
 Fax: +33.(0).4.76.18.99.63
 e-mail: info@stemcellfrance.com

August 2007

FOR RESEARCH USE ONLY

#28952

Catalog #18681

For labeling 60 mL whole blood

Components:

- EasySep® Human Whole Blood CD15 Positive Selection Cocktail 3 x 1.0 mL
- EasySep® Magnetic Nanoparticles 3 x 1.0 mL
- EasySep® RBC Lysis Buffer 10X Concentrate 10 mL

**Product Information Sheet****REQUIRED EQUIPMENT:**

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Human Whole Blood CD15 Positive Selection Cocktail and EasySep® Whole Blood Magnetic Nanoparticles label CD15⁺ cells for magnetic separation. These positive selection reagents are designed to positively select CD15⁺ cells (cells expressing the CD15 antigen) from fresh whole blood.

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 flow cytometric analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep® procedure (reverse side).

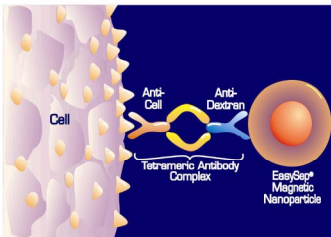


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

EasySep® RBC Lysis Buffer. Lysis buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least 1 hour before use by adding 1 part 10X lysis buffer to 9 parts distilled or Type 1 water. Mix gently and completely before use.

Recommended Medium. The recommended medium is 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.

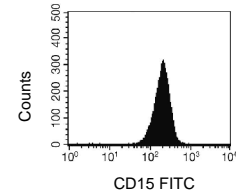
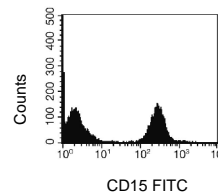
Donor Variability. Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic nanoparticles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population on FSC vs. SSC plots during flow cytometry analysis of the enriched fraction. This population consists solely of particles, with no cells or platelets present, as determined by staining with fluorescently-labeled antibodies against dextran, CD41 and CD45.

Potential aggregation can be avoided by washing away the donor plasma. Dilute the sample 2-fold in the recommended medium, and centrifuge at 300 x g for 10 minutes. Remove as much plasma as possible without disturbing the white and red blood cells, then resuspend sample to original volume with recommended medium before beginning the separation procedure.

If the samples have not been washed, any aggregates can be gated out during flow cytometry analysis of the enriched fraction based on their FSC vs. SSC characteristics, or by their lack of CD45 expression.

Assessing Purity. The CD15 Positive Selection Cocktail uses the anti-CD15 antibody clone W6D3. We recommend the MMA antibody clone (e.g. Catalog #10407) to assess purity by flow cytometry.

A fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG₁ can also be used to assess purity.

TYPICAL EASYSEP® CD15 SELECTION PROFILE:Start*: 38.7% CD15⁺ CellsSelected: 99.8% CD15⁺ Cells

Starting with fresh whole blood, the CD15⁺ cell content of the enriched fraction typically ranges from 97.5 - 99.8%.

*Red blood cells were removed by lysis prior to flow cytometry.

COMPONENT DESCRIPTIONS:**EasySep® Human Whole Blood CD15 Positive Selection Cocktail****code #18681C**

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 CD15 and dextran. The mouse monoclonal antibody subclass is IgG₁. This cocktail 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® Magnetic Nanoparticles**code #18150**

A suspension of magnetic dextran iron particles in water.

EasySep® RBC Lysis Buffer 10X Concentrate**code #20110**

Concentrated buffer used to lyse red blood cells prior to cell labeling and separation.

STABILITY AND STORAGE:**EasySep® Human CD15 Positive Selection 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.

EasySep® 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.

EasySep® RBC Lysis Buffer 10X Concentrate

10X concentrate is stable at room temperature for 2 years. Store at room temperature. 1X Lysis Buffer is stable at 4°C for 3 months. Store at 2 - 8°C. Do not freeze.

StemCell Technologies**In North America**

Tel: 1.604.877.0713
 Fax: 1.604.877.0704
 Toll Free Tel: 1.800.667.0322
 Toll Free Fax: 1.800.567.2899
 e-mail: info@stemcell.com
www.stemcell.com

In the United Kingdom

Tel: +44.(0).20.7691.3561
 Fax: +33.(0).4.76.18.99.63
 Toll Free within United Kingdom: e-mail: info@stemcellfrance.com
 Tel: 0800.731.27.14
 Fax: 0800.731.27.13
 e-mail: info@stemcellgb.com

In Europe

Tel: +33.(0).4.76.04.75.30
 Fax: +33.(0).4.76.18.99.63

FOR RESEARCH USE ONLY**#28952**