

PROCEDURE **Negative Selection**

+EasySep

Human B Cell Enrichment Kit

Version 3.0.2

CATALOG #19054

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

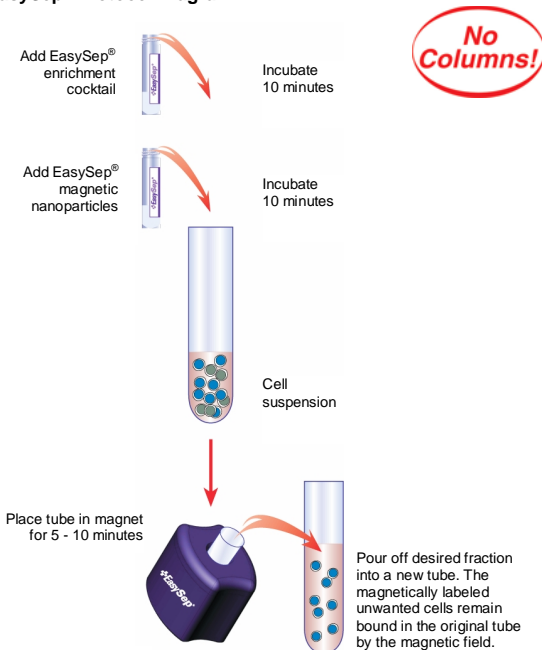
A) Fully Automated Protocol Using RoboSep[®] (Catalog #20000).

This procedure is used for processing **500 µL - 8.5 mL** of sample (up to 4.25×10^6 cells).

1. Prepare nucleated cell suspension at a concentration of 5×10^7 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.
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Becton Dickinson, Catalog #352057) are recommended.
2. Select the appropriate RoboSep[®] protocol:
RoboSep[®] protocols can be optimized for high B cell purity or high B cell recovery. Select one of the protocols listed below as appropriate.
 - "Human B Cell Negative Selection 19054-high purity".
 - "Human B Cell Negative Selection 19054-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 vigorously pipetting 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 tube containing the enriched cells from the RoboSep[®] 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[®] 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 1×10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 5×10^7 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 (Becton Dickinson, Catalog #352058) are recommended.
2. Add EasySep[®] Negative Selection Human B 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**.
3. Mix EasySep[®] 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 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**.
5. 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 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.* The negatively selected, enriched cells in the new tube, are now ready for use or for a second round of separation (see Notes below).

Additional Notes:

- I. For some applications it may be desirable to perform a second round of magnetic separation. This will increase purity but may reduce recovery. Remove the first tube from the EasySep[®] Magnet and place the new tube containing the desired cells into the magnet and set aside for **5 minutes**. Repeat Step 5.
- II. Some of the desired cells may be left behind in the original tube after pouring off the desired fraction. These cells may be recovered by resuspending the magnetically labeled cells in 2.5 mL of recommended medium and performing an additional 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 low start percentages.

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 4.25×10^6 cells).

1. Prepare mononuclear cell suspension at a concentration of 5×10^7 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 (Becton Dickinson, Catalog #352057) are recommended.
2. Add EasySep[®] Negative Selection Human B 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**.
3. 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 **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 $1 - 4.25 \times 10^8$ 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 **10 minutes** at room temperature.
5. 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 the 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.* The negatively selected enriched cells in the new tube are now ready for use (see Notes below).

Additional Notes:

- I. For some applications it may be desirable to perform a second round of magnetic separation. This will increase purity, but may reduce recovery. Remove the first tube from the EasySep[®] magnet and place the new tube containing the desired cells into the magnet and set aside for **10 minutes** at room temperature. Repeat Step 5.
- II. Some of the desired cells may be left behind in the original tube after pouring off the desired fraction in Step 5. These cells may be recovered by resuspending the magnetically labeled cells in 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 low start percentages.

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September 2008

FOR RESEARCH USE ONLY

#28744

Catalog #19054For labeling 10^9 total cells

Components:

- EasySep[®] Negative Selection Human B Cell Enrichment Cocktail 1.0 mL
- EasySep[®] Magnetic Nanoparticles 2 x 1.0 mL

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

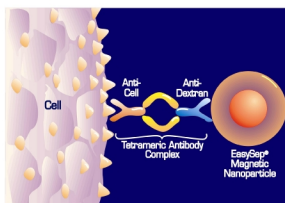
EasySep[®] Magnet (Catalog #18000), or "The Big Easy" EasySep[®] Magnet (Catalog #18001), or RoboSep[®] (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] Negative Selection Human B Cell Enrichment Cocktail and EasySep[®] Magnetic Nanoparticles label non-B cells for magnetic separation. These reagents are designed to enrich B cells from normal fresh or previously frozen peripheral blood mononuclear cells by depletion of non-B cells. For isolation of B cells from blood or other tissues of patients with B cell leukemia or lymphoma, or with other disease states in which B cells may express CD43, we recommend the EasySep[®] Human B Cell Enrichment Kit without CD43 Depletion (Catalog #19154).

EASYSEP[®] 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[®] procedure (reverse side).

**Figure 1.**

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

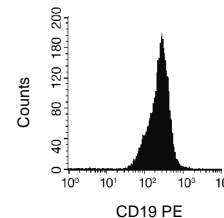
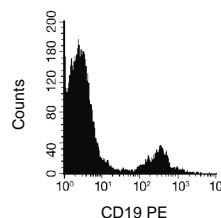
NOTES AND TIPS:

Preparing a Mononuclear Cell Suspension. Prepare a mononuclear cell suspension from whole peripheral blood by Ficoll-Paque[™] 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 5×10^7 cells per separation may result in sub-optimal performance.

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

Assessing Purity. Purity of B cells can be measured by flow cytometry after staining with a fluorochrome-conjugated antibody against CD19 (e.g. FITC anti-CD19, Catalog #10409), CD20 or other B cell marker.

TYPICAL EASYSEP[®] B CELL ENRICHMENT PROFILE:Start: 13% CD19⁺ CellsEnriched: 99% CD19⁺ Cells

Starting with frozen mononuclear cells, the CD19⁺ cell content of the enriched fraction typically ranges from 95 - 99%.

COMPONENT DESCRIPTIONS:

EasySep[®] Negative Selection Human B Cell Enrichment Cocktail 19054C.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 cell surface antigens on human blood cells (CD2, CD3, CD14, CD16, CD36, CD43, CD56, glycoporphin A) and dextran. The mouse monoclonal antibody subclass is IgG₁. 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[®] Negative Selection Human B 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.

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.

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