PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

HUMAN B CELL ENRICHMENT KIT WITHOUT CD43 DEPLETION

CATALOG #19154

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 B Cell Negative Selection without CD43 19154-high purity
   - Human B Cell Negative Selection without CD43 19154-high recovery

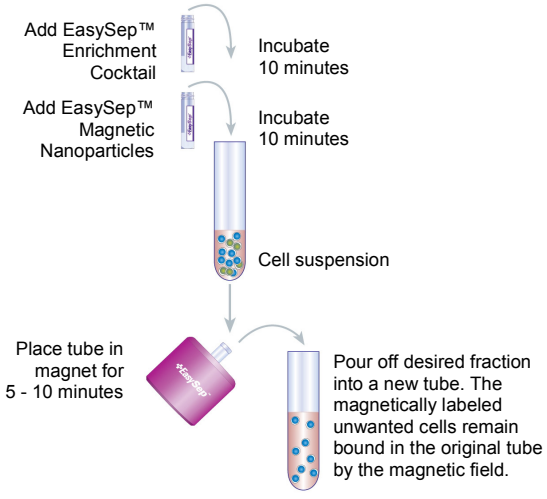
   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

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 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 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 B Cell Enrichment Cocktail w/o CD43 Depletion 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 **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.

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**.

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. The isolated 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 6.

II. Some of the desired cells may be left behind in the original tube after pouring off the desired fraction 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 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 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 B Cell Enrichment Cocktail w/o CD43 Depletion 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 **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.

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**.

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. The isolated cells in the new tube are now ready for use.

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. Repeat Step 6.

II. Some of the desired cells may be left behind in the original tube after pouring off the desired fraction in Step 6. 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.

CATALOG #19154 For labeling up to 10^9 total cells

Components:

- EasySep™ Human B Cell Enrichment Cocktail w/o CD43 Depletion 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 B Cell Enrichment Cocktail without CD43 Depletion and EasySep™ Magnetic Nanoparticles label non-B cells for depletion. This product is recommended for enrichment 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, CD123 or CD36. For isolation of CD43 negative B cells from normal samples we recommend the EasySep™ Human B Cell Enrichment Kit (Catalog #19054). To isolate all B cells including plasma cells from non-leukemia or lymphoma samples use the EasySep™ Human Pan-B Cell Enrichment Kit (Catalog #19554).

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).

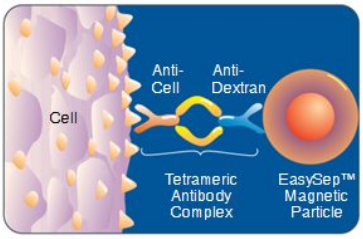


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING THE CELL SUSPENSION

FROM WHOLE PERIPHERAL BLOOD

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.

FROM PERIPHERAL BLOOD APHERESIS (LEUKOPAK)

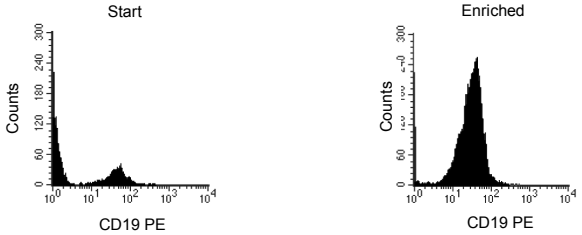
If working with large volumes (> 150 mL), concentrate Leukopak cells first by centrifuging at 500 x *g* for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (150 mL or less), add the Ammonium chloride solution (Catalog #07800/07850) directly to the cell suspension.

1. Add an equal volume of ammonium chloride solution to the Leukopak suspension (e.g. for 5 mL of Leukopak suspension, add 5 mL ammonium chloride solution).
2. Incubate 15 minutes on ice.
3. Centrifuge at 500 x *g* for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x *g* for 10 minutes at room temperature (15 - 25°C) with the brake off. Carefully remove the supernatant.
5. Repeat the wash step one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend cells at recommended cell concentration, in the recommended medium.

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++ and Mg++ free.

ASSESSING PURITY. Purity of B cells can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD19 antibody (Catalog #60005), CD20 (Catalog #60008) or other B cell marker.

TYPICAL EASYSEP™ HUMAN B CELL W/O CD43 DEPLETION ENRICHMENT PROFILE:



Starting with previously frozen normal peripheral blood mononuclear cells, the CD19+ cell content of the enriched fraction typically ranges from 87 to 98%. In the example above, the final purities of the start and enriched fractions are 11% and 96% respectively.

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN B CELL ENRICHMENT COCKTAIL W/O CD43 DEPLETION **CODE #19154C**

This cocktail contains a combination of monoclonal antibodies bound in bispecific TACs which are directed against cell surface antigens on human blood cells (CD2, CD3, CD14, CD16, CD56, glycophorin A) 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 B CELL ENRICHMENT COCKTAIL W/O CD43 DEPLETION

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.