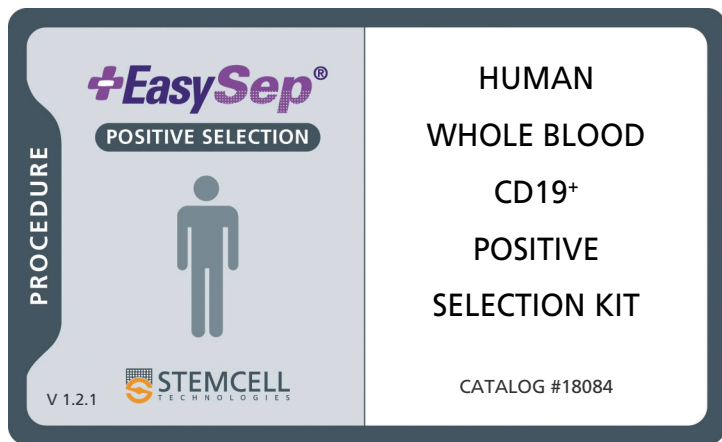




THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP® (SECTION A) OR "THE BIG EASY" SILVER EASYSEP® MAGNET (SECTION B, NORMAL SAMPLES, OR SECTION C, ABNORMAL SAMPLES).

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 (BD, 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. Select the appropriate RoboSep® protocol:

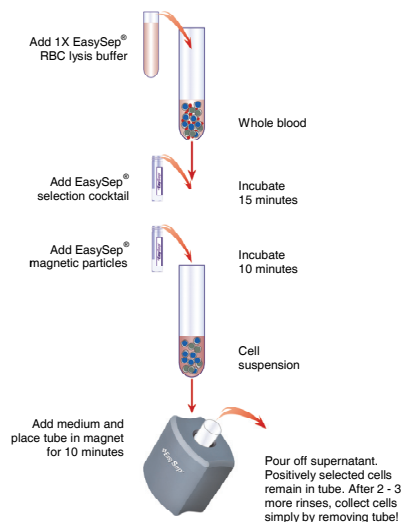
- For most normal samples, select the protocol entitled "Human CD19 WB Positive Selection 18084-high purity".
- For samples with an abnormally low proportion of CD19⁺ cells, or in which B cells express CD19 at a low level (e.g. for chimerism analysis following bone marrow transplantation), select the protocol entitled "Human CD19 WB Positive Selection 18084-chimerism".

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. 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®.

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 SILVER "THE BIG EASY" EASYSEP® MAGNET (CATALOG #18001) WITH NORMAL SAMPLES

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 (BD, 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 µL/mL** 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 (15 – 25°C) for **15 minutes**.

4. 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 **25 µL/mL** 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 (15 – 25°C) for **10 minutes**.

5. If total volume is less than 2.5 mL, add the recommended medium to 5 mL, otherwise add the recommended medium to 10 mL (see Notes and Tips). 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 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.*

7. Remove the tube from the magnet and add 10 mL of the 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**.

8. Repeat Steps 6 and 7, and then Step 6 once more, for a total of 1 x 10-minute and 2 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 SILVER "THE BIG EASY" EASYSEP® MAGNET (CATALOG #18001) FOR SAMPLES WITH AN ABNORMALLY LOW PROPORTION OF CD19⁺ CELLS, OR IN WHICH B CELLS EXPRESS CD19 AT A LOW LEVEL (E.G. FOR CHIMERISM ANALYSIS FOLLOWING BONE MARROW TRANSPLANTATION).

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 (BD, 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 µL/mL** 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 (15 – 25°C) for **15 minutes**.

4. 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 **25 µL/mL** 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 (15 – 25°C) for **10 minutes**.

5. If total volume is less than 2.5 mL, add the recommended medium to **5 mL**, otherwise add the recommended medium to **10 mL** (see Notes and Tips). 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 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.*

7. Remove the tube from the magnet and add 10 mL of the 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**.

8. Repeat Steps 6 and 7 twice, and then Step 6 once more, for a total of 1 x 10-minute and 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.

CATALOG #18084

For labeling 60 mL whole blood

Components:

• EasySep® Human Whole Blood CD19 Positive Selection Cocktail	3 x 1.0 mL
• EasySep® Magnetic Nanoparticles	3 x 1.0 mL
• EasySep® RBC Lysis Buffer 10X Concentrate	10 mL


POSITIVE SELECTION**REQUIRED EQUIPMENT:**

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep® Human Whole Blood CD19 Positive Selection Cocktail and EasySep® Whole Blood Magnetic Nanoparticles label CD19⁺ cells for magnetic separation. These positive selection reagents are designed to positively select human CD19⁺ cells (cells expressing the CD19 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 FACS analysis. Magnetically labeled cells are then separated from unlabeled cells using the EasySep® procedure (reverse side).

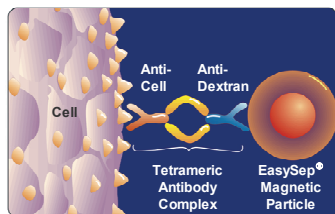


Figure 1.
Schematic Drawing of EasySep® TAC
Magnetic Labeling of Human Cells.

NOTES AND TIPS:

EASYSEP® RBC LYSIS BUFFER. Lysis buffer is supplied as a 10X concentrate. Prepare 1X lysis buffer at least one 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 PBS containing 2% FBS (Catalog #07905) and 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free.

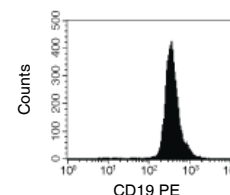
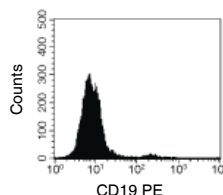
DONOR VARIABILITY. Certain donors express one or more soluble serum factors that can cause cross-linking with magnetic nanoparticles. This may result in visible aggregates in the enriched cell fraction following positive selection. These aggregates may appear as a distinct, high side-scatter population 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 CD19 Positive Selection Cocktail uses the anti-CD19 antibody clone AE-1. We recommend one of the following antibody clones to assess purity by flow cytometry: 4G7 (e.g. Catalog #10409), FMV63, HIB19 (all partially blocking). One of the following methods can also be used to assess purity:

1. Use an alternative marker such as fluorochrome-conjugated anti-CD20 (e.g. Catalog #10410). Note that CD20 may underestimate CD19-positive purity by up to 15%.
2. Use a fluorochrome-conjugated secondary antibody, such as a FITC-labeled sheep anti-mouse IgG.

TYPICAL EASYSEP® WHOLE BLOOD CD19 SELECTION PROFILE:Start*: 2.1% CD19⁺ cellsSelected: 99.5% CD19⁺ cells

Starting with fresh whole blood, the CD19⁺ cell content of the enriched fraction typically ranges from 94.3 - 99.6%.

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

COMPONENT DESCRIPTIONS:**EASYSEP® HUMAN WHOLE BLOOD CD19 POSITIVE SELECTION COCKTAIL****CODE #18084C.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 CD19 and dextran. The mouse monoclonal antibody subclass is IgG₁. This cocktail is supplied in phosphate buffered saline and contains an antibody against human Fc receptor. 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 WHOLE BLOOD CD19 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. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

EASYSEP® 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.

EASYSEP® RBC LYSIS BUFFER 10X CONCENTRATE

10X concentrate stable at room temperature (15 - 25°C) until expiry date as indicated on label. Store at room temperature (15 - 25°C). 1X Lysis Buffer is stable at 2 - 8°C for 3 months. Store at 2 - 8°C. Do not freeze.

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