PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

HUMAN PAN-GRANULOCYTE ISOLATION KIT

CATALOG #19259

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 **0.5 - 8 mL** of sample (up to 4 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:

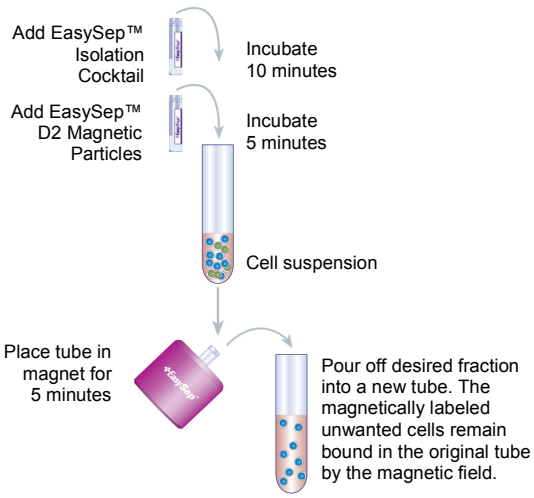
   - Human Pan-Granulocyte Isolation 19259

   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. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates. 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 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 **0.25 - 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 Pan-Granulocyte Isolation Cocktail 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. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ D2 Magnetic Particles 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 **5 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.

C) MANUAL EASYSEP™ PROTOCOL USING "THE BIG EASY" SILVER EASYSEP™ MAGNET (CATALOG #18001)

This procedure is used for processing **0.5 - 8 mL** of sample (up to 4 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 Pan-Granulocyte Isolation Cocktail 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. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ D2 Magnetic Particles 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 **5 minutes**.

5. Bring the cell suspension up to a total volume of **5 mL** (for ≤ 2 x 10^8 cells) or **10 mL** (for > 2 - 4 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**.

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.

CATALOG #19259 — For labeling 10^9 total cells

Components:

- EasySep™ Human Pan-Granulocyte Isolation Cocktail — 1 mL
- EasySep™ D2 Magnetic Particles — 2 x 1 mL

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REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Human Pan-Granulocyte Isolation Cocktail and EasySep™ D2 Magnetic Particles label non-granulocytes for magnetic separation. These reagents are designed to enrich for all granuloctyes (neutrophils, eosinophils, basophils) from whole peripheral blood by depletion of non-granulocytes.

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).

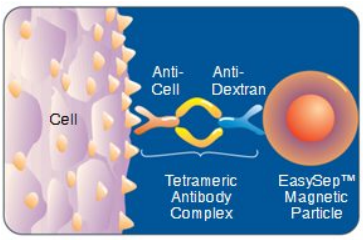


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING THE CELL SUSPENSION

Note: Do not use dextran sedimentation to prepare cells.

Preparation of Whole Blood Using Red Blood Cell Lysis (preferred for slightly higher recovery). Collect whole blood in a blood collection tube containing heparin or another anticoagulant. Add 4 parts Ammonium Chloride Solution (Catalog #07800) to 1 part whole blood and mix well. Incubate on ice for 15 minutes then centrifuge at 300 x *g* for 10 minutes. Wash pellet once by filling tube with recommended medium and centrifuging at 120 x *g* for 10 minutes with the brake off. Discard supernatant and resuspend cells at 5 x 10^7 cells/mL in recommended medium.

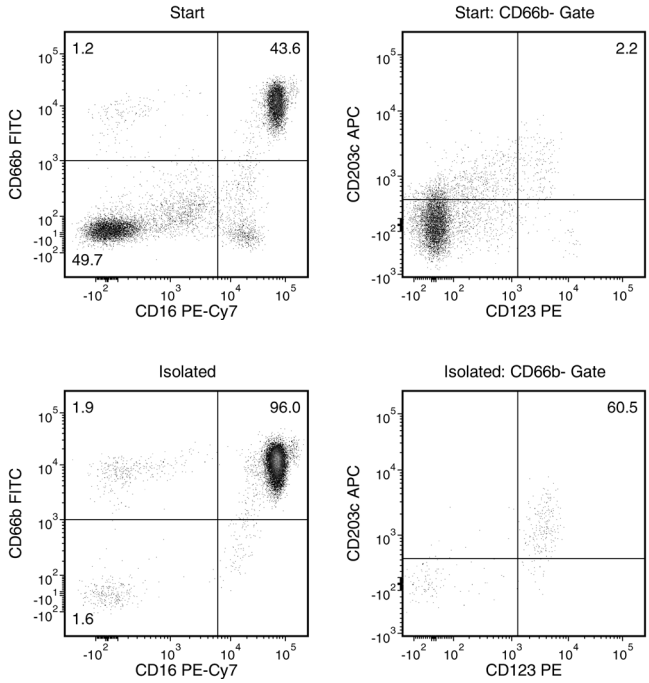
Preparation of Whole Blood Using HetaSep™ Red Blood Cell Sedimentation (preferred for lysis-free sample processing).

1. Collect whole blood in a blood collection tube containing heparin or other anticoagulant. Add 1 part HetaSep™ (Catalog #07906) to 5 parts whole blood and mix well. Use the minimum sized tube for the total volume of HetaSep™:blood sample. A 14mL tube is the maximum size recommended for optimal leukocyte recovery.
2. Centrifuge sample at 90 x *g* for 2 minutes (total volume ≤ 5 mL) or 5 minutes (total volumes of > 5 mL) at room temperature (15 - 25°C) with the brake off.
3. Remove tube from centrifuge and let sit undisturbed for 10 minutes.
4. Harvest the leukocyte-rich supernatant (everything above the red blood cell fraction) into a 50 mL tube and resuspend this fraction using at least a 4-fold volume of recommended medium.
5. Centrifuge at 300 x *g* for 10 minutes at room temperature (15 - 25°C).
6. Discard supernatant and wash a second time to remove excess platelets, centrifuging at 120 x *g* for 10 minutes at room temperature (15 - 25°C) with the brake off.
7. Discard supernatant and resuspend cells at 5 x 10^7 cells/mL in recommended medium.

RECOMMENDED MEDIUM. The recommended medium is RoboSep™ Buffer (Catalog #20104), or EasySep™ Buffer (Catalog #20144), or PBS + 2% FBS with 1 mM EDTA. Medium should be Ca^{2+} and Mg^{2+}.

ASSESSING PURITY. Purity of granulocytes can be measured by flow cytometry after staining with fluorochrome-conjugated antibodies directed against neutrophils (CD66b+CD16+), eosinophils (CD66b+CD16- and low in forward scatter but high in side scatter), and basophils (CD66-CD123+IgE+ or CD66-CD123+CD203c^{low}). Alternatively, purity may be assessed by performing a cytospin of the enriched cells followed by Wright's or May-Grünwald staining (e.g. Sigma-Aldrich Catalog #W0625 or #205435, respectively).

TYPICAL EASYSEP™ HUMAN PAN-GRANULOCYTE ISOLATION PROFILE:



Starting with whole peripheral blood, the total granulocyte content of the isolated fraction typically ranges from 97 - 99%. As shown in the examples above, neutrophils are typically CD66b+CD16+. Eosinophils are typically CD66b+CD16- and basophils are CD66b- and can be further defined as CD203c+CD123+ and IgE+ (not shown).

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN PAN-GRANULOCYTE ISOLATION COCKTAIL — CODE #19259C

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, CD19, CD36, CD45RA, CD56 and 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™ D2 MAGNETIC PARTICLES — CODE #19650

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:

EASYSEP™ HUMAN PAN-GRANULOCYTE ISOLATION COCKTAIL

EASYSEP™ D2 MAGNETIC PARTICLES

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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