PROCEDURE

EasySep™

NEGATIVE SELECTION

HUMAN MONOCYTE ENRICHMENT KIT

CATALOG #19059

V 4.6.1

STEMCELL TECHNOLOGIES

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 Technical Support at techsupport@stemcell.com.

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 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 (BD Biosciences, Catalog #352057) are recommended.

2. Select the appropriate RoboSep™ protocol:

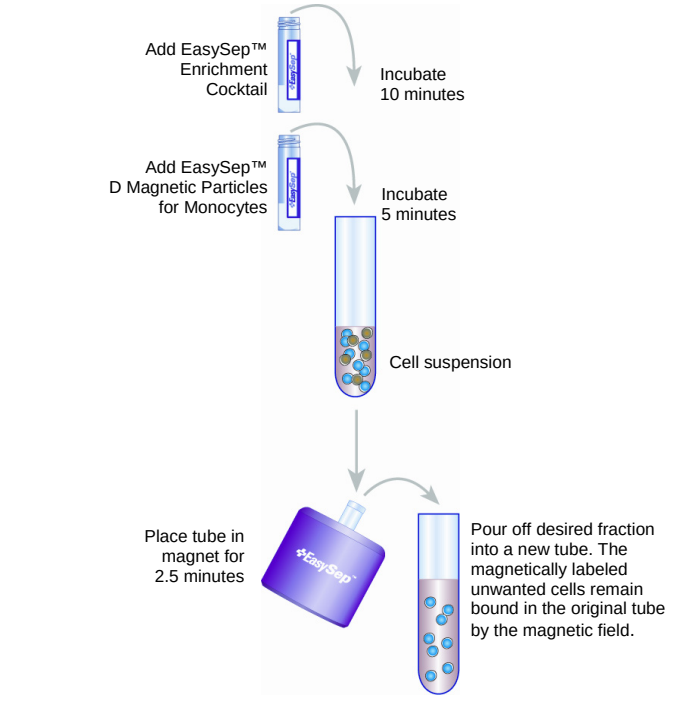
   - Human Monocyte Negative Selection 19059 - high recovery

   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. **Vortex the EasySep™ D Magnetic Particles for Monocytes 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 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 **500 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 (BD Biosciences, Catalog #352058) are recommended.

2. Add the EasySep™ Human Monocyte Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 2 - 8°C for **10 minutes.**

3. Vortex the EasySep™ D Magnetic Particles for Monocytes for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ D Magnetic Particles for Monocytes at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of magnetic particles). Mix well and incubate at 2 - 8°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 **2.5 minutes** at room temperature (15 - 25°C).

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 negatively selected, enriched 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 Purple EasySep™ Magnet and place the new tube containing the desired cells into the magnet and set aside for **2.5 minutes** at room temperature (15 - 25°C). 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 (BD Biosciences, Catalog #352057) are recommended.

2. Add the EasySep™ Human Monocyte Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 2 - 8°C for **10 minutes**.

3. Vortex the EasySep™ D Magnetic Particles for Monocytes for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

4. Add the EasySep™ D Magnetic Particles for Monocytes at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of magnetic particles). Mix well and incubate at 2 - 8°C for **5 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 **2.5 minutes** at room temperature (15 - 25°C).

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 negatively selected, enriched 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 Silver EasySep™ Magnet and place the new tube containing the desired cells into the magnet and set aside for **2.5 minutes** at room temperature (15 - 25°C). 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 (for <10^8 cells) or 10 mL (for 1 - 4.25 x 10^8 cells) 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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CATALOG #19059 — For labeling 10^9 total cells

Components:

- EasySep™ Human Monocyte Enrichment Cocktail — 1.0 mL
- EasySep™ D Magnetic Particles for Monocytes — 1.0 mL

EasySep™ NEGATIVE SELECTION

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Human Monocyte Enrichment Cocktail and EasySep™ D Magnetic Particles for Monocytes label non-monocyte cells for magnetic separation. These reagents are designed to enrich monocytes from fresh or previously frozen peripheral blood mononuclear cells or ammonium chloride-lysed leukapheresis by depletion of non-monocyte cells. This cocktail also depletes the small subset of monocytes that express CD16. For applications where $CD16^+$ monocytes are desired, we recommend the Human Monocyte Enrichment Kit without CD16 Depletion (Catalog #19058).

EASYSEP™ LABELING OF HUMAN CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles 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).

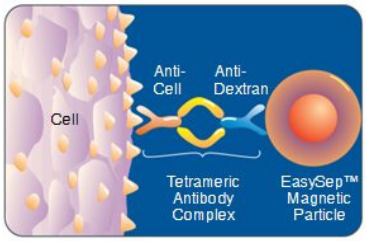


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING THE CELL SUSPENSION

FROM WHOLE PERIPHERAL BLOOD

Prepare a mononuclear cell suspension from whole peripheral blood by density gradient centrifugation. Following density centrifugation, platelets should be removed by resuspending the cells in recommended medium and centrifuging for 10 minutes at 120 x *g* at room temperature (15 - 25°C), with the brake off. Carefully remove the supernatant, which contains the platelets, and resuspend the cell pellet in fresh buffer. Repeat this twice, for a total of 2 washes at 120 x *g* at room temperature (15 - 25°C).
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 on ice prior to labeling and separation. Filter clumpy suspensions through a 30 µm mesh nylon filter 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.

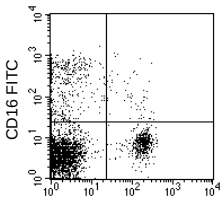
1. Add an equal volume of Ammonium Chloride Solution to the Leukopak suspension (e.g. for 5 mL of Leukopak suspension, add 5 mL Ammonium Chloride Solution).
2. Incubate 15 minutes on ice.
3. Centrifuge at 500 x *g* for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells 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 RoboSep™ Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) with 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free. For optimal performance it is recommended to store the medium at 2 - 8°C prior to use.

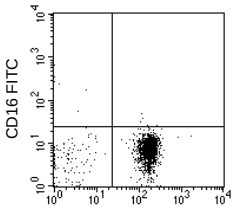
ASSESSING PURITY. Purity of monocytes can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-CD14 antibody (e.g. Anti-Human CD14, Clone M5E2, Catalog #60004, or Anti-Human CD14, Clone MoP9, FITC-conjugated, Catalog #10406), and optionally a fluorochrome-conjugated anti-Human CD16 antibody.

TYPICAL EASYSEP™ HUMAN MONOCYTE ENRICHMENT PROFILE:

Start: 14% $CD14^+16^-$ Cells



Enriched: 94% $CD14^+CD16^-$ Cells



Starting with previously frozen peripheral blood mononuclear cells, the monocyte content of the enriched fraction typically ranges from 83 - 95%.

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN MONOCYTE ENRICHMENT COCKTAIL — **CODE #19059C.2**

This cocktail contains a combination of monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes (TAC) which are directed against cell surface antigens on human blood cells (CD2, CD3, CD16, CD19, CD20, CD56, CD66b, CD123, glycophorin A) and dextran. The mouse monoclonal antibody subclass is IgG_1. The cocktail also contains an FcR blocker to prevent non-specific binding of monocytes. The mouse monoclonal antibody subclass of the FcR blocker is IgG_{2b}. 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™ D MAGNETIC PARTICLES FOR MONOCYTES — **CODE #19550**

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:

EASYSEP™ HUMAN MONOCYTE ENRICHMENT COCKTAIL

EASYSEP™ D MAGNETIC PARTICLES FOR MONOCYTES

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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