PROCEDURE

EasySep®

NEGATIVE SELECTION

HUMAN MONOCYTE ENRICHMENT KIT WITHOUT CD16 DEPLETION

CATALOG #19058

STEMCELL TECHNOLOGIES

V 4.2.1

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).

A) FULLY AUTOMATED PROTOCOL USING ROBOSEP® (CATALOG #20000).

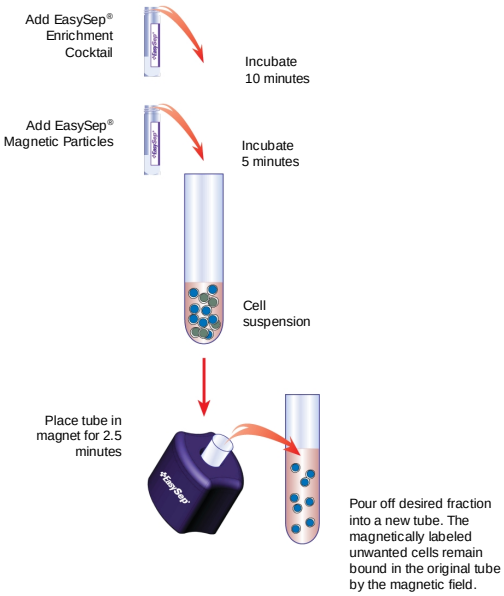
This procedure is used for processing **500 µL - 8.5 mL** of sample (up to 4.25 x 10^8 cells).

1. Prepare mononuclear cell suspension (see Notes and Tips, reverse side) at a concentration of 5 x 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 (BD, Catalog #352057) are recommended.
2. Select the appropriate RoboSep® protocol:
   - For most normal samples, select the protocol entitled "Human Monocyte Negative Selection 19058-MP & 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 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, 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 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 mononuclear 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, Catalog #352058) are recommended.
2. Add EasySep® Human Monocyte Enrichment Cocktail without CD16 Depletion at **50 µL/mL** cells (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 4°C for **10** minutes.
3. Vortex EasySep® D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the magnetic particles at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of magnetic particles). Mix well and incubate at 4°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 tube, held by the magnetic field of the 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.

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 **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 mononuclear 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 magnet.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
2. Add EasySep® Human Monocyte Enrichment Cocktail without CD16 Depletion at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 4°C for **10** minutes.
3. Vortex EasySep® D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
4. Add the magnetic particles at **50 µL/ mL cells** (e.g. for 2 mL of cells, add 100 µL of magnetic particles). Mix well and incubate at 4°C for **5** minutes.
5. Bring the cell suspension 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 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

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 **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 #19058 For labeling up to 10^9 total cells

Components:

- EasySep® Negative Selection Human Monocyte Enrichment Cocktail without CD16 Depletion 1.0 mL
- EasySep® D Magnetic Particles 1.0 mL

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 Monocyte Enrichment Cocktail without CD16 Depletion and EasySep® D Magnetic Particles are designed to enrich monocytes from fresh or previously frozen peripheral blood mononuclear cells by depletion of non-monocyte cells by magnetic separation. The enriched monocyte preparation will include the small subset of monocytes that express CD16. For applications in which removal of all $CD16^+$ cells is desired, we recommend the Human Monocyte Enrichment Kit (Catalog #19059), which contains anti-CD16.

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).

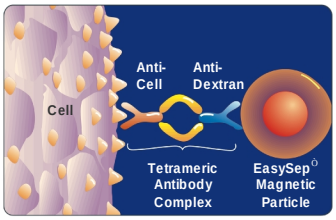


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION. Prepare a mononuclear cell suspension from whole peripheral blood by Ficoll-Paque™ PLUS density separation (Catalog #07957). 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 for a total of 2 slow washes.

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.

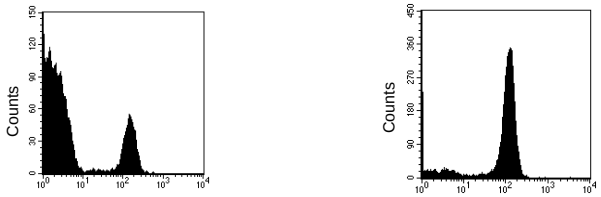
OPTIMAL CELL NUMBER. The use of fewer than 5 x 10^7 cells per separation may result in sub-optimal performance.

RECOMMENDED MEDIUM. The recommended medium is RoboSep® Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) with 2% FBS (Catalog # 07905) and 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. FITC anti-CD14, Catalog #10406 or PE anti-CD14, Catalog #10506).

TYPICAL EASYSEP® MONOCYTE ENRICHMENT PROFILE:

Start: 14% $CD14^+$ Cells Enriched: 80% $CD14^+$ Cells



Starting with freshly prepared peripheral blood mononuclear cells, the $CD14^+$ cell content of the enriched fraction typically ranges from 73 - 81%. Slightly lower $CD14^+$ cell purities may be obtained from samples that contain a large number of $CD16^+$ cells.

COMPONENT DESCRIPTIONS:

EASYSEP® NEGATIVE SELECTION HUMAN MONOCYTE ENRICHMENT COCKTAIL WITHOUT CD16 DEPLETION code #19058C.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, 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 to 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 code #19250

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:

EASYSEP® NEGATIVE SELECTION HUMAN MONOCYTE ENRICHMENT COCKTAIL WITHOUT CD16 DEPLETION

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® D 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.