PROCEDURE

EasySep™

NEGATIVE SELECTION

MOUSE MONOCYTE ENRICHMENT KIT

CATALOG #19761

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).

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

This procedure is used for processing **500 µL – 6.0 mL** of sample (up to 6 x 10^8 cells).

1. Prepare cell suspension at a concentration of 1 x 10^8 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. Add the Normal Rat Serum (provided) at **50 µL/mL of cells** (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Corning, Catalog #352057) are recommended.

2. Add the EasySep™ Mouse Monocyte Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.

3. Wash cells by topping up the sample tube with RoboSep™ Buffer and centrifuge at 300 x *g* for **10 minutes**. Discard the supernatant and resuspend the cells at 1 x 10^8 cells/mL.

4. Select the appropriate RoboSep™ protocol:

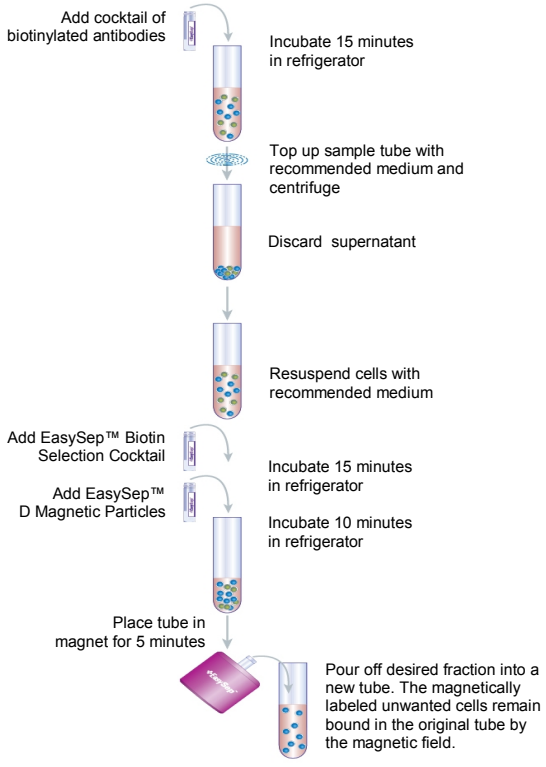
   - Mouse Monocyte Negative Selection 19761

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

5. 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™.

6. 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 – 2.0 mL** of sample (up to 2 x 10^8 cells).

1. Prepare cell suspension at a concentration of 1 x 10^8 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. Add the Normal Rat Serum (provided) at **50 µL/mL of cells** (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).

   Falcon™ 5 mL Polystyrene Round-Bottom Tubes (Corning, Catalog #352058) are recommended.

2. Add the EasySep™ Mouse Monocyte Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.

3. Wash the cells by topping up the sample tube with the recommended medium and centrifuge at 300 x *g* for **10 minutes**. Discard the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium.

4. Add the EasySep™ Biotin Selection Cocktail at **60 µL/mL cells** (e.g. for 2 mL of cells, add 120 µL of selection cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.

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

6. Add the EasySep™ D Magnetic Particles at **150 µL/mL cells** (e.g. for 2 mL of cells, add 300 µL of magnetic particles). Mix well and incubate in refrigerator (2 - 8°C) for **10 minutes**.

7. Bring the cell suspension up to a **total volume** of **2.5 mL** by adding recommended medium without rat serum. 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**.

8. 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 enriched 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 **500 µL – 6.0 mL** of sample (up to 6 x 10^8 cells).

1. Prepare cell suspension at a concentration of 1 x 10^8 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. Add the Normal Rat Serum (provided) at **50 µL/mL of cells** (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Corning, Catalog #352057) are recommended.

2. Add the EasySep™ Mouse Monocyte Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**

3. Wash the cells by topping up the sample tube with the recommended medium and centrifuge at 300 x *g* for **10 minutes**. Discard the supernatant and resuspend the cells at 1 x 10^8 cells/mL in recommended medium

4. Add the EasySep™ Biotin Selection Cocktail at **60 µL/mL of cells** (e.g. for 2 mL of cells, add 120 µL of selection cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes.**

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

6. Add the EasySep™ D Magnetic Particles at **150 µL/mL cells** (e.g. for 2 mL of cells, add 300 µL of magnetic particles). Mix well and incubate in refrigerator (2 - 8°C) for **10 minutes**.

7. Bring the cell suspension to a **total volume** of **2.5 mL** (for ≤2 x 10^8 cells) or **10 mL** (for 2 - 6 x 10^8 cells) by adding recommended medium without rat serum. 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**.

8. 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 enriched cells in the new tube are now ready for use.

CATALOG #19761 For labeling 1 x 10^9 total cells

Components:

- EasySep™ Mouse Monocyte Enrichment Cocktail 0.5 mL
- EasySep™ Biotin Selection Cocktail 1.0 mL
- EasySep™ D Magnetic Particles 2 x 1.0 mL
- Normal Rat Serum 2.0 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™ Mouse Monocyte Enrichment Cocktail, EasySep™ Biotin Selection Cocktail and EasySep™ D Magnetic Particles are designed to enrich mouse monocytes from mouse bone marrow and blood cell suspensions by depletion of T cells, B cells, NK cells, dendritic cells, progenitors, granuloctyes and red blood cells.

EASYSEP™ LABELING OF MOUSE CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles using biotinylated antibodies against cell surface antigens expressed on the unwanted cells and bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and biotin (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).

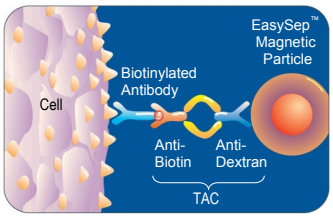


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

PREPARING A CELL SUSPENSION

FROM BONE MARROW Harvest bone marrow by preferred method. Crush bones in recommended medium using a mortar and pestle. Alternatively, flush bone marrow cells from femur and tibia into recommended medium using a syringe equipped with a 23-gauge needle. Disperse clumps by gently passing the cell suspension through the syringe several times.

With either method, remove remaining clumps of cells and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x *g* for 6 minutes, discard supernatant and resuspend cells at 1 x 10^8 cells/mL in recommended medium with 5% Normal Rat Serum added.

FROM BLOOD Collect blood into sodium heparin anticoagulant. Blood should be lysed prior to use. Mix 1 part blood with 9 parts Ammonium Chloride Solution (Catalog # 07800) and incubate on ice for 15 minutes until lysis is complete. Centrifuge at 300 x *g* for 6 minutes. Discard supernatant and wash pellet 1X with recommended medium. Discard supernatant and resuspend at 1 x 10^8 cells/mL in recommended medium with 5% Normal Rat Serum added.

OPTIMAL CELL NUMBER. The use of fewer than 5 x 10^7 cells per separation may result in sub-optimal performance. However, if <5 x 10^7 cells are used when starting with lysed blood, prepare cell suspension at 1 x 10^8 cells/mL with recommended medium and top up small volumes to 500 µL before starting Step 2 of protocol (reverse side).

RECOMMENDED MEDIUM. The recommended medium is EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS) and 1 mM EDTA. Hanks' Balanced Salt Solution (Hanks' BSS; Catalog #37250) can be used in place of PBS. Medium should be Ca^{++} and Mg^{++} free.

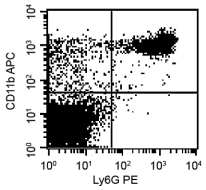
ASSESSING PURITY. To date, an exclusive marker for mouse bone marrow and peripheral blood monocytes has not been identified. However, monocytes are known to express CD11b, F4/80, CD115 (M-CSFR), Gr-1, and Ly-6C.

The recommended antibody clones for staining are M1/70 (Anti-Mouse CD11b Antibody, Clone M1/70, Catalog #60001) and 1A8 (Anti-Mouse Ly-6G Antibody, Clone 1A8, APC, Catalog #60031), where the monocytes will be CD11b positive and Ly-6G negative.

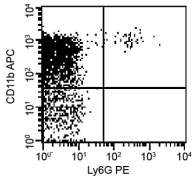
TYPICAL EASYSEP™ MOUSE MONOCYTE ENRICHMENT PROFILE:

Bone Marrow

Start: 8.8% $CD11b^+Ly\text{-}6G^-$ Cells

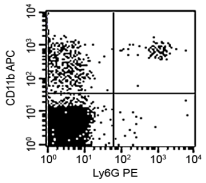


Enriched: 88.8% $CD11b^+Ly\text{-}6G^-$ Cells

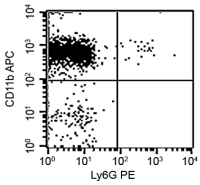


Blood

Start: 9.7% $CD11b^+Ly\text{-}6G^-$ Cells*



Enriched: 95.1% $CD11b^+Ly\text{-}6G^-$ Cells



The $CD11b^+Ly\text{-}6G^-$ cell content of the enriched cells typically ranges from 80 - 93% (Bone Marrow) and 92 - 98% (Blood).

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

COMPONENT DESCRIPTIONS:

EASYSEP™ MOUSE MONOCYTE ENRICHMENT COCKTAIL **CODE #19761C.1**

This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens (T cells, B cells, NK cells, dendritic cells, progenitors, granulocytes and red blood cells) on mouse cells. This cocktail is supplied in PBS. 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™ BIOTIN SELECTION COCKTAIL **CODE #19153**

This cocktail is a combination of two mouse IgG_1 monoclonal antibodies directed against biotin and dextran. These antibodies are bound in bispecific Tetrameric Antibody Complexes (TAC) by rat monoclonal antibodies directed against mouse IgG_1. This cocktail is supplied in PBS. 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.

NORMAL RAT SERUM **CODE #13551**

This normal rat serum is used to prevent non-specific binding of rat antibodies to mouse cells. Serum has been certified by the manufacturer to be mycoplasma-free.

STABILITY AND STORAGE:

EASYSEP™ MOUSE MONOCYTE ENRICHMENT COCKTAIL

EASYSEP™ BIOTIN SELECTION COCKTAIL

EASYSEP™ D MAGNETIC PARTICLES

Product stable at 2 - 8°C until expiry date as indicated on label. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

NORMAL RAT SERUM

Product stable at -20°C until expiry date as indicated on label. Stable for at least 2 months when stored at 2 - 8°C.