PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

MOUSE HEMATOPOIETIC PROGENITOR CELL ENRICHMENT KIT

CATALOG #19756

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™

This procedure is used for processing **500 µL – 8.0 mL** of sample (up to 8.0 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 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 (BD Biosciences, Catalog #352057) are recommended.

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

3. Wash cells and resuspend at 1 x 10^8 cells/mL in recommended medium.

4. Select the appropriate RoboSep™ protocol:

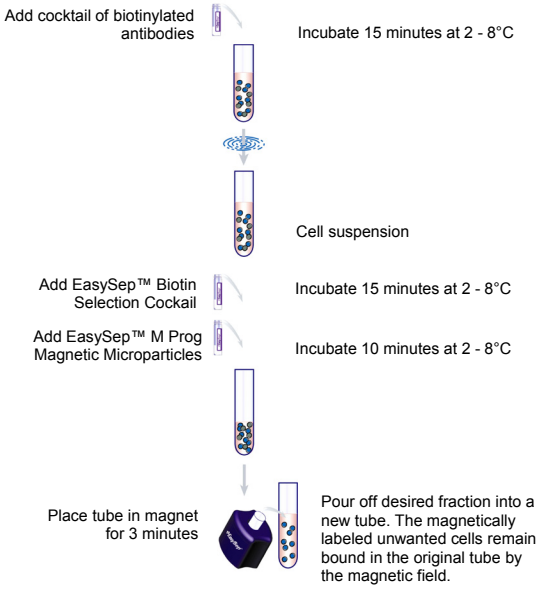
   - For high purity, select the protocol titled "Mouse Progenitor Negative Selection 19756 – high purity"
   - For high recovery, select the protocol titled "Mouse Progenitor Negative Selection 19756 – high recovery"

   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 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 **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 (BD Biosciences, Catalog #352058) are recommended.

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

3. Wash cells and resuspend at 1 x 10^8 cells/mL in recommended medium.

   Note: The wash step is recommended if a high depletion of lineage antigen positive cells is required and the start number of desired cells is low. However, if high recovery is more desirable than high purity, it is recommended to leave out the wash step.

4. Add the EasySep™ Biotin Selection Cocktail at **100 µL/mL of cells** (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate in the refrigerator (2 - 8°C) for **15 minutes**.

5. Vortex the EasySep™ Mouse Progenitor (M Prog) Magnetic Microparticles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

6. Add the 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 in the refrigerator (2 - 8°C) for **10 minutes**.

   Note: For increased depletion of lineage antigen positive cells, magnetic particles may be added at 75 µL/mL of cells. This will increase purity but will decrease recovery of cells.

7. 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 **3 minutes** at room temperature (15 - 25°C).

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 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 **500 µL – 8.5 mL** of sample (up to 8.5 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 (BD Biosciences, Catalog #352057) are recommended.

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

3. Wash cells and resuspend at 1 x 10^8 cells/mL in recommended medium.

   Note: The wash step is recommended if a high depletion of lineage antigen positive cells is required and the start number of desired cells is low. However, if high recovery is more desirable than high purity, it is recommended to leave out the wash step.

4. Add the EasySep™ Biotin Selection Cocktail at **100 µL/mL of cells** (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate in the refrigerator (2 - 8°C) for **15 minutes**.

5. Vortex the EasySep™ Mouse Progenitor (M Prog) Magnetic Microparticles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

6. Add the 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 in the refrigerator (2 - 8°C) for **10 minutes**.

   Note: For increased depletion of lineage antigen positive cells, magnetic particles may be added at 75 µL/mL of cells. This will increase purity but will decrease recovery of cells.

7. Bring the cell suspension up to a total volume of **5 mL** (for < 4 x 10^8 cells) or **10 mL** (for 4 - 8.5 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 **3 minutes** at room temperature (15 - 25°C).

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 isolated cells in the new tube are now ready for use.

STEMCELL TECHNOLOGIES INC.'S QUALITY MANAGEMENT SYSTEM IS CERTIFIED TO ISO 13485 MEDICAL DEVICE STANDARDS.
FOR RESEARCH USE ONLY. NOT INTENDED FOR HUMAN OR ANIMAL DIAGNOSTIC OR THERAPEUTIC USES.

CATALOG #19756 For labeling up to 10^9 total cells

Components:

- EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Cocktail 0.5 mL
- EasySep™ Biotin Selection Cocktail 1.0 mL
- EasySep™ Mouse Progenitor (M Prog) Magnetic Microparticles 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™.

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Mouse Hematopoietic Progenitor Cell Isolation Cocktail, EasySep™ Biotin Selection Cocktail and EasySep™ Mouse Progenitor (M Prog) Magnetic Microparticles label lineage antigen (CD5, CD11b, CD19, CD45R, Ly-6G/C, TER119, 7-4) positive cells for magnetic separation. These reagents are designed to enrich hematopoietic stem and progenitor cells from mouse bone marrow cell suspensions by depletion of lineage positive 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 (TACs). These complexes recognize both dextran and biotin (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep™ procedure (reverse side).

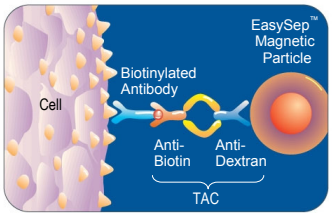


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

BONE MARROW. 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. Alternatively, crush bones using a mortar and pestle. Remove remaining clumps of cells and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge at 300 x g for 10 minutes, discard supernatant and resuspend cells at 1 x 10^8 cells/mL in recommended medium. Add 5% rat serum (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).

OPTIMAL CELL NUMBER. We do not recommend the use of fewer than 5 x 10^7 cells per separation as this may result in sub-optimal performance.

RECOMMENDED MEDIUM. The recommended medium is RoboSep™ Buffer (Catalog #20104), EasySep™ buffer (Catalog #20144) or phosphate-buffered saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905) and 1 mM EDTA. Medium should be Ca^{2+} and Mg^{2+} free. Hanks' Balanced Salt Solution can be used in place of PBS.

ASSESSING PURITY. The first step to isolation of mouse hematopoietic stem cells (HSCs) and progenitor cells from bone marrow (BM), consists of removing mature cells that express "lineage" (Lin) antigens specific to terminally differentiated blood cells. Lineage antigens are absent or weakly expressed on HSCs and progenitors. Lineage antigens include CD3, CD11b, CD19, CD45R/B220, Gr-1, TER119. In many mouse strains, HSCs and primitive progenitors are positive for Sca1 (Ly-6A/E) and c-Kit (the receptor for SCF, also known as CD117) and referred to as LSK (Lin-Sca1+c-Kit+).[1,2,3,4] LSK cells which make up < 0.1% of nucleated BM cells, contain most repopulating stem cells but are depleted of more mature erythroid, myeloid and megakaryocyte cells including most CFUs which are Lin-Sca1-/^{low}c-Kit+.[5] Mouse HSCs and progenitors are heterogeneous for other antigens, e.g. CD34 and Thy-1.

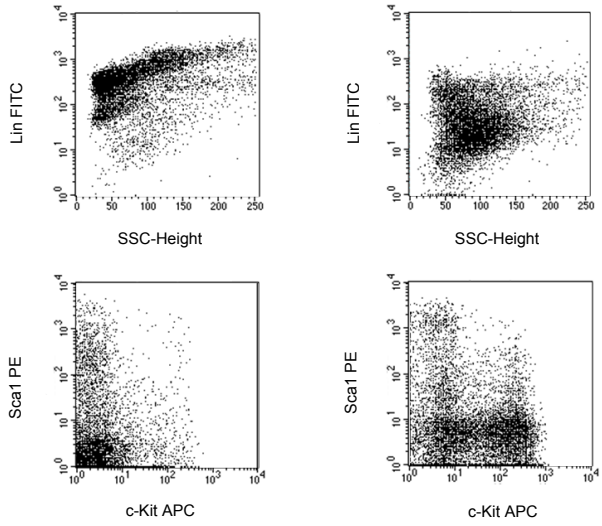
The purity of these subsets after progenitor enrichment can be assessed by flow cytometry after staining with a cocktail of fluorescently-labeled antibodies against lineage antigens, c-KIT, Sca1, CD34 and/or THY1.1. The recommended antibodies for lineage antigen staining are fluorochrome-conjugated anti-CD3 (clone 145-2C11, Catalog #60015), anti-Mac-1/CD11b (clone M1/70, Catalog #60001), anti-CD19 (clone 6D5, Catalog #60006), anti-CD45R/B220 (clone RA3-6B2, Catalog #60019), anti-Gr-1 (clone RB6-8C5, Catalog #60028), TER119/Ly-76 (clone TER-119, Catalog #60033).

REFERENCES:

1. Spangrude GJ, et al. Purification and characterization of mouse hematopoietic stem cells. Science 241(4861): 58-62, 1988
2. Uchida N, Weissman IL. Searching for hematopoietic stem cells: evidence that Thy-1.1lo Lin- Sca1+ cells are the only stem cells in C57BL/Ka-Thy-1.1 bone marrow. J Exp Med 175(1): 175-184, 1992
3. Okada S et al. In vivo and in vitro stem cell function of c-kit and Sca-1-positive murine hematopoietic cells. Blood 80(12): 3044-3050, 1992
4. Osawa M et al. In vivo self-renewal of c-Kit+ Sca-1+ Lin(low/-) hemopoietic stem cells. J Immunol 156(9):3207-3214, 1996
5. Akashi K, et al. A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. Nature 404(6774): 193-197, 2000

TYPICAL EASYSEP™ MOUSE HEMATOPOIETIC CELL ENRICHMENT PROFILE:

Start: 10% Lineage Negative Cells Enriched: 72% Lineage Negative Cells



Starting with mouse bone marrow, the lineage antigen negative cell content of the enriched fraction typically ranges from 60 - 90%.

COMPONENT DESCRIPTIONS:

EASYSEP™ MOUSE HEMATOPOIETIC PROGENITOR CELL ISOLATION COCKTAIL **CODE #19856C**
This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens on mouse cells of hematopoietic origin (CD5, CD11b, CD19, CD45R, Ly-6G/C(Gr-1), TER119, 7-4). 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 contains a combination of monoclonal antibodies purified by affinity chromatography. These antibodies are bound in bispecific Tetrameric Antibody Complexes (TAC) which are directed against biotin and dextran. The mouse monoclonal antibody subclass is 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™ MOUSE PROGENITOR (M PROG) MAGNETIC MICROPARTICLES **CODE #19350**
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 HEMATOPOEITIC PROGENITOR CELL ISOLATION COCKTAIL

EASYSEP™ BIOTIN SELECTION COCKTAIL

EASYSEP™ MOUSE PROGENITOR (M PROG) MAGNETIC MICROPARTICLES
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.

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. Contents have been sterility tested.

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