

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® (CATALOG #20000).

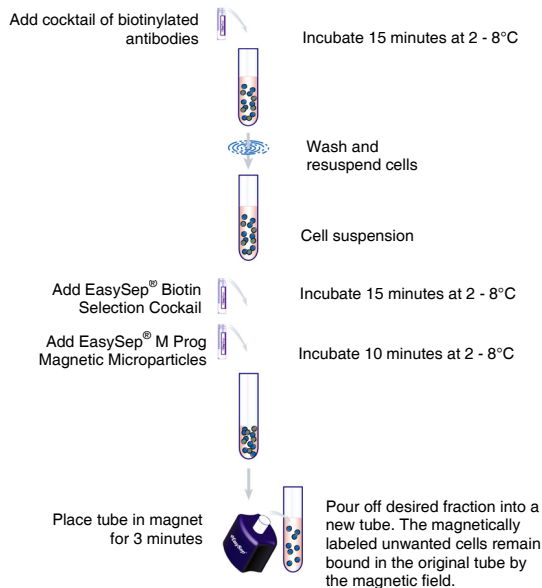
This procedure is used for processing **500 µL – 8.0 mL** of sample (up to 8.0×10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^8 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. Add Normal Rat Serum (provided) at 50 µL per mL of cell suspension (e.g. for 2 mL of cell suspension, add 100 µL of serum).
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
2. Add EasySep® Mouse Hematopoietic Progenitor Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 2 - 8°C for **15 minutes**.
3. Wash cells and resuspend at 1×10^8 cells/mL in recommended medium.
4. Select the appropriate RoboSep® protocol:
 - For high purity, select the protocol entitled "Mouse Progenitor Negative Selection 19756 – high purity".
 - For high recovery, select the protocol entitled "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 remaining 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



WWW.STEMCELL.COM

B) MANUAL EASYSEP® PROTOCOL USING PURPLE EASYSEP® MAGNET (CATALOG #18000).

This procedure is used for processing **500 µL – 2.0 mL** of sample (up to 2×10^8 cells)

1. Prepare single nucleated cell suspension at a concentration of 1×10^8 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 Normal Rat Serum (provided) at 50 µL per mL of cell suspension (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).
Falcon™ 5 mL (BD, Catalog #352058) Polystyrene Round-Bottom Tubes are recommended.
2. Add EasySep® Mouse Hematopoietic Progenitor Cell Enrichment Cocktail at **50 µL/mL** of cells (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at 2 - 8°C for **15 minutes**.
3. Wash cells and resuspend at 1×10^8 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 EasySep® Biotin Selection Cocktail at **100 µL/mL** of cells (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate at 2 - 8°C for **15 minutes**.
5. Vortex EasySep® Mouse Progenitor (M Prog) Magnetic Microparticles for 30 seconds to 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 at 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 to a total volume of **2.5 mL** by adding recommended medium without rat serum. Mix the cells in the tube by pipetting gently 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **3 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 magnet. Leave the magnet and the tube inverted 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 – 8.5 mL** of sample (up to 8.5×10^8 cells).

1. Prepare nucleated cell suspension at a concentration of 1×10^8 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 magnet. Add Normal Rat Serum (provided) at 50 µL per mL of cell suspension (e.g. for 2 mL of cell suspension, add 100 µL of rat serum).
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
2. Add EasySep® Mouse Hematopoietic Progenitor Cell 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 **15 minutes**.
3. Wash cells and resuspend at 1×10^8 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 EasySep® Biotin Selection Cocktail at **100 µL/mL** cells (e.g. for 2 mL of cells, add 200 µL of selection cocktail). Mix well and incubate at 2 - 8°C for **15 minutes**.
5. Vortex EasySep® Mouse Progenitor (M Prog) Magnetic Microparticles for 30 seconds to ensure that the particles are in a uniform suspension with no visible aggregates.
6. Add the magnetic particles at **50 µL/mL** cells (e.g. for 2 mL of cells add **100 µL** of particles). Mix well and incubate at 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 to a total volume of 5 mL (for $< 4 \times 10^8$ cells) or 10 mL (for $4 - 8.5 \times 10^8$ cells) by adding recommended medium without rat serum. Mix the cells in the tube by pipetting gently 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **3 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 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 inverted 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 are now ready for use.

Components:

• EasySep [®] Mouse Hematopoietic Progenitor Enrichment 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



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 Hematopoietic Progenitor Enrichment Cocktail, EasySep[®] Biotin Selection Cocktail and EasySep[®] Mouse Progenitor 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 cells 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 (TAC). These complexes recognize both dextran and biotin (Figure 1). Magnetically labeled cells are then separated from unlabeled target cells using the EasySep[®] procedure (reverse side).

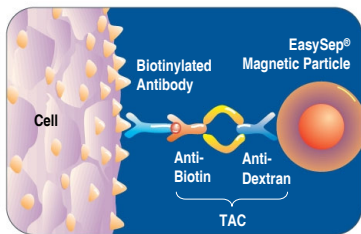


Figure 1.
Schematic Drawing of EasySep[®] TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

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 μ m mesh nylon strainer. Centrifuge at 300 x g for 10 minutes, discard supernatant and resuspend cells at 1×10^8 cells/mL in recommended medium. Add 5% rat serum (e.g. for 2 mL of cell suspension, add 100 μ L of rat serum).

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

RECOMMENDED MEDIUM. The recommended medium is RoboSep[®] Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905) and 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free. Hank's Balanced Salt Solution can be used in place of PBS.

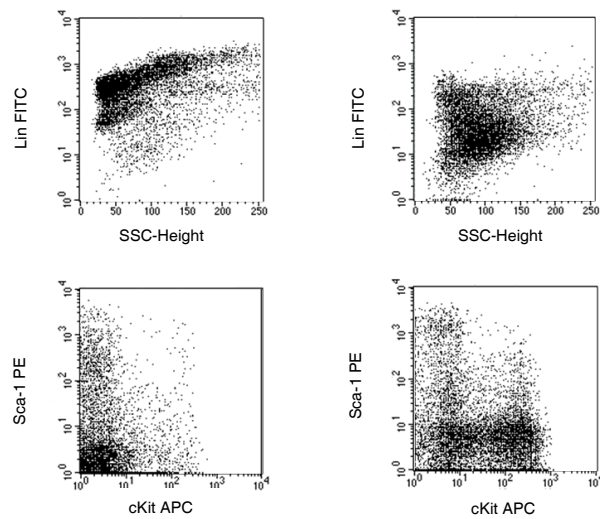
ASSESSING PURITY. Mouse hematopoietic stem cells (HSCs) and closely related primitive progenitors are distinguished from the majority of bone marrow cells by their lack of expression of markers specific for maturing blood cells (lineage antigens, e.g. CD3, CD11b, CD19, CD45R, GR1, and TER119). In many mouse strains, HSCs and primitive progenitors are positive for SCA1 (Ly-6A/E) and cKIT (Lin⁻SCA1⁺KIT⁺ phenotype).^{1,2} More mature erythroid, myeloid and megakaryocyte progenitor cells are also Lin⁻ and cKIT⁺, but negative for SCA1 (Lin⁻SCA1⁻KIT⁺ phenotype).⁴ Mouse HSCs and progenitors are heterogeneous for other antigens, e.g., CD34 and THY1. 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, cKIT, SCA1, CD34 and/or THY1.1. The recommended antibody clones for lineage antigen staining are 145-2C11 (CD3), RA3-6B2 (CD45R/B220, Catalog #10711), RB6-8C5 (GR1), Catalog #10717), M1/70 (MAC1/CD11b, Catalog #10705), 1D3 (CD19, Catalog #10707), TER-119 (TER119/Ly-76, Catalog #10729).

References:

- Spangrude GJ, Heimfeld S, Weissman IL: Purification and characterization of mouse hematopoietic stem cells. *Science* 241: 58, 1988
- 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: 175, 1992
- Osawa M, Hanada K, Hamada H, Nakauchi H: Long-term lymphohematopoietic reconstitution by a single CD34⁻ low/negative hematopoietic stem cell. *Science* 273: 242, 1996
- Akashi K, Traver D, Miyamoto T: A clonogenic common myeloid progenitor that gives rise to all myeloid lineages. *Nature* 404: 193, 2000

TYPICAL EASYSEP[®] MOUSE HEMATOPOIETIC CELL ENRICHMENT PROFILE:

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



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

COMPONENT DESCRIPTIONS:**EASYSEP[®] MOUSE HEMATOPOIETIC CELL ENRICHMENT COCKTAIL****CODE #19756C.1**

This cocktail contains a combination of biotinylated monoclonal antibodies purified from rat ascites fluid or hybridoma culture supernatant. The monoclonal antibodies are purified by affinity chromatography using Protein A or Protein G Sepharose. These antibodies are directed against cell surface antigens on mouse cells of hematopoietic origin (CD5, CD11b, CD19, CD45R, Ly-6G/C(GR1), TER119, 7-4). This cocktail is supplied in Phosphate Buffered Saline. 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 IgG₁ monoclonal antibodies against biotin and dextran purified from hybridoma culture supernatant. These antibodies are bound in bispecific Tetrameric Antibody Complexes by rat monoclonal antibodies against mouse IgG₁. 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. It has been certified by the manufacturer to be mycoplasma-free.

STABILITY AND STORAGE:**EASYSEP[®] MOUSE HEMATOPOIETIC CELL ENRICHMENT 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 2 - 8°C until expiry date as indicated on label. Stable for at least 2 years when stored at -20°C. Contents have been sterility tested.