

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 8.5×10^8 cells).

1. Prepare single 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. For samples containing 5×10^7 cells or fewer, resuspend in 500 µL.

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

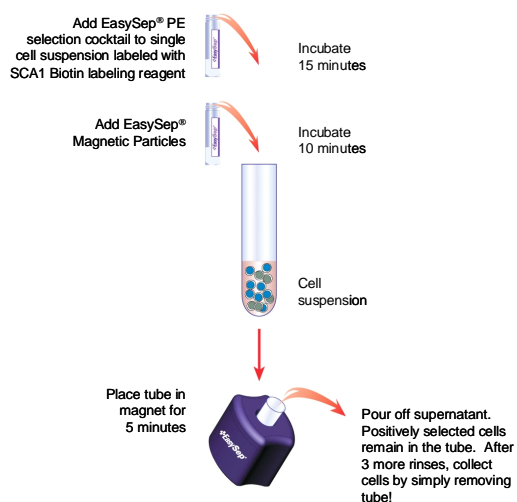
2. Select the appropriate RoboSep® protocol:

For most normal samples, select the protocol entitled "Mouse SCA1 Biotin Positive Selection 18856-high purity".

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. Mix EasySep® Magnetic Nanoparticles before loading to ensure that they are in a uniform suspension by pipetting up and down vigorously more than 5 times. 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 tube containing the isolated cells from the magnet. The positively selected 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 **100 µL - 2 mL** of sample (up to 2×10^8 cells).

1. Prepare single 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 EasySep® Magnet. For samples containing 10^7 cells or fewer, resuspend in 100 µL.
Falcon™ 5 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352058) are recommended.
2. Add SCA1 Biotin Labeling Reagent at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of labeling reagent). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
3. Add EasySep® Biotin Selection Cocktail at **70 µL/mL cells** (e.g. for 2 mL of cells, add 140 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
4. Mix Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously 5 times. Add the nanoparticles at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
5. Bring the cell suspension 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 **5 minutes**.
6. Pick up the EasySep® magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled cells will remain inside the tube, held by the magnetic field of the EasySep® Magnet. Hold 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.**
7. Remove the tube from the magnet and add 2.5 mL of recommended medium. Mix the cell suspension by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.
8. Repeat Steps 6 and 7 twice, then Step 6 once more, for a total of 4 x 5-minute separations in the magnet. Remove tube from the magnet and resuspend cells in an appropriate amount of desired medium. These positively selected cells are now ready for use.

C) MANUAL EASYSEP® PROTOCOL USING "THE BIG EASY" SILVER EASYSEP® MAGNET (CATALOG #18001).

This procedure is used for processing **250 µL - 8 mL** of sample (up to 8×10^8 cells).

1. Prepare single 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 fit properly into the silver magnet. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 µL.
Falcon™ 14 mL Polystyrene Round-Bottom Tubes (BD, Catalog #352057) are recommended.
2. Add SCA1 Biotin Labeling Reagent at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of labeling reagent). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
3. Add EasySep® Biotin Selection Cocktail at **70 µL/mL cells** (e.g. for 2 mL of cells, add 140 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.
4. Mix Magnetic Nanoparticles to ensure that they are in a uniform suspension by pipetting vigorously 5 times. Add the nanoparticles at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of nanoparticles). Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
5. Bring the cell suspension to a **total volume** of 5.0 mL (for $< 4 \times 10^8$ cells) or 10 mL (for $4 - 8 \times 10^8$ 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 **5 minutes**.
Note: decreasing the top-up volume will increase cell recovery, but may slightly reduce cell purity.
6. Pick up the EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the supernatant fraction. The magnetically labeled cells will remain inside the 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.**
7. Remove the tube from the magnet and add 5.0 mL (for $< 4 \times 10^8$ cells) or 10 mL (for $4 - 8 \times 10^8$ cells) of recommended medium. Mix the cell suspension by gently pipetting up and down 2 - 3 times. Place the tube back in the magnet and set aside for **5 minutes**.
Note: decreasing the top-up volume will increase cell recovery, but may slightly reduce cell purity.
8. Repeat Steps 6 and 7, then Step 6 once more, for a total of 4 x 5-minute separations in the magnet. Remove the tube from the magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

Mouse SCA1 Biotin Positive Selection Kit Components:

• EasySep [®] Mouse SCA1 Biotin Labeling Reagent	1.0 mL
• EasySep [®] Biotin Selection Cocktail	2 x 1.0 mL
• EasySep [®] Magnetic Nanoparticles	1.0 mL



POSITIVE SELECTION

REQUIRED EQUIPMENT:

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

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] Biotin Selection Cocktail and EasySep[®] Magnetic Nanoparticles are designed to positively select cells labeled with EasySep[®] SCA1 Biotin Labeling Reagent.

EASYSEP[®] LABELING OF HUMAN CELLS:

Cells specifically targeted with Biotin Labeling Reagent are then labeled with EasySep[®] dextran-coated magnetic nanoparticles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the biotin molecule on the Biotin Labeling Reagent (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep[®] procedure (reverse side).

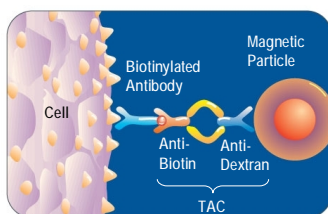


Figure 1.
Schematic Drawing of EasySep[®] TAC
Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

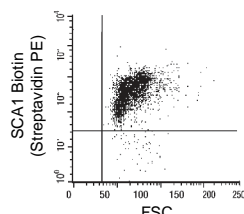
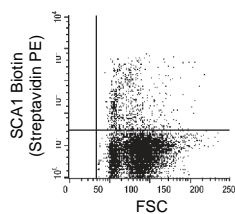
PREPARING A SINGLE CELL SUSPENSION.

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, discard supernatant and resuspend cells at 1×10^8 cells/mL.

RECOMMENDED MEDIUM. RoboSep[®] Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS, Catalog #07905) with 1 mM EDTA. Medium should be Ca⁺⁺ and Mg⁺⁺ free.

MOUSE STRAINS. Most hematopoietic stem cells from BALB/c and BALB/c-derived mouse strains (e.g. some NOD/SCID strains) do not express SCA1 (see e.g. Spangrude & Brooks 1993, Blood 82:3327 - 3332). Alternative markers should be used to select hematopoietic stem and progenitor cells from these mice; see Technical Tip (opposite) for more information.

ASSESSING PURITY. The biotin-labeled selected cells can be stained with a suitable secondary reagent. We recommend using streptavidin-PE at a final concentration of 1 μ g/mL.

TYPICAL EASYSEP[®] SCA1 BIOTIN SELECTION PROFILE:Start: 8% SCA1⁺ CellsSelected: 98% SCA1⁺ Cells

The SCA1⁺ cell content of the selected cells typically ranges from 80 - 98%. Hematopoietic stem and progenitor cells are present in the Lin⁻SCA1⁺cKIT⁺ population. See technical tip (opposite) for more information.

COMPONENT DESCRIPTIONS:

EASYSEP[®] MOUSE SCA1 BIOTIN LABELING REAGENT CODE #18856C

Supplied in aqueous buffer containing 0.1% bovine serum albumin (BSA). Also contains an unlabeled antibody directed against mouse CD16/32 (Fc γ III/II Receptor).

EASYSEP[®] BIOTIN SELECTION COCKTAIL CODE #18153

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 biotin and dextran. The mouse monoclonal antibody subclass is IgG₁. 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[®] MAGNETIC NANOPARTICLES CODE #18150

A suspension of magnetic dextran iron particles in water.

STABILITY AND STORAGE:

EASYSEP[®] BIOTIN SELECTION COCKTAIL

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[®] MAGNETIC NANOPARTICLES

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[®] MOUSE SCA1 BIOTIN LABELING REAGENT

Product stable at 2 - 8°C until expiry date as indicated on label. Protect from light. 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.



Technical Tip

Hematopoietic Stem Cells (HSCs) and closely related primitive progenitors in mice are distinguished from the majority of the cells in hematopoietic tissues by their lack of expression of markers specific to maturing blood cells (i.e. CD3, CD11b, CD45R/B220, GR1, TER119). In many mouse strains, HSCs are positive for SCA1 (Ly-6A/E) and cKIT (CD117) (Lin⁻SCA1⁺cKIT⁺ phenotype) (Spangrude, Heimfeld, *et al.* 1988; Uchida & Weissman 1992). More mature erythroid, myeloid and megakaryocyte progenitor cells are also Lin⁻ and cKIT⁺, but negative for SCA1 (Lin⁻SCA1⁻cKIT⁺ phenotype) (Akashi, Traver, *et al.* 2000). The various subsets can be significantly enriched by depletion of lineage⁺ cells using the StemSep[®] or EasySep[®] Mouse Progenitor Cell Enrichment Cocktails, or by positive selection of SCA1⁺ or cKIT⁺ cells with EasySep[®].

