

Positive Selection

EasySep

PROCEDURE

Mouse

CD117 (cKIT)

Selection

Cocktail

CATALOG #18757

Version 3.0.1



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 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 (Becton Dickinson, Catalog #352057) are recommended.

2. Select the appropriate RoboSep[®] protocol:

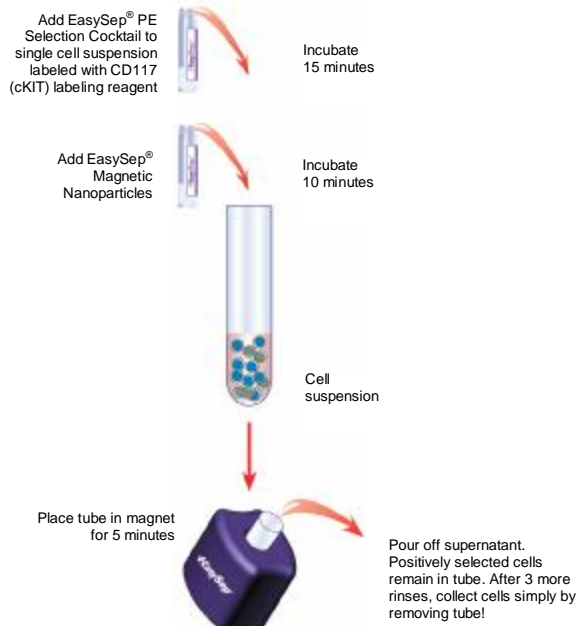
For most normal samples, select the protocol entitled "Mouse CD117 (cKIT) Positive Selection 18757-high purity".

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 the 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 purple EasySep[®] Magnet. For samples containing 10^7 cells or fewer, resuspend in 100 µL.

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

2. Add CD117 (cKIT) PE 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 for **15** minutes.
3. Add EasySep[®] PE 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 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 for **10** minutes.
5. Bring the cell suspension to a **total volume** of 2.5 mL with 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, and then Step 6 once more, for a total of 4 x 5-minute separations in the magnet. Remove tube from magnet and resuspend cells in an appropriate amount of desired medium. The 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 - 8.5 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 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. For samples containing 2.5×10^7 cells or fewer, resuspend in 250 µL.

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

2. Add CD117 (cKIT) PE 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 for **15** minutes.
3. Add EasySep[®] PE 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 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 for **10** minutes.
5. 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. 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 mL (for $< 4 \times 10^8$ cells) or 10 mL (for $4 - 8.5 \times 10^8$ 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.

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October 2007

FOR RESEARCH USE ONLY

#28857

Catalog #18757For labeling 2×10^9 total cells

Mouse CD117 (cKIT) Positive Selection Kit Components:

- EasySep[®] Mouse CD117 (cKIT) PE Labeling Reagent 1.0 mL
- EasySep[®] PE Selection Cocktail 2 x 1.0 mL
- EasySep[®] Magnetic Nanoparticles 1.0 mL

REQUIRED EQUIPMENT:

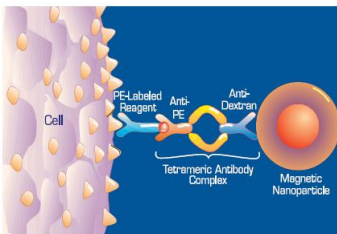
EasySep[®] Magnet (Catalog #18000), or "The Big Easy" EasySep[®] Magnet (Catalog #18001), or RoboSep[®] (Catalog #20000).

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep[®] PE Selection Cocktail and EasySep[®] Magnetic Nanoparticles are designed to positively select CD117⁺ (cKIT⁺) cells (cells labeled with the EasySep[®] Mouse CD117 (cKIT) PE-labeling reagent).

EASYSEP[®] LABELING OF CELLS:

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

**Figure 1.**

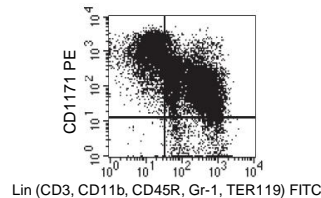
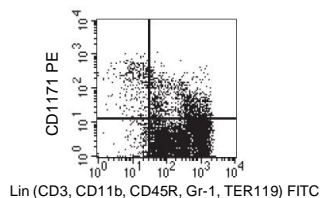
Schematic Drawing of EasySep[®] TAC Magnetic Labeling of Mouse Cells.

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 1×10^6 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.

Assessing Purity. Since the positively selected cells have already been PE-labeled, purity can be assessed directly by flow cytometry.

TYPICAL PROFILE OF EASYSEP MOUSE CD117⁺ (cKIT⁺) CELL SELECTION FROM BONE MARROWStart: 18.9% CD117⁺ CellsSelected: 92.8% CD117⁺ Cells

The CD117⁺ cell content of the selected cells typically ranges from 88 -95%.

Hematopoietic stem and progenitor cells are present in the Lin-Sca1⁺CD117⁺ population. Please see technical tip (opposite) for more information.

COMPONENT DESCRIPTIONS:**EasySep[®] Mouse CD117 (cKIT) PE Labeling Reagent****code #18757C.1**

Supplied in aqueous buffer containing 0.1% sodium azide. Also contains an unlabeled antibody directed against mouse CD16/32 (Fc γ III/II receptor).

EasySep[®] PE Selection Cocktail**code #18151**

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 PE (Phycoerythrin) 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[®] Mouse CD117 (cKIT) PE Labeling Reagent.**

Store undiluted at 4°C. Protect from light. Do not freeze this product. This product may be shipped at room temperature and should be refrigerated upon receipt.

Hazardous Ingredient: Sodium Azide. Avoid exposure to skin and eyes, ingestion and contact with heat, acids and metals. Wash exposed skin with soap and water. Flush eyes with water. Dilute with running water before discharging into plumbing.

EasySep[®] PE Selection Cocktail.

Stable at 4°C for 2 years. Do not freeze this product. This product may be shipped at room temperature, and should be refrigerated upon receipt.

EasySep[®] Magnetic Nanoparticles.

Stable at 4°C for 2 years. This product may be shipped at room temperature, and should be refrigerated upon receipt.

See Material Safety Data Sheet for more information (available on our website at www.stemcell.com/technical/msds.aspx).

**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[®], EasySep[®] or SpinSep[®] Mouse Progenitor Cell Enrichment Cocktails, or by positive selection of SCA1⁺ or cKIT⁺ cells with EasySep[®].

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