PROCEDURE

EasySep™

POSITIVE SELECTION

MOUSE CD49b POSITIVE SELECTION KIT

CATALOG #18755

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 **250 µL - 8.0 mL** of sample (up to 8 x 10^8 cells).

1. Preparea single 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. For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL.

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

2. Select the appropriate RoboSep™ protocol:

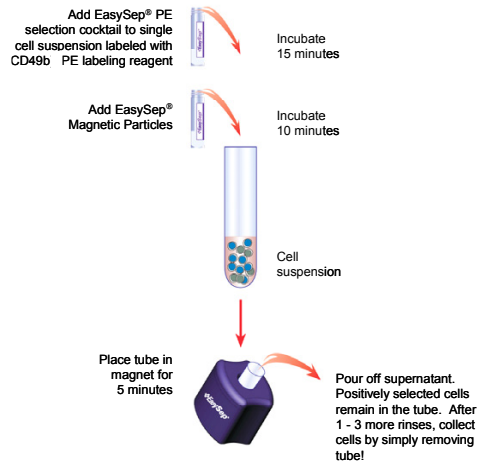
   - Mouse CD49b Positive Selection 18755-high purity

   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 for processing **100 µL – 2.5 mL** of sample (up to 2.5 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). For samples containing 10^7 cells or fewer, resuspend in 100 µL. Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to properly fit into the purple EasySep™ Magnet.

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

2. Add the EasySep™ Mouse CD49b 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 (15 - 25°C) for **15 minutes**.

3. Add EasySep™ PE Selection Cocktail at **100 µL/mL** of cells (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.

4. Mix EasySep™ Magnetic Nanoparticles to ensure that they are in uniform suspension by pipetting vigorously up and down 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 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.*

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 (without cap) into the magnet. Set aside for **5 minutes**.

8. Repeat Steps 6 and 7, and then Step 6 once more, for a total of 3 x 5 minute separations in the magnet (see Notes and Tips, reverse side). 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 x 10^8 cells).

1. Prepare a single cell suspension at a concentration of 1 x 10^8 cells/mL in recommended medium (see Notes and Tips, reverse side). For samples containing 2.5 x 10^7 cells or fewer, resuspend in 250 µL. Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the Silver EasySep™ magnet.

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

2. Add the EasySep™ Mouse CD49b 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 **100 µL/mL** of cells (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **15 minutes**.

4. Mix EasySep™ Magnetic Nanoparticles to ensure that they are in uniform suspension by pipetting vigorously up and down 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 **5.0 mL** (for <3 x 10^8 cells) or **10 mL** (for 3 - 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 **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 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.*

7. Remove the tube from the magnet and add 5.0 mL (for <3 x 10^8 cells) or 10.0 mL (for 3 - 8.5 x 10^8 cells) of recommended medium. Mix the cell suspension by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **5 minutes**.

8. Repeat Steps 6 and 7, and then Step 6 once more, for a total of 3 x 5 minute separations in the magnet (see Notes and Tips, reverse side). Remove tube from magnet and resuspend cells in an appropriate amount of desired medium. The positively selected cells are now ready for use.

CATALOG #18755 For labeling 2 x 10^9 total cells

Mouse CD49b Positive Selection Kit Components:

• EasySep™ PE Selection Cocktail	2 x 1.0 mL
• EasySep™ Magnetic Nanoparticles	1.0 mL
• EasySep™ Mouse CD49b Labeling Reagent	1.0 mL

EasySep™ 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™ PE Selection Cocktail and EasySep™ Magnetic Nanoparticles are designed to positively select cells labeled with EasySep™ PE-labeling reagent.

EASYSEP™ LABELING OF HUMAN 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).

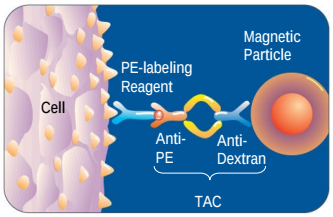


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

PREPARING A SINGLE CELL SUSPENSION Disrupt spleen into 5 mL of recommended medium. Further disperse clumps by gently pipetting up and down several times. Remove remaining clumps of cells and debris by passing cell suspension through a 70 µm mesh nylon strainer. Centrifuge, discard supernatant and resuspend cells at 1 x 10^8 cells/mL. Ammonium chloride treatment is not recommended when preparing the cells for separation.

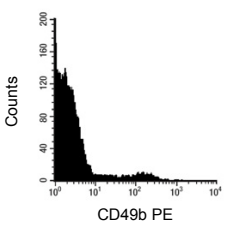
RECOMMENDED MEDIUM Phosphate buffered saline (PBS) + 2% fetal bovine serum (FBS) (Catalog #07905) with 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

OPTIMIZING PURITY AND RECOVERY To avoid selecting unwanted cells, work quickly and do not prolong incubation times. High purity will be achieved with 3 separations in most cases but for increased purity repeat Steps 6 and 7 once more. Cell recovery will decrease with each additional round of separation.

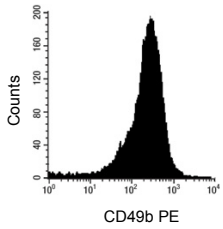
ASSESSING PURITY Since the positively selected cells have already been PE-labeled, purity can be assessed directly by flow cytometry.

TYPICAL EASYSEP™ CD49b PE SELECTION PROFILE:

Start: 6.1% CD49b$^+$ (DX5) Cells



Selected: 96.5% CD49b$^+$ (DX5) Cells



Starting with mouse splenocytes, the CD49b$^+$ (DX5) cell content of the selected cells typically ranges from 88 - 97%.

COMPONENT DESCRIPTIONS:

EASYSEP™ PE SELECTION COCKTAIL **CODE #18154**

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 IgG_1. This cocktail is supplied in PBS with 0.1% bovine serum albumin (BSA). 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.

EASYSEP™ MOUSE CD49b PE LABELING REAGENT **CODE #18755C.1**

Supplied in PBS with 0.1% BSA and 0.1% sodium azide. Contains an antibody directed against mouse CD16/32 (Fcγ III/II receptor).

STABILITY AND STORAGE:

EASYSEP™ PE 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 CD49b PE 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.

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.*

Refer to Material Safety Data Sheet for additional information.

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