PROCEDURE

EasySep® POSITIVE SELECTION

MOUSE CD93 (AA4.1) POSITIVE SELECTION KIT

CATALOG #18762

V 1.1.0

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 - 8.5 mL** of sample (up to 8.5 x 10^8 cells).

1. Prepare single cell suspension at a concentration of 1 x 10^8 cells/mL (bone marrow) or 5 x 10^7 cells/mL (fetal liver) in RoboSep® Buffer (Catalog #20104). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to fit properly 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, Catalog #352057) are recommended.

2. Select the appropriate RoboSep® protocol:

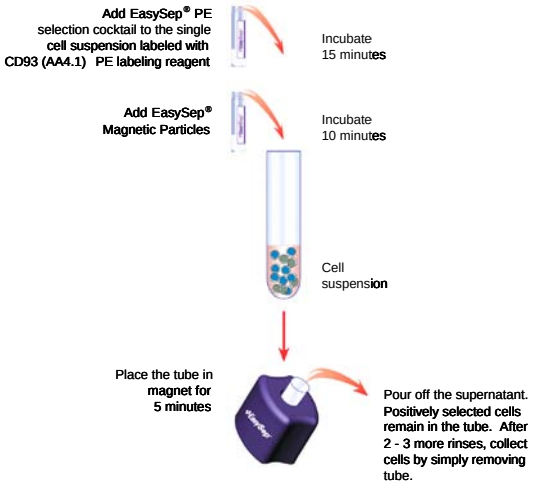
   For most normal samples, select the protocol entitled "Mouse AA4.1 Positive Selection 18762-high purity".

   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 x 10^8 cells).

1. Prepare a single cell suspension at a concentration of 1 x 10^8 cells/mL (bone marrow) or 5 x 10^7 cells/mL (fetal liver) in the recommended medium (see Notes and Tips, reverse side). Cells must be placed in a 5 mL (12 x 75 mm) polystyrene tube to fit properly into the EasySep® Magnet. For samples containing 1 x 10^7 cells or fewer, resuspend in 100 µL.

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

2. Add CD93 (AA4.1) PE 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® 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 (15 – 25°C) for **15** minutes.

4. Mix EasySep® 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 with the 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 the 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 once (for bone marrow) or twice (for fetal liver), then step 6 once more, for a total of 3 x 5-minute (bone marrow) or 4 x 5-minute (fetal liver) separations in the magnet. Remove tube from the 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 mL** of sample (up to 8 x 10^8 cells).

1. Prepare a single cell suspension at a concentration of 1 x 10^8 cells/mL (bone marrow) or 5 x 10^7 cells/mL (fetal liver) in the 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 x 10^7 cells or fewer, resuspend in 250 µL.

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

2. Add CD93 (AA4.1) 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 **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 EasySep® 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 mL (for < 4 mL) or 10 mL (for 4 - 8 mL) by adding the 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 mL) or 10 mL (for 4 - 8 mL) of the 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 once (for bone marrow) or twice (for fetal liver), then Step 6 once more, for a total of 3 x 5-minute (bone marrow) or 4 x 5-minute (fetal liver) 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.

CATALOG #18762 For labeling up to 2 x 10^9 total cells

Mouse CD93 (AA4.1) Positive Selection Kit Components:

• EasySep® Mouse CD93 (AA4.1) PE Labeling Reagent	1.0 mL
• EasySep® PE Selection Cocktail	2 x 1.0 mL
• EasySep® Magnetic Nanoparticles	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® Mouse CD93 (AA4.1) 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).

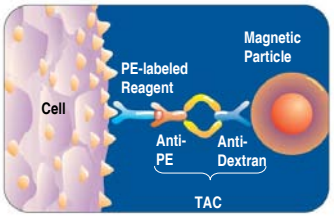


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

PREPARING A SINGLE CELL SUSPENSION.

Bone marrow: Flush bone marrow cells from femur and tibia into the 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, discard supernatant and resuspend cells at 1 x 10^8 cells/mL.

Fetal liver: Dissect fetal livers from mouse embryos. Resuspend to a single cell suspension by pipetting cells up and down, then filter through a 70 µm mesh nylon strainer. Resuspend cells at 5 x 10^7 cells/mL.

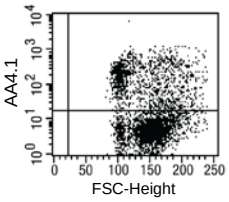
RECOMMENDED MEDIUM. Phosphate Buffered Saline (PBS) + 2% Fetal Bovine Serum (FBS) (Catalog #07905) with 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

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

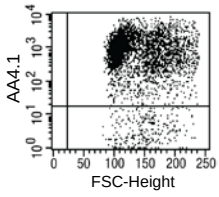
TYPICAL EASYSEP® CD93 (AA4.1) PE SELECTION PROFILE:

Bone marrow

Start: 39.7% AA4.1$^+$ Cells



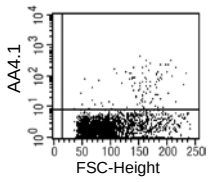
Selected: 94.4% AA4.1$^+$ Cells



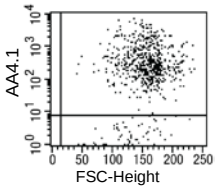
The CD93$^+$ (AA4.1$^+$) cell content of the selected cells typically ranges from 86.7 - 98.5%.

Fetal liver

Start: 2.7% AA4.1$^+$ Cells



Selected: 91.3% AA4.1$^+$ Cells



The CD93$^+$ (AA4.1$^+$) cell content of the selected cells typically ranges from 74.4 - 97.0%.

COMPONENT DESCRIPTIONS:

EASYSEP® MOUSE CD93 (AA4.1) PE LABELING REAGENT **CODE #18762C**

Supplied in aqueous buffer containing 0.1% sodium azide. Also contains an unlabeled antibody directed against mouse CD16/32 (Fcγ 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 IgG_1. 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® 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 CD93 (AA4.1) 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.*

See Material Safety Data Sheet for more information.