PROCEDURE

EasySep™

NEGATIVE SELECTION

MOUSE PAN-DC ENRICHMENT KIT

CATALOG #19763

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™.

This procedure is used for processing **500 µL – 6.5 mL** of sample (up to 6.5 x 10^8 cells).

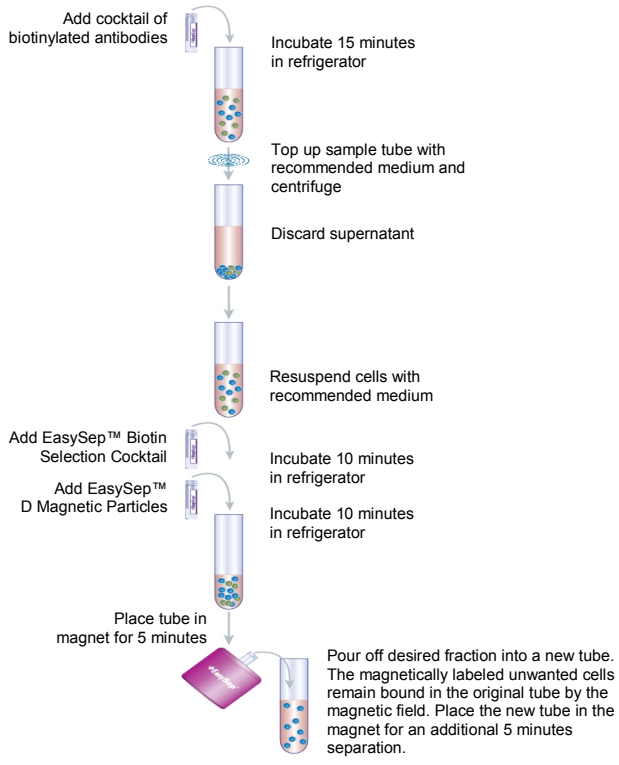
1. Prepare cell suspension at a concentration of 1 x 10^8 cells/mL in RoboSep™ Buffer (Catalog #20104) (see Notes and Tips, reverse side). Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the RoboSep™ carousel.

   Falcon™ 14 mL Polystyrene Round-Bottom Tubes (Corning Catalog #352057) are recommended.
2. Add the EasySep™ Mouse Pan-DC Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
3. Wash cells by topping up the sample tube with RoboSep™ Buffer and centrifuge at 300 x *g* for **10 minutes**. Discard the supernatant and resuspend the cells in the original start volume.
4. Select the appropriate RoboSep™ protocol:
   - Mouse Pan-DC Negative Selection 19763-small volume (for sample volumes between 0.5 - 4.0 mL)
   - Mouse Pan-DC Negative Selection 19763-large volume (for sample volumes > 4.0 mL)

   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 particles are in a uniform suspension with no visible aggregates.**

   IMPORTANT NOTE: These protocols require that **two** vials of EasySep™ D Magnetic Particles (Catalog #19250) be loaded onto the carousel for a single run. Place the first vial of particles in the ▲ (triangle) slot, and the second particle vial in the ● (circle) slot of the same quadrant.
6. When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep™.
7. When cell separation is complete, remove the enriched cells in the 50 mL tube located to the left of the tip rack in the second quadrant. The enriched cells are now ready for use.

MANUAL EASYSEP™ PROTOCOL DIAGRAM



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

This protocol is used for processing **250 µL – 2.0 mL** of sample (up to 2 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). 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 (Corning Catalog #352058) are recommended.
2. Add the EasySep™ Mouse Pan-DC Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes.**
3. Wash cells by topping up the sample tube with recommended medium and centrifuge at 300 x *g* for **10 minutes**. Discard the supernatant and resuspend the cells in the original start volume.
4. Add the 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 in refrigerator (2 - 8°C) for **10 minutes.**
5. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
6. Add the EasySep™ D Magnetic Particles at **75 µL/mL cells** (e.g. for 2 mL of cells, add 150 µL of magnetic particles). Mix well and incubate in refrigerator (2 - 8°C) for **10 minutes**.
7. Bring the cell suspension up 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**.
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 EasySep™ Magnet. Leave 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.*
9. Remove the original tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet and set aside for **5 minutes**.
10. Repeat Step 8 for a total of 2 separations in the magnet (**2 x 5 minutes**). The negatively selected, 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 – 8.0 mL** of sample (up to 8 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). 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 (Corning Catalog #352057) are recommended.
2. Add the EasySep™ Mouse Pan-DC Enrichment Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in refrigerator (2 - 8°C) for **15 minutes**.
3. Wash cells by topping up the sample tube with recommended medium and centrifuge at 300 x *g* for **10 minutes**. Discard the supernatant and resuspend the cells in the original start volume.
4. Add the 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 in refrigerator (2 - 8°C) for **10 minutes**.
5. Vortex the EasySep™ D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.
6. Add the EasySep™ D Magnetic Particles at **125 µL/mL cells** (e.g. for 2 mL of cells, add 250 µL of magnetic particles). Mix well and incubate in refrigerator (2 - 8°C) for **10 minutes**.
7. Bring the cell suspension up to a **total volume** of **2.5 mL** (for ≤2 x 10^8 cells) or **10 mL** (for >2.0 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**.
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 polystyrene 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 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.*
9. Remove the original tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet and set aside for **5 minutes**.
10. Repeat Step 8 for a total of 2 separations in the magnet (**2 x 5 minutes**). The negatively selected, enriched cells in the new tube are now ready for use.

CATALOG #19763 — For labeling 2 x 10^9 total cells

Components:

- EasySep™ Mouse Pan-DC Enrichment Cocktail — 1.0 mL
- EasySep™ Biotin Selection Cocktail — 2 x 1.0 mL
- EasySep™ D Magnetic Particles — 4 x 1.0 mL

EasySep™ NEGATIVE SELECTION

REQUIRED EQUIPMENT:

EasySep™ Magnet (Catalog #18000), or "The Big Easy" EasySep™ Magnet (Catalog #18001), or RoboSep™.

PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Mouse Pan-DC Enrichment Cocktail, EasySep™ Biotin Selection Cocktail and EasySep™ D Magnetic Particles are designed to enrich all dendritic cells (DCs) (including conventional and plasmacytoid DCs: pan-DCs) from mouse spleen cell suspensions by depletion of non-DCs.

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). The small size of the magnetic dextran iron particles allows for efficient binding to the TAC-labeled cells. Magnetically labeled cells are then separated from unlabeled target cells using the EasySep™ procedure (reverse side).

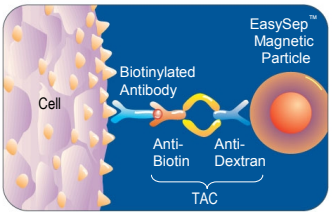


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

NOTES AND TIPS:

PREPARING A SINGLE CELL SUSPENSION. For maximum recovery, we recommend digesting the spleen at 37°C using Spleen Dissociation Medium (Catalog #07915). Refer to the Product Information Sheet (Document #29636) for the Spleen Dissociation Medium for more information. Ammonium chloride treatment is not recommended when preparing the cells for separation.

RECOMMENDED MEDIUM. The recommended medium is EasySep™ Buffer (Catalog #20144), RoboSep™ Buffer (Catalog #20104), or phosphate-buffered saline (PBS) containing 2% fetal bovine serum (FBS) and 1 mM EDTA. Hanks' Balanced Salt Solution (Hanks' BSS; Catalog #37250) can be used in place of PBS. Medium should be free of Ca^{++} and Mg^{++}.

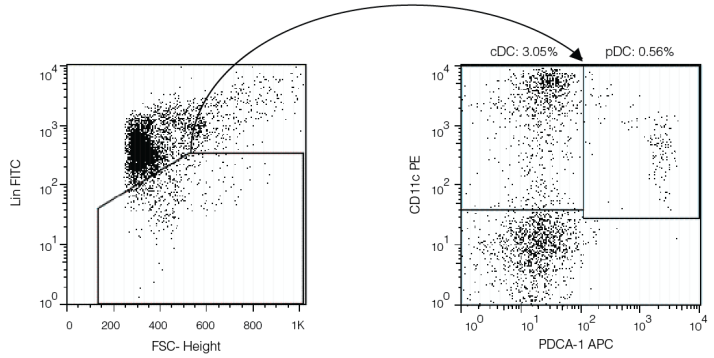
ASSESSING PURITY. Conventional dendritic cells (cDCs) express high levels of CD11c, whereas plasmacytoid dendritic cells (pDCs) express lower levels of CD11c. PDCA-1 (BST-2) is specifically expressed by pDCs. cDCs are defined as $Lin^-CD11c^+PDCA\text{-}1^-$, whereas pDCs are $Lin^-CD11c^{low}PDCA\text{-}1^+$.

Purity of EasySep™ enriched pan-DCs can be assessed by flow cytometry using a combination of fluorochrome-conjugated antibodies against non-DC lineage markers, CD11c (e.g. Anti-Mouse CD11c Antibody, Clone N418; Catalog #60002) and PDCA-1. Recommended antibodies to stain non-DC lineage cells are:

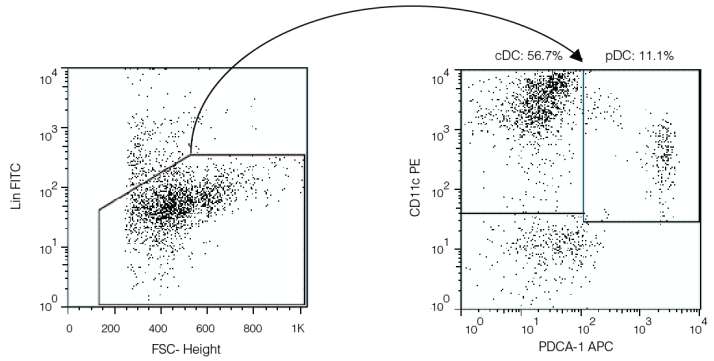
- Anti-Mouse CD3e Antibody, Clone 145-2C11 (Catalog #60015), and
- Anti-Mouse CD19 Antibody, Clone 1D3 (Catalog #60112), and
- Anti-Mouse F4/80 Antibody, Clone BM8 (Catalog #60027), and
- Anti-Mouse Ly-6G Antibody, Clone 1A8 (Catalog #60031), and
- Anti-Mouse NK1.1 (CD161) Antibody, Clone PK136 (Catalog #60103), and
- Anti-Mouse TER119 Antibody, Clone TER-119 (Catalog #60033), and
- Anti-IgM antibody, clone 1B4B1

TYPICAL EASYSEP™ MOUSE PAN-DC ENRICHMENT PROFILE:

Start: 3.61% Pan-Dendritic Cells



Enriched: 67.8% Pan-Dendritic Cells



The dendritic cell content of the enriched fraction is typically 65 ± 11%.

COMPONENT DESCRIPTIONS:

EASYSEP™ MOUSE PAN-DC ENRICHMENT COCKTAIL — **CODE #19763C**

This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens on mouse cells of non-DC lineage. To prevent, non-specific binding of antibodies to mouse cells, a Fc receptor blocking antibody has been added to this cocktail. 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™ BIOTIN SELECTION COCKTAIL — **CODE #19153**

This cocktail is a combination of two mouse IgG_1 monoclonal antibodies bound in bispecific Tetrameric Antibody Complexes (TAC) by rat monoclonal antibodies against mouse IgG_1. 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™ D MAGNETIC PARTICLES — **CODE #19250**

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:

EASYSEP™ MOUSE PAN-DC ENRICHMENT COCKTAIL

EASYSEP™ BIOTIN SELECTION COCKTAIL

EASYSEP™ D MAGNETIC NANOPARTICLES

Product stable at 2 - 8°C until expiry date as indicated on label. Do not freeze this product. This product may be shipped at room temperature (15 - 25°C), and should be refrigerated upon receipt.

STEMCELL™ TECHNOLOGIES

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