PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL™ TECHNOLOGIES

# MOUSE PLASMACYTOID DC ISOLATION KIT

CATALOG #19764

**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).**

If using other EasySep™ Magnets, please visit www.stemcell.com to download the magnet-specific Product Information Sheet or contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

## A) FULLY AUTOMATED PROTOCOL USING ROBOSEP™ (CATALOG #20000).

This procedure is used for processing **1 - 8 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 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 (BD Biosciences, Catalog #352057) are recommended.*

2. Add the EasySep™ Mouse Plasmacytoid DC Isolation Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in the refrigerator (2 - 8°C) for **15 minutes**.

3. Wash cells by topping up the 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 Plasmacytoid DC Negative Selection 19764-small volume (for sample volumes between 1 - 4 mL)
   - Mouse Plasmacytoid DC Negative Selection 19764-large volume (for sample volumes between > 4 - 8 mL)

   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.

5. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates.

   **IMPORTANT NOTE:** These protocols require that two vials of EasySep™ D2 Magnetic Particles (Catalog #19650) 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. Load the RoboSep™ carousel as directed by the on-screen prompts. 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 isolated cells in the new tube are now ready for use.

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

This procedure is used for processing **0.5 - 2 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 (BD Biosciences, Catalog #352058) are recommended.*

2. Add the EasySep™ Mouse Plasmacytoid DC Isolation Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in the refrigerator (2 - 8°C) for **15 minutes**.

3. Wash cells by topping up the 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 of cells** (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate in the refrigerator (2 - 8°C) for **10 minutes**.

5. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

6. Add the EasySep™ D2 Magnetic Particles at **37.5 µL/mL of cells** (e.g. for 2 mL of cells, add 75 µL of magnetic particles). Mix well and incubate in the 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 at room temperature (15 - 25°C) 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. Re-vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

10. Add **7 µL** of EasySep™ D2 Magnetic Particles to the new tube containing the poured off (enriched) sample. Mix well and incubate in the refrigerator (2 - 8°C) for **5 minutes**.

11. Place the tube (without cap) into the magnet for a second round of separation. Set aside at room temperature (15 - 25°C) for **5 minutes**.

12. 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.* The isolated 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 **0.5 - 8.5 mL** of sample (up to 8.5 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). 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 Plasmacytoid DC Isolation Cocktail at **50 µL/mL of cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate in the refrigerator (2 - 8°C) for **15 minutes**.

3. Wash cells by topping up the 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 of cells** (e.g. for 2 mL of cells, add 200 µL of cocktail). Mix well and incubate in the refrigerator (2 - 8°C) for **10 minutes**.

5. Vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

6. Add the EasySep™ D2 Magnetic Particles at **37.5 µL/mL of cells** (e.g. for 2 mL of cells, add 75 µL of magnetic particles). Mix well and incubate in the refrigerator (2 - 8°C) for **10 minutes**.

7. Bring the cell suspension up to a total volume of **5 mL** (for ≤ 4 x $10^8$ cells) or **10 mL** (for > 4 - 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 at room temperature (15 - 25°C) 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. Re-vortex the EasySep™ D2 Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

10. Add **25 µL** (for ≤ 4 x $10^8$ cells) or **50 µL** (for > 4 - 8.5 x $10^8$ cells) of EasySep™ D2 Magnetic Particles to the new tube containing the poured off (enriched) sample. Mix well and incubate in the refrigerator (2 - 8°C) for **5 minutes**.

11. Place the tube (without cap) into the magnet for a second round of separation. Set aside at room temperature (15 - 25°C) for **5 minutes**.

12. 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.* The isolated cells in the new tube are now ready for use.

CATALOG #19764 — For labeling 2 x $10^9$ total cells

Components:

- EasySep™ Mouse Plasmacytoid DC Isolation Cocktail — 1 x 1 mL
- EasySep™ Biotin Selection Cocktail — 2 x 1 mL
- EasySep™ D2 Magnetic Particles — 4 x 1 mL

EasySep™ NEGATIVE SELECTION

## REQUIRED EQUIPMENT:

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

## PRODUCT DESCRIPTION AND APPLICATIONS:

EasySep™ Mouse Plasmacytoid DC Isolation Cocktail, EasySep™ Biotin Selection Cocktail and EasySep™ D2 Magnetic Particles label non-plasmacytoid dendritic cells (pDCs) for magnetic separation. These reagents are designed to isolate pDCs from single cell suspensions of splenocytes or other tissues by depletion of non-pDCs.

## 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 (TACs). These complexes recognize both dextran and biotin (Figure 1). Magnetically labeled cells are then separated from unlabeled cells using the EasySep™ procedure (reverse side).

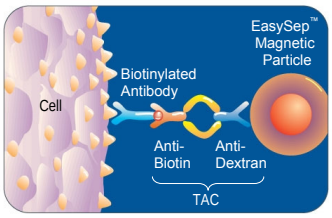



**Figure 1.**
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Mouse Cells.

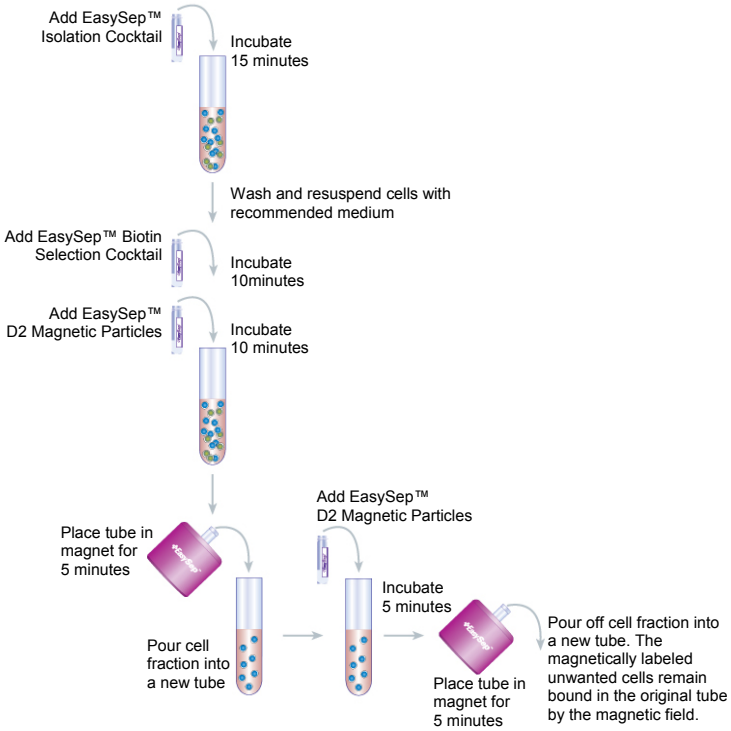
## NOTES AND TIPS:

**PREPARING A SINGLE CELL SUSPENSION** Disrupt spleen in phosphate-buffered saline (PBS) or Hank's balanced salt solution (HBSS) plus 2% fetal bovine serum (FBS). Centrifuge at 300 x *g* for 10 minutes and resuspend at 1 x $10^8$ nucleated cells/mL in recommended medium. Ammonium chloride treatment or enzymatic digestion are not recommended when preparing the cells for separation.

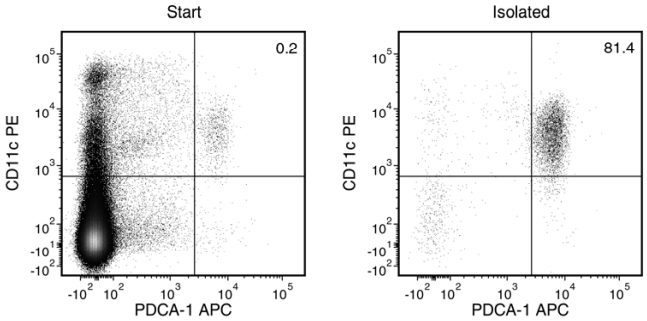
**RECOMMENDED MEDIUM** The recommended medium is RoboSep™ Buffer (Catalog #20104), or EasySep™ Buffer (Catalog #20144), or PBS + 2% FBS with 1 mM EDTA. HBSS can be used in place of PBS. Medium should be Ca++, Mg++ free.

**ASSESSING PURITY** Purity of pDCs can be measured by flow cytometry after staining with a fluorochrome-conjugated anti-PDCA-1 antibody and anti-CD11c antibody.

## MANUAL EASYSEP™ PROTOCOL DIAGRAM




## TYPICAL EASYSEP™ MOUSE PLASMACYTOID DC ISOLATION PROFILE:




Starting with mouse splenocytes, the pDC content (PDCA-1+CD11c+) of the isolated fraction typically ranges from 62 - 94%.

## COMPONENT DESCRIPTIONS:

**EASYSEP™ MOUSE PLASMACYTOID DC ISOLATION COCKTAIL** **CODE #19764C**
This cocktail contains a combination of biotinylated monoclonal antibodies directed against cell surface antigens on mouse cells of non-pDC origin. To prevent non-specific binding of antibodies to mouse cells, an Fc receptor blocking antibody (anti-CD16/32) 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 IgG1 monoclonal antibodies against biotin and dextran purified from hybridoma culture supernatant. These antibodies are bound in bispecific TACs by rat monoclonal antibodies against mouse IgG1. 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™ D2 MAGNETIC PARTICLES** **CODE #19650**
A suspension of magnetic dextran iron particles in TRIS buffer.

## STABILITY AND STORAGE:

**EASYSEP™ MOUSE PLASMACYTOID DC ISOLATION COCKTAIL**

**EASYSEP™ BIOTIN SELECTION COCKTAIL**

**EASYSEP™ D2 MAGNETIC PARTICLES**
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.