PROCEDURE

EasySep®

NEGATIVE SELECTION

HUMAN PLASMACYTOID DC ENRICHMENT KIT

CATALOG #19062

STEMCELL TECHNOLOGIES

V 1.0.0

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).

Please note that dendritic cells (DC) are very sensitive to handling conditions. Please follow all steps of this optimized protocol exactly.

This procedure is used for processing **500 µL – 8.0 mL** of sample (up to 4.0 x 10^8 cells).

1. Prepare mononuclear cell suspension as described in Notes and Tips (reverse side) at a concentration of 5 x 10^7 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.

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

2. Add Anti-Human CD32 (Fcγ RII) Blocker at **30 µL/mL**. *Note: Blocker addition is optional. Please see Notes and Tips for details.*

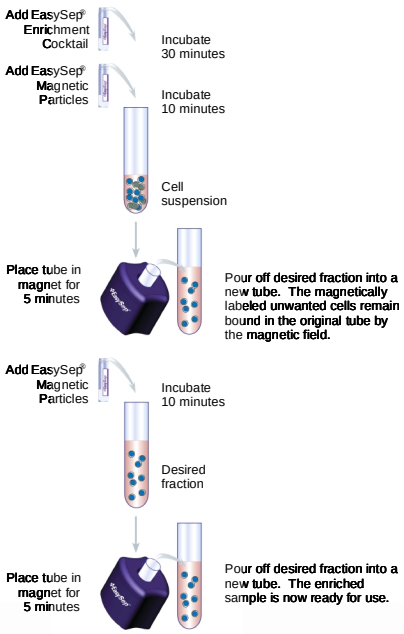
3. Select the appropriate RoboSep® protocol, based on sample volume:
   - "Human pDC Enrichment 19062 0.5 – 2.0mL - small volume"
   - "Human pDC Enrichment 19062 2.1 – 5.0mL – large volume"
   - "Human pDC Enrichment 19062 5.1 – 8.0mL – mega volume"

4. Load the RoboSep® carousel as directed by the on-screen prompts. ***Vortex the EasySep® D Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates.*** When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep®.

   Note: The protocol requires that **two** vials of D Magnetic Particles be loaded onto the carousel – one in standard magnetic particle slot • (triangle) and the other in slot ˜ (circle).

5. When cell separation is complete, collect the enriched cells as prompted in the 50 mL tube located in the second quadrant (of the two-quadrant protocol). The enriched cells are now ready for use.

MANUAL EASYSEP® PROTOCOL DIAGRAM



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

Please note that dendritic cells (DC) are very sensitive to handling conditions. Please follow all steps of this optimized protocol exactly.

This procedure is used for processing **250 µL - 2 mL** of sample (up to 1 x 10^8 cells).

1. Prepare mononuclear cell suspension as described in Notes and Tips (reverse side) at a concentration of 5 x 10^7 cells/mL in recommended medium. 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 (BD, Catalog #352058) Polystyrene Round-Bottom Tubes are recommended.

2. Add Anti-Human CD32 (Fcγ RII) Blocker at **30 µL/mL.** *Note: Blocker addition is optional. Please see Notes and Tips for details.*

3. Add EasySep® Human Plasmacytoid DC Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **30** minutes.

4. Vortex EasySep® D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

5. Add the EasySep® D Magnetic Particles at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10** minutes.

6. Bring the cell suspension up to a **total volume** of 2.5 mL by adding recommended medium. Mix the suspension by gently pipetting up and down 2 - 3 times. Place this tube (without cap) into the magnet. Set aside for **5** minutes.

7. Pick up the EasySep® Magnet, and in one continuous motion invert the magnet and tube, pouring off the desired unlabeled fraction into a new 5 mL polystyrene tube. The magnetically labeled unwanted cells will remain inside the original tube, held by the magnetic field of the EasySep® magnet. Leave the magnet and the 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*.

8. Re-vortex the EasySep® D Magnetic Particles ensuring a uniform suspension and add **100 µL** of magnetic particles to the enriched sample in the new 5 mL polystyrene tube. Mix well and incubate at room temperature (15 - 25°C) for **10** minutes.

9. Place the tube (without cap) into the magnet for a second round of magnetic separation. Set aside for **5** minutes.

10. 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. Leave the magnet and the 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 negatively selected enriched plasmacytoid DC 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 – 5.0 mL** of sample (up to 2.5 x 10^8 cells).

1. Prepare mononuclear cell suspension as described in Notes and Tips (reverse side) at a concentration of 5 x 10^7 cells/mL in recommended medium. Cells must be placed in a 14 mL (17 x 100 mm) polystyrene tube to properly fit into the silver magnet.

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

2. Add Anti-Human CD32 (Fcγ RII) Blocker at **30 µL/mL.** *Note: Blocker addition is optional. Please see Notes and Tips for details.*

3. Add EasySep® Human Plasmacytoid DC Enrichment Cocktail at **50 µL/mL cells** (e.g. for 2 mL of cells, add 100 µL of cocktail). Mix well and incubate at room temperature (15 - 25°C) for **30** minutes.

4. Vortex EasySep® D Magnetic Particles for 30 seconds. Ensure that the particles are in a uniform suspension with no visible aggregates.

5. Add the EasySep® D Magnetic Particles at **100 µL/mL cells** (e.g. for 2 mL of cells, add 200 µL of magnetic particles). Mix well and incubate at room temperature (15 - 25°C) for **10** minutes.

6. Bring the cell suspension to a **total volume** of 3.5 mL (for ≤1 x 10^8 start cells) or 6.5 mL (for >1 x 10^8 start cells) by adding recommended medium. Mix the suspension by gently pipetting up and down 2 - 3 times. Place the tube (without cap) into the magnet. Set aside for **5** minutes at room temperature (15 - 25°C).

7. 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 inside the original tube, held by the magnetic field of the EasySep® magnet. Leave the magnet and the 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***.**

8. Re-vortex the EasySep® D Magnetic Particles ensuring a uniform suspension and add **200 µL** of magnetic particles to the enriched sample (if 3.5 mL volume), or **400 µL** (if 6.5 mL volume). Mix well and incubate at room temperature (15 - 25°C) for **10** minutes.

9. Place the tube (without cap) into the magnet for a second round of separation. Incubate for **5** minutes.

10. 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. Leave the magnet and the 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 negatively selected enriched plasmacytoid DC in the new tube are now ready for use.

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CATALOG #19062 For labeling up to 10^9 total cells

Components:

- EasySep® Human Plasmacytoid DC Enrichment Cocktail 1.0 mL
- EasySep® D Magnetic Particles 4 x 1.0 mL
- Anti-Human CD32 (Fcγ RII) Blocker 0.8 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® Human Plasmacytoid DC Enrichment Cocktail and EasySep® D Magnetic Particles label myeloid dendritic cells (mDCs) and non-DCs for magnetic separation. These reagents are designed to enrich plasmacytoid dendritic cells (pDC) from fresh or previously frozen peripheral blood mononuclear cells (PBMC) by depletion of all other non-pDCs.

EASYSEP® LABELING OF HUMAN CELLS:

Unwanted cells are specifically labeled with dextran-coated magnetic particles using bispecific Tetrameric Antibody Complexes (TAC). These complexes recognize both dextran and the cell surface antigen expressed on the unwanted cells (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).

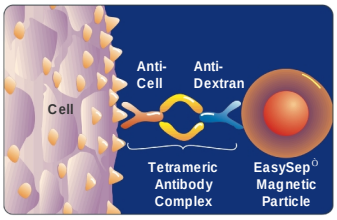


Figure 1.
Schematic Drawing of EasySep® TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING A MONONUCLEAR CELL SUSPENSION.

For fresh samples:

- Prepare a nucleated cell suspension from whole blood, using Ficoll-Paque™ PLUS density separation (Catalog #07957). Resuspend cells at a concentration of 5 x 10^7 cells/mL in recommended medium (see below).

Note: We strongly recommend the use of freshly harvested whole blood for optimal results. Use of day old blood will result in reduced pDC purities and recoveries.

For previously frozen samples:

- We recommend washing up to 5 x 10^8 cells in 50 mL of Phosphate Buffered Saline (PBS) with 20% FBS and 1 mM EDTA. Centrifuge at 200 x *g* for 10 minutes. Wash the cells a second time in recommended medium and centrifuge at 200 x *g* for 10 minutes. Due to the sensitive nature of pDCs, **we do not recommend the use of DNase I to treat cell suspensions.** Resuspend cells at a concentration of 5 x 10^7 cells/mL in recommended medium.

RECOMMENDED MEDIUM. The recommended medium is RoboSep® Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) with 2% FBS (Catalog #07905) and 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

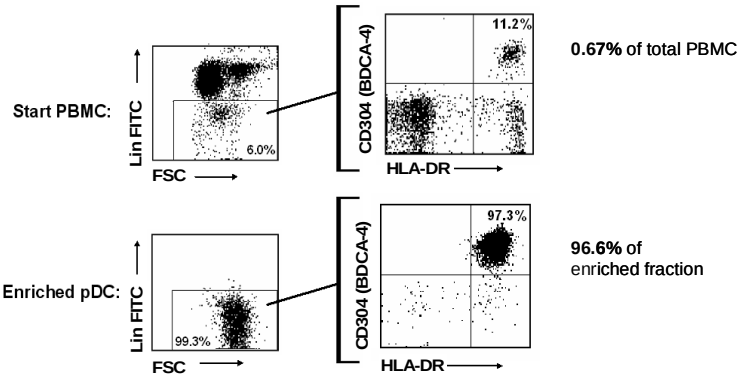
EASYSEP® D MAGNETIC PARTICLES. If extra D Magnetic Particles (Catalog #19250) are required in addition to the four vials provided in the EasySep® Human Plasmacytoid DC Enrichment Kit (Catalog #19062), please contact STEMCELL Technologies' techsupport at techsupport@stemcell.com to place an order.

ANTI-HUMAN CD32 (FCg RII) BLOCKER. Addition of Anti-Human CD32 (Fcγ RII) Blocker is optional but improves product performance by preventing non-specific depletion of dendritic cells. Use of the Anti-Human CD32 (Fcγ RII) Blocker may prevent subsequent attempts at cross-linking CD32 molecules on the surface of enriched cells to trigger signaling through these receptors. It may therefore be desirable to omit CD32 addition to the cell suspension for such studies.

ASSESSING PURITY. pDCs are described as Lineage (CD3, CD14, CD16, CD19, CD20, CD34, CD56) negative, HLA-DR positive, and CD304 (BDCA-4) positive.

TYPICAL EASYSEP® pDC ENRICHMENT PROFILE:

Start: **0.67%** $Lin^-BDCA\text{-}4^+HLA\text{-}DR^+$ of total peripheral blood mononuclear cells (PBMC)
Enriched: **96.6%** $Lin^-BDCA\text{-}4^+HLA\text{-}DR^+$ of enriched fraction



Starting with 0.2 - 0.9% pDC in PBMC, the pDC content of the enriched fraction typically ranges from 87 - 97% purity based on the pDC phenotype of Lineage (CD3, CD14, CD16, CD19, CD20, CD34, CD56) negative, HLA-DR positive, and CD304 (BDCA-4) positive.

COMPONENT DESCRIPTIONS:

EASYSEP® HUMAN PLASMACYTOID DC ENRICHMENT COCKTAIL **CODE #19062C**

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 cell surface antigens on human blood cells and dextran. It should be noted that this product is a biological reagent, and as such cannot be completely characterized or quantified. Some variability is unavoidable.

ANTI-HUMAN CD32 (FCg RII) BLOCKER **CODE #14551C**

A mouse IgG2b monoclonal antibody purified from concentrated hybridoma culture supernatant by affinity chromatography using Protein A or Protein G Sepharose. CD32 (Fcγ RII) is a 40 kD receptor for the Fc region of the Immunoglobulin G (IgG), and is expressed on the surface of dendritic cells. CD32 binds weakly to the Fc portion of monomeric IgG, but efficiently to IgG aggregates and immune complexes.

EASYSEP® D MAGNETIC PARTICLES **CODE #19250**

A suspension of magnetic dextran iron particles in TRIS buffer.

STABILITY AND STORAGE:

EASYSEP® HUMAN PLASMACYTOID DC ENRICHMENT 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® D 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.

ANTI-HUMAN CD32 (FCg RII) BLOCKER

Product stable at 2 - 8°C until expiry date as indicated on label. Contents have been sterility tested. No preservative has been added. Do not freeze this product.

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