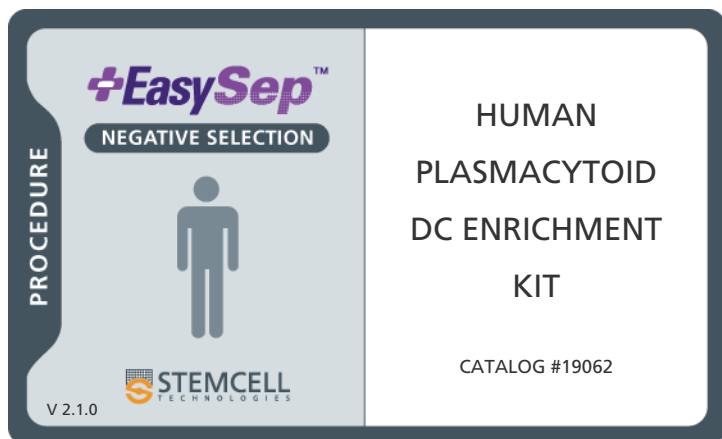




THIS PRODUCT INFORMATION SHEET IS PROVIDED FOR USE WITH ROBOSEP™ (SECTION A), THE PURPLE EASYSEP™ MAGNET (SECTION B) OR "THE BIG EASY" SILVER EASYSEP™ MAGNET (SECTION C).

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

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 4.0×10^6 cells).

1. Prepare cell suspension at a concentration of 5×10^7 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 Anti-Human CD32 (Fcγ RII) Blocker at **30 µL/mL cells**.

Note: This step is optional. Please see Notes and Tips (reverse side) for details.

3. Select the appropriate RoboSep™ protocol, based on sample volume:

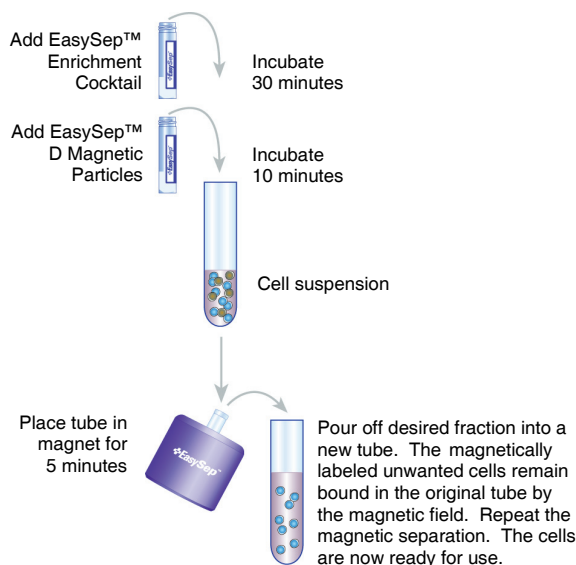
- Human pDC Enrichment 19062-small volume (0.5 – 2.0 mL)
- Human pDC Enrichment 19062-large volume (2.1 – 5.0 mL)
- Human pDC Enrichment 19062-mega volume (5.1 – 8.0 mL)

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, 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).

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 1×10^6 cells).

1. Prepare cell suspension at a concentration of 5×10^7 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 (BD Biosciences, Catalog #352058) Polystyrene Round-Bottom Tubes are recommended.

2. Add the Anti-Human CD32 (Fcγ RII) Blocker at **30 µL/mL cells**.

Note: This step is optional. Please see Notes and Tips (reverse side) for details.

3. Add the 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 the 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 **200 µL/mL cells** (e.g. for 2 mL of cells, add 400 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 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 unlabeled 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.*

8. Place the new tube containing the negatively selected fraction (without cap) into the magnet for a second round of magnetic separation. Set aside for **5 minutes**.

9. 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 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 cells in the new tube are now ready for use.

C) MANUAL EASYSEP™ PROTOCOL USING "THE BIG EASY" SILVER EASYSEP™ MAGNET (CATALOG #18001).

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 4.0×10^6 cells).

1. Prepare cell suspension at a concentration of 5×10^7 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 (BD Biosciences, Catalog #352057) Polystyrene Round-Bottom Tubes are recommended.

2. Add the Anti-Human CD32 (Fcγ RII) Blocker at **30 µL/mL cells**.

Note: This step is optional. Please see Notes and Tips (reverse side) for details.

3. Add the 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 the 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 **200 µL/mL cells** (e.g. for 2 mL of cells, add 400 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 3.5 mL** (for $\leq 1 \times 10^6$ start cells), **6.5 mL** (for $1 \times 10^6 - 2.5 \times 10^6$ start cells), or **10 mL** (for $>2.5 \times 10^6$ 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 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.*

8. Place the new tube containing the negatively selected fraction (without cap) into the magnet for a second round of separation. Incubate for **5 minutes** at room temperature (15 - 25°C).

9. 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 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 cells in the new tube are now ready for use.

Components:

• EasySep™ Human Plasmacytoid DC Enrichment Cocktail	1.0 mL
• EasySep™ D Magnetic Particles	4 x 1.0 mL
• Anti-Human CD32 (Fc γ RII) Blocker	0.8 mL

**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 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).

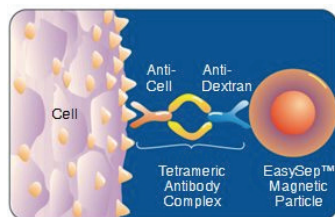


Figure 1.
Schematic Drawing of EasySep™
TAC Magnetic Labeling of Human
Cells.

NOTES AND TIPS:**PREPARING THE CELL SUSPENSION.****FROM WHOLE PERIPHERAL BLOOD**

Prepare a mononuclear cell suspension from whole peripheral blood, using density gradient centrifugation.

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.

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

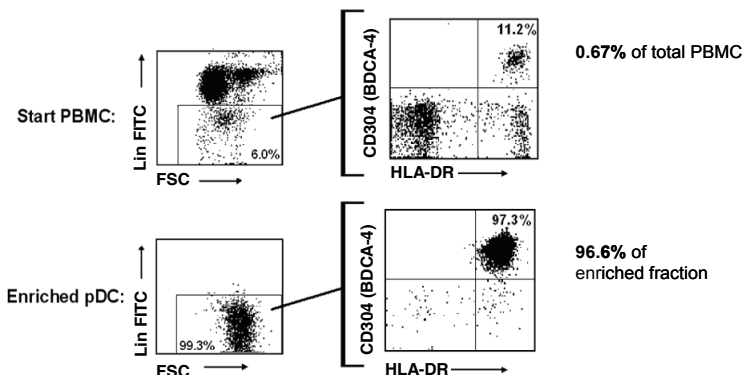
EASYSEP™ D MAGNETIC PARTICLES. If extra EasySep™ D Magnetic Particles (Catalog #19250) are required in addition to the four vials provided in the EasySep™ Human Plasmacytoid DC Enrichment Kit (Catalog #19062), please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com to place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

ANTI-HUMAN CD32 (Fc γ RII) BLOCKER. Addition of Anti-Human CD32 (Fc γ RII) Blocker (Catalog #14551C) is optional but may improve product performance by preventing non-specific depletion of dendritic cells. Use of the Anti-Human CD32 (Fc γ 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™ HUMAN pDC ENRICHMENT PROFILE:

Start: **0.67%** Lin⁺BDCA-4⁺HLA-DR⁺ of total peripheral blood mononuclear cells (PBMC)
Enriched: **96.6%** Lin⁺BDCA-4⁺HLA-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 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 (Fc γ RII) BLOCKER**CODE #14551C**

A mouse IgG2b monoclonal antibody. CD32 (Fc γ 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. The Fc/FcR interactions may result in non-specific labeling in antibody-based detection and cell separation experiments. This antibody is recommended to block Fc/FcR interaction during negative cell selection.

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****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 (Fc γ 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.