PROCEDURE

EasySep™

NEGATIVE SELECTION

STEMCELL TECHNOLOGIES

V 1.0.0

HUMAN MYELOID DC ENRICHMENT KIT

CATALOG #19061

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).

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

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

This procedure is used for processing **500 µL – 8.5 mL** of sample (up to 4.25 x 10^8 cells).

1. Prepare cell suspension at a concentration of 5 x 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 **15 µL/mL cells**.

   Note: Blocker addition is highly recommended.

3. Select the appropriate RoboSep™ protocol:

   - Human mDC Negative Selection 19061

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

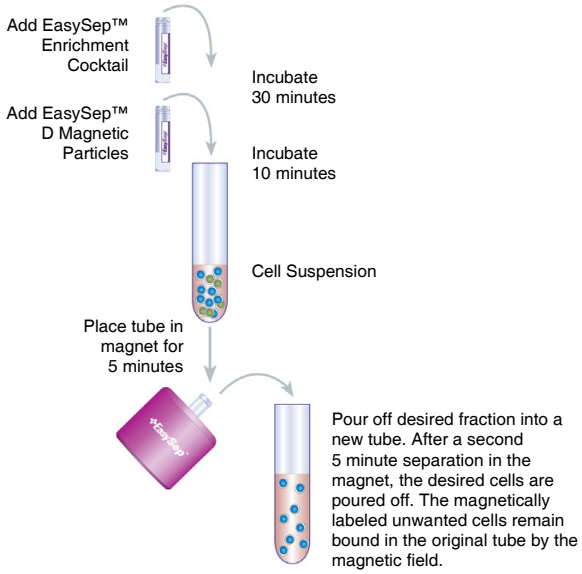
4. Load the RoboSep™ carousel as directed by the on-screen prompts. **Vortex the EasySep™ D Magnetic Particles for 30 seconds before loading. Ensure that the particles are in a uniform suspension with no visible aggregates.**

   IMPORTANT NOTE: This protocol requires 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.

5. When all desired quadrants are loaded, press the green "Run" button. All cell labeling and separation steps will be performed by RoboSep™.

6. When cell separation is complete, remove the enriched cells 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 THE PURPLE EASYSEP™ MAGNET (CATALOG #18000).

This procedure is used for processing **500 µL – 2.0 mL** of sample (up to 1.0 x 10^8 cells).

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

2. Add the Anti-Human CD32 (Fcγ RII) Blocker at **15 µL/mL cells**.

   Note: Blocker addition is highly recommended.

3. Add the EasySep™ Human Myeloid 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 **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 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**.

7. 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.*

8. Remove the original tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet. Set aside for **5 minutes**.

9. Repeat Step 7 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 **1.0 mL – 8.5 mL** of sample (up to 4.25 x 10^8 cells).

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

2. Add the Anti-Human CD32 (Fcγ RII) Blocker at **15 µL/mL of cells**.

   Note: Blocker addition is highly recommended.

3. Add the EasySep™ Human Myeloid 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 **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 **5 mL** (for ≤ 2 x 10^8 cells) or **10 mL** (for > 2 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**.

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. Remove the original tube from the EasySep™ Magnet and place the new tube containing the desired cells into the magnet. Set aside for **5 minutes**.

9. Repeat Step 7 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 #19061 — For labeling 2 x 10^9 total cells

Components:

• EasySep™ Human Myeloid DC Enrichment Cocktail	2 x 1.0 mL
• EasySep™ D Magnetic Particles	6 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 Myeloid DC Enrichment Cocktail and EasySep™ D Magnetic Particles label plasmacytoid dendritic cells (pDC) and non-DC for magnetic separation. These reagents are designed to enrich myeloid dendritic cells (mDC) from fresh peripheral blood mononuclear cells (PBMC), buffy coat, or ammonuium chloride-lysed leukapheresis by depletion of non-mDC.

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).

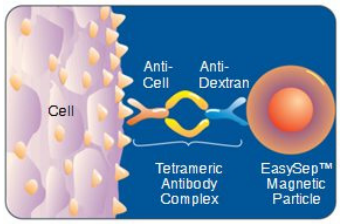


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING THE CELL SUSPENSION

FROM WHOLE PERIPHERAL BLOOD

Prepare a mononuclear cell suspension from whole peripheral blood by 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 mDC purities and recoveries.

FROM PERIPHERAL BLOOD APHERESIS (LEUKOPAK)

If working with large volumes (>150 mL), concentrate Leukopak cells first by centrifuging at 500 x *g* for 10 minutes. Remove the supernatant and resuspend the cells in 1/10th of the original Leukopak volume with recommended medium (e.g. for 300 mL of cells, resuspend in 30 mL of recommended medium). For small volumes (150 mL or less), add the Ammonium Chloride Solution (Catalog #07800/07850) directly to the cell suspension.

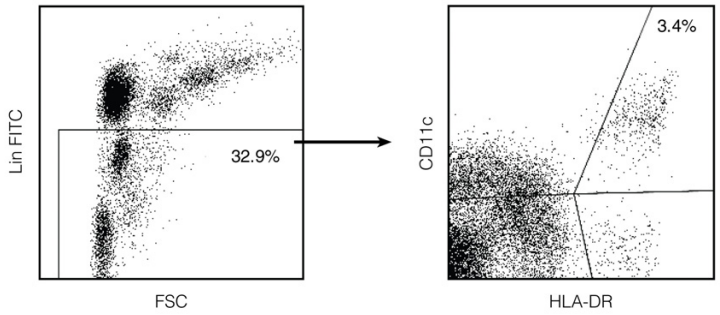
1. Add an equal volume of Ammonium Chloride Solution to the Leukopak suspension (e.g. for 5 mL of Leukopak suspension, add 5 mL Ammonium Chloride Solution).
2. Incubate 15 minutes on ice.
3. Centrifuge at 500 x *g* for 10 minutes at room temperature (15 - 25°C). Remove the supernatant.
4. Wash the cells by topping up the tube with recommended medium. Centrifuge the cells at 150 x *g* for 10 minutes at room temperature (15 - 25°C) with the brake off. Carefully remove the supernatant.
5. Repeat the wash step one or more times until most of the platelets have been removed (indicated by a clear supernatant).
6. Resuspend cells at recommended cell concentration, in the recommended medium.

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.

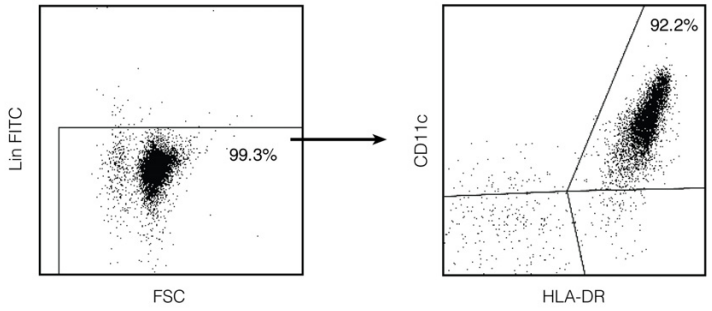
ASSESSING PURITY. Purity of mDC can be measured by flow cytometry after staining with fluorochrome-conjugated antibodies. mDC are described as Lineage (CD3, CD14, CD19, CD20, CD34, CD56) negative, HLA-DR positive and CD11c positive.

TYPICAL EASYSEP™ MYELOID DC ENRICHMENT PROFILE:

Start: 1.1% $Lin^-CD11c^+HLA\text{-}DR^+$ of total nucleated cells



Enriched: 91.6% $Lin^-CD11c^+HLA\text{-}DR^+$ of enriched fraction



Starting with 0.6 - 1.8% mDC in fresh peripheral blood nucleated cells, the mDC content of the enriched fraction typically ranges from 79 - 94% purity based on the mDC phenotype of Lineage (CD3, CD14, CD19, CD20, CD34, CD56) negative, HLA-DR positive, and CD11c positive.

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN MYELOID DC ENRICHMENT COCKTAIL — **CODE #19061C**

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 (Fcγ RII) BLOCKER — **CODE #14551C**

A mouse IgG2b monoclonal antibody. 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. 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 MYELOID 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 (Fcγ 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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