PROCEDURE

EasySep™

NEGATIVE SELECTION

HUMAN PAN-DC PRE-ENRICHMENT KIT

CATALOG #19251

STEMCELL TECHNOLOGIES

V 3.2.0

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).

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.

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

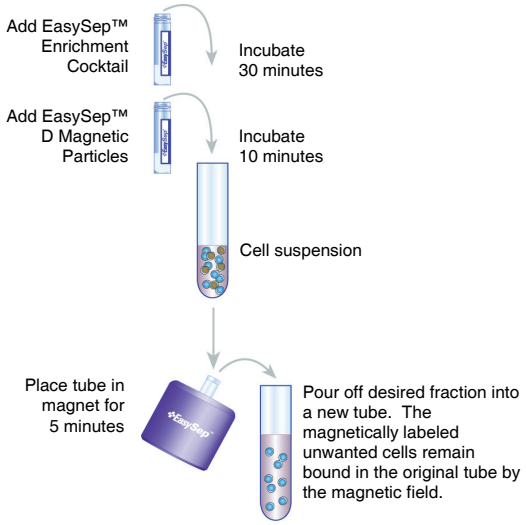
A) FULLY AUTOMATED PROTOCOL USING ROBOSEP™ (CATALOG #20000).

This procedure is used for processing **500 µL - 6.5 mL** of sample (up to 3.3 x 10^8 cells).

1. Prepare mononuclear 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 **30 µL/mL cells**.
   Note: This step is optional. Please see Notes and Tips (reverse side) for details.
3. Select the appropriate RoboSep™ protocol:
   - Human panDC pre-Enrichment Negative Selection 19251 - high recovery

   If a modified RoboSep™ protocol is required, please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com.
   Note: The above protocol has been optimized for maximal dendritic cell recovery. To increase dendritic cell purity in the pre-enriched sample, an alternate RoboSep™ protocol may be performed, but may result in reduced cell recovery. Use of this protocol requires additional EasySep™ D Magnetic Particles. Please contact STEMCELL Technologies Technical Support at techsupport@stemcell.com for more information and to request additional particles.
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™.
5. When cell separation is complete, remove the enriched cells in the 50 mL tube located to the left of the tip rack. 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 **250 µL - 2 mL** of sample (up to 1 x 10^8 cells).

1. Prepare mononuclear 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 **30 µL/mL cells**.
   Note: This step is optional. Please see Notes and Tips (reverse side) for details.
3. Add the EasySep™ Human Pan DC Pre-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.* The negatively selected, enriched cells in the new tube are now ready for use.
   NOTE: The above protocol has been optimized for maximal dendritic cell recovery. Further enrichment of specific dendritic cell subsets by fluorescence-activated cell sorting may be required. To increase dendritic cell purity in the pre-enriched sample, Steps 8 - 10 may be performed, but may result in reduced cell recovery. Use of this protocol requires additional EasySep™ D Magnetic Particles. Please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com for more information on this protocol and to request additional particles.
8. Repeat Step 4 and add **100 µL** of EasySep™ D Magnetic Particles to the enriched sample. Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
9. Place the tube (without cap) into the magnet. Set aside for **5 minutes**.
10. Repeat Step 7 for a total of two 5-minute incubations in the magnet (**2 x 5 minutes**).

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

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

1. Prepare mononuclear 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 **30 µL/mL cells**.
   Note: This step is optional. Please see Notes and Tips (reverse side) for details.
3. Add the EasySep™ Human Pan DC Pre-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 **5 mL** (for <10^8 cells) or **10 mL** (for 1 - 4.25 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.* The negatively selected, enriched cells in the new tube are now ready for use.
   NOTE: The above protocol has been optimized for maximal dendritic cell recovery. Further enrichment of specific dendritic cell subsets by fluorescence-activated cell sorting may be required. To increase dendritic cell purity in the pre-enriched sample, Steps 8 - 10 may be performed, but may result in reduced cell recovery. Use of this protocol requires additional EasySep™ D Magnetic Particles. Please contact STEMCELL Technologies' Technical Support at techsupport@stemcell.com for more information on this protocol and to request additional particles.
8. Repeat Step 4 and add **200 µL** (for <10^8 cells), or **400 µL** (1 - 4.25 x 10^8 cells) of EasySep™ D Magnetic Particles to the enriched sample. Mix well and incubate at room temperature (15 - 25°C) for **10 minutes**.
9. Place the tube (without cap) into the magnet. Incubate for **5 minutes**.
10. Repeat Step 7 for a total of two 5-minute incubations in the magnet (**2 x 5 minutes**).

IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899 T. +1 604 877 0713 F. +1 604 877 0704 E. INFO@STEMCELL.COM
IN EUROPE TOLL-FREE T. 00 800 7836 2355 TOLL-FREE F. 00 800 7836 2300 E. INFO.EU@STEMCELL.COM
IN AUSTRALIA TOLL-FREE T. 1 800 060 350 F. +61 (03) 9338 4320 E. INFO.AUS@STEMCELL.COM
IN SINGAPORE T. +(65) 6776 7754 F. +(65) 6776 7114 E. INFO.SG@STEMCELL.COM

CATALOG #19251 — For labeling 10^9 total cells

Components:

• EasySep™ Human Pan DC Pre-Enrichment Cocktail	1.0 mL
• EasySep™ D Magnetic Particles	2 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 Pan DC Pre-Enrichment Cocktail and EasySep™ D Magnetic Particles label non-dendritic cells for magnetic separation. These reagents are designed to pre-enrich all dendritic cells (including myeloid and plasmacytoid dendritic cells) from fresh or previously frozen peripheral blood mononuclear cells by depletion of non-dendritic cells.

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.

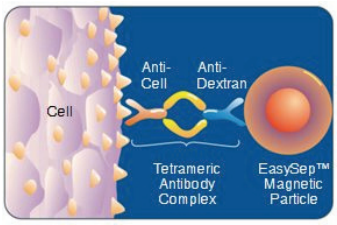


Figure 1.
Schematic Drawing of EasySep™ TAC Magnetic Labeling of Human Cells.

NOTES AND TIPS:

PREPARING THE CELL SUSPENSION.

FOR FRESH SAMPLES

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

FOR PREVIOUSLY FROZEN SAMPLES

For previously frozen mononuclear cells, we recommend washing up to 5 x 10^8 cells in 50 mL of Phosphate Buffered Saline (PBS) + 20% FBS with 1 mM EDTA added. 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. **We do not recommend the use of DNase I to treat cell suspensions.**

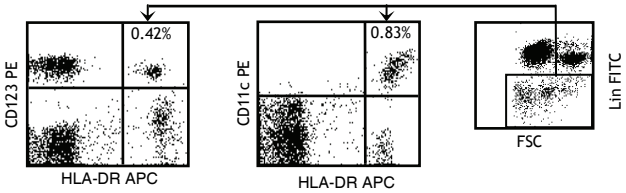
RECOMMENDED MEDIUM. The recommended medium is RoboSep™ Buffer (Catalog #20104), or Phosphate Buffered Saline (PBS) + 2% FBS (Catalog #07905) with 1 mM EDTA. Medium should be Ca^{++} and Mg^{++} free.

ANTI-HUMAN CD32 (FCγ RII) BLOCKER. Addition of Anti-Human CD32 (Fcγ 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 (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 addition to the cell suspension for such studies.

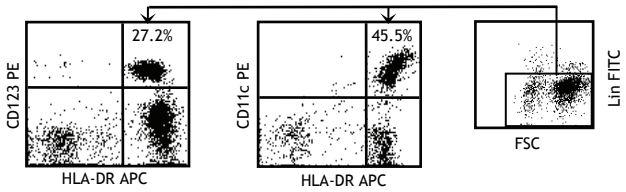
ASSESSING PURITY. Dendritic cells are defined as either Lin^- ($TCR\alpha\beta^-CD14^-CD16^-CD20^-CD56^-$)$HLA\text{-}DR^+CD123^+$ or Lin^- ($TCR\alpha\beta^-CD14^-CD16^-CD20^-CD56^-$)$HLA\text{-}DR^+CD11c^+$. The purity of dendritic cells after EasySep™ pre-enrichment can be measured by flow cytometry after staining with a combination of suitable fluorochrome-conjugated antibodies. Further enrichment of specific dendritic cell subsets by fluorescence-activated cell sorting may be required.

TYPICAL EASYSEP™ HUMAN PAN-DC PRE-ENRICHMENT PROFILE:

Start: 1.25% $Lin^-HLA\text{-}DR^+CD123^+$ or $CD11c^+$



Enriched: 72.7% $Lin^-HLA\text{-}DR^+CD123^+$ or $CD11c^+$



Starting with peripheral blood mononuclear cells, the dendritic cell content of the enriched fraction typically ranges from 40 - 80%.

COMPONENT DESCRIPTIONS:

EASYSEP™ HUMAN PAN-DC PRE-ENRICHMENT COCKTAIL — **CODE #19251C.1**

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 (CD3, CD9, CD14, CD16, CD19, CD34, CD56, CD66b, glycophorin A) 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 PAN DC PRE-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.

IN NORTH AMERICA TOLL-FREE T. 1 800 667 0322 TOLL-FREE F. 1 800 567 2899 T. +1 604 877 0713 F. +1 604 877 0704 E. INFO@STEMCELL.COM
IN EUROPE TOLL-FREE T. 00 800 7836 2355 TOLL-FREE F. 00 800 7836 2300 E. INFO.EU@STEMCELL.COM
IN AUSTRALIA TOLL-FREE T. 1 800 060 350 F. +61 (03) 9338 4320 E. INFO.AUS@STEMCELL.COM
IN SINGAPORE T. +(65) 6776 7754 F. +(65) 6776 7114 E. INFO.SG@STEMCELL.COM

V3.2.0
#29038