CELL SEPARATION PRODUCTS

For HLA Analysis

STEMCELL™ TECHNOLOGIES

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Cell Separation Solutions for HLA Laboratories

Product Listing

Scientists Helping Scientists™

STEMCELL Technologies is a leader in the development of specialty cell culture media, cell separation products, and accessory reagents for life science research. Driven by science, we deliver over 1500 products to more than 70 countries worldwide. To learn more about how STEMCELL Technologies helps to make research work, visit www.stemcell.com.

Cell Separation Solutions for HLA Laboratories

The role of the HLA (Human Leukocyte Antigen) complex is of critical importance in immune responses and transplant medicine. Since the late 1960s, research on human organ and tissue transplantation has reinforced the importance of HLA matching for graft survival and transplantation efficiency.

The specific and varied distribution of HLA antigens requires precise, accurate, and consistent HLA testing. In a busy HLA lab, finding powerful tools that facilitate reliable HLA testing while processing a high number of samples is of great importance.

At STEMCELL Technologies, we provide highly optimized cell separation solutions for a variety of HLA applications to facilitate high-volume sample processing and clear, reliable test results. Our products have been formulated for the isolation of highly purified cells to be used in:

- Crossmatch Analysis (see pages 4-5)
- HLA Serological Typing (see pages 8-9)
- Chimerism Analysis (see pages 10-11)

Cell Isolation Systems Available for HLA Tests:

EasySep™

Fast and Easy Immunomagnetic Cell Isolation

EasySep™ isolates cells quickly and easily without the use of columns (see pages 14 - 15). Most EasySep™ kits can be completely automated using **RoboSep™**, the fully automated cell separator (see pages 16 - 17).

RosetteSep™

Unique Immunodensity Cell Isolation

RosetteSep™ isolates highly purified cells directly from human whole blood during density gradient centrifugation (see page 19). RosetteSep™ is easily combined with **SepMate™**, the specialized density gradient tube, for fast, easy, and consistent cell isolation (see page 18).

Our cell isolation systems are particularly suitable for HLA labs, as all procedures are:

GENTLE. Obtain viable, functional cells without the need for columns.

FAST AND EASY. Isolate cells with easy-to-follow protocols.

CONVENIENT. Perform cell isolations at room temperature.

Cell Separation Solutions for Crossmatch Analysis

To facilitate solid organ transplant, several screening tests, such as the complement-dependent cytoxicity (CDC) assay and the flow cytometric crossmatch (FCXM) assay, are performed to assess whether the organ recipient has donor specific antibodies (DSA) that may contribute to transplant rejection. When performing any type of crossmatch assay to test for the presence of DSA in recipient serum, it is important that cell preparations are clean and maintain cell viability. Any damage to or activation of the cells during separation may compromise the cell membrane or alter antigen expression, possibly causing erroneous results and non-specific, high background staining.

EasySep™, RoboSep™, and RosetteSep™ have been optimized for use in downstream HLA tests, including the CDC and FCXM assays, and can be performed at room temperature for easier sample processing.

EasySep™

- EasySep™ is a column-free immunomagnetic cell isolation technology to isolate cells from a variety of sample sources (see pages 14 - 15).
- EasySep™ Direct products isolate untouched cells directly from whole blood, spleen, or lymph node samples without density gradient centrifugation or red blood cell lysis.
- EasySep™ is ideal for obtaining highly purified lymphocytes that can immediately be used in the CDC and FCXM assays.

RoboSep™

- RoboSep™ instruments offer true walk-away automation of cell separation using EasySep™ reagents (see pages 16 - 17).
- Cells are isolated from up to 16 samples simultaneously.
- Separation protocols are easy to run and can be standardized for high throughput labs.
- Laboratory personnel save time by automating sample processing.

RosetteSep™

- RosetteSep™ is a fast and easy immunodensity procedure for the isolation of untouched cells directly from whole blood (see page 19).
- Desired T and/or B cells are purified during the standard density gradient centrifugation step.
- Easily combined with SepMate™ (see page 18) to maximize reproducibility and minimize the need for user training.

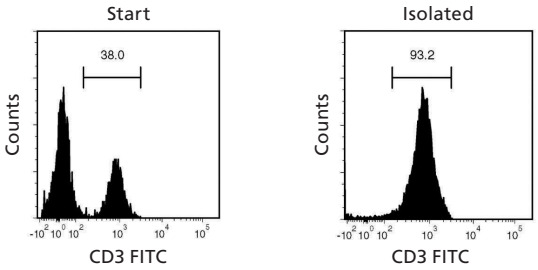


Figure 1. EasySep™ Direct HLA Crossmatch T Cell Isolation Kit (Catalog #19671)

Starting with human whole blood from normal healthy donors, the typical T cell ($CD3^+$) content of the non-lysed final isolated fraction is 97 ± 3% (gated on CD45). In the above example, the T cell ($CD3^+$) content of the lysed whole blood start sample and non-lysed final isolated fraction is 38.0% and 93.2% (gated on CD45), respectively.

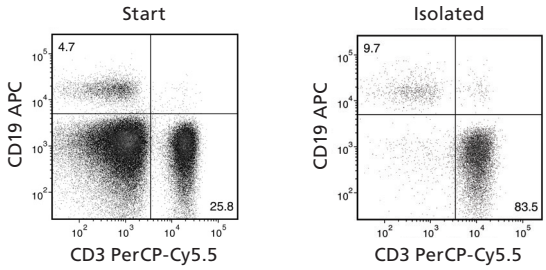


Figure 2. EasySep™ Direct Human Total Lymphocyte Isolation Kit (Catalog #19655)

Starting with human whole blood from normal healthy donors, the typical total lymphocyte ($CD3^-CD19^+$ and $CD3^+CD19^-$) content of the non-lysed final isolated fraction is 96.7 ± 1.5% (gated on CD45) or 95.8 ± 2.2% (not gated on CD45). In the above example, the total lymphocyte ($CD3^-CD19^+$ and $CD3^+$) content of the lysed whole blood start sample and non-lysed final isolated fraction is 30.5% and 93.2% (gated on CD45), respectively.

Cell Separation Products for Crossmatch Analysis

Cell Type	Selection	EasySep™ / RoboSep™ (Catalog #)		rosetteSep™ (Catalog #)
		Whole Blood[1], Spleen	Lymph Node and MNC[2]	Whole Blood[1]
T Cells	Negative	19671, 89671		15061, 15061HLA
B Cells	Negative	19684, 89684		15064, 15064HLA
Total Lymphocytes	Negative	19655	19961HLA	15263, 15263HLA

1. Kit also works on other red blood cell containing samples (i.e. cord blood, buffy coat).
2. Kit works on mononuclear cells (MNCs) isolated from peripheral blood or bone marrow. MNC: Mononuclear Cell

Case Study

Development and Validation of a Rapid Optimized Flow Cytometric Crossmatch (FCXM) Assay

HLA Laboratory, Department of Pathology, Dalhousie University, Halifax, Canada

"It is about time."

Dr. Robert Liwski, MD, PhD, from the Deparment of Pathology at Dalhouse University commenting on the need for faster and standardized protocols for the FCXM assay.

Background

To facilitate solid organ transplantation, the flow cytometric crossmatch (FCXM) assay is used as part of a pre-transplant risk assessment to determine whether the organ recipient has donor specific antibodies (DSA).[1] Performing the FCXM assay is time consuming and the various steps in the protocol, including the donor lymphocyte enrichment step, are not performed consistently across laboratories. This lack of standardization may lead to inconsistent results.

In order to optimize the standard FCXM assay, Dr. Robert Liwski from Dalhousie University in Halifax, developed, optimized and validated two FCXM procedures, the Halifax, and Halifaster FCXM protocols.[2,3] The different parameters that were optimized, included the cell isolation method, assay platform, cell number, serum volume, and incubation times. In this case study we focus on the different cell isolation methods followed by these protocols.

Methods

The FCXM assay can be divided in two parts. The first part consists of the donor lymphocyte isolation and pronase and DNAse treatment. The second part consists of the crossmatch assay. The FCXM assays were performed following the standard, Halifax, and Halifaster protocols as previously described.[2,3] In the standard and Halifax protocols, cells were isolated from whole blood by density gradient centrifugation using the density gradient medium Lympholyte® (~45 minutes/sample). The lymphocyte purity obtained using density gradient centrifugation varies between 15-90% depending on the donor and sample quality.[4] In the Halifaster protocol, cells were isolated directly from whole blood with the EasySep™ Direct Human Total Lymphocyte Isolation Kit Catalog #19655 (~25 minutes/sample) following instructions on the product information sheet (see schematic on page 15). This lymphocyte isolation method is fast, and the typical total lymphocyte purity is 95.8 ± 2.2% (not gated on CD45). The results of the FCXM assays following the different protocols were compared.

In addition, the impact of lymphocyte purity on FCXM results was assessed.[5] Briefly, lymphocytes (Ly), neutrophils (Nu), and monocytes (Mo) were isolated from 5 volunteer donors using the corresponding EasySep™ Direct kits (Catalog #19655, #19666, and #19669, respectively). Whole leukocyte (WL) preparations were obtained by adding Ly, Nu, and Mo cells in equal proportions (1/3 of each). The results of the FCXM assays using WL (low lymphocyte purity) and Ly (high lymphocyte purity) preparations were compared.

Results

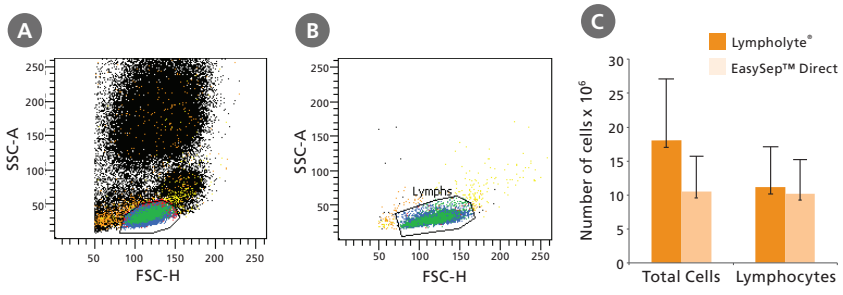


Figure 3. Using EasySep™ Direct Human Total Lymphocyte Isolation Kit (Catalog #19655) Increases Lymphocyte Purity While Maintaining Cell Yield

Cells were isolated from the same deceased donor whole blood sample using (A) the density gradient separation medium Lympholyte® (~45 minutes/sample) or (B) EasySep™ Direct (~25 minutes/sample) and analyzed by flow cytometry. Cleaner samples were obtained with EasySep™ Direct. (C) Samples isolated using EasySep™ Direct contained fewer contaminating cells and yet maintained overall number of T ($CD3^+$) and B ($CD19^+$) cells compared to samples isolated using Lympholyte®. Each column with error bar represents the mean ± SD from 24 mL whole blood (n = 20 donors). Data kindly provided by Dr. Robert Liwski.

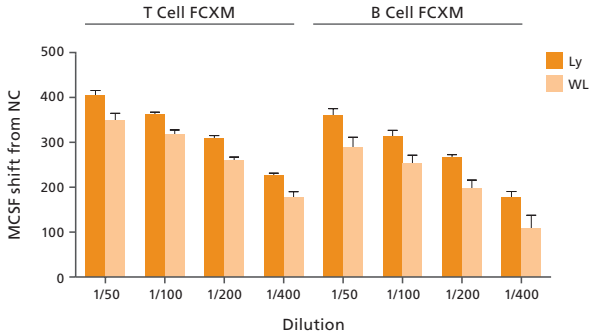


Figure 4. Use of Highly Enriched Lymphocytes Isolated with EasySep™ Direct Improves DSA Detection Compared to Whole Leukocyte Cell Preparations

Lymphocytes (Ly), neutrophils (Nu), and monocytes (Mo) were isolated from volunteer donors (n=5) using EasySep™ Direct Catalog #19655, #19666, and #19669, respectively. Whole leukocyte (WL) preparations were obtained by adding Ly, Nu, and Mo cells in equal propotions. WL (low lymphocyte purity) and Ly (high lymphocyte purity) preparations were treated with pronase and then used to perform the FCXM assay against negative control (NC) sera or several dilutions of positive control sera. The median channel fluorescence shifts (MCFS) were generated by using the negative control sera samples as a baseline. The MCFS between WL and Ly were then compared. Each column with error bars represents the mean ± SEM (n = 5 donors). Data kindly provided by Dr. Robert Liwski.

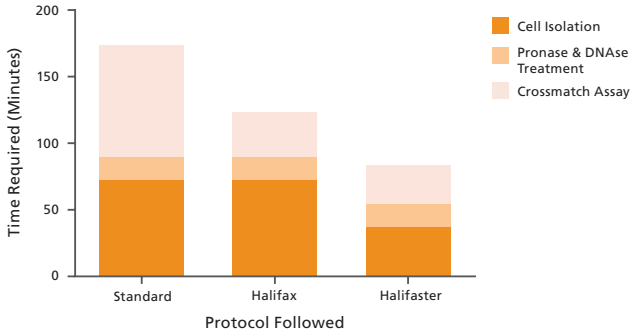


Figure 6. Following the Halifaster FCXM Protocol Reduces the Overall Time to Complete the FCXM assay to Less Than 2 Hours

The cell preparation part for the FCXM assay consists of the donor lymphocyte isolation step followed by treatment with pronase and DNAse. The Halifaster FCXM protocol reduced the time required for the cell preparation by almost 40% (from 90 to 55 minutes). This was achieved by using EasySep™ Direct technology for the lymphocyte isolation step. This technology is significantly faster than the density gradient centrifugation method used by the standard and Halifax FCXM protocols. The approximate total times it takes to complete the FCXM assay (including cell preparation) when following the standard, Halifax, and Halifaster FCXM protocols is 175 minutes, 125 minutes, and 85 minutes, respectively.[3] Data kindly provided by Dr. Robert Liwski.

Data kindly provided by Dr. Robert Liwski, HLA Laboratory, Department of Pathology, Dalhousie University, Halifax, Canada. Robert.Liwski@nshealth.ca

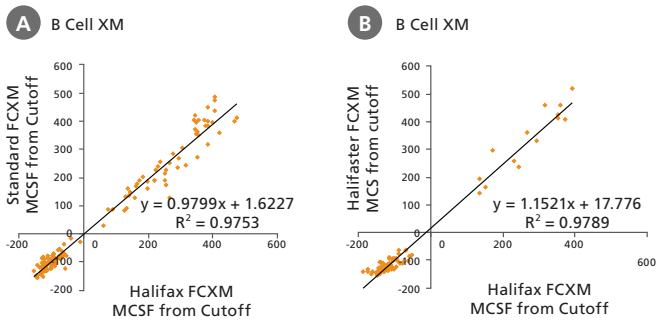


Figure 5. Isolation of Lymphocytes from Whole Blood Using EasySep™ Direct Does Not Compromise Sensitivity of the FCXM Assay

B cell FCXM assays were performed in parallel using cells isolated with EasySep™ Direct (Halifaster Protocol) or with the density gradient separation medium Lympholyte® (standard and Halifax Protocol). FCXM results were compared between (A) standard and Halifax and (B) Halifaster and Halifax FCXM protocols. Linear regression analysis of median channel fluorescence shifts (MCFS) showed an excellent correlation for the B cell and T cell (not shown here) FCXM assays between the two different isolation protocols. Data are expressed as MCSF from the cutoff level defined as the mean + three standard deviations. Data kindly provided by Dr. Robert Liwski.

Summary

- The Halifaster protocol incorporates EasySep™ Direct for the isolation of lymphocytes. This resulted in fewer contaminating cells compared to using the density gradient centrifugation method with Lympholyte®.
- Performing FCXM assays with highly enriched lymphocytes isolated with EasySep™ Direct improved detection of DSA and reduced the variability of FCXM results.
- The Halifaster FCXM protocol reduced the overall time to complete the FCXM assay to less than 2 hours without compromising quality or sensitivity. This was in part by reducing the lymphocyte isolation step to less than 30 minutes.

Cited References

1. Rebibou JM et al. (2004) T-cell flow-cytometry crossmatch and long-term renal graft survival. Clin Transplant 18(5): 558–563.
2. Liwski RS et al. (2015) Development and Validation of a Rapid Optimized Flow Cytometry Crossmatch (FCXM) Assay, the Halifax and Halifaster FCXM Protocols. ASHI Quarterly (Third Quarter 2015): 19–25.
3. Liwski RS et al. (2018) Rapid optimized flow cytometric crossmatch (FCXM) assays: The Halifax and Halifaster protocols. Hum Immunol 79(1): 28–38.
4. Hamrick C & Lebeck L. (2000) Flow cytometric T and B cell crossmatching. ASHI Lab Man 4th Ed.(Philadelphia: American Society for Histocompatibility and Immunogenetics): 41–45.
5. Liwski R et al. (2016) P099 The impact of lymphocyte purity on flow cytometry crossmatch (FCXM) assay. It's not purely theoretical. Hum Immunol 77, Supple: 110–111.

Cell Separation Solutions for HLA Serological Typing

For clear and reliable HLA serological typing, RosetteSep™ kits are the ideal solution for isolating clean cell preparations that are compatible with HLA typing reagents.

RosetteSep™ is a fast, easy, and cost-effective immunodensity procedure for the isolation of untouched cells directly from whole blood. Cells are isolated during the density gradient centrifugation step, which reduces sample handling time and maximizes convenience.

RosetteSep™

- RosetteSep™ is a fast, easy, and cost-effective cell isolation technology for obtaining untouched cells directly from whole blood (see page 19).
- Desired T and/or B cells are purified during the standard density gradient centrifugation step without columns or magnets.
- Cells are viable, untouched, and immediately ready for downstream testing.
- Cell purities of up to 95% allow for accurate serological HLA testing.
- Can be used with SepMate™ for consistent and fast cell separations.

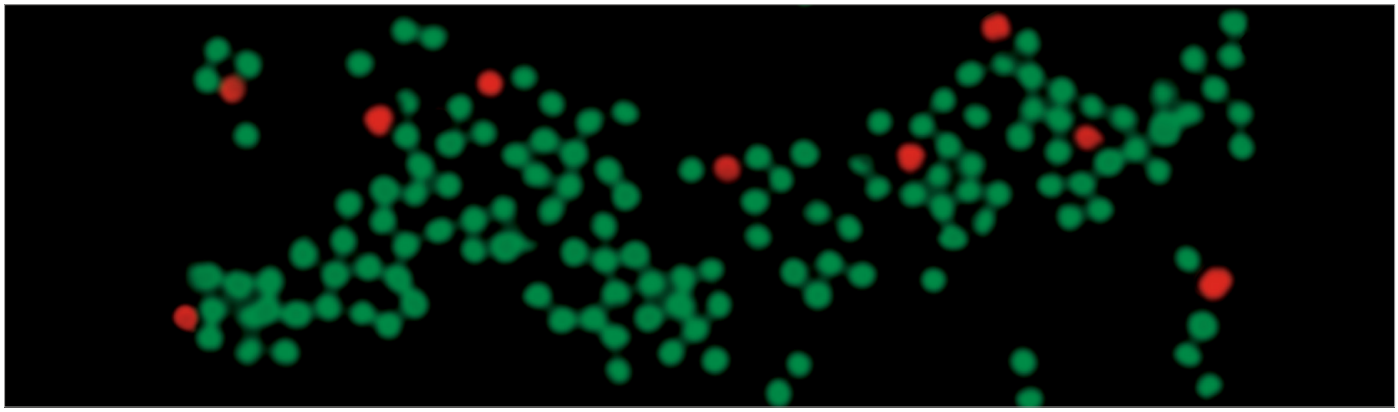


Figure 7. Results of a Complement-Dependent Cytotoxicity Assay

Cells were separated using STEMCELL Technologies' HLA reagents and analysed in a complement-dependent cytotoxicity (CDC) assay.

Typical Performance Data for Kits Used for Serological HLA Testing

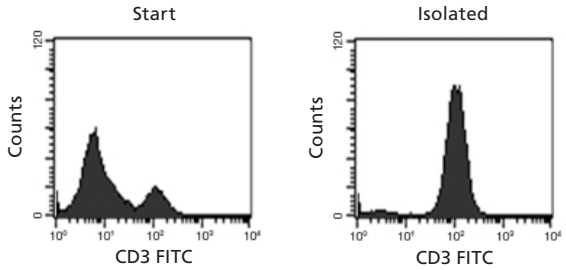


Figure 8. RosetteSep™ HLA T Cell Enrichment Cocktail (Catalog #15061HLA)

Starting with human fresh whole blood the $CD3^+$ cell content of the non-lysed final isolated fraction typically ranges from 90% - 97% (gated on CD45). In the above example, the T cell ($CD3^+$) content of the lysed whole blood start sample and non-lysed final isolated fraction is 20% and 96% (gated on CD45), respectively.

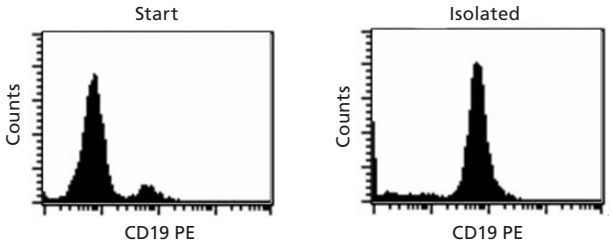


Figure 9. RosetteSep™ HLA B Cell Enrichment Coktail (Catalog #15064HLA)

Starting with human fresh whole blood, the typical B cell ($CD19^+$) content of the non-lysed final isolated fraction typicall ranges from 59.3% - 97.2% (gated on CD45). In the above example, the B cell ($CD19^+$) content of the lysed whole blood start sample and non-lysed final isolated fraction is 3.8% and 94.6% (gated on CD45), respectively.

Cell Separation Products for Serological HLA Typing

Cell Type	Selection	RosetteSep™ (Catalog #)
		Whole Blood[1]
T Cells	Negative	15061, 15061HLA
B Cells	Negative	15064, 15064HLA

1. Kit also works on other red blood cell containing samples (i.e. cord blood, buffy coat).

Cell Separation Solutions for Chimerism Analysis

Chimerism analysis is important for detecting and monitoring the establishment of donor leukocytes in a recipient following bone marrow transplantation.[1,2] Investigating chimerism within a specific cell population, also referred to as lineage-specific chimerism, can increase sensitivity over analyzing the entire leukocyte population.[3,4] However, lineage-specific chimerism requires cell isolation techniques that result in high cell purity.
In addition, cross-contamination between samples is always a concern since the presence of even a few non-target cells or cells from another sample can alter the integrity of downstream genetic analyses. For reliable lineage-specific chimerism analysis, EasySep™ provides a fast and easy method to obtain highly purified cells and flexible protocols to isolate multiple cell types from the same sample. To save time and increase laboratory throughput, EasySep™ can be automated with RoboSep™, an instrument that offers full automation of immunomagnetic cell separation.

EasySep™

- EasySep™ positive selection kits provide highly purified cells ready for downstream lineage-specific chimerism analysis (see pages 14 - 15).
- Flexible protocols allow for sequential isolations to maximize cell recovery of multiple cell types from a single sample.
- Cells can be isolated directly from a variety of sources including buffy coat or whole blood.

RoboSep™

- RoboSep™ instruments offer true walk-away automation of immunomagnetic cell separation using EasySep™ reagents (see pages 16 - 17).
- Disposable tips and a column-free system eliminate the risk of sample cross-contamination.
- Protocols for simultaneous and sequential cell isolations allow for automated sample processing of up to 16 samples.

RosetteSep™

- RosetteSep™ allows for efficient cell isolations directly from whole blood (see page 19).
- Desired cells are isolated during density gradient centrifugation and are immediately ready for downstream applications.

Typical Performance Data for Kits Used by Chimerism Laboratories

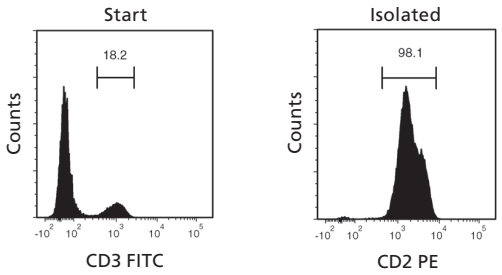


Figure 11. EasySep™ HLA Whole Blood CD3$^+$ Positive Selection Kit (Catalog #17871)

Starting with human whole blood, the CD3$^+$ cell content of the isolated fraction (as assessed by staining the start and isolated fractions with anti-CD3 or anti-CD2 antibodies, respectively) typically ranges from 96 - 99%. In the above example, the T cell content of the lysed whole blood start sample and the non-lysed final isolated fraction is 18.2% and 98.1% (gated on CD45), respectively.

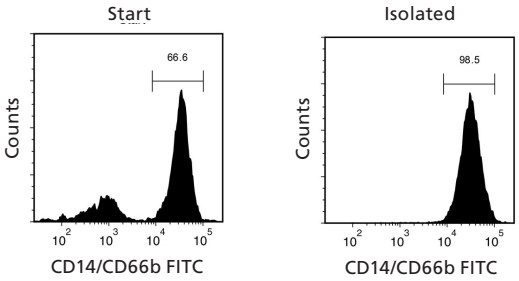


Figure 12. EasySep™ HLA Chimerism Whole Blood Myeloid Cell Positive Selection Kit (Catalog #17884)

Starting with human whole blood, the CD14$^+$/CD66b$^+$ cell content of the isolated fraction is typically 94.5 ± 4.1%. In the above example, the myeloid cell (CD14$^+$/CD66b$^+$) content of the lysed whole blood start sample and the non-lysed final isolated fraction is 66.6% and 98.5% (gated on CD45), respectively.

Cell Separation Products for Chimerism Analysis

Cell Type	EasySep™ / RoboSep™ (Catalog #)			RosetteSep™ (Catalog #)
	Positive Selection			Negative Selection
	Selection Marker	Whole Blood[1]	MNC[2]	Whole Blood[1]
T Cells	CD3	17871	17851	15271, 15271HLA
B Cells	CD19	17874	17854	–
	CD19/ CD20	17886	–	–
Myeloid Cells	CD15	17881	18651	15272, 15272HLA
	CD33	17885	17876	
	CD33/66b	17884	–	
Granulocytes	CD66b	17882	–	–
Monocytes	CD14	17878	17858	–
NK Cells	CD56	17875	17855	–
Hematopoietic Progenitor Cells	CD34	17879	17856	–

For automated cell isolation EasySep™ kits are available as RoboSep™ Reagent kits (RF).
1. Kit also works on other red blood cell containing samples (i.e. cord blood, buffy coat).
2. Kit works on mononuclear cells (MNCs) isolated from peripheral blood or bone marrow. MNC: Mononuclear Cell

Cited References

1. Bader P et al. (2005) How and when should we monitor chimerism after allogeneic stem cell transplantation? Bone Marrow Transplant 35(2): 107–19.
2. Levrat E et al. (2015) Very long term stability of mixed chimerism after allogeneic hematopoietic stem cell transplantation in patients with hematologic malignancies. Bone Marrow Res 2015: 176526.
3. Breuer S et al. (2012) Early recipient chimerism testing in the T- and NK-cell lineages for risk assessment of graft rejection in pediatric patients undergoing allogeneic stem cell transplantation. Leukemia 26(3): 509–19.
4. Rupa-Matysek J et al. (2011) Correlation between the kinetics of CD3$^+$ chimerism and the incidence of graft-versus-host disease in patients undergoing allogeneic hematopoietic stem cell transplantation. Transplant Proc 43(5): 1915–23.

Case Study

Fully Automated Sequential Cell Isolation of Four Different Cell Types from a Single Sample with RoboSep™-S

Florida Hospital Tissue Typing Laboratory, Orlando, Florida

Background

Many analyses, such as chimerism testing, are often performed on small blood samples (e.g. pediatric samples). As a result, analysis of purified cell subsets requires techniques that can isolate more than one cell type from an undivided starting sample. Here we describe a method used by the Florida Tissue Typing Laboratory to sequentially isolate B cells, T cells, myeloid cells, and NK cells starting from a single sample of HetaSep™-treated blood for their chimerism analysis.

Methods

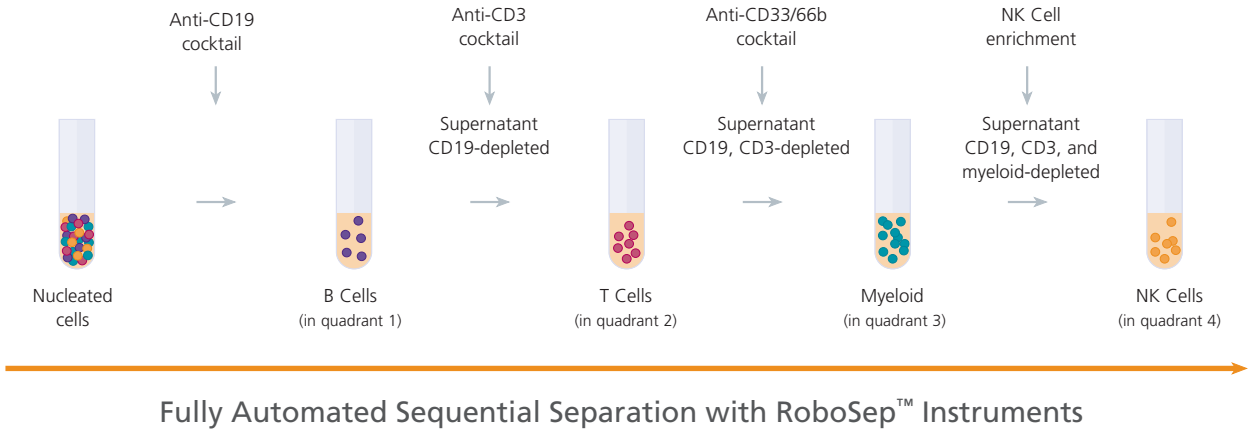


Figure 13. Automated Sequential Separation of B Cells, T Cells, Myeloid Cells, and NK Cells from a Single Sample of Hetasep™-Treated Blood

Results

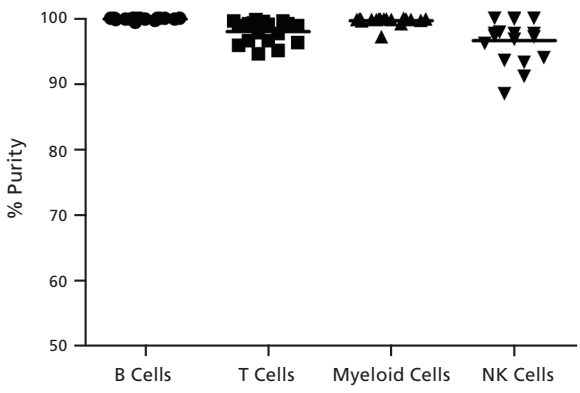


Figure 14. Purity of Four Different Cell Types Isolated from 18 Different Samples Using RoboSep™-S Sequential Separation

Data kindly provided by Max Marschner, Supervisor, Florida Hospital Tissue Typing Lab.

Why Use RoboSep™ for Sequential Isolation of Immune Cells?

FLEXIBLE. Perform sequential cell isolations of 4 cell types from a single sample using RoboSep™-S or from four samples using RoboSep™-16.

FULLY AUTOMATED. Perform all isolations during a single machine cycle with minimal "hands-on" time.

EFFICIENT. Isolate cells from small volumes (0.5 - 4.5 mL) of blood to run DNA analysis or other down stream applications.

VERSATILE. Customize protocols for any cell type and sample source.

INCREASED THROUGHPUT. Streamline cell isolations and increase laboratory throughput.

The Importance of Purity Assessment in Chimerism Analysis

Lineage-specific chimerism analysis is an important tool for monitoring the outcome of allogeneic hematopoietic cell transplantations (allo-HCT). Chimerism status in transplant recipients can indicate the potential dynamics of disease relapse, graft-versus-host disease (GVHD), graft versus tumor (GVT) effects, and other outcomes.[1-5]

Therefore, investigating chimerism within specific cell subsets, particularly in recipients of non-myeloablative allo-HCT[6,7], is an increasingly common practice that offers the following advantages over analyzing the entire leukocyte population:

- **Increased assay sensitivity.** If a patient has mixed chimerism in only one or a few cell lineages, the overall proportion of host cells may be too low for detection by whole blood assays.[8]
- **The significance of mixed chimerism differs between cell subsets.** For instance, one recent study found that the degree of mixed chimerism within T and NK cells, but not myeloid cells, allowed patients to be classified into different risk groups for graft rejection.[9] In another study, full donor T cell chimerism consistently preceded GVT effects.[13] Recent literature suggests that this type of lineage-specific analysis is highly informative for chimerism labs.[14]

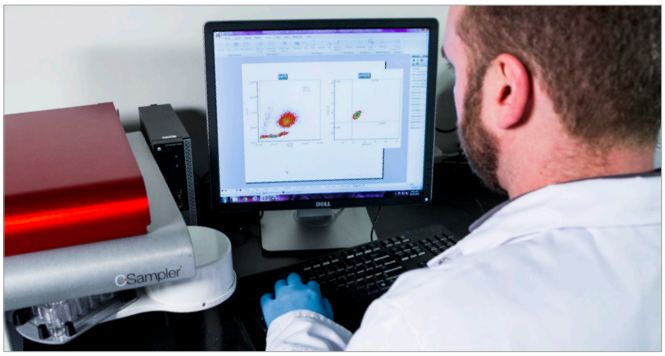
The benefits of lineage-specific chimerism analysis depend upon the purity of isolated cell subsets, as contamination by non-target cells decreases the reliability of the results. **Assessing the purity of isolated cell populations is therefore an essential quality control step.** The official European Federation for Immunogenetics (EFI) and American Society for Histocompatibility and Immunogenetics (ASHI) guidelines stipulate that the purity of sorted cell populations must be documented and taken into account when results are analyzed.

When HCE (Haemopoietic Chimaerism and Engraftment) testing is performed on cellular subsets isolated by cell sorting, the purity of the sorted population must be documented and taken into account in the analysis of the results. If this is not possible it must be clearly stated in the report.

EFI STANDARDS VERSION 6.3 (2015), SECTION I6.9

Document the purity obtained if processing involves isolation of cell subsets. If purity is not assessed, this must be documented on the test report.

ASHI STANDARDS 2016, SECTION D.5.3.4.1.8



To read the complete review on the importance of lineage-specific chimerism analysis and guidelines for assessing purity using flow cytometry visit **www.stemcell.com/chimerism-purity-assessment**.

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1. McCann SR et al. (2005) Hemopoietic chimerism following stem cell transplantation. Transfus Apher Sci 32(1): 55–61.
2. Khan F et al. (2004) Significance of chimerism in hematopoietic stem cell transplantation: new variations on an old theme. Bone Marrow Transplant 34(1): 1–12.
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6. Childs R et al. (1999) Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood 94(9): 3234–41.
7. Urbano-Ispizua A et al. (2001) The number of donor CD3(+) cells is the most important factor for graft failure after allogeneic transplantation of CD34(+) selected cells from peripheral blood from HLA-identical siblings. Blood 97(2): 383–7.
8. Antin JH et al. (2001) Establishment of complete and mixed donor chimerism after allogeneic lymphohematopoietic transplantation: recommendations from a workshop at the 2001 Tandem Meetings of the International Bone Marrow Transplant Registry and the American Society of Blood an. Biol Blood Marrow Transplant 7(9): 473–85.
9. Breuer S et al. (2012) Early recipient chimerism testing in the T- and NK-cell lineages for risk assessment of graft rejection in pediatric patients undergoing allogeneic stem cell transplantation. Leukemia 26(3): 509–19.
10. Jens G et al. (2011) Mixed Chimerism after Allogeneic Stem Cell Transplantation - Focus on Double Cord Blood Transplantation. Emma W (Ed.). J Blood Disord Transfus 2(S1).
11. Moratto D et al. (2011) Long-term outcome and lineage-specific chimerism in 194 patients with Wiskott-Aldrich syndrome treated by hematopoietic cell transplantation in the period 1980-2009: an international collaborative study. Blood 118(6): 1675–84.
12. Goh R-Y et al. (2011) Lineage-specific chimerism analysis in nucleated cells, T cells and natural killer cells after myeloablative allogeneic hematopoietic stem cell transplantation. Korean J Hematol 46(1): 18–23.
13. Bornhäuser M et al. (2009) Monitoring of donor chimerism in sorted CD34+ peripheral blood cells allows the sensitive detection of imminent relapse after allogeneic stem cell transplantation. Haematologica 94(11): 1613–7.
14. Buño I et al. (2015) Mixed Chimerism in T Cells after Allogeneic SCT Allows Early Diagnosis of Graft Rejection and Successful Treatment with Immunosuppression Withdrawal and/or Donor Leukocyte Infusion. Blood 106(11): 5289 LP-5289.

EasySep™

Rapid Immunomagnetic Cell Isolation

EasySep™ is a powerful immunomagnetic cell selection procedure that combines the specificity of monoclonal antibodies with the simplicity of a column-free magnetic system for the effortless isolation of highly purified cells. Cells are targeted for either depletion (negative selection) or selection (positive selection) using antibody complexes directed to specific cell surface antigens. The antibody complexes link targeted cells to magnetic particles. Labeled cells are pulled to the sides of the tube when the sample is placed in an EasySep™ magnet.

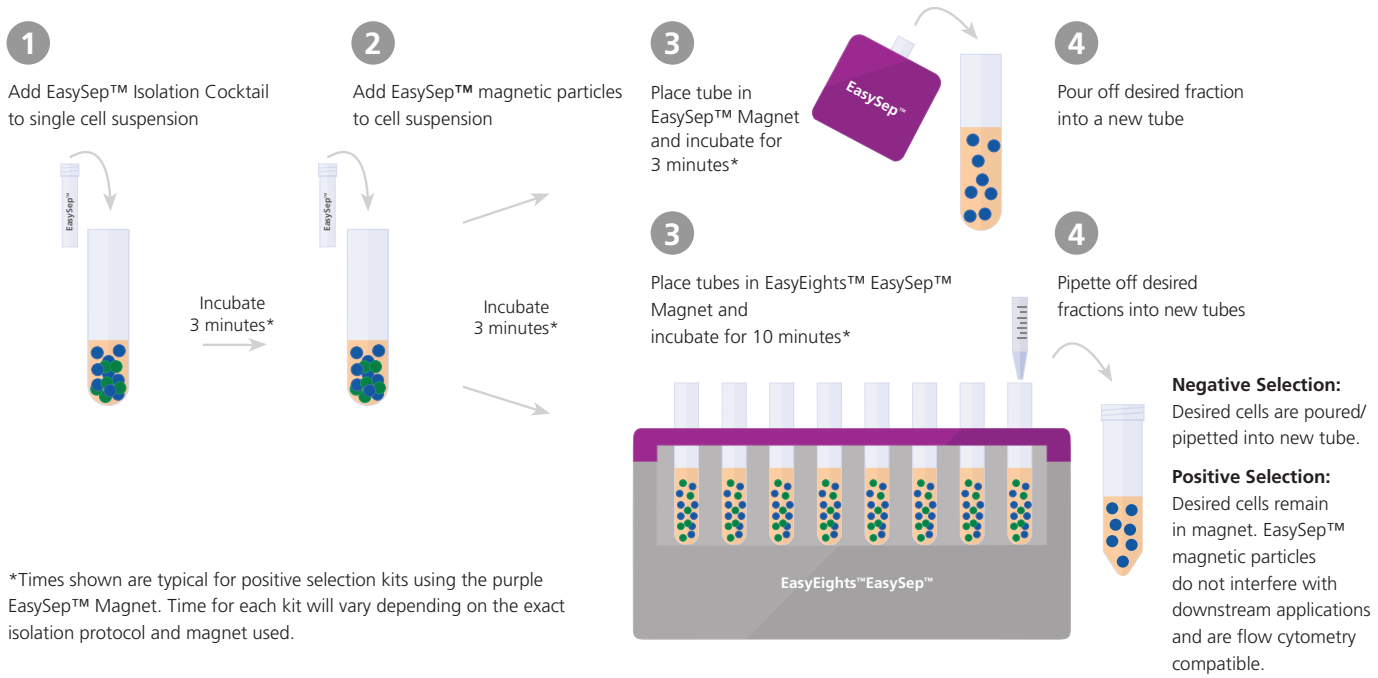
EasySep™ kits are available for the isolation of human T cells, B cells, granulocytes, lymphoid, or myeloid cells from a variety of sample sources, including whole blood, spleen, lymph nodes, and mononuclear cells (MNCs) from whole blood or bone marrow.

Manual or Automated Cell Isolation

Automate cell isolation to save hands-on time and increase laboratory throughput with RoboSep™ instruments (see pages 16 - 17).

For manual cell separation choose from a wide range of EasySep™ magnets that provide flexibility in the sample volume and throughput of cells (see page 23).

Typical EasySep™ Human Positive Selection Protocol



*Times shown are typical for positive selection kits using the purple EasySep™ Magnet. Time for each kit will vary depending on the exact isolation protocol and magnet used.

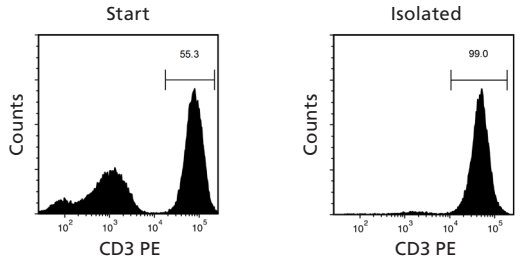


Figure 15. EasySep™ Human CD3 Positive Selection Kit II (Catalog #17851)

Starting with a single cell suspension of human peripheral blood mononuclear cells, the $CD3^+$ cell content of the isolated fraction is typically 99.2 ± 0.2% (mean ± SD using the purple EasySep™ Magnet). In this example, the total $CD3^+$ cell content of the start sample and isolated fraction is 55.3% and 99.0%, respectively.

EasySep™ Direct

Cell Isolation Directly from Whole Blood

EasySep™ Direct isolates untouched and highly purified cells directly from whole blood, spleen, or lymph nodes without density gradient centrifugation, RBC lysis, or other pre-processing steps.

Cells are immediately ready for downstream applications, including flow cytometry crossmatch and gene expression analysis. Individual samples of 0.5 – 30 mL can be processed in as little as 20 minutes.

Why Use EasySep™ Direct?

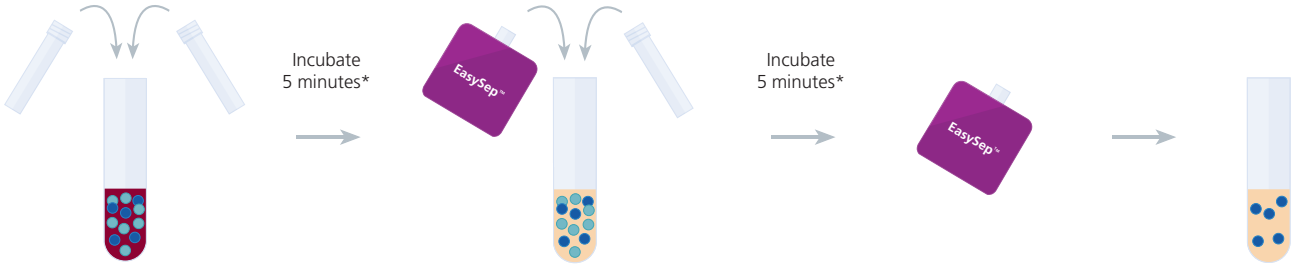
FAST. Isolate highly purified total lymphocytes, T cells, or B cells directly from whole blood, spleen, or lymph nodes without lysis or centrifugation.

HIGH PURITY. Obtain high cell purity with no RBC contamination, even on older blood samples.

CONVENIENT. Automate cell isolations and minimize sample handling with RoboSep™ instruments.

Typical EasySep™ Direct Protocol for Whole Blood and Buffy Coat

1. Add EasySep™ Direct Isolation Cocktail and EasySep™ Direct RapidSpheres™ to whole blood
2. Place tube in EasySep™ Magnet and incubate for 5 minutes*
3. Pour off desired fraction into a new tube and add EasySep™ Direct RapidSpheres™
4. Place tube in EasySep™ Magnet and incubate for 5 minutes*
5. Pour off desired fraction into a new tube



*Times for each kit will vary depending on sample source and isolation protocol. Please refer to the product information sheet of each product for sample compatibility and specific isolation protocol.

VIDEO
Learn how EasySep™ Direct works
www.EasySepDirect.com

RoboSep™

Fully Automated Immunomagnetic Cell Isolation

RoboSep™ instruments offer true walk-away automation of immunomagnetic cell separation. Using EasySep™ reagents, RoboSep™-S and RoboSep™-16 perform all cell labeling and magnetic isolation steps for up to four and sixteen samples, respectively. Sample handling is minimized and the use of disposable tips in these column-free systems ensures that isolated cells of interest are immediately available for any downstream application.

RoboSep™-S

The compact design of RoboSep™-S brings the convenience of automated cell isolation to any busy laboratory.

RoboSep™-16

The enhanced liquid-handling capabilities of RoboSep™-16 allow high-volume users to efficiently isolate desired cells with speed and confidence.

Why Use RoboSep™?

FULLY AUTOMATED. Just load reagents and samples and walk away.

SIMULTANEOUS OR SEQUENTIAL CELL ISOLATION. Perform simultaneous cell isolations for up to 4 samples using RoboSep™-S and 16 samples using RoboSep™-16 or sequentially isolate different cell types from the same sample.

NO CROSS-CONTAMINATION. Eliminate the risk of cross-contamination with a column-free system and single-use disposable tips.

VERSATILE. Use EasySep™ technology to isolate virtually any cell type from any species or sample, including whole blood. Programs are cell type-specific and customizable.

How RoboSep™ Works

RoboSep™-S and RoboSep™-16 fit easily into the workflow of any lab that needs the multi-sample processing capacity, speed, reliability, and convenience of automated cell isolation. Start your cell isolation protocol with as little as 5 minutes of "hands-on" time with RoboSep™-S and RoboSep™-16. Both pre-programmed routine protocols and customizable protocols are available to meet your unique cell isolation needs.

Typical RoboSep™-S Protocol

1. Select protocol. Load sample, EasySep™ reagents, buffer, and tips in carousel.

2. Press "Run".

3. Return in 25 to 60 minutes to collect your separated cells.

Typical RoboSep™-16 Protocol

1. Select protocol. Load sample, EasySep™ reagents, buffer, and tips.

2. Press "Run".

3. Return in 25 to 60 minutes to collect your separated cells.

"We like the reliability of the RoboSep™, the minimization/elimination of specimen handling by the tech during subset separation, and the low maintenance of the instrument. These factors are important to us with such a high throughput of samples processed."

Wendy Leong, Senior Clinical Laboratory Scientist
PATHOLOGY/BLOOD CENTER LABORATORY

PRODUCT TOUR
See RoboSep™ Instruments in Action
www.RoboSep.com

SepMate™

Hassle-Free PBMC Isolation

SepMate™ is a specialized tube for fast and easy PBMC isolation in just 15 minutes. The SepMate™ tube contains a unique insert that prevents the density gradient medium[1] (e.g., Ficoll-Paque® or Lymphoprep™) and blood sample from mixing. The density gradient medium is pipetted through a central hole in the insert and the sample is poured or rapidly pipetted on top of the insert. This eliminates the need to carefully layer the sample directly onto the density gradient medium, an otherwise time-consuming and highly laborious step. Only 10 minutes of centrifugation are required, and this step can be performed with the brake on, further reducing the total time necessary for separation. After centrifugation, plasma and PBMCs are simply poured into a new tube.

SepMate™ is registered as an In Vitro Diagnostic (IVD)[2] device in select regions.

Why Use SepMate™?

EASY. Avoid the need for slow and laborious sample layering over the density gradient medium.

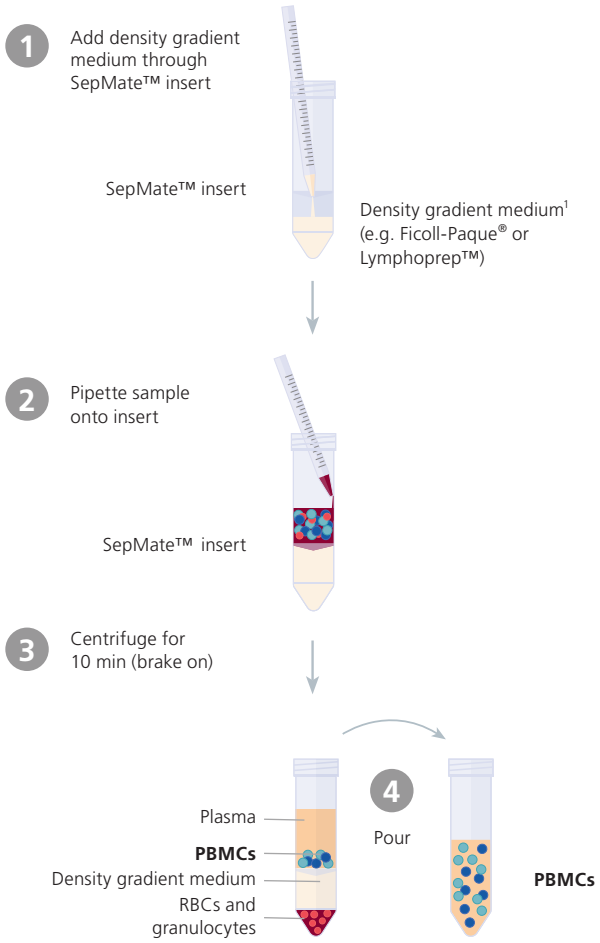
FAST. Centrifuge for just 10 minutes with the brake on and simply pour off PBMCs into a new tube.

CONSISTENT. Eliminate errors and minimize variability between users.

VERSATILE. Combine with RosetteSep™ to isolate purified cell subsets from whole blood in 25 minutes.

REGISTERED. Use with whole blood or bone marrow samples for In Vitro Diagnostic (IVD) applications.[2]

Typical SepMate™ Protocol



Products

Product	Catalog #	Blood Volume Processed (mL)	Unit Size
SepMate™-15 (IVD[2])	85415	0.5 - 5	100 tubes
SepMate™-15 (RUO[3])	86415		
SepMate™-50 (IVD[2])	85450	4 - 17	
SepMate™-50 (RUO[3])	86450		

Product	Catalog #	Density	Unit Size
Lymphoprep™	07801 07851 07811 07861	1.077 g/mL[1]	250 mL 500 mL 4 x 250 mL 6 x 500 mL

1. Lymphoprep™ has the same density as Ficoll-Paque® and can be substituted for Ficoll-Paque® without any need to change your existing protocols.
2. SepMate™ 85415/85450 is available in Australia, Canada, Europe, and the United States of America, where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells from human whole blood, cord blood, and bone marrow by density gradient centrifugation. This product is also available in China where it is considered a non-medical device by the China Food and Drug Administration (CFDA) and should therefore be used as general laboratory equipment.
3. SepMate™ 86415/86450 is intended for Research Use Only (RUO) and is available in regions where SepMate™ 85415/85450 is not available.

VIDEO
How to Use SepMate™
www.stemcell.com/SepMateVideo

RosetteSep™

Unique Immunodensity Cell Isolation

RosetteSep™ is a fast and easy immunodensity procedure for the isolation of untouched cells directly from whole blood. By crosslinking unwanted cells to red blood cells (RBCs) present in the sample, RosetteSep™ eliminates the need for a separate magnetic separation step because cells are purified during standard density gradient centrifugation. This approach significantly reduces sample handling time and maximizes convenience. For a complete list of RosetteSep™ products visit **www.RosetteSep.com**.

Typical RosetteSep™ Protocol

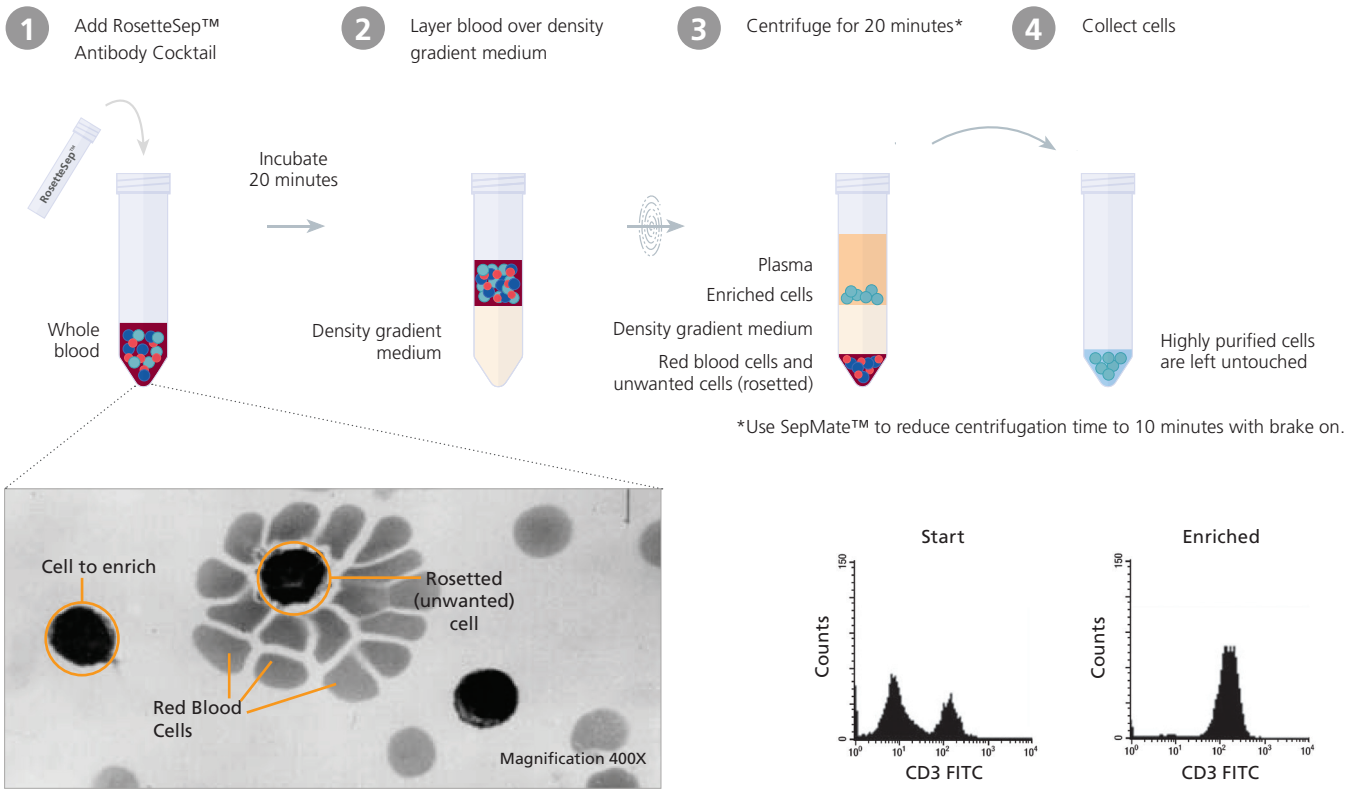


Figure 16. Picture of a Blood Sample After Addition of the RosetteSep™ Cocktail and Prior to Density Gradient Centrifugation

Figure 17. RosetteSep™ HLA T Cell Enrichment Cocktail (Catalog #15061HLA)

Starting with fresh whole blood the $CD3^+$ cell content of the enriched fraction typically ranges from 90% - 97%. In the example above, the T cell ($CD3^+$) content of the lysed whole blood start sample and the non-lysed final isolated fraction is 20% and 96%, respectively.

RosetteSep™ and SepMate™

Simplified and Standardized Cell Isolation

RosetteSep™ is easily combined with SepMate™ to rapidly and reproducibly isolate PBMC subsets from whole blood. By using the unique SepMate™ tube, sample throughput is increased and errors associated with improper sample layering are eliminated. This allows even users with minimal training to consistently perform cell isolation by density gradient centrifugation in a busy laboratory environment.

Verified and Reliable Antibodies

Take the Guesswork Out of Finding the Right Antibody

Using compatible antibodies is an essential component for your research. Antibodies from different vendors can provide variable and potentially sub-optimal staining profiles. STEMCELL Technologies offers a line of high-quality primary and secondary antibodies that have been verified to work with our cell isolation and cell culture reagents in specific applications, ensuring that your downstream cell analysis, including phenotyping and purity assessments, works consistently.

Why Use STEMCELL's Antibodies?

LARGE PORTFOLIO. Large selection of primary and secondary antibodies and isotype controls.

VERSATILE. Proven performance for multiple applications.

RELIABLE. Trusted by our dedicated team of research scientists.

Recommended Antibodies for Purity Assessment

Cell Type	Recommended Labeling Antibodies		
	Marker	Antigen, Clone	Catalog #
Leukocytes	CD45	Anti-Human CD45, HI30	60018
T Cells	CD2	Anti-Human CD2, RPA-2.10	60007
	CD3	Anti-Human CD3, UCHT1	60011
		Anti-Human CD3, SK7	60127
	CD4	Anti-Human CD4, OKT4	60016
	CD5	Anti-Human CD5, UCHT2	60082
	CD8	Anti-Human CD8a, RPA-T8	60022
B Cells	CD19	Anti-Human CD19, HIB19	60005
	CD20	Anti-Human CD20, 2H7	60008
	CD22	Anti-Human CD22, HIB22	60083
	CD43	Anti-Human CD43, CD43-10G7	60085
NK Cells	CD56	Anti-Human CD56 (NCAM), HCD56	60021
Myeloid Cells	CD15	Anti-Human SSEA-1 (CD15), MC-480	60060
	CD33	Anti-Human CD33, HIM3-4	60096
	CD66b	Anti-Human CD66b, G10F5	60086
Monocytes	CD14	Anti-Human CD14, M5E2 Anti-Human CD14, MoP9	60004 60124
	CD36	Anti-Human CD36, FA6-152	60084
Granulocytes	CD66b	Anti-Human CD66b, G10F5	60086
Hematopoietic Progenitor Cells	CD34	Anti-Human CD34, 581	60013
Antigen Presenting Cells	HLA-DR	Anti-Human HLA-DR, LN3	60164

Secondary Antibodies

Target Antigen	Host Species	Type	Conjugation	Catalog #
Mouse IgG (H + L)	Goat	Polyclonal	FITC	60138

New antibodies are added to our growing inventory on a continuing basis. To find labeling antibodies compatible with a specific cell isolation kit, please refer to the corresponding product information sheet. For a complete list of our available antibodies visit **www.stemcell.com/antibodies**.

Selected Publications

Selected RoboSep™ Publications

1. Decot V et al. (2008) Chimerism analysis following nonmyeloablative stem cell transplantation using a new cell subset separation method: Robosep. Biomed Mater Eng 18 (1 Suppl): S19–26.
2. Eggimann L et al. (2015) Kinetics of peripheral blood chimerism for surveillance of patients with leukemia and chronic myeloid malignancies after reduced-intensity conditioning allogeneic hematopoietic SCT. Bone Marrow Transplant 50(5): 743–5.
3. Fernandez-Bango C et al. (2017) P230 Automation for flow cytometry crossmatch (FCXM) lymphocyte isolation using robosep. Hum Immunol 78: 224.
4. Hanson V et al. (2013) Assessment of the purity of isolated cell populations for lineage-specific chimerism monitoring post haematopoietic stem cell transplantation. Tissue Antigens 82(4): 269–275.
5. Lee HC et al. (2015) Mixed T Lymphocyte Chimerism after Allogeneic Hematopoietic Transplantation Is Predictive for Relapse of Acute Myeloid Leukemia and Myelodysplastic Syndromes. Biol Blood Marrow Transplant 21(11): 1948–54.
6. McQueen KL et al. (2013) 115-P: Automated High Throughput Isolation of Lymphoid and Myeloid Cells Directly from Whole Blood using RoboSep-HT. Hum Immunol 74(Supplement): 129.
7. Rennert H et al. (2011) Bone Marrow Engraftment Analysis BT - Diagnostic Molecular Pathology in Practice: A Case-Based Approach. In I. Schrijver, ed. Diagnostic Molecular Pathology in Practice. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 147–157.
8. Szewczyk K et al. (2016) Flow cytometry crossmatch reactivity with pronase-treated T cells induced by non-HLA autoantibodies in human immunodeficiency virus-infected patients. Hum Immunol 77(6): 449–55.
9. van Besien K et al. (2017) Cord blood chimerism and relapse after haplo-cord transplantation. Leuk Lymphoma 58(2): 288–297.

Selected EasySep™ and RosetteSep™ Publications

1. Alheim M et al. (2013) Evaluation of a new flow cytometry crossmatch procedure for simultaneous detection of cytotoxicity and antibody binding. Tissue Antigens 82(2): 125–30.
2. Alheim M et al. (2015) Improved flow cytometry based cytotoxicity and binding assay for clinical antibody HLA crossmatching. Hum Immunol 76(11): 849–57.
3. Aljurf M et al. (2016) Chimerism Analysis of Cell-Free DNA in Patients Treated with Hematopoietic Stem Cell Transplantation May Predict Early Relapse in Patients with Hematologic Malignancies. Biotechnol Res Int 2016: 8589270.
4. Crespo E et al. (2015) Pre-Transplant Donor-Specific T-Cell Alloreactivity Is Strongly Associated with Early Acute Cellular Rejection in Kidney Transplant Recipients Not Receiving T-Cell Depleting Induction Therapy. PLoS One 10(2): e0117618.
5. Falbo DK et al. (2015) Flow cytometric crossmatch results using lymphocytes isolated from donor peripheral blood and spleen tissue on five consecutive days. Hum Immunol 76(Supplement): 62.
6. Fidler SJ. (2012) Crossmatching by complement-dependent lymphocytotoxicity. Methods Mol Biol 882: 359–77.
7. Lemp NA et al. (2014) 1016-LBP: RosetteSep HLA – An exceptional method for isolation of lymphocytes from peripheral blood. Hum Immunol 75(6): 487.
8. Liwski RS et al. (2012) 30-OR: Canada-Wide Evaluation of Rapid Optimized Flow Crossmatch (ROFCXM) Protocol. Hum Immunol 73: 26.
9. Liwski RS et al. (2018) Rapid optimized flow cytometric crossmatch (FCXM) assays: The Halifax and Halifaster protocols. Hum Immunol 79(1): 28–38.
10. Park MH. (2014) P022: Flow Cytometric Crossmatch for Deceased Donor Transplant Candidates Using Small Number of Cells and Serum Volume in Microplate. Hum Immunol 75(Supplement): 64.

Product Listing

Cell Isolation Products

Catalog Numbers of Kits Commonly Used for HLA Applications

Cell Type	Selection		Crossmatch			Serological Typing	Chimerism	
			Whole Blood[1]	Whole Blood[1], Spleen	MNC[2], LN[3]	Whole Blood[1]	Whole Blood[1]	MNC[2], LN[3]
Total Lymphocytes	Negative		15263 15263HLA	19655	19961HLA	–	–	–
T Cells	Positive	CD3	–	–	–	–	17871	17851
	Negative		15061 15061HLA	19671 89671	19671 89671	15061 15061HLA	–	–
		Lymphoid (CD3+)	–	–	–	–	15271 15271HLA	–
B Cells	Positive	CD19	–	–	–	–	17874	17854
		CD19/CD20	–	–	–	–	17886	–
	Negative		15064 15064HLA	19684 89684	19684 89684	15064 15064HLA	–	–
Myeloid Cells/ Granulocytes	Positive	CD15	–	–	–	–	17881	18651
		CD33	–	–	–	–	17885	17876
		CD33/66b	–	–	–	–	17884	–
		CD66b	–	–	–	–	17882	–
	Negative	Myeloid	–	–	–	–	15272 15272HLA	–
Monocytes	Positive	CD14	–	–	–	–	17878	17858
NK Cells	Positive	CD56	–	–	–	–	17875	17855
	Negative		–	–	–	–	–	17955
Hematopoietic Progenitors	Positive	CD34	–	–	–	–	17879	17856

EasySep™/RoboSep™ kits RosetteSep™ kits

1. Kit also works on other red blood cell containing samples (i.e. cord blood, buffy coat).
2. Kit works on mononuclear cells (MNCs) isolated from peripheral blood or bone marrow. MNC: Mononuclear Cell
3. LN: Lymph Node

Our commitment to your research does not end when you purchase our products. STEMCELL Technologies' Technical Support experts and comprehensive RoboSep™ service packages offer top quality support and maintenance, freeing you to focus on your science. Please visit **www.RoboSep.com** to learn more.

Cell Separation Magnets and Accessories

EasySep™ Magnets for Manual Cell Isolation

	The Iconic Column-Free Cell Isolation Platform	For Larger Column-Free Cell Isolations	For Very Large Volume Cell Isolations	For Simultaneous Processing of Multiple Samples	For Simultaneous Small Volume Cell Isolations
	EasySep™ Magnet 	**"The Big Easy" EasySep™ Magnet** 	**Easy 50 EasySep™ Magnet** 	**EasyEights™ EasySep™ Magnet** 	**EasyPlate™ EasySep™ Magnet** 
Catalog #	18000	18001	18002	18103	18102
Number of Samples	1	1	1	8 on each side = 16 total	96
Working Volume[1]	0.1 - 2.5 mL	0.2 - 10 mL	0.5 - 40 mL	0.25 - 2.0 per 5 mL tube 0.5 - 8.0 per 14 mL tube	0.05 - 0.2 mL
Collection Method	Pour off	Pour off	Pipette off	Pipette off	Pipette off
Recommended Plasticware	Polystyrene 5 mL Tube (Catalog #38007)	Polystyrene 14 mL Tube (Catalog #38008)	Polystyrene 50 mL Tube (Catalog #38010)	Polystyrene 5 mL Tube (Catalog #38007) Polystyrene 14 mL Tube (Catalog #38008)	96-Well Round-Bottom Microplate (Catalog #38018)

RoboSep™ Instruments & Accessories

Product	Catalog #
RoboSep™-S	21000
RoboSep™-16	23000
RoboSep™ Buffer[2] (250 mL)	20104
RoboSep™ Buffer 5X Concentrate (250 mL)	20124
RoboSep™ Filter Tip Racks[2] (1 box of 8 racks) for RoboSep™-S	20125
Sterile Filtered Conductive Tips for RoboSep™-16	23101
Non-Sterile Filtered Conductive Tips for RoboSep™-16	23102

Density Media

Product	Catalog #	Unit Size
Lymphoprep™	07801 07851 07811 07861	250 mL 500 mL 4 x 250 mL 6 x 500 mL
HetaSep™	07806 07906	20 mL 100 mL
RosetteSep™ DM-L	15705	100 mL
RosetteSep™ DM-M	15725	100 mL
SpinSep™ Density Medium	17531	100 mL

SepMate™ Products

Product	Catalog #	Blood Volume Processed (mL)	Unit Sizte
SepMate™-15 (IVD[3])	85415	0.5 - 5	100 tubes
SepMate™-15 (RUO[4])	86415		
SepMate™-50 (IVD[3])	85450	4 - 17	
SepMate™-50 (RUO[4])	86450		

1. Please refer to the product information sheet of each product for the corresponding volume range and cell concentration that can be processed with each EasySep™ magnet.
2. RoboSep™ Buffer and 1 - 2 boxes of RoboSep™ Filter Tip Racks are included with every purchase of a RoboSep™ Reagent Kit.
3. SepMate™ 85415/85450 is available in Australia, Canada, Europe and the United States of America, select regions where it is registered as an In Vitro Diagnostic (IVD) device for the isolation of mononuclear cells from human whole blood, cord blood and bone marrow by density gradient centrifugation. This product is also available in China where it is considered a non-medical device by the China Food and Drug Administration (CFDA), and should therefore be used as general laboratory equipment.
4. SepMate™ 86415/86450 is intended for Research Use Only (RUO) and is available in regions where SepMate™ 85415/85450 is not available.

CELL SEPARATION PRODUCTS

For HLA Analysis

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FOR GLOBAL CONTACT DETAILS VISIT WWW.STEMCELL.COM

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