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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 cytotoxicity (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.
### Cell Type Selection

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Selection</th>
<th>EasySep™ / RoboSep™ (Catalog #)</th>
<th>rosetteSep™ (Catalog #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td>1, Spleen</td>
<td>19671, 89671&lt;sup&gt;4&lt;/sup&gt;</td>
<td>15061, 15061HLA&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lymph Node and MNC</td>
<td>2</td>
<td>19684, 89684&lt;sup&gt;4&lt;/sup&gt;</td>
<td>15064, 15064HLA&lt;sup&gt;4&lt;/sup&gt;</td>
</tr>
<tr>
<td>Total Lymphocytes</td>
<td>Negative</td>
<td>19655</td>
<td>19961HLA</td>
</tr>
</tbody>
</table>

1. Kit also works on other red blood cell containing samples (i.e. cord blood, buffy coat).
2. Kit works on mononuclear cells (MNC) isolated from peripheral blood or bone marrow. MNC: Mononuclear Cell
3. This kit has been CE-marked as a Class I In Vitro Diagnostic (IVD) product in the European Union according to the 98/79/EC In Vitro Diagnostic Device Directive and complies with its requirements.
4. This kit carries the CE mark and is available as a Class I IVD device in Australia, Canada, and the European Union. To learn more about the regulatory status of this product in your specific region, contact info@stemcell.com.

### In Vitro Diagnostic (IVD) Reagents

Several of our EasySep™ Direct and RosetteSep™ reagents have been CE-marked as in vitro diagnostic devices in specific regions. We strive to offer reagents you can trust and demonstrate our commitment to the highest standard of quality control.

---

**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<sup>+</sup>) content of the non-lysed final isolated fraction is 97 ± 3% (gated on CD45). In the above example, the T cell (CD3<sup>+</sup>) 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<sup>+</sup>CD19<sup>+</sup> and CD3<sup>+</sup>CD19<sup>-</sup>) 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<sup>+</sup>CD19<sup>+</sup> and CD3<sup>+</sup>) content of the lysed whole blood start sample and non-lysed final isolated fraction is 30.5% and 93.2% (gated on CD45), respectively.
Case Study

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

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

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). 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. 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. 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. 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. 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.
The approximate total times it takes to complete the FCXM assay density gradient centrifugation method used by the standard and Halifax FCXM protocols. The approximate total times it takes to complete the FCXM assay 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 (including cell preparation) when following the standard, Halifax, and Halifaster FCXM protocols. The median channel fluorescence shifts (MCFS) were generated by using the negative control sera samples as a baseline. The MCFS of positive control sera. The median channel fluorescence shifts (MCFS) were 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 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

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 Cocktail (Catalog #15064HLA)

Starting with human fresh whole blood, the typical B cell (CD19+) content of the non-lysed final isolated fraction typically 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

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Selection</th>
<th>RosetteSep™ (Catalog #)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole Blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T Cells</td>
<td>Negative</td>
<td>15061, 15061HLA^2</td>
</tr>
<tr>
<td>B Cells</td>
<td>Negative</td>
<td>15064, 15064HLA^2</td>
</tr>
</tbody>
</table>

1. Kit also works on other red blood cell containing samples (i.e. cord blood, buffy coat).
2. This kit has been CE-marked as a Class I IVD product in the European Union according to the 98/79/EC In Vitro Diagnostic Device Directive and complies with its requirements.
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. Investigating chimerism within a specific cell population, also referred to as lineage-specific chimerism, can increase sensitivity over analyzing the entire leukocyte population. 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

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>EasySep™ / RoboSep™ (Catalog #)</th>
<th>RosetteSep™ (Catalog #)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive Selection</td>
<td>Negative Selection</td>
</tr>
<tr>
<td></td>
<td>Selection Marker</td>
<td>Whole Blood¹</td>
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<tr>
<td>T Cells</td>
<td>CD3</td>
<td>17871</td>
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<tr>
<td>B Cells</td>
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<td>17874</td>
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<td></td>
<td>CD19/CD20</td>
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<td>Myeloid Cells</td>
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<td>17881</td>
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<td></td>
<td>CD33</td>
<td>17885</td>
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<tr>
<td></td>
<td>CD33/66b</td>
<td>17884</td>
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<tr>
<td>Granulocytes</td>
<td>CD66b</td>
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<tr>
<td>Monocytes</td>
<td>CD14</td>
<td>17878</td>
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<tr>
<td>NK Cells</td>
<td>CD56</td>
<td>17875</td>
</tr>
<tr>
<td>Hematopoietic Progenitor Cells</td>
<td>CD34</td>
<td>17879</td>
</tr>
</tbody>
</table>

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.
3. This kit has been CE-marked as a Class I IVD product in the European Union according to the 98/79/EC In Vitro Diagnostic Device Directive and complies with its requirements.

### Cited References

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

Anti-CD19 cocktail

Supernatant CD19-depleted

Nucleated cells

B Cells (in quadrant 1)

Anti-CD3 cocktail

Supernatant CD19, CD3-depleted

T Cells (in quadrant 2)

Anti-CD33/66b cocktail

Supernatant CD19, CD3, and myeloid-depleted

Myeloid (in quadrant 3)

NK Cell enrichment

NK Cells (in quadrant 4)

Fully Automated Sequential Separation with RoboSep™ Instruments

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

Results

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 downstream applications.

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

INCREASED THROUGHPUT. Streamline cell isolations and increase laboratory throughput.

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.
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-HCT6,7, 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.10 Recent literature suggests that this type of lineage-specific analysis is highly informative for chimerism labs.11

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 16.9**

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](http://www.stemcell.com/chimerism-purity-assessment).

Cited References

**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.

**Typical EasySep™ Human Positive Selection Protocol**

1. Add EasySep™ Isolation Cocktail to single cell suspension
2. Add EasySep™ magnetic particles to cell suspension
3. Place tube in EasySep™ Magnet and incubate for 3 minutes*
4. Pour off desired fraction into a new tube
5. Incubate 3 minutes*
6. Place tubes in EasyEights™ EasySep™ Magnet and incubate for 10 minutes*
7. Pipette off desired fractions into new tubes

*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.

**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).

**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

1. **Add density gradient medium through SepMate™ insert**

2. **Pipette sample onto insert**

3. **Centrifuge for 10 min (brake on)**

4. **Pour**

### Products

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog #</th>
<th>Blood Volume Processed (mL)</th>
<th>Unit Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>SepMate™-15 (IVD)</td>
<td>85415</td>
<td>0.5 - 5</td>
<td>100 tubes</td>
</tr>
<tr>
<td>SepMate™-15 (RUO)</td>
<td>86415</td>
<td></td>
<td></td>
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<tr>
<td>SepMate™-50 (IVD)</td>
<td>85450</td>
<td>4 - 17</td>
<td></td>
</tr>
<tr>
<td>SepMate™-50 (RUO)</td>
<td>86450</td>
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<table>
<thead>
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<th>Density Unit Size</th>
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<td>07801</td>
<td>1.077 g/mL</td>
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<tr>
<td></td>
<td>07851</td>
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</tr>
<tr>
<td></td>
<td>6 x 500 mL</td>
<td></td>
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</tbody>
</table>

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.
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. Select kits have been CE-marked as Class I IVD products in the European Union. For a complete list of RosetteSep™ products visit www.RosetteSep.com.

Typical RosetteSep™ Protocol

1. Add RosetteSep™ Antibody Cocktail
2. Layer blood over density gradient medium
3. Centrifuge for 20 minutes*
4. Collect cells

*Use SepMate™ to reduce centrifugation time to 10 minutes with brake on.

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.

Recommended Antibodies for Purity Assessment

<table>
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<tr>
<th>Cell Type</th>
<th>Recommended Labeling Antibodies</th>
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<td></td>
<td>Marker</td>
<td>Antigen, Clone</td>
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<tr>
<td></td>
<td>CD45</td>
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<td>Leukocytes</td>
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<td>CD2</td>
<td>Anti-Human CD2, RPA-2.10</td>
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<tr>
<td></td>
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<td>Anti-Human CD3, SK7</td>
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<tr>
<td></td>
<td>CD4</td>
<td>Anti-Human CD4, OKT4</td>
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<tr>
<td></td>
<td>CD5</td>
<td>Anti-Human CD5, UCHT2</td>
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<tr>
<td></td>
<td>CD8</td>
<td>Anti-Human CD8a, RPA-T8</td>
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<td>T Cells</td>
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<tr>
<td></td>
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<td>Anti-Human CD19, HIB19</td>
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<td></td>
<td>CD20</td>
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<td>Anti-Human CD22, HIB22</td>
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<td>CD56</td>
<td>Anti-Human CD56 (NCAM), HCD56</td>
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<td>B Cells</td>
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<td></td>
<td>CD15</td>
<td>Anti-Human SSEA-1 (CD15), MC-480</td>
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<td>CD33</td>
<td>Anti-Human CD33, HiM3-4</td>
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<td>CD66b</td>
<td>Anti-Human CD66b, G10F5</td>
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<tr>
<td>NK Cells</td>
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<tr>
<td></td>
<td>CD56</td>
<td>Anti-Human CD56 (NCAM), HCD56</td>
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<tr>
<td>Myeloid Cells</td>
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<tr>
<td></td>
<td>CD15</td>
<td>Anti-Human SSEA-1 (CD15), MC-480</td>
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<td></td>
<td>CD33</td>
<td>Anti-Human CD33, HiM3-4</td>
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<td>CD66b</td>
<td>Anti-Human CD66b, G10F5</td>
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<td>Monocytes</td>
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<td>Anti-Human CD14, MoP9</td>
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<td></td>
<td>CD36</td>
<td>Anti-Human CD36, FA6-152</td>
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<td>Granulocytes</td>
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<tr>
<td></td>
<td>CD66b</td>
<td>Anti-Human CD66b, G10F5</td>
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<tr>
<td>Hematopoietic Progenitor Cells</td>
<td>CD34</td>
<td>Anti-Human CD34, S81</td>
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<td>Antigen Presenting Cells</td>
<td>HLA-DR</td>
<td>Anti-Human HLA-DR, LN3</td>
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Secondary Antibodies

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<th>Target Antigen</th>
<th>Host Species</th>
<th>Type</th>
<th>Conjugation</th>
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<td>Mouse IgG (H + L)</td>
<td>Goat</td>
<td>Polyclonal</td>
<td>FITC</td>
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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


Selected EasySep™ and RosetteSep™ Publications


## Product Listing

### Cell Isolation Products

Catalog Numbers of Kits Commonly Used for HLA Applications

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Selection</th>
<th>Crossmatch</th>
<th>Serological Typing</th>
<th>Chimerism</th>
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<tr>
<td></td>
<td></td>
<td>Whole Blood, Spleen</td>
<td>MNC&lt;sup&gt;2&lt;/sup&gt;, LN&lt;sup&gt;3&lt;/sup&gt;</td>
<td>Whole Blood</td>
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<tr>
<td>Total Lymphocytes</td>
<td>Negative</td>
<td>15263, 15263HLA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>19655</td>
<td>19961HLA −</td>
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<tr>
<td></td>
<td>Positive</td>
<td>CD3 − − − −</td>
<td>15061 15061HLA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>19671 89671&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td></td>
<td></td>
<td>Lymphoid (CD&lt;sup&gt;3&lt;/sup&gt;) − − − −</td>
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<tr>
<td></td>
<td>Positive</td>
<td>CD19 − − − −</td>
<td>15064 15064HLA&lt;sup&gt;4&lt;/sup&gt;</td>
<td>19684 89684&lt;sup&gt;1&lt;/sup&gt;</td>
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<tr>
<td></td>
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<td>CD19/CD20 − − − −</td>
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<td>Myeloid Cells/</td>
<td>Positive</td>
<td>CD15 − − − −</td>
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<td>−</td>
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<tr>
<td>Granulocytes</td>
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<td>CD33 − − − −</td>
<td>−</td>
<td>−</td>
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<td></td>
<td></td>
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<td>CD66b − − − −</td>
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<tr>
<td></td>
<td>Negative</td>
<td>Myeloid − − − −</td>
<td>−</td>
<td>−</td>
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<tr>
<td>Monocytes</td>
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<td></td>
<td>Negative</td>
<td>− − − − −</td>
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<td>−</td>
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<td>Hematopoietic</td>
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<td>CD34 − − − −</td>
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<td>−</td>
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<tr>
<td>Progenitors</td>
<td></td>
<td>− − − − −</td>
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<td>−</td>
</tr>
</tbody>
</table>

- 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
4. This kit has been CE-marked as a Class I In Vitro Diagnostic (IVD) product in the European Union according to the 98/79/EC In Vitro Diagnostic Device Directive and complies with its requirements.
5. This kit carries the CE mark and is available as a Class I IVD device in Australia, Canada and the European Union. To learn more about the regulatory status of this product in your specific region, contact info@stemcell.com.

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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](http://www.RoboSep.com) to learn more.
Cell Separation Magnets and Accessories

EasySep™ Magnets for Manual Cell Isolation

<table>
<thead>
<tr>
<th>Product</th>
<th>Catalog #</th>
<th>Number of Samples</th>
<th>Working Volume 1</th>
<th>Collection Method</th>
<th>Recommended Plasticware</th>
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<tbody>
<tr>
<td>EasySep™ Magnet</td>
<td>18000</td>
<td>1</td>
<td>0.1 - 2.5 mL</td>
<td>Pour off</td>
<td>Polystyrene 5 mL Tube (Catalog #38007)</td>
</tr>
<tr>
<td>EasySep™ 50</td>
<td>18001</td>
<td>1</td>
<td>0.2 - 10 mL</td>
<td>Pour off</td>
<td>Polystyrene 14 mL Tube (Catalog #38008)</td>
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<tr>
<td>EasySep™ Magnet EasyEights™</td>
<td>18002</td>
<td>1</td>
<td>0.5 - 40 mL</td>
<td>Pipette off</td>
<td>Polystyrene 50 mL Tube (Catalog #38010)</td>
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<tr>
<td>EasySep™ Magnet EasyPlate™</td>
<td>18103</td>
<td>8</td>
<td>0.25 - 2.0 per 5 mL tube</td>
<td>Pipette off</td>
<td>Polystyrene 5 mL Tube (Catalog #38007)</td>
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<tr>
<td>EasySep™ Magnet EasyPlate™</td>
<td>18102</td>
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<td>0.5 - 8.0 per 14 mL tube</td>
<td>Pipette off</td>
<td>Polystyrene 14 mL Tube (Catalog #38008)</td>
</tr>
<tr>
<td>SepMate™-15 (IVD)</td>
<td>85415</td>
<td></td>
<td>0.5 - 5</td>
<td>100 tubes</td>
<td>96-Well Round-Bottom Microplate (Catalog #38018)</td>
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<td>SepMate™-15 (RUO)</td>
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<tr>
<td>SepMate™-50 (IVD)</td>
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<td>SepMate™-50 (RUO)</td>
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</tbody>
</table>

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™-15 (RUO) 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™-15 (IVD) is intended for Research Use Only (RUO) and is available in regions where SepMate™-15 (RUO) is not available.

RoboSep™ Instruments & Accessories

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<th>Blood Volume Processed (mL)</th>
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<td>RoboSep™-16</td>
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<td>RoboSep™ Buffer (250 mL)</td>
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<td>RoboSep™ Buffer SX Concentrate (250 mL)</td>
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<td>RoboSep™ Filter Tip Racks (1 box of 8 racks) for RoboSep™-S</td>
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<td>Sterile Filtered Conductive Tips for RoboSep™-16</td>
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<td>Non-Sterile Filtered Conductive Tips for RoboSep™-16</td>
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SepMate™ Products

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<th>Blood Volume Processed (mL)</th>
<th>Unit Size</th>
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<td>SepMate™-15 (IVD)</td>
<td>85415</td>
<td>0.5 - 5</td>
<td>100 tubes</td>
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<td>SepMate™-15 (RUO)</td>
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<td>SepMate™-50 (IVD)</td>
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2. RoboSep™ Buffer and 1 - 2 boxes of RoboSep™ Filter Tip Racks are included with every purchase of a RoboSep™ Reagent Kit.
3. SepMate™-15 (IVD) 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™-15 (RUO) is intended for Research Use Only (RUO) and is available in regions where SepMate™-15 (RUO) is not available.

Density Media

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<tr>
<td>Lymphoprep™</td>
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<td></td>
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<tr>
<td></td>
<td>07861</td>
<td>6 x 500 mL</td>
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<tr>
<td>HetaSep™</td>
<td>07806</td>
<td>20 mL</td>
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<td></td>
<td>07906</td>
<td>100 mL</td>
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<tr>
<td>RosetteSep™ DM-L</td>
<td>15705</td>
<td>100 mL</td>
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<td>RosetteSep™ DM-M</td>
<td>15725</td>
<td>100 mL</td>
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<tr>
<td>SpinSep™ Density Medium</td>
<td>17531</td>
<td>100 mL</td>
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