

# Fully Automated, High Purity Cell Isolation for Chimerism Labs

In this technical bulletin, we describe a method to automate isolation of lymphoid (T and B cells) and myeloid lineages from human whole blood samples for chimerism analysis.

## Background

Lineage-specific chimerism analysis is an important tool for monitoring the outcome of allogeneic hematopoietic cell transplantations (allo-HCT). It involves investigating the presence of donor leukocytes within specific cell subsets of the host's peripheral blood or bone marrow.<sup>1,2</sup> As patients can show complete chimerism in one cell lineage and partial chimerism in another, lineage-specific chimerism analysis is becoming an increasingly common practice that offers several advantages over analyzing the entire leukocyte population.<sup>3,4</sup> Performing chimerism analysis requires sensitive and reliable techniques, and ensuring that cross-contamination between samples does not occur is critical to the success of downstream chimerism analyses.

Chimerism analysis is typically performed on small blood samples, and analysis of purified cell subsets often requires isolation of more than one cell type from a single starting sample. Chimerism analysis can be facilitated with EasySep™, a column-free immunomagnetic cell separation platform, which provides a fast and easy method to obtain highly purified cells (Figure 1). In addition, EasySep™ reagents have flexible protocols to isolate multiple cell types from the same sample.

To help large routine laboratories save time and increase throughput, EasySep™ can be automated using RoboSep™ instruments. RoboSep™ uses individual separation magnets and disposable pipette tips to eliminate and provide highly purified cells in sufficient quantities for downstream flow cytometry and DNA analyses.



RoboSep™-S

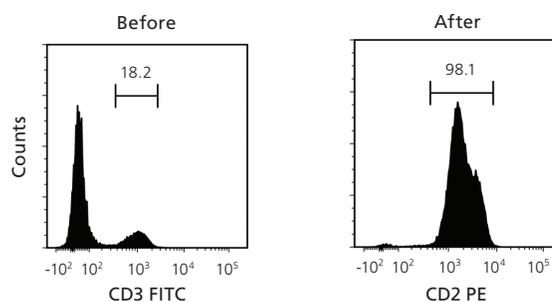
## Why Use RoboSep™ for Chimerism Analysis?

**HIGH PURITY.** Achieve purities of up to 99% in as little as 25 minutes.

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

**EFFICIENT.** Isolate multiple cell subsets from a single, low-volume sample with minimal hands-on time.

**NO CROSS-CONTAMINATION.** Maintain sample integrity through processing with individual magnets and pipette tips.



**Figure 1.** Cells Before and After Cell Isolation with EasySep™ HLA Chimerism Whole Blood CD3<sup>+</sup> Positive Selection Kit (Catalog #17871)

Starting with human whole blood from healthy donors, the CD3<sup>+</sup> 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 92.4 - 99.8%. 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. EasySep™ cell isolations can be automated using RoboSep™ instruments.

## Method: Automated Separation of Multiple Cell Types from a Single Sample

Below, we describe a method to sequentially isolate lymphoid (T and B cells) and myeloid lineages from human whole blood samples using RoboSep™-S, the fully automated cell isolation platform. The RoboSep™-S instrument uses EasySep™ column-free immunomagnetic technology and automates all the labeling and separation steps. After completion of the cell separation cycle, the cells of interest are immediately available for any application.

The carousel inside the RoboSep™-S instrument is divided into four quadrants (see page 3, bottom), each containing an EasySep™ magnet and designated space for loading sample and waste tubes and EasySep™ reagents, as well as a filter tip rack. The quadrant system allows RoboSep™-S to be programmed to separate cells simultaneously from up to four samples or to sequentially isolate up to four different cell types from a single starting sample.

### Sample Preparation

Samples of whole blood need to be prepared before loading them into the RoboSep™-S instrument for automated cell isolation, which can be performed on whole blood or buffy coat. To prepare whole blood, first collect your sample in a blood collection tube containing anticoagulant. Transfer a maximum of 4.5 mL of whole blood to a 14 mL polystyrene round-bottom tube and add 1X EasySep™ RBC Lysis Buffer (Catalog #20110) at a ratio of 1:1. The sample is now ready to be loaded into the RoboSep™-S instrument.

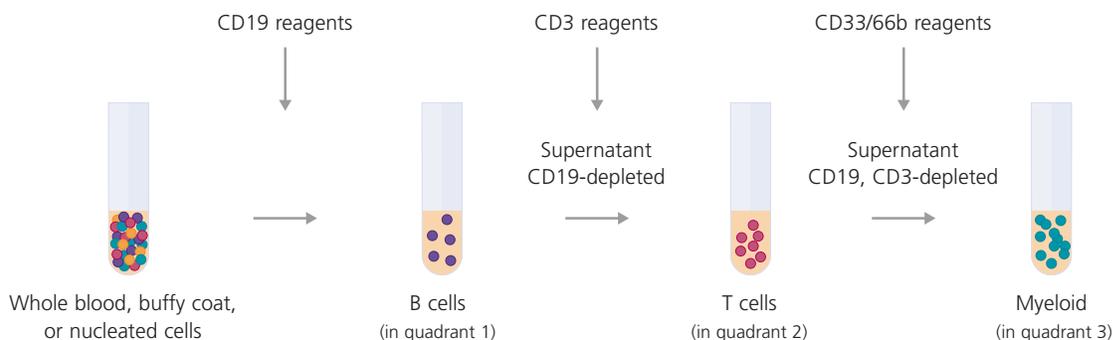
To prepare a buffy coat sample, add an equal volume of buffer (PBS with 2% FBS and 1mM EDTA) to the whole blood. Centrifuge the tube at 800 x g for 10 minutes at room temperature (15 - 25°C) with the brake off. Collect the concentrated leukocyte band (this is the buffy coat), plus a small portion of the plasma and concentrated red blood cells (RBCs). The target is to concentrate the leukocytes approximately 5-fold while maintaining the same hematocrit (e.g. collect 2 mL of buffy coat when starting with 10 mL of whole blood). Proceed with transferring and diluting the buffy coat sample, as described above for whole blood.

### Automated Cell Isolations with RoboSep™

The labeling and sequential separation of B cells (CD19<sup>+</sup>), T cells (CD3<sup>+</sup>), and myeloid cells (CD33<sup>+</sup>CD66b<sup>+</sup>) can be fully automated with RoboSep™ (see Figure 2). This 3-step process uses EasySep™ reagents for positive selection from an undivided sample of whole blood or buffy coat. First, the cells in the sample in quadrant 1 are labeled with antibody complexes targeting CD19<sup>+</sup> cells. After a short incubation, magnetic particles are added to the sample, followed by another short incubation. The sample with labeled CD19<sup>+</sup> cells linked to magnetic particles is then transferred to the tube sitting inside the magnet in quadrant 1. The labeled cells are held in the tube by the magnetic field and the supernatant containing unlabeled cells is transferred to the sample tube in quadrant 2. The fraction left behind in the tube sitting inside the magnet in quadrant 1 now contains the isolated CD19<sup>+</sup> cells.

Next, the CD19-depleted supernatant in quadrant 2 is labeled with anti-CD3 antibody complexes and magnetic particles, and transferred to the tube sitting inside the magnet in quadrant 2. After a short incubation of the sample in the magnet, the supernatant, which is now depleted of CD19 and CD3 cells, is transferred to the sample tube in quadrant 3, leaving highly purified CD3<sup>+</sup> cells in the tube sitting inside the magnet in quadrant 2.

Finally, the last fraction containing the myeloid cells is labeled with a combination of anti-CD33, anti-CD66b antibody complexes and magnetic particles, and is transferred to the tube sitting inside the magnet in quadrant 3. Unlabeled cells are removed to the waste tube, leaving highly enriched myeloid cells in the tube sitting inside the magnet in quadrant 3. To perform the cell isolation as described above, use the following reagents: EasySep™ HLA Chimerism Whole Blood CD19 Positive Selection Kit (Catalog #17874), EasySep™ HLA Chimerism Whole Blood CD3 Positive Selection Kit (Catalog #17871), and EasySep™ HLA Chimerism Whole Blood Myeloid Positive Selection Kit (Catalog #17884).

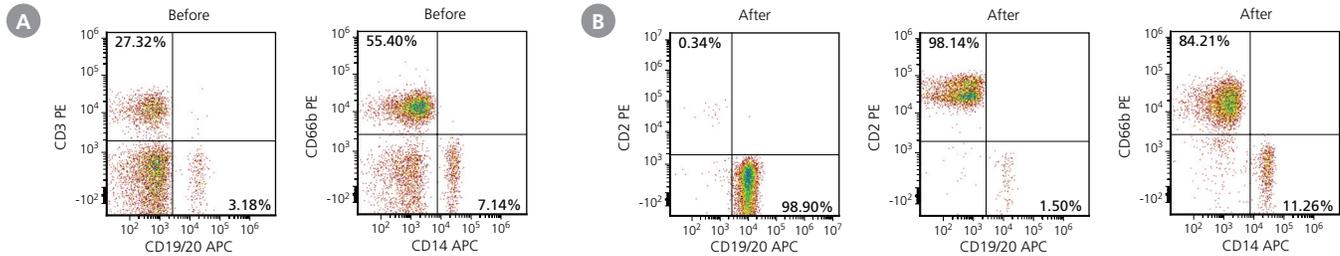


**Figure 2.** Fully Automated Sequential Separation of B Cells, T Cells, and Myeloid Cells From a Single Sample Using RoboSep™

The above illustration is an example of an automated sequential cell isolation protocol. RoboSep™-S has flexible protocols that allow for isolation of various cell types, as well as the separation of up to four different cell types from a single sample. The specific EasySep™ reagents and protocol will depend on the desired cells to be isolated.

## Results

Automated sequential cell isolations with RoboSep™-S from a single sample provides final isolated fractions with high cell purity and recovery rates (Figure 3, Table 1). Small starting samples can provide sufficient genomic DNA for downstream chimerism analysis (Table 2).



**Figure 3.** Typical Flow Cytometry Plots Before and After Enrichment of Selected Cells

In the above example, the B cell (CD19/20<sup>+</sup>), T cell (CD2<sup>+</sup> CD19/20<sup>+</sup>), and myeloid cell (CD14/CD66b<sup>+</sup>) content (A) in the starting human blood sample before isolation corresponded to 3.18%, 27.32% and 62.54%, respectively. (B) After cell isolation, the B cell (CD19/20<sup>+</sup>), T cell (CD2<sup>+</sup> CD19/20<sup>+</sup>), and myeloid cell (CD14/CD66b<sup>+</sup>) content in the isolated fraction corresponded to 98.90%, 98.14%, and 95.47%, respectively. Some antibodies used in positive selection may fully or partially block the primary cell surface marker. Therefore, after cell isolation, B cells were stained with anti-CD19 and anti-CD20 antibodies, T cells were stained with anti-CD2 antibodies, and myeloid cells were stained with anti-CD14 and anti-CD66b antibodies. Plots show viable (PI<sup>-</sup>) cells gated on CD45<sup>+</sup>.

**Table 1.** Percent Purity and Percent Recovery of B Cells, T Cells, and Myeloid Cells Isolated From a Single Whole Blood Sample Using RoboSep™

Catalog #	Cell Type	% Before Isolation	% After Isolation
17874	B Cells	7.2 ± 3.1	98.6 ± 0.9
17871	T Cells	23.7 ± 3.0	99.1 ± 0.6
17884	Myeloid Cells	62.6 ± 8.9	95.5 ± 1.5

B Cells (CD19/20<sup>+</sup>), T Cells (CD2<sup>+</sup> CD19/20<sup>+</sup>) and Myeloid Cells (CD14/CD66b<sup>+</sup>) were isolated from 1 mL of whole blood, and cell purities were determined by flow cytometry and gated on leukocytes (CD45<sup>+</sup>) and viable (PI<sup>-</sup>) cells. Values are expressed as means ± SD (n=3).

**Table 2.** Amount of Genomic DNA Isolated from Cells Obtained From a Single Whole Blood Sample Using RoboSep™-S

Catalog #	Cell Type	Average Number of Cells Recovered per mL of Whole Blood	DNA (μg) Recovered per mL WB
17874	B Cells	1.8 x 10 <sup>5</sup>	0.8
17871	T Cells	8.0 x 10 <sup>5</sup>	1.6
17884	Myeloid Cells	2.2 x 10 <sup>6</sup>	9.0

B Cells (CD19/20<sup>+</sup>), T Cells (CD2<sup>+</sup> CD19/20<sup>+</sup>) and Myeloid Cells (CD14/CD66b<sup>+</sup>) were isolated from 1 mL of whole blood using EasySep™ Positive Selection on RoboSep™-S. The number of B, T and myeloid cells recovered per mL of whole blood were determined by cell counting using a hemocytometer. DNA was isolated from the cells using a QIAamp DNA Blood Mini Kit (Qiagen) following the manufacturer's instructions and spin columns were spun at maximum speed at all steps. Results are the average of 3 donors (n=3).

## The RoboSep™ Advantage



### Simultaneous or Sequential Cell Isolation

The carousel, which is divided into four quadrants, allows for simultaneous cell isolations from up to 4 samples or sequential isolation of up to 4 cell types from the same sample.



### Simple Reagent and Experiment Tracking

Integrated barcode reader and End-of-Run reports track user, reagent, and protocol details.



### No Daily Maintenance

No daily washing or decontamination is required.



### Minimal Sample Handling with No Cross-Contamination

Automated sample handling with disposable pipette tips reduces the risk of exposure to dangerous pathogens and eliminates the risk of sample cross-contamination.



### Quick Start Options

Pre-program routine protocols and start a separation in just 5 minutes with minimal "hands-on" time.

# Product Listing

## Cell Separation Products for Chimerism Analysis

Cell Type	Selection Marker	EasySep™ Kit (Catalog #)		Compatible Anti-Human Staining Antibodies <sup>3</sup>
		Whole Blood <sup>1</sup>	MNC <sup>2</sup>	
T Cells	CD3	17871	17851	CD2, Clone RPA-2.10 (Catalog #60007) CD5, Clone UCHT2 (Catalog #60082) CD20, Clone 2H7 (Catalog #60008)
B Cells	CD19	17874	17854	CD19, Clone HIB19 (Catalog #60005) CD20, Clone 2H7 (Catalog #60008)
	CD19/ CD20	17886	–	CD19, Clone HIB19 (Catalog #60005) CD22, Clone HIB22 (Catalog #60083)
Myeloid Cells	CD15	17881	18651	CD15, Clone HI98 CD45, Clone HI30 (Catalog #60018)
	CD33	17885	17876	CD66b, Clone G10F5 (Catalog #60086) CD14, Clone M5E2 (Catalog #60004)
	CD33/66b	17884	–	
Granulocytes	CD66b	17882	–	CD66b, Clone G10F5(Catalog #60086)
Monocytes	CD14	17878	17858	CD14, Clone M5E2 (Catalog #60004) CD36, Clone FA6-152 (Catalog #60084)
NK Cells	CD56	17875	17855	CD56, Clone HCD56 (Catalog #60021)
Hematopoietic Progenitor Cells	CD34	17879	17856	CD34, Clone 581 (Catalog #60013) CD34, Clone 8G12 (Catalog #60121)

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.
3. To assess cell purity by flow cytometry, gate on viable and CD45<sup>+</sup> cells. Refer to the product information sheet for more information on fully or partially blocked markers and recommended staining antibodies.

## RoboSep™ Instruments

Product	Catalog #
RoboSep™-S	21000
RoboSep™-16	23000

## References

1. Bader P et al. (2005) How and when should we monitor chimerism after allogeneic stem cell transplantation? Bone Marrow Transplant 35(2): 107–119.
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<sup>+</sup> chimerism and the incidence of graft-versus-host disease in patients undergoing allogeneic hematopoietic stem cell transplantation. Transplant Proc 43(5): 1915–23.

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