Isolation of Very Pure Lymphoid (CD3+, CD19+ or CD56+) and Myeloid (CD15+ or CD33/CD66b+) Cell Subsets in as Little as 15 Minutes for Use in Lineage-Specific Chimerism Analysis

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Introduction

Chimerism analysis is used to monitor the presence of donor leukocytes in a recipient following hematopoietic cell transplantation. Lineage-specific chimerism can increase sensitivity compared to analysing the entire leukocyte population, however it requires the isolation of highly purified cell subsets, as even a few contaminating cells can compromise the integrity of the assay. We describe a method (EasySep™) to rapidly isolate very pure lymphoid (CD3+, CD19+ or CD56+) and myeloid (CD15+ or CD33/CD66b+) cell subsets directly from whole blood (WB) or buffy coat (BC) in as little as 15 minutes. The separation procedure can be fully automated with RoboSep™ and is compatible with downstream DNA isolation methods. These new EasySep™ kits provide immunogenetics laboratories a fast and easy method to obtain highly purified cells for use in lineage-specific chimerism testing.

Methods

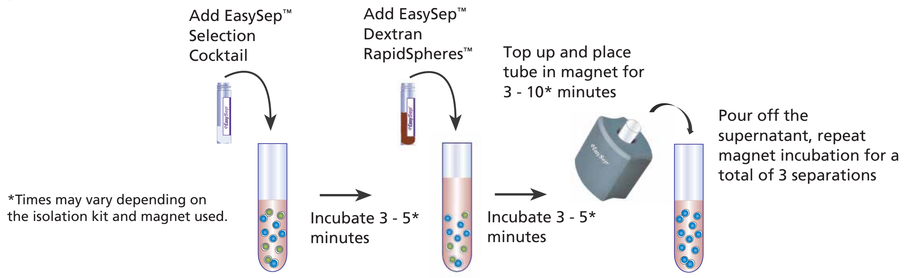
Samples:

Whole blood (WB): Peripheral blood, 24 or 48-hour post draw, was obtained commercially.
Buffy coat (BC): BC were generated following manufacturer's instructions on the Product Information Sheet.

Cell Isolation Strategy: WB or BC was diluted with an equal volume of EasySep™ Red Blood Cell Lysis Buffer (Catalog #20110) and cells were immunomagnetically labelled and then placed in a magnet. Target cells were retained in the magnet while unwanted cells were poured or pipetted off.

EasySep™ HLA Chimerism WB Positive Selection kits examined: CD3+ WB (Catalog #17871); CD19+ WB (Catalog #17874); CD56+ BC (Catalog #17875); CD33/CD66b+ Myeloid WB (Catalog #17884); CD15+ WB (Catalog #17881).

Figure 1. EasySep™ positive selection procedure

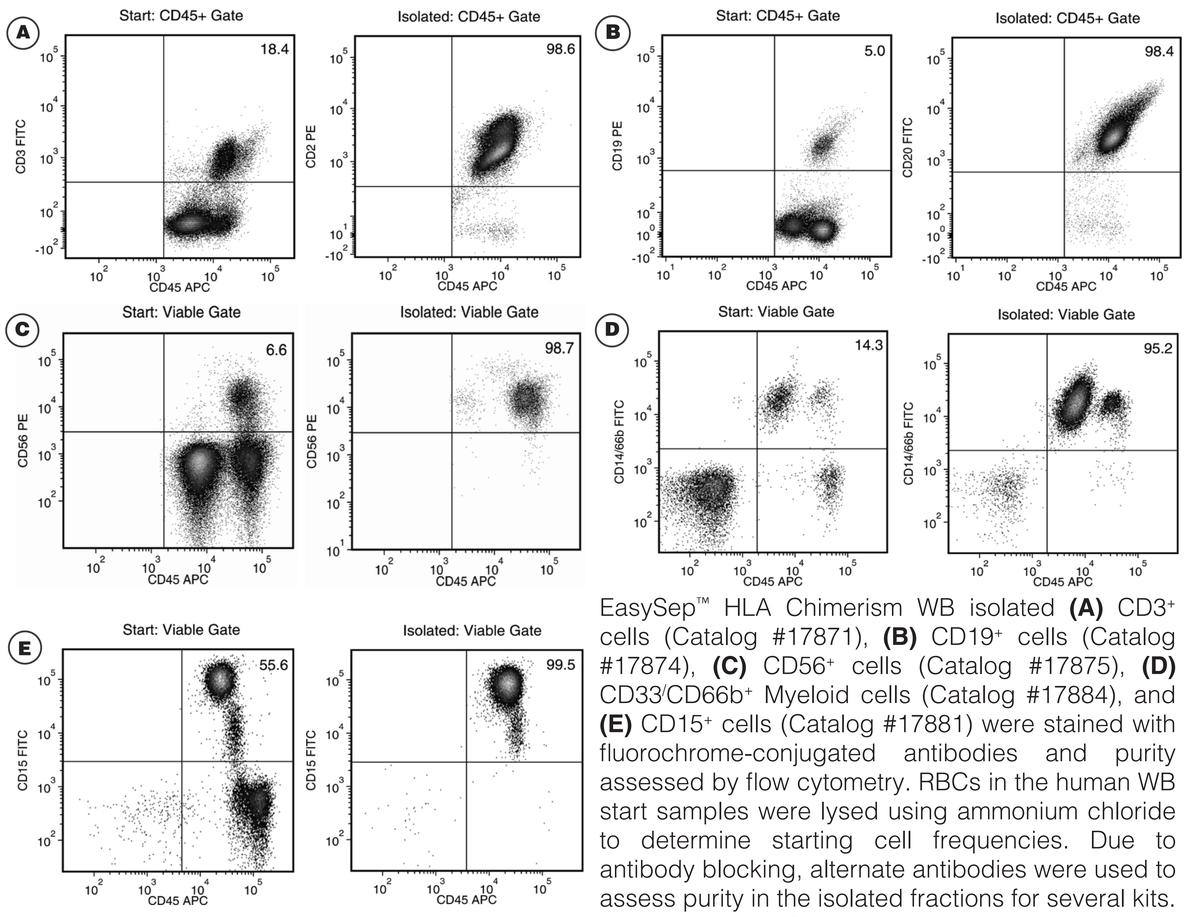


Purity Assessment: The percentage of cells isolated by each EasySep™ HLA Chimerism Positive Selection kit was determined by flow cytometry using fluorochrome-conjugated antibodies against CD45 and either CD3 and CD2 (for CD3+); CD19 and CD20 (for CD19+); CD56 (for CD56+); CD33, CD14 and CD66b (for CD33/CD66b+ Myeloid); or CD15 (for CD15+). Cells were also stained with the viability dye 7AAD.

Assessment of Cell Recovery: The number of CD3+, CD19+, CD56+, CD33/CD66b+ Myeloid and CD15+ cells per mL of WB or BC were determined by cell counting using a hemocytometer.

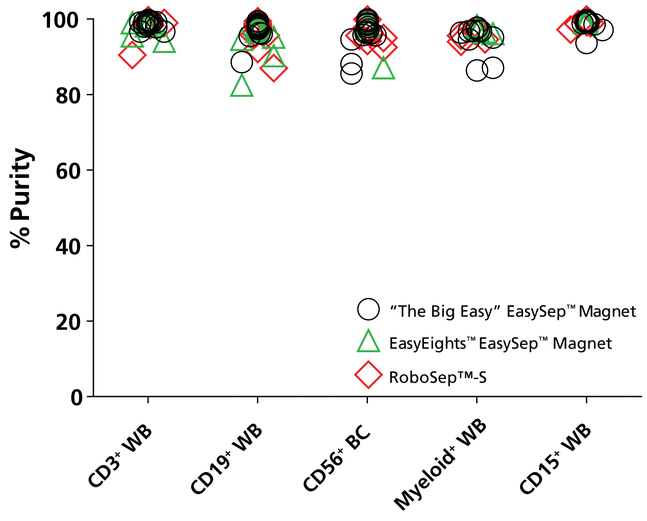
Results

Figure 2. Representative flow cytometry data of cells isolated using five different EasySep™ HLA Chimerism WB kits



EasySep™ HLA Chimerism WB isolated **(A)** CD3+ cells (Catalog #17871), **(B)** CD19+ cells (Catalog #17874), **(C)** CD56+ cells (Catalog #17875), **(D)** CD33/CD66b+ Myeloid cells (Catalog #17884), and **(E)** CD15+ cells (Catalog #17881) were stained with fluorochrome-conjugated antibodies and purity assessed by flow cytometry. RBCs in the human WB start samples were lysed using ammonium chloride to determine starting cell frequencies. Due to antibody blocking, alternate antibodies were used to assess purity in the isolated fractions for several kits. For example, CD3 purity in the isolated fraction was assessed using anti-CD2, CD19 purity was assessed using anti-CD20, and CD33/CD66b purity was assessed using anti-CD14 and CD66b.

Figure 2. All five EasySep™ HLA Chimerism WB Positive Selection kits yield average purities above 95% regardless of the isolation platform used



Average purities (± SD) for each isolation kit on three different magnet platforms are as follows: CD3+ WB (Catalog #17871) 99.0 ± 0.9 (n = 21, "The Big Easy" magnet), 98.4 ± 1.9 (n = 11, EasyEights™ magnet), 98.2 ± 2.9 (n = 9, RoboSep™-S); CD19+ WB (Catalog #17874) 97.5 ± 2.3 (n = 20, "The Big Easy" magnet), 96.1 ± 4.6 (n = 14, EasyEights™ magnet), 96.4 ± 3.4 (n = 13, RoboSep™-S); CD56+ BC (Catalog #17875) 96.4 ± 3.9 (n = 17, "The Big Easy" magnet), 96.2 ± 5.1 (n = 5, EasyEights™ magnet), 95.9 ± 2.4 (n = 7, RoboSep™-S); Myeloid+ WB (Catalog #17884) 94.8 ± 4.1 (n = 11, "The Big Easy" magnet), 97.5 ± 0.8 (n = 9, EasyEights™ magnet), 95.7 ± 1.4 (n = 5, RoboSep™-S); CD15+ WB (Catalog #17881) 99 ± 1.5 (n = 18, "The Big Easy" magnet), 99.4 (n = 5, EasyEights™ magnet), 99.4 ± 0.7 (n = 16, RoboSep™-S). Purities are based on samples gated on CD45+ cells.

Figure 3. Average numbers of cells (mean ± SD) recovered from 1 mL of WB or BC for the lymphoid or myeloid EasySep™ HLA Chimerism WB positive selection kits

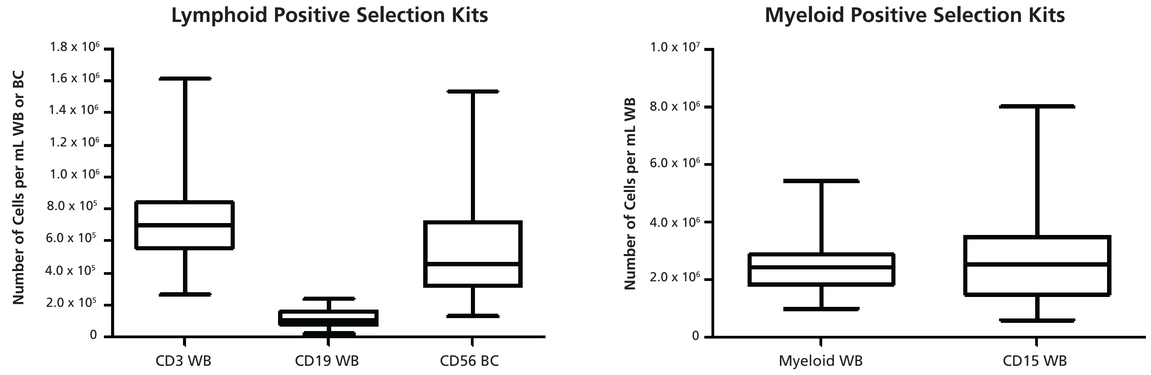


Table 1. Percent purity and number of cells recovered from 1 mL of WB or BC following isolation with the new EasySep™ HLA Chimerism WB Positive Selection kits

EasySep™ HLA Chimerism Kit	Kit Catalog #	n	% Purity of Isolated Cells	Number of Cells Recovered from 1 mL of WB or BC (mean ± SD)
CD3 WB Positive	17871	41	98.6 ± 1.8	$7.2 \times 10^5 \pm 2.7 \times 10^5$
CD19 WB Positive	17874	47	96.8 ± 3.4	$1.2 \times 10^5 \pm 5.7 \times 10^4$
CD56 BC Positive	17875	29	96.2 ± 3.7	$5.8 \times 10^5 \pm 3.5 \times 10^5$
Myeloid WB Positive	17884	25	95.9 ± 3.0	$2.6 \times 10^6 \pm 9.5 \times 10^5$
CD15 WB Positive	17881	39	99.2 ± 1.0	$2.7 \times 10^6 \pm 2.7 \times 10^5$

Average purity and cell recovery (mean ± SD) were compiled from all magnet platforms combined.

Summary

- Lymphoid (CD3+, CD19+ or CD56+) or myeloid (CD33/CD66b+ or CD15+) cells can be rapidly isolated using the new EasySep™ HLA Chimerism WB kits.
- Average cell purities greater than 95% can be achieved for each kit, regardless of the magnet platform used.
- On average, between 1 - 7 x 10^5 lymphoid cells and 2 x 10^6 myeloid cells can be recovered from 1 mL of blood or buffy coat, depending on the cell type.
- Cell isolations can be automated using RoboSep™.