Isolation of Highly Purified Lymphocyte Subsets Directly from Whole Blood

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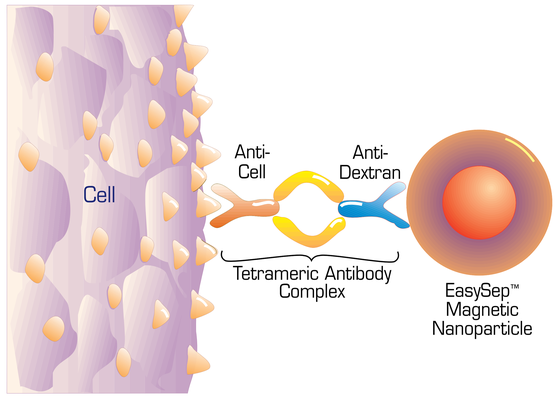
Introduction

Previous efforts to purify subpopulations of nucleated cells (NCs) directly from whole blood have been hindered by the abundance of RBCs and the correspondingly low frequency of NCs (<0.1%). Most techniques require a time-consuming prepatory step to remove RBCs and concentrate nucleated cells. This is especially true for manual column-based separations where preparation of mononuclear cell (MNC) suspensions using Ficoll® density separation takes 40 minutes and typically incurs a 40% loss of lymphocytes. To obviate these time and cell loss problems we have used recent advances in magnetic particle technology and magnet design to develop a column-free magnetic cell separation technique (EasySep™) in order to positively select lymphocytes directly from whole blood.

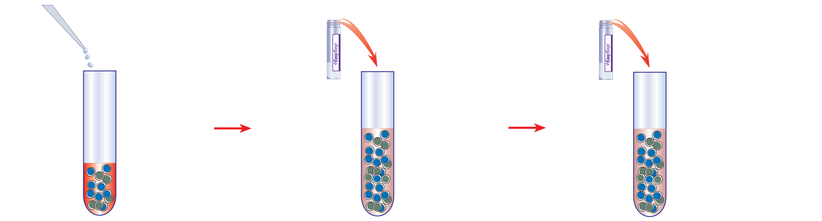
Figure 1. Schematic drawing of EasySep™ magnetic labeling of human cells



Method

Heparinized whole blood from a normal donor in a standard 14 mL centrifuge tube is first diluted 1:1 with a RBC lysis buffer solution, and then incubated for 15 minutes at room temperature with bi-specific (*i.e.* anti-cell x anti-dextran) antibody complexes, followed by dextran-coated submicron superparamagnetic nanoparticles for 10 minutes at room temperature (Figure 1). The tube containing the cells is then placed in an EasySep™ magnet. After a minimum of 5 minutes the supernatant containing the unlabeled cells is poured off. The tube is then removed from the magnet and the selected cells are resuspended in cell culture medium. To improve the purity of the targeted cells, the magnetic separation procedure is repeated for 5 minutes twice more, for a total of three rounds of separation (Figure 2). Cells from the start and enriched fractions were analyzed using standard FACS procedures.

Figure 2. Procedure for EasySep™ positive cell selection from whole blood



Add RBC lysis buffer solution at a 1:1 ratio of buffer to whole blood

Immediately add EasySep™ positive selection cocktail to diluted whole blood

Incubate for 15 minutes at room temperature

Add EasySep™ magnetic particles

Incubate 10 minutes at room temperature

Place tube in magnet for a minimum of 5 minutes

Pour off supernatant

Positively selected cells remain in tube

Remaining cells are resuspended and the tube is placed back in the magnet for two additional 5 minute rounds of separation

Results

We used EasySep™ to positively select cells based on a range of common lymphocyte markers (CD3, CD4, CD8 and CD19). Table 1 shows that high purity was obtained for these target cells (in all cases >95%). In addition, the recovery is high for all target cell types. Table 2 highlights the benefit to recovery by comparison to MACS® positive selection from MNC suspensions. Target cell recovery was calculated from the initial whole blood sample, thus recoveries reported for the Ficoll®/MACS® method include cell losses due to Ficoll® density separation (typically 40% loss).

In a subset of enriched samples (originating from 6 donors of a 21 donor pool) the light scatter dot plots obtained by flow cytometry consistently exhibited a smeared population that was low in forward scatter and high in side scatter (Figure 4C). The smear appears to consist only of particles and not cells or platelets, as determined by staining with fluorescently labeled antibodies against dextran, CD45 and CD41 respectively. Using a slow spin to remove plasma and platelets or a fast spin to remove just plasma prior to addition of the magnetic labeling reagents eliminated the smear. It is therefore presumed that the smear is due to particle aggregates induced by an unknown plasma factor present in a fraction of donors. When assessing purity, any aggregates were gated out based on light scatter characteristics and/or a lack of CD45 expression.

Figure 3. FACS profiles from a typical EasySep™ CD8⁺ cell selection from whole blood.
Viability is assessed based on PI exclusion.

Start:
5.4% CD8⁺ cells (of viable)

Enriched:
99.0% CD8⁺ cells (of viable)

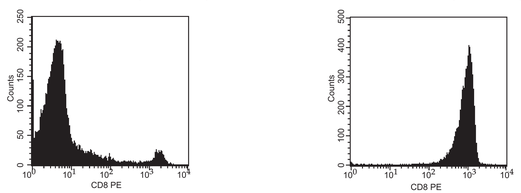
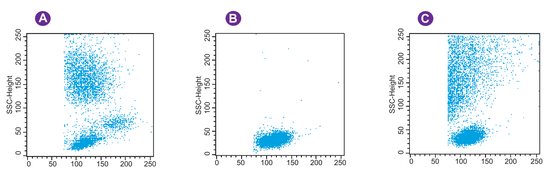


Figure 4. FSC/SSC properties of a typical sample of unseparated whole blood (A), a typical enriched cell sample (B) and an atypical enriched sample showing a smear of particles (C). RBC in the unseparated sample were lysed prior to FACS using an ammonium chloride solution.



Typical Start | Typical Enriched Fraction | Atypical Enriched fraction showing Particle Aggregates

Table 1. Purity and recovery of lymphocytes selected from whole blood using EasySep™. Purity is reported as percentage of viable (PI negative) cells. Results are expressed as mean ± 1 SD.

cell type	n	% purity in start	% purity in enriched	% recovery target cells	# target cells recovered per mL whole blood (x 10^5 cells)
CD3⁺	4	27.7 ± 12.8	99.3 ± 0.4	48 ± 26	6.9 ± 3.6
CD4⁺	4	25.8 ± 10.3	98.9 ± 1.2	48 ± 14	7.4 ± 2.7
CD8⁺	27	9.3 ± 4.0	99.3 ± 0.6	51 ± 17	3.4 ± 2.0
CD19⁺	7	4.5 ± 1.6	97.9 ± 1.9	42 ± 14	1.2 ± 0.3

Table 2. Target cell recovery is higher using EasySep™ selection directly from whole blood than for MACS® selection following MNC preparation by Ficoll® density separation.

	EasySep™		MACS®	
cell type	n	% recovery	n	% recovery
CD3⁺	4	48 ± 26	13	37 ± 14
CD4⁺	4	48 ± 14	14	40 ± 15
CD8⁺	27	51 ± 17	10	31 ± 8
CD19⁺	7	42 ± 14	15	40 ± 16

Conclusions & Benefits

- Cells are isolated at high purity directly from whole blood
- Cell recovery is higher than for separations from MNC suspensions
- No centrifuge or columns are required
- Cells isolated in less than an hour - eliminating Ficoll® step reduces processing time by approximately 40 minutes
- Cells recovered in a small volume and are immediately ready for further use

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