A Rapid Method to Enrich Specific Lymphocyte Populations (T cells, B cells, Total Lymphocytes) from Whole Blood

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Summary

The isolation of specific lymphocyte populations is essential for many HLA applications. While density centrifugation with Ficoll™ is used by most laboratories, it is a lengthy process and can result in lymphocyte loss of up to 50%. Isolation of specific cell types directly from whole blood requires a red blood cell lysis/wash step which is time consuming and is often toxic to certain cell subsets.

We have developed a rapid (20 minute) immunomagnetic cell separation system (EasySep®) that enriches for T cells, B cells or total lymphocytes from whole blood. This method does not require Ficoll™ density centrifugation or lysis to remove the red blood cell burden from the sample, but instead relies on hetastarch sedimentation (HetaSep™) which is less toxic, produces a high yield of lymphocytes and typically can be performed in 15 minutes.

Assessment by flow cytometry yields average purities greater than 90% for all cell types. As the cells of interest are not labeled with antibody, they are immediately available for all downstream HLA applications.

Methods

Preparation of sample using HetaSep™:

Whole blood was collected in a blood collection tube containing heparin or ACD. One part HetaSep™ (Catalog #07806) was added to 5 parts whole blood and mixed. The sample was placed in a 37°C incubator and allowed to settle until the red blood cell interface was at approximately 40% of the total volume. The supernatant was then harvested, washed once and centrifuged at room temperature at 120 x g for 10 minutes with the brake off. Cells were resuspended in 1/10th the original starting volume of whole blood.

The starting cell number:

From a starting blood volume of 5.0 mL, the number of nucleated cells used per experiment ranged from $7.5x10^6$ – $3.0x10^7$.

EasySep® enrichment kits used to isolate specific lymphocyte subsets from whole blood:

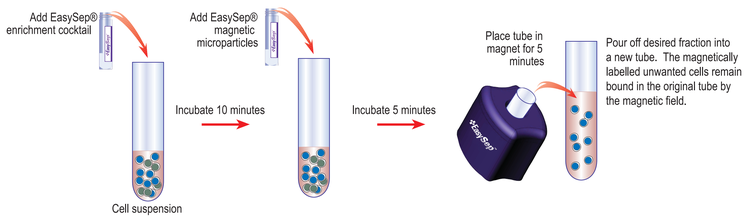
Total Lymphocyte Enrichment: Catalog #19961HLA
T Cell Enrichment: Catalog # 19951HLA
B Cell Enrichment: Catalog # 19954HLA

Following HetaSep™ treatment of the whole blood, cells are labeled with a cocktail of antibodies targeting unwanted cells. Cells are then coupled to magnetic particles and the sample is placed in a magnet. Labeled unwanted cells remain in the sample tube in the magnet, while the unlabeled cells of interest are removed to a new tube.

Cell isolation can be performed manually, or can be automated using RoboSep®.

Purity of selected cell populations was determined by flow cytometry. Total lymphocytes were defined as $CD3^+/CD19^+$; T cells were defined as $CD3^+$; B cells were defined as $CD19^+$. Only nucleated cells ($CD45^+$) were included in this assessment.

Figure 1: Schematic Representation of the Manual EasySep® Procedure.



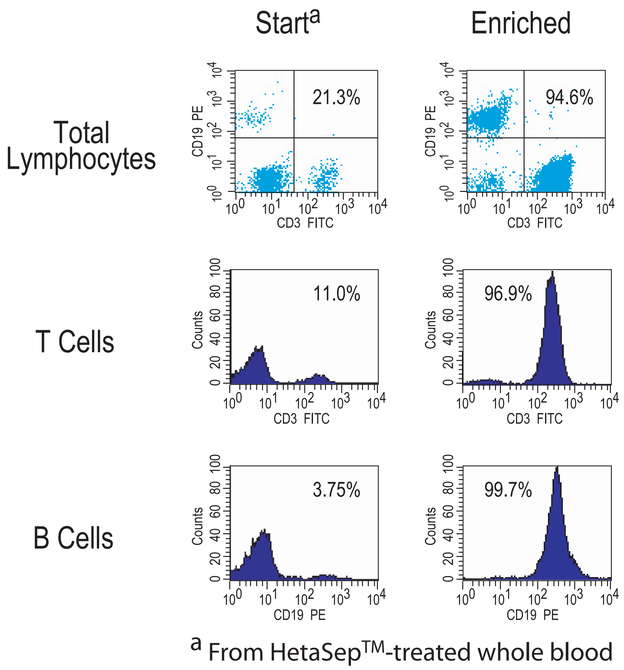
Results

Table 1. Percent purity, recovery and average number of enriched cells obtained from 5.0 mL of whole blood using the EasySep® Total Lymphocyte (TL), T cell and B cell enrichment kits. Purities were determined by flow cytometry. All samples were gated on $CD45^+$, viable (PI negative) cells. Values are expressed as means +/- 1 standard deviation.

	n	% Start [a]	% Purity	% Recovery [a]	# Enriched Cells ($x10^6$) [b]
TL	8	26.9 ± 8.8	93.6 ± 2.3	73.4 ± 12.6	2.6
T cell	16	21.6 ± 8.6	96.6 ± 1.6	76.8 ± 25.5	2.2
B cell	17	4.1 ± 1.6	96 ± 5.6	58.5 ± 20.3	0.4

[a] From HetaSep™ - treated whole blood
[b] From 5.0 mL of whole blood starting sample

Figure 2. Typical FACS plots before and after enrichment of selected cells (gated on $CD45^+$ cells).



[a] From HetaSep™-treated whole blood

Table 2. Direct comparison between the number of purified cells obtained from 10 mL of whole blood using either EasySep® enrichment or a competitor's negative selection system. EasySep® HLA enrichment was performed on HetaSep™-treated blood, whereas the competitor's enrichment was performed on a mononuclear cell suspension. In both systems, 10 mL of whole blood from the same donor was used as the starting sample.

	Average Number of Enriched Cells ($x10^6$)		
Cell Type	EasySep®	Competitor	Fold Difference
T Cells [a]	6.5	3.5	1.8
B Cells [b]	0.9	0.43	2.6

[a] average from 3 experiments
[b] average from 6 experiments

Conclusions

- **Total lymphocytes, T cells or B cells can be rapidly isolated in approximately 20 minutes**
- **The red blood cell burden of the sample is reduced prior to cell isolation by hetastarch sedimentation using HetaSep™**
- **No layering of Ficoll™ or post-enrichment lysis of the sample is required**
- **Purity of total lymphocytes ranges from 90.2 – 96.9%, purity of T cells ranges from 93.1 – 98%, while purity of B cells ranges from 81.5 – 99.4%**
- **A 5.0 mL whole blood start sample yields enough cells for many HLA applications**
- **Purified cells are unlabeled (no antibodies or magnetic particles) and are immediately available for all HLA applications**

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