



The Sequential Isolation of Both T Cells and B Cells from a Single Sample of Mononuclear Cells or Whole Blood

Introduction

Clinical laboratories often need to isolate different lymphocyte subsets from small samples. This report describes a method to isolate both T cells and B cells from a single sample of either mononuclear cells or whole blood using EasySep® selection. As this procedure does not require division of the start sample, the number of T and B cells that can be isolated is maximized. The isolated T and B cell populations can then be used individually in downstream applications.

Methods

Preparation of mononuclear cells

Mononuclear cells are isolated from peripheral blood by density centrifugation using Ficoll™-Hypaque. Cells are resuspended at 1×10^8 cells/ml prior to EasySep® separation.

Preparation of whole blood using HetaSep™

This method does not require Ficoll™ density centrifugation or lysis to remove the red blood cell burden from whole blood, but instead relies on hetastarch sedimentation (HetaSep™).

In brief, whole blood is collected in a blood collection tube containing heparin or ACD. One part HetaSep™ (Catalog #07806) is added to 5 parts whole blood and mixed. The sample is placed in a 37°C incubator and allowed to settle until the red blood cell interface is at approximately 40% of the total volume (~15-30 min). The supernatant is then harvested, washed once and centrifuged at room temperature at 120 x g for 10 minutes with the brake off. Cells are resuspended in 1/10th the original starting volume of whole blood prior to EasySep® separation.

Table 1. EasySep® kits used to sequentially isolate T cells and B cells from mononuclear cells or whole blood.

Product	Catalog #
CD3 Positive Selection	18051HLA
B Cell Negative Selection	19954HLA

Summary of sequential separation procedure (see Figure 1)

Following isolation of mononuclear cells or HetaSep™-treatment of whole blood, cells are labeled with an antibody cocktail targeting CD3⁺ cells. Cells are then coupled to magnetic nanoparticles and the sample is placed in a

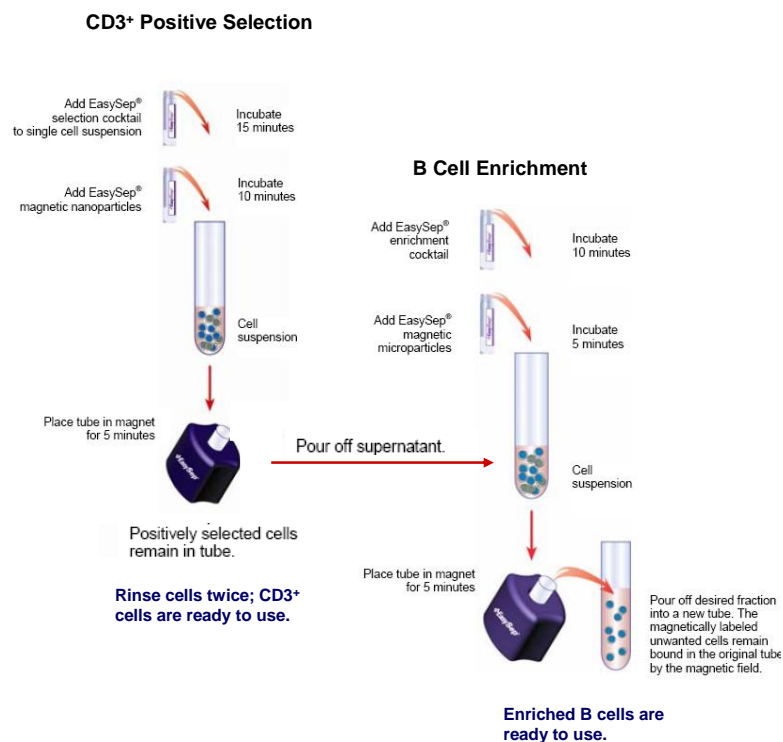
magnet. The labeled cells of interest (T cells) remain in the sample tube in the magnet, while the supernatant containing the unlabeled cells (the B cell-containing fraction) is removed to a new tube. The supernatant is then labeled with a cocktail of antibodies targeting unwanted cells and magnetic microparticles and placed in a magnet. Labeled unwanted cells remain in the sample tube in the magnet, while the unlabeled B cells are then transferred to a new tube.

Sequential cell isolation can be performed manually, or can be fully automated using RoboSep®.

Assessment of start and enriched cell populations

The purity of each cell population is determined by flow cytometry.

Figure 1. Schematic representation of the manual EasySep® T cell and B cell sequential separation procedure.



Results

The results of a typical experiment are shown below. T cells in the start population are defined as CD3⁺; T cells in the enriched fraction are defined as CD2⁺; and B cells are defined as CD19⁺. Only nucleated, viable cells (CD45⁺/PI-negative) are included in this assessment.

Table 2. Percent purity and average number of T cells and B cells isolated from a single sample of mononuclear cells or whole blood.

Start Sample	n	T Cells		B Cells	
		% Purity	# Enriched Cells [x10 ⁶]	% Purity	# Enriched Cells [x10 ⁶]
PBMC ^a	4	97.0 - 99.2	0.93 - 1.69	88.0 - 96.5	0.15 - 0.27
WB ^b	11	87.8 - 98.3	0.96 - 3.43	86.9 - 94.9	0.1 - 0.52

a - from 5x10⁷ PBMC freshly isolated by density centrifugation

b - from 5.0 ml whole blood treated with HetaSep™

Figure 2. Typical FACS plots before and after the sequential isolation of T cells and B cells from 5x10⁷ mononuclear cells. The B cell start purity is based on the initial sample of mononuclear cells.

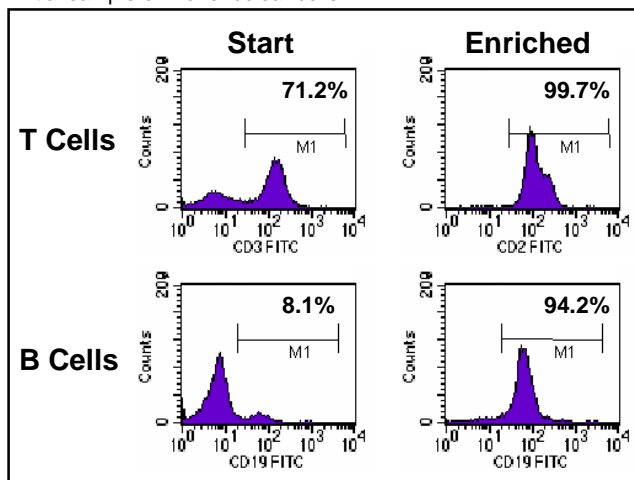
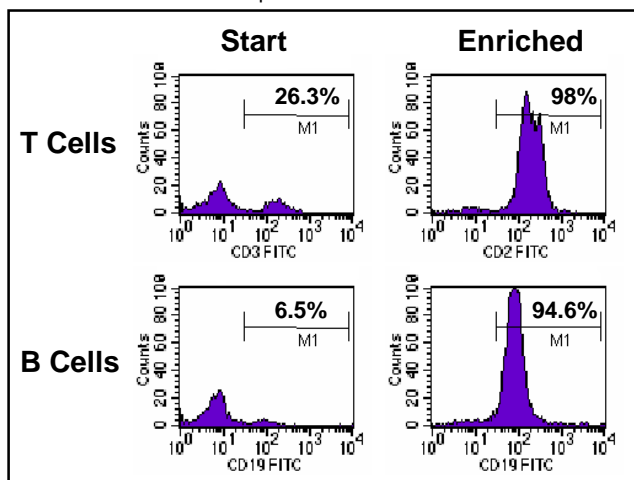


Figure 3. Typical FACS plots before and after the sequential isolation of T cells and B cells from 5.0ml of whole blood treated with HetaSep™. The B cell start purity is based on the initial sample of whole blood.



Summary

- Labeled T cells and unlabeled B cells can be sequentially isolated from a single sample of either mononuclear cells or whole blood.
- Purity of T cells from mononuclear cells ranges from 97.0 – 99.2%, while purity of B cells ranges from 88.0 – 96.5%.
- Purity of T cells from whole blood ranges from 87.8 – 98.3% while purity of B cells ranges from 86.9 – 94.9%.
- When starting from 5x10⁷ mononuclear cells, an average of 1.3x10⁶ purified T cells and 2.1x10⁵ purified B cells are recovered.
- When starting from 5.0 ml of whole blood treated with HetaSep™, an average of 2.4x10⁶ purified T cells and 3.7x10⁵ purified B cells are recovered.

For a detailed protocol please contact StemCell Technologies' Technical Support at techsupport@stemcell.com.