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 FicollTM 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 #072006) 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.

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.

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>EasySep®</th>
<th>Competitor</th>
<th>Fold Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>T Cells</td>
<td>6.5</td>
<td>3.5</td>
<td>1.8</td>
</tr>
<tr>
<td>B Cells</td>
<td>0.9</td>
<td>0.43</td>
<td>2.6</td>
</tr>
</tbody>
</table>

* average from 3 experiments

** 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 – 90.4%
- 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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