A Simple and Fast Method for the Isolation of Mouse Lymphoid Progenitors from Bone Marrow

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Abstract

The expression of Lin-7/fluorescent-activated cell sorting (Cd127) in the common lymphoid progenitor (CLP) population marks initiation and commitment to the lymphoid lineage. CLPs hold potential for only B, T, and NK cell lymphoid lineages and are defined as Lin-Cd127-rK-Fsc-a-P. Study of lymphoid development largely relies on access to CLPs or other Cd127+ lymphoid progenitors. Lin-7/fluorescent-activated cell sorting (FACS) commonly used to isolate lymphoid progenitors is costly, time-consuming, and possibly detrimental to cell viability. We report here a simple EasySep™ cell separation method for enrichment of mouse lymphoid progenitors in two steps. The first step (negative selection) is to remove lineage positive cells. Subsequently, Cd127+ cells are positively selected. After selection, the purity of Lin-Cd127+ lymphoid progenitors reaches 95 ± 8%. The purity of more defined Lin-Cd127+ rK-Fsc-a-P CLPs increases from 0.07 ± 0.05% in starting whole bone marrow (BM) to 4.9 ± 2.3% in the enriched fraction (fold enrichment: 69). Limiting dilution assays using enriched cells by negative followed by positive selection show the enrichment of T, B, and NK progenitors as compared to the whole BM.

Methods

FIGURE 1: EasySep™ procedure for column-free enrichment of lymphoid progenitors

- **Negative Selection**: Cells are passed over EasySep™ magnetic beads to remove lineage positive cells.
- **Positive Selection**: The remaining enriched cells are collected and expanded.

The EasySep™ mouse lymphoid progenitor enrichment kit is designed to isolate lymphoid progenitors from mouse BM. Briefly, BM cells were prepared by crushing femur and tibia from C57BL/6 mice with a mortar and pestle. Clumps of cells and debris were removed by passing cell suspension through a 70µm mesh strainer. BM cells were collected and resuspended at 1 x 10^6 cells/ml in PBS ± 2% FBS and 1 mM EDTA with 5% newborn calf serum added. A representative experiment has been shown. Total viable cells are used for the analysis. To analyze CLPs, the Lin-Cd127+ (CD3, CD19, B220, NK1.1, Ter119, Gr-1, CD11b) Cd127+ cells (left panels) are first gated with subsequent gating on -CD35+CD14+ cells (right panel). The percentage of Lin-Cd127+ rK-Fsc-a-P CLPs is calculated from total viable cells and shown in the right panel. The rare Lin-Cd127+ rK-Fsc-a-P CD127+ cells (right panel) are positively selected using EasySep™ magnetic beads. Lin-Cd127+ rK-Fsc-a-P CLPs are enriched approximately 41-fold in the final purified fraction in this experiment.

Results

FIGURE 2: EasySep™ labeling of mouse bone marrow cells

Linage-positive cells are labeled with biotinylated antibodies and coated with magnetic particles using biotinated specific antibody cocktails (TAC). The unattached magnetically labeled cells are removed using the EasySep™ magnet. For positive selection, the lineage depleted cells are labeled with Cd127 followed by TAC and magnetic particles. Using the magnet, the desired cells are retained and separated from unwanted cells in the suspension.

TABLE 1: Purity, recovery, and fold enrichment of lymphoid progenitors enriched from bone marrow by EasySep™ negative selection followed by positive selection

<table>
<thead>
<tr>
<th>Cell Subset</th>
<th>Start Bone Marrow</th>
<th>Negative Selection (Linage depletion)</th>
<th>Negative Selection Followed by CD127 PE Positive Selection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lin-Cd127-</td>
<td>17 ± 10^6</td>
<td>0.6 ± 0.25</td>
<td>3 ± 0.1 ± 0.25</td>
</tr>
<tr>
<td>Lin-CD127=-rK-Fsc-a-P</td>
<td>0.07 ± 0.05</td>
<td>6.4 ± 0.25</td>
<td>6.4 ± 0.25</td>
</tr>
</tbody>
</table>
| Values expressed as mean ± SD. Purity determined by flow cytometry. Cell viability expressed by PI or DAPl negative gate and/or scatter profile. Viable typically ranges from 80-95%.

Conclusions

- Lin-Cd127+ lymphoid progenitors can be rapidly isolated from whole bone marrow using a column-free, EasySep™ negative enrichment followed by positive selection.
- 2 log enrichment of target cells (Table 1) with purity up to 35% can be achieved using this method.
- EasySep™ purified cells are enriched for progenitors with B, T, and NK cell differentiation potential.