A Simple Immunomagnetic, Column-Free Method for the Enrichment of Lymphoid Progenitors from Mouse Bone Marrow

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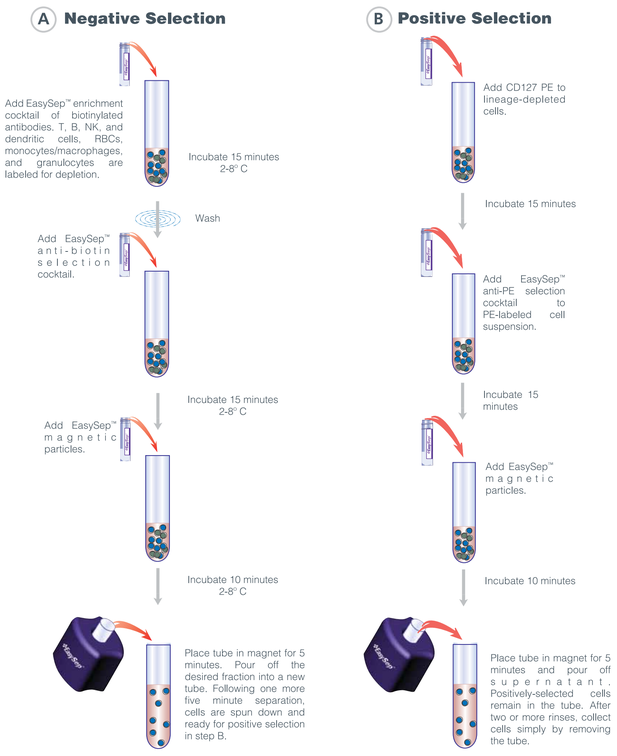
Introduction

Lymphocytes are derived from hematopoietic stem cells through an important developmental intermediate called a common lymphoid progenitor (CLP). The expression of IL-7Rα (CD127) in CLP population marks initiation and/or commitment to the lymphoid lineage. CLPs hold potential for B, T, and NK cell lymphoid lineages but lack myeloid and erythroid potential, and are defined as lineage negative (Lin^-)$CD127^+c\text{-}Kit^{lo}Sca\text{-}1^{lo}$. We describe here a simple immunomagnetic, column-free cell separation method (EasySep™) for the enrichment of lymphoid progenitors from mouse bone marrow (BM) in two steps. The first step (negative selection) is to deplete lineage positive cells by cross-linking them to magnetic particles using biotinylated antibodies. Unwanted cells are separated using the EasySep™ magnet. The lineage-depleted fraction will then be subjected to positive selection via labeling of $CD127^+$ cells. After selection, the purity of Lin^-CD127^+ lymphoid progenitors as assessed by flow cytometry averages 35 ± 8% (n=17). The purity of more defined $Lin^-CD127^+c\text{-}Kit^{lo}Sca\text{-}1^{lo}$ CLPs increases from 0.07 ± 0.05% in starting whole BM to 4.9 ± 2.3 % in the enriched fraction (fold enrichment: 69). Limiting dilution assays using cells enriched by negative followed by positive selection show the enrichment of T, B, and NK progenitors as compared to the whole BM. This easy and convenient system to enrich lymphoid progenitors is invaluable to the field of developmental immunology.

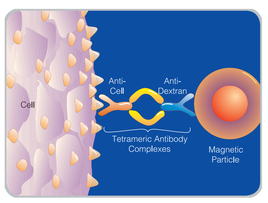
Methods

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



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 70 µm mesh nylon strainer. BM cells were collected and resuspended at 1 x 10^8 cells/ml in PBS + 2% FBS and 1 mM EDTA with 5% normal rat serum added.

FIGURE 2: EasySep™ labeling of mouse bone marrow cells



Lineage-positive cells are labeled with biotinylated antibodies and cross-linked to magnetic particles using bispecific tetrameric antibody complex (TAC). The unwanted 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 will be retained and separated from unwanted cells in the suspension.

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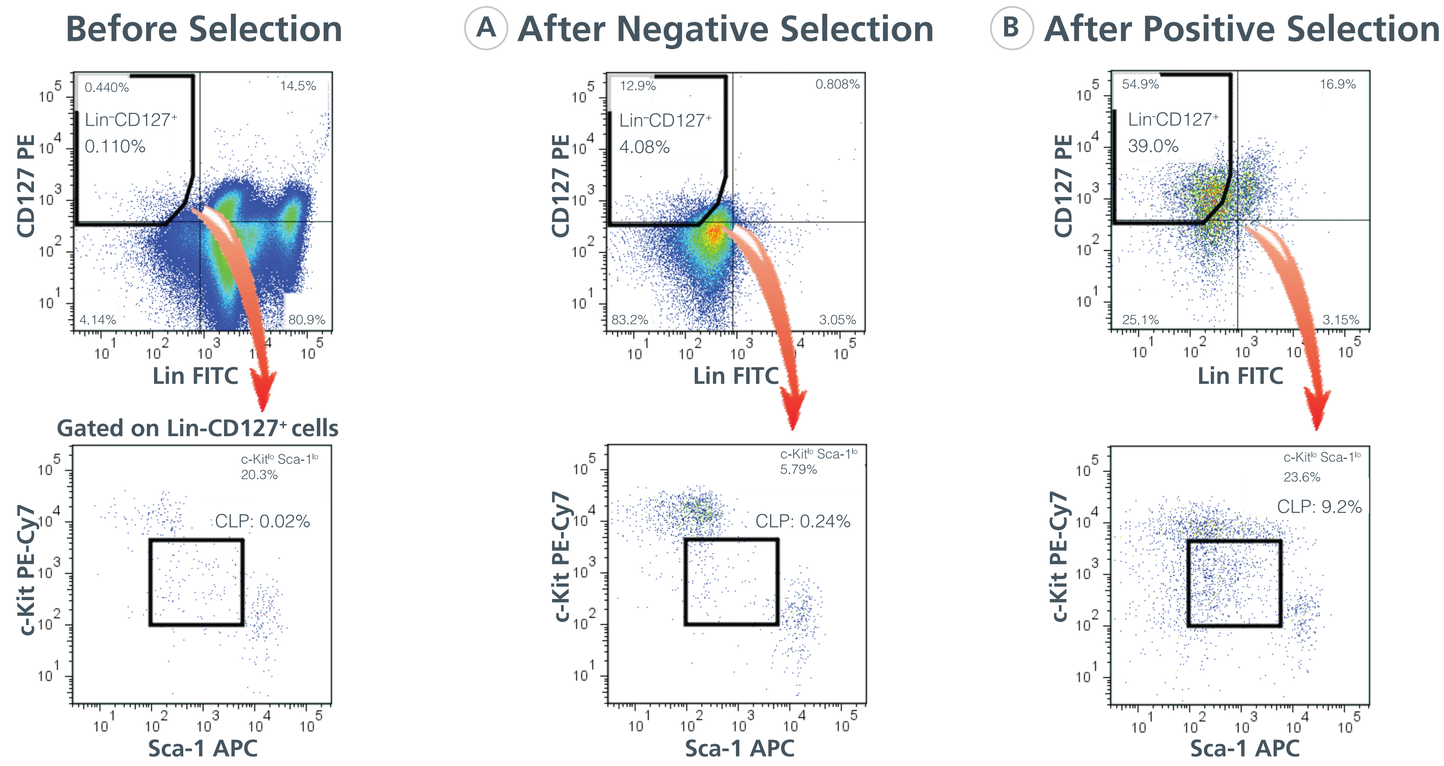
Results

FIGURE 3: FACS profiles before and after enrichment of lymphoid progenitors using EasySep™



A representative experiment has been shown. Total viable cells are used for the analysis. To analyse CLPs, the Lin⁻ (CD3, CD19, B220, NK1.1, Ter119, Gr-1, CD11b) $CD127^+$ cells (top panels) are first gated with subsequent gating on $c\text{-}Kit^{lo}Sca\text{-}1^{lo}$ cells (bottom panels). The percentage of $Lin^-CD127^+c\text{-}Kit^{lo}Sca\text{-}1^{lo}$ CLPs is calculated from total viable cells and shown in the bottom panels. The rare $Lin^-CD127^+c\text{-}Kit^{lo}Sca\text{-}I^{lo}$ CLPs are enriched approximately 418-fold in the final purified fraction in this experiment.

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

		Start Bone Marrow		Negative Selection (Lineage depletion)			Negative Selection followed by CD127 PE Positive Selection		
Cell Subset	n	Avg. total cell no.	% Purity	Avg. total cell yield	% Purity	Fold enrichment	Avg. total cell yield	% Purity	Fold enrichment[1]
Lin^-CD127^+	17	1 x 10^8	0.4 ± 0.23	1.0 x 10^6 ± 4.2 x 10^5	3.6 ± 2.0	9	8.4 x 10^4 ± 5.4 x 10^4	35 ± 7.7	87
$Lin^-CD127^+c\text{-}Kit^{lo}Sca\text{-}1^{lo}$			0.07 ± 0.05		0.42 ± 0.25	6		4.9 ± 2.3	69

Values expressed as mean ± SD. Purity determined by flow cytometry. Viable cells gated using PI or DAPI staining (PI or DAPI negative gate) and/or scatter profile. Viability typically ranges from 80-95%.

[1]Fold enrichment for positive selection is based on start purity.

TABLE 2: Limiting dilution assay showing progenitor frequencies within start bone marrow and EasySep™ enriched cell populations

	Start Bone Marrow			Negative Selection			Negative Selection followed by CD127 PE Positive Selection			
	n	Average frequency	Range	n	Average frequency	Range	n	Average frequency	Range	Fold enrichment
B progenitors	10	1/461	1/2392 - 1/204	10	1/425	1/2664 - 1/114	16	1/80	1/295 - 1/36	6
T progenitors	8	1/817	1/1984 - 1/411	8	1/51	1/368 - 1/16	9	1/16	1/40 - 1/9	51
NK progenitors	5	1/542	1/1342 - 1/290	4	1/846	1/2478 - 1/432	6	1/65	1/142 - 1/49	8

Various dilutions of start BM cells as well as cells enriched by negative selection or by sequential negative and positive selection were transferred onto 96-well plates containing either OP9 (B and NK cell assay) or OP9-DL1 (T cell assay) stromal cell lines. B and T cell cultures were supplemented with cytokines IL-7 and Flt3-L while IL-2 and IL-15 were added to NK cell cultures. After two weeks, cultures were analysed by flow cytometry to score for B cells ($CD19^+$), T cells ($CD25^+$) and NK cells ($NK1.1^+$). The statistical analysis to determine progenitor frequencies was performed using L-Calc™ software (STEMCELL Technologies).

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.