Abstract #1067789

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

Nooshin Tabatabaei-Zavareh, Maureen A. Fairhurst, and Terry E. Thomas

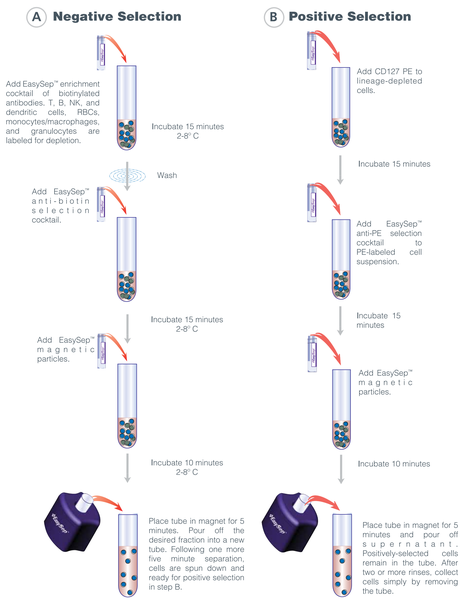
STEMCELL Technologies Inc., Vancouver, BC, Canada

Introduction

The expression of IL-7Rα (CD127) in the common lymphoid progenitor (CLP) population marks initiation and/or commitment to the lymphoid lineage. CLPs hold potential for only B, T, and NK cell lymphoid lineages and are defined as Lin$^-$CD127$^+$c-Kit^{lo}Sca-1^{lo}. Study of lymphocyte development largely relies on access to CLPs or other CD127$^+$ lymphoid progenitors. Fluorescence-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 the 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 35 ± 8%. The purity of more defined Lin$^-$CD127$^+$c-Kit^{lo}Sca-I^{lo} 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 cells enriched by negative followed by positive selection show the enrichment of T, B and NK progenitors as compared to the whole BM.

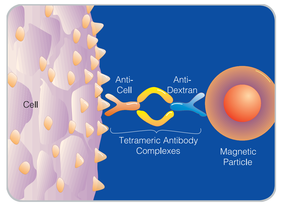
Methods

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



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.

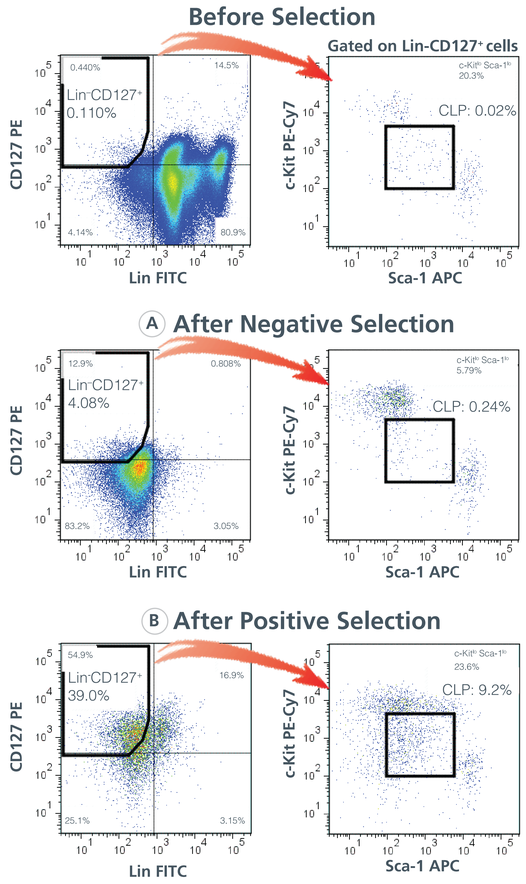
FIGURE 2: EasySep™ labeling of mouse bone marrow cells



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.

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 (left panels) are first gated with subsequent gating on c-Kit^{lo}Sca-1^{lo} cells (right panels). The percentage of Lin$^-$CD127$^+$c-Kit^{lo}Sca-1^{lo} CLPs is calculated from total viable cells and shown in the right panels. The rare Lin$^-$CD127$^+$c-Kit^{lo}Sca-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	3.6 ± 2.0	9	8.4 x 10^4	35 ± 7.7	87
Lin$^-$CD127$^+$c-Kit^{lo}Sca-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 BM 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	1/847	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 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.**